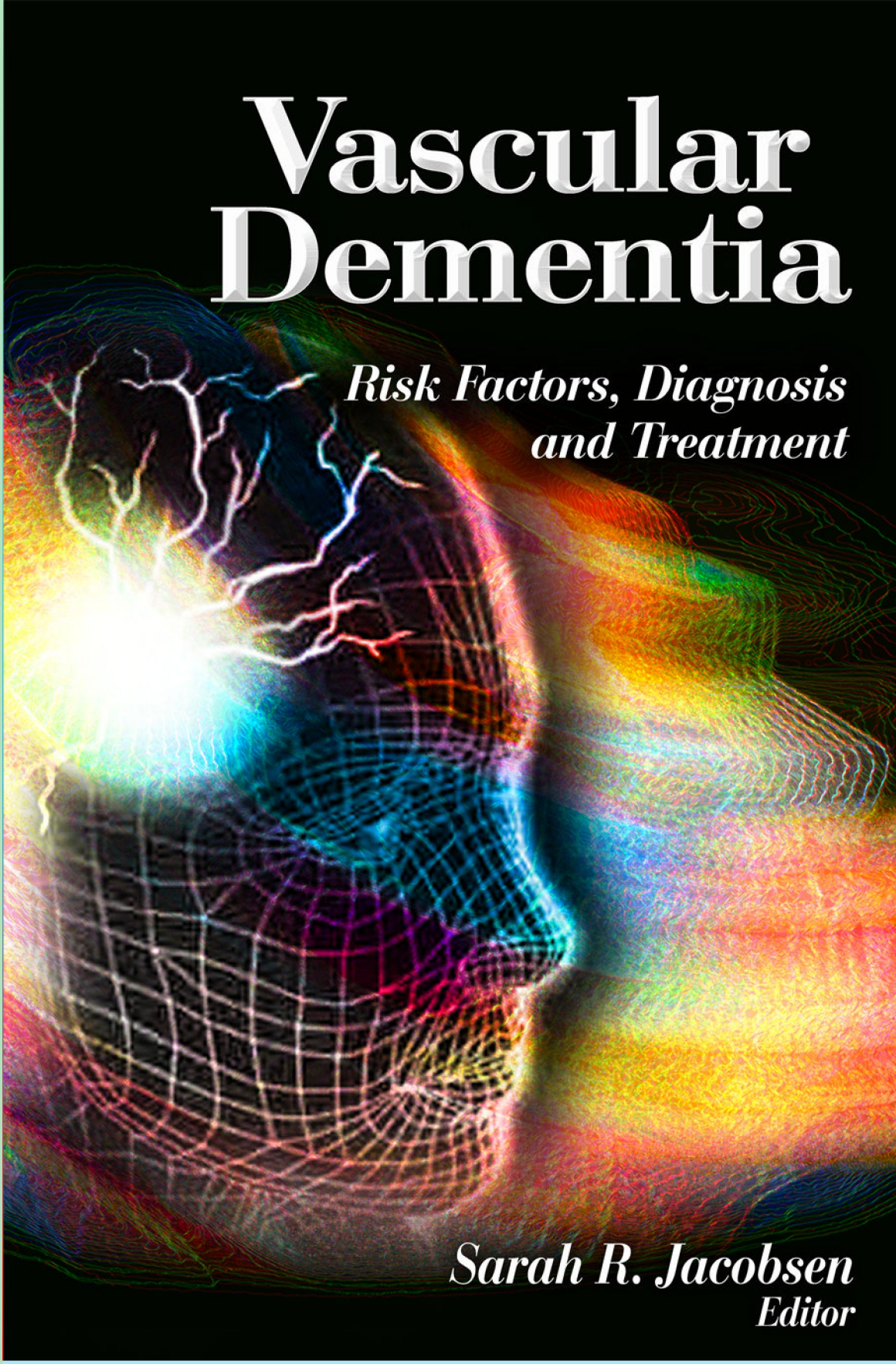
Vascular Dementia

Risk Factors, Diagnosis and Treatment



Sarah R. Jacobsen
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VASCULAR DEMENTIA: RISK FACTORS, DIAGNOSIS AND TREATMENT

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Contents

Preface

Chapter 1 – In modern societies, lifestyle changes and the acquisition of sedentary habits have been linked to the rise in the prevalence of vascular risk factors, which may increase the risk of central nervous deterioration in aging. Previous studies have associated cerebrovascular changes occurring in later life with cognitive and structural brain characteristics. The detection of initial states of vascular dysregulation in the brain could prevent the progression to pathological conditions such as vascular cognitive impairment. Accordingly, the concept of brain-at-vascular-risk highlights the importance of detecting prodromal states of unsuccessful aging. Today most vascular risk factors are treatable, and so it should be possible to prevent, postpone, or mitigate vascular cognitive impairment as well as the vascular exacerbation of Alzheimer disease.

This chapter describes the scientific evidence of the effect of vascular risk factors in structural brain changes, and their influence on cognition in aging. Today, a limited percentage of the population present isolated vascular risk factors such as type 2 diabetes, hypertension, obesity and dyslipidemia, or in many cases a combination of these factors. Some authors have proposed that a cluster of vascular risk factors known as the Metabolic Syndrome has a synergistic effect. At present, the prevalence of Metabolic Syndrome is between 20% and 30% of the world's population, and increases during aging. The increasing presence of Metabolic Syndrome during aging highlights the importance of establishing its effects on the central nervous system(CNS) during this stage of life and its influence on the development of cerebrovascular pathology.

Metabolic Syndrome has been linked to dementia, fronto-subcortical symptoms and poorer cognitive performance associated with aging. It has also been related to white matter lesions as a sign of small vessel disease. Their previous findings provide evidence that Metabolic Syndrome is related to subtle white matter alterations and a specific neuropsychological profile suggesting that it has an influence on the CNS probably due to the chronic state of vascular dysregulation in the brain. Metabolic Syndrome may be a prodromal state of vascular cognitive impairment.

In summary, this chapter reviews the effects of vascular risk factors in the brain and cognition, and their influence in unsuccessful aging.

Chapter 2 – Mitochondrial dysfunction may be a principal underlying event in aging, including age-associated brain degeneration. Mitochondria provide energy for basic metabolic processes. Their decay with age impairs cellular metabolism and leads to a decline of cellular function. Alzheimer disease (AD) and cerebrovascular accidents (CVAs) are two leading

causes of age-related dementia. Increasing evidence strongly supports the theory that oxidative stress, largely due to reactive oxygen species (ROS), induces mitochondrial damage, which arises from chronic hypoperfusion and is primarily responsible for the pathogenesis that underlies both disease processes. Mitochondrial membrane potential, respiratory control ratios and cellular oxygen consumption decline with age and correlate with increased oxidant production. The sustained hypoperfusion and oxidative stress in brain tissues can stimulate the expression of nitric oxide synthases (NOSs) and brain endothelium probably increase the accumulation of oxidative stress products, which therefore contributes to blood brain barrier (BBB) breakdown and brain parenchymal cell damage. Determining the mechanisms behind these imbalances may provide crucial information in the development of new, more effective therapies for stroke and AD patients in the near future.

Chapter 3 – Increasing evidence points to vascular damage as an early contributor to the development of two leading causes of age-associated dementia, namely Alzheimer disease (AD) and AD-like pathology such as stroke. This review focuses on the role of G protein-coupled receptor kinases, particularly GRK2 as they relate to dementia and how the cardiovasculature is involved in cerebrovascular pathogenesis. Any possible involvement of GRKs in AD pathogenesis is an interesting notion, whose exploration may help bridge the gap in their understanding of the heart-brain connection in relation to neurovisceral damage and vascular complications in AD. The a priori basis for this inquiry stems from the fact that kinases of this family regulate numerous receptor functions in the brain, the myocardium and elsewhere. The aim of this review is to discuss the finding of GRK2 overexpression in the context of the early AD pathogenesis, since increased levels of GRK2 immunoreactivity were found in vulnerable neurons from AD patients and from a two-vessel occlusion (2-VO) mammalian model of cerebral ischemia. Also, the authors consider the consequences for this overexpression as a loss of G-protein coupled receptor (GPCR) regulation, as well as suggest a potential role for GPCRs and GRKs in a unifying theory of AD pathogenesis and cerebrovascular disease. The authors therefore synthesize this newer information in an attempt to put it into context with GRKs as regulators of diverse physiological cellular functions. The complex mechanisms, which underlie regulation of GRK expression, degradation and function now are being elucidated and the levels of these kinases have been described to be altered in several pathological situations, such as cardiac failure, hypertension, inflammation and cancer. The authors suggest that GRKs may contribute to the development of pathology, making these proteins potential diagnostic and therapeutic targets for future pharmacological intervention.

Chapter 4 – Age-related dementias such as Alzheimer disease (AD) have been linked to vascular disorders like hypertension, diabetes and atherosclerosis. These risk factors are known to cause ischemia, inflammation, oxidative damage and consequently reperfusion, which is largely due to reactive oxygen species (ROS) that are believed to induce mitochondrial damage. At higher concentrations, ROS can cause cell injury and death which occurs during the aging process, where oxidative stress is incremented due to an accelerated generation of ROS and a gradual decline in cellular antioxidant defense mechanisms. Neuronal mitochondria are especially vulnerable to oxidative stress due to their role in energy supply and use, causing a cascade of debilitating factors such as the production of giant and/or vulnerable young mitochondrion who’s DNA has been compromised. Therefore, mitochondria selective antioxidants such as acetyl-l-Carnitine (ALCAR) and R-alpha-Lipoic acid (LA) seem to be potential treatments for AD as they target the factors that damage

mitochondria and reverse its effect, thus eliminating the imbalance seen in energy production and amyloid beta oxidation, making these antioxidants very powerful alternate strategies for the treatment of AD.

Chapter 5 – Several vitamin deficiencies have been repeatedly reported to be associated with cognitive impairment, though there is not a consensus about all of these associations. Some vitamins, like vitamin A, vitamin C and vitamin E were investigated for their prevention from neuronal death as being antioxidant agents. Deficiencies of several B vitamins have been reported to be associated with cognitive dysfunction in many studies. More recently, deficiencies of folate (vitamin B9) and cobalamine (vitamin B12) have been studied in relation to their causing to hyperhomocysteinemia. As homocysteine is a proatherogenic and protrombotic factor, it is natural the deficiencies of these vitamins to cause vascular events in brain and resultant vascular dementia. The most recent reported vitamins related to cognitive functioning are vitamin D and vitamin K. Vitamin D has vasculoprotective effects through various mechanisms and a vitamin K dependent receptor can protect neurons against apoptosis. Also, there is a possibility that vitamin K may decrease brain damage produced by cerebrovascular disease.

Most of the evidence about the association of vitamin deficiencies and vascular dementia is based on cross-sectional studies, which can not prove whether a nutrional deficit is the cause or the result of an impaired cognive status. In fact, cognitive impairment can also determine changes in dietary habits and and consequently cause vitamin deficiencies. In this section, the relations between vascular dementia and vitamin deficiencies have been discussed under the light of literature knowledge and potential of vitamin supplementation to prevent or treat vascular dementia has been tried to be evaluated. It has been realized that though there are promising, positive results about vitamin supplementation; well designed studies with larger number of participants are needed to clarify the subject.

Chapter 6 – Vascular risk factors (VaRF) have increasingly being recognized as important contributors for late-onset Alzheimer´s disease (AD). There is growing evidence implying neurovascular dysfunction as an integral part of AD. Both AD and vascular dementia show similar microvascular and cerebral amyloid angiopathic pathological changes, suggesting that they are closely related entities. VaRF may lower the threshold for clinically manifested AD/mixed type dementia by decreasing cognitive reserve. VaRF may increase the risk of AD by increasing both β-amyloid and hyperphosphorized tau burden in the brain through several mechanisms, such as high levels of oxidative stress and impairment to the blood-brain barrier. A chronic ischemic brain state also seems an uncontrolled and desperate attempt to increase neuroplasticity. However, this sustained state may favor amyloid oligomerization and trigger tau hyperphosphorylati to induce neuroplastic failure, as increased β-amyloid expression on the brain might be on, promoting neurodegeneration. Several VaRF have already being associated with increased risk for AD. High cholesterol levels may also induce β-amyloid oligomerization and deposition. Hypertension produces vascular abnormalities contributing to arteriolar lipohyalinosis and WMLs. Insulin resistance and hyperinsulinism promote endothelial proliferation, microvascular disease, and chronic neuronal oligemia. Sustained hyperglycemia increases the formation of Advanced Glycated Endproducts (AGEs), which, in turn, also promotes β-amyloid misfolding and tau hyperphosporilation. Obesity itself may increase oxidative stress and cerebral biological aging, contributing to accelerate AD pathology. All of these risk factors may contribute to amyloid angiopathy and microbleeding, further decreasing cognitive reserve. For these

reasons, AD-vascular dementia has being considered by many authors as the most common dementia subtype.

Chapter 7 – The concept of Mild Cognitive Impairment (MCI) refers to a group of patients with a degree of cognitive decline that cannot yet characterize dementia because it still does not interfere with the Activities of Daily Living. Virtually, MCI may be caused by all types of dementia, including the reversible ones. In this sense, it may assume from the beginning (sub)clinical features of the subjacent pathophysiological process. The diagnosis of vascular MCI (vMCI) is made when the patient with MCI has higher degree cerebrovascular ischemic lesions than it would be expected from normal aging only. This is especially true for small-vessel disease vMCI.

MCI may be classified into four basic subtypes, according to type and number of altered cognitive domains: single-domain amnestic MCI (saMCI), single-domain non-amnestic MCI (snMCI), multi-domain amnestic MCI (maMCI), and multi-domain non-amnestic MCI (mnMCI). saMCI is typical of Alzheimer´s disease and it is not a common presentation in vMCI. All other three forms may be related to vMCI, depending on the site and number of ischemic lesions.

A dysexecutive syndrome is the most common initial presentation of snMCI in vMCI. It may be clinically differentiated from preclinical stages of frontotemporal dementia by the lack of disinhibition and/or language problems of cortical subtype in vMCI. Besides, vMCI is often accompanied by pyramidal and extrapyramidal signs, including small-stepped gait. In most advanced vMCI stages, both mnMCI and maMCI get more common, but this means that the physician should start considering the diagnosis of Vascular Dementia (VaD).

The diagnosis of post-stroke MCI does not poses diagnostic doubts. The most difficult scenario is when the onset of vMCI is insidious and depression coexists. Depression may be considered a reversible cause of MCI. In another hand, vascular depression is often associated with vMCI, since both are often caused by lesions in similar frontostriatal networks, specially the dorsomesial circuit. Even though apathy is often a manifestation of v-MCI, coexistent vascular depression should be identified not only in order to alleviate suffering, but also because it may improve cognition itself and help clarifying differential diagnosis.

vMCI is not reversible, but progression to VaD may be. Appropriately identifying and treating vascular risk factors in vMCI may decelerate or even avoid progression to VaD.

Chapter 8 – The coexistence of Alzheimer disease (AD) and cerebrovascular lesions in postmortem studies has been so frequently reported that some authors have begun to consider this pathology as the most common type of dementia, especially in very old persons. An association between the coexistence of those lesions and severity of cognitive impairment has also been reported. Nevertheless, by searching “mixed dementia” in Pubmed (April 17, 2010) only 55 reviews were found (against 1063 for “vascular dementia” (VD)).

Traditionally, patients who showed a combination of Alzheimer disease and vascular dementia (AD+VD patients) were included as VD in epidemiologic studies. Subsequently the attention was focused on AD, which was considered the main cause of dementia. Nowadays, the traditional view has been revitalized with new findings and the re-examination of the vascular hypothesis of AD. However, the term “mixed dementia” is still avoided and the validity of VD is still under discussion.

The concept of mixed dementia only could only have serious foundations if distinctive cognitive patterns in quality, not quantity, were found. Besides, there is an epistemological problem for the taxonomy of AD+VD, and VD. If it is assumed that: a) distinctive and

synergistic neuropsychological patterns exist for AD and VD, and b) these patterns can simultaneously or successively be present in a certain patient or group of patients, then the dementia risk (severity and/or progression) should increase. However, as rapid progression is considered a VD characteristic for antemortem studies while VD pathology represents an essential component of AD+VD for postmortem studies, then, the concept of AD+VD for antemortem studies should include the possibility of rapid progression. If AD+VD dementia is defined by the presence of cerebrovascular disease and only gradual progression, some kind of antagonism between AD+VD seems to be unjustifiably assumed. In addition, abrupt onset and stepwise progression has not been consistently found in patients with vascular lesions. So, and in order to avoid misdiagnosis, the concept of VD for antemortem studies should include the possibility of a slow progression.

If AD+VD condition seems to be the most common type of dementia in postmortem studies, then that combined condition should be the first option to categorize dementia in antemortem studies. But in order to do that, and considering that dementia is not just an anatomical question, distinctive cognitive outputs should be empirically and consistently demonstrated for AD and VD. If not, may be the authors should only speak about dementia or cognitive impairment.

Chapter 9 – Vascular dementia (VaD) is a heterogeneous disease including several different vascular mechanisms in the brain as well as various clinical manifestations.

Biomarkers to aid in the early and precise diagnosis of vascular dementia (VaD) are in great need. The differential diagnosis between vascular dementia and Alzheimer's disease (AD) or mixed dementia is not always easy in clinical practice. There can also be overlap in symptoms with other neurodegenerative diseases, especially in the early phases of the disease. Biochemical diagnostic markers, which reflect the pathogenetic processes in the brain, would add to the accuracy of the diagnosis for the differentiation between VaD and healthy aging but also for the differential diagnosis between VaD, AD and other neurodegenerative disorders.

This chapter examines the biofluid markers that have been described in the literature as potential markers for VaD.

Cerebrospinal fluid (CSF) levels of amyloid beta 1-42, tau and phosphorylated tau are used routinely to aid in the diagnosis of AD. These markers reflect plaque and tangle pathology and have also shown promise in the differentiation between AD and VaD.

Furthermore, an elevated CSF/serum albumin ratio has been described in VaD, probably reflecting the increased extent of blood-brain barrier damage in VaD.

Circulating inflammatory markers such as C-reactive protein and IL-6 are increased in plasma of VaD patients compared to both AD and healthy controls. In addition, blood levels of coagulation markers such as fibrinogen, factor VIII and fibrin D-dimer were increased in the same manner. Also, elevated levels of the amino acid homocysteine in plasma have been described in patients with VaD compared to both AD patients and healthy controls.

The current available clinical studies describe a wide array of promising markers. There are, however, limitations to many of these studies due to the heterogeneity of definitions of VaD, small population sizes and differences in analytical methodologies. Ongoing and future validation studies will significantly narrow down the present list of markers with the most robust and reproducible remaining for possible implementation in clinical practice.

Chapter 10 – Behavioral and psychological symptoms such as apathy negatively affect cognitive performance, mood, functional status, and prognosis of patients with dementia.

Apathy appears to be common in many diseases of the brain, and is associated with distress in caregivers. Apathetic behavior of minor degree seems less problematic than other agitated behaviors such as irritability and restlessness. Nonetheless, apathy has a significant impact on quality of life and activity of daily living in patients with cognitive impairment. Although apathetic behavior in healthy elderly subjects has not been paid much attention until recently, brain magnetic resonance imaging (MRI) studies revealed that silent or subclinical brain ischemic lesions are the basis for apathetic behavior in elderly subjects without dementia. Furthermore, vascular risk factors such as hypertension or vascular diseases per se are known to produce apathy in old age. In this short review, the authors will discuss about the relationship between apathy and vascular factors in healthy elderly subjects.

Chapter 11 – There has been recent interest in the basal ganglia as structural components of small-scale neural networks that may be strategically affected in cerebrovascular disease. The neural connectivity, blood and metabolic requirements of the caudate nucleus may make this structure particularly vulnerable to cerebrovascular disease related neurodegeneration. The authors have demonstrated cross-sectional caudate nucleus atrophy in a Stroke sample, and were interested to investigate whether longitudinal atrophy occurred in a cohort with cerebrovascular disease, to study the disease trajectory. Accordingly, the authors sought to compare two subsets of longitudinal epidemiological studies investigating confirmed cerebrovascular disease in the form of leukoaraiosis, and a group of healthy age-matched controls.

The authors manually measured caudate nucleus volume via MRI at baseline and at three year follow-up, in the Stockholm subset of the Leukoaraioisis and Disability in the elderly Study (LADIS). The authors found that normalized bilateral caudate volume was significantly smaller on follow-up in the LADIS group; and there was an approximately twice higher annual rate of atrophy in LADIS than in healthy age and gender matched controls from Stockholm (Swedish National Study on Aging and Care – Kungsholmen subset) without white matter disease. Leukoaraiosis may be associated with increased caudate nucleus atrophy, a finding which has implications for cognitive and behavioural functions served by this structure. In the context of previous findings of reduced caudate volume associated with white matter hyperintensities in the Sydney Stroke Cohort, these findings support a potential aetiological role for the caudate in cerebrovascular disease.

Chapter 12 – In this chapter, the epidemiology of vascular dementia (VaD) is reviewed; it's prevalence, risk factors, incidence, and prognosis.

Chapter 13 – Dementia is on the increase worldwide, and developing countries are expected to carry the burden of this. Relatively little is known about dementia prevalence in Indonesia. This chapter discusses two short screening tests to assess dementia in rural and urban Indonesian cohorts.

Method

At baseline in 2006/7, 719 elderly were included from rural and urban sites on Java. Large differences appeared in dementia prevalence in those over 60 years of age between urban (3%) and rural sites (7-16%) employing two dementia screening tests also used in Oxfordshire with the same cut-offs. An in depth study was performed on the rural sample from East Java to validate the cut-offs of the tests. For this study, Javanese Indonesian elderly from 4 villages around Borobudur were asked to participate. 113 agreed to participate and these were tested in a health center by medical experts and trained research assistants. The screening test cut-offs were validated against consensus based clinical dementia diagnoses by

an expert psychiatrist, nurses and GP, which were based on a gold standard diagnostic instrument for dementia diagnoses from Cambridge. In addition, a sub sample of these participants was tested in depth by another psychiatrist using questions from their expert dementia diagnostic system developed at Oxford University.

Results

The adapted memory screening test was shown to have similar cut-offs for dementia (19.5 for controls and 14.5 for cases) as in Oxfordshire and the Mini Mental Status Examination (MMSE) had optimal sensitivity (100%) using a similar cut-off of 24. However, for optimal specificity, the MMSE was shown to require a lower cut-off of 21.5 and MMSE scores were also affected significantly by educational level. It was unclear how many of these cases had vascular dementia (VaD), as stroke, transient ischaemic attack and myocardial infract assessed by self-report were rare (n=1-2) and were only reported by controls, suggesting a recall bias. There was also no difference in diabetes mellitus or (high) blood pressure between cases and controls which could have increased risk. Physical examination suggested no other morbidity driving the dementia (e.g. infectious or lung disease). The cut-offs were also tested by another expert psychiatrist on a sub sample of these participants using her clinical assessment and aided by questions from their expert dementia diagnostic system. Agreement between psychiatrists was high (79%) on diagnoses of these 28 participants, with only 6 disagreed on. Of these, only diagnoses of 2 participants were disagreed on whether these had dementia or were controls. Of 59 elderly patients from the villages who were tested in depth by the second psychiatrist, 17 were thought to have dementia, with most (53%) having Alzheimer's disease (AD) and 6 (35%) suspected of having VaD, with only one mixed (with stroke) case. There was no clear indication of other types of dementia, but two cases with dementia (12%) were thought to be related to systemic disease. The 19 preclinical cases (possible dementia) all had memory complaints, but scored significantly higher on the adapted memory test and MMSE than those with dementia and scored lower than controls (but not significantly so on the memory test), independent of age and gender. Optimal cut-offs for dementia on the memory test were again 19.5 for the total immediate recall (100% sensitivity and 78% specificity) and 24 for the MMSE (88% sensitivity and 96% specificity).

Discussion

This study showed that two short cognitive tests can be used for dementia screening in rural Java. It has been hypothesized that VaD is more prevalent than AD in East Asian countries, but the authors could not substantiate this. Future studies should investigate in more detail the prevalence of vascular and secondary dementias (due to thyroid or infectious disease, nutritional deficiency etc.). Use of this screening instrument in other ethnic groups in other developing countries also needs to be explored.

Chapter 14 – According to several longitudinal studies, hypertension appears to predispose individuals to the development of cognitive impairment, dementia and Alzheimer's disease, after a period varying from a few years to several decades. Antihypertensive drug treatment, according to preliminary evidence, may help to reduce the rates of such incidences. Such findings wait to be confirmed by more large therapeutic trials. Understanding the effect of hypertension on cognition is a work in progress. There are several mechanisms of impairment of cognition in hypertensive state. Cerebral hypoperfusion and chronic oxygen deprivation appear to play a pivotal role in cognition deficit and AD pathophysiology due to hypertension. Chronic hypertension alters cerebral endothelium by causing microvascular degeneration. Further, hypertension induces proliferation of smooth

muscle cells, basal lamina alterations, luminal narrowing, endothelial hyalinosis, and fibrosis which leads to hypoperfusion, chronic cerebral oxygen insufficiency and deranged glucose homeostasis such as in AD. There are alterations in neurovascular coupling and autoregulatory system which further causes cerebral hypoperfusion. Chronic hypertension also alters peripheral as well as brain renin angiotensin system (RAS) and nitric oxide (NO) pathways which also contribute in cerebral hypoperfusion and hypometabolism. As pathophysiology of hypertension emerges as a contributing risk factor for dementia and AD, it appears that measures directed to control blood pressure will enhance cognitive reserve.

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Chapter I

Vascular Risk Factors, Brain Changes and Cognition: The Role of Metabolic Syndrome

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Abstract

In modern societies, lifestyle changes and the acquisition of sedentary habits have been linked to the rise in the prevalence of vascular risk factors, which may increase the risk of central nervous deterioration in aging. Previous studies have associated cerebrovascular changes occurring in later life with cognitive and structural brain characteristics. The detection of initial states of vascular dysregulation in the brain could prevent the progression to pathological conditions such as vascular cognitive impairment. Accordingly, the concept of brain-at-vascular-risk highlights the importance of detecting prodromal states of unsuccessful aging [1]. Today most vascular risk factors are treatable, and so it should be possible to prevent, postpone, or mitigate vascular cognitive impairment as well as the vascular exacerbation of Alzheimer disease [2].

This chapter describes the scientific evidence of the effect of vascular risk factors in structural brain changes, and their influence on cognition in aging. Today, a limited percentage of the population present isolated vascular risk factors such as type 2 diabetes, hypertension, obesity and dyslipidemia, or in many cases a combination of these factors. Some authors have proposed that a cluster of vascular risk factors known as the Metabolic Syndrome has a synergistic effect. At present, the prevalence of Metabolic Syndrome is between 20% and 30% of the world's population, and increases during aging [3]. The increasing presence of Metabolic Syndrome during aging highlights the importance of establishing its effects on the central nervous system(CNS) during this stage of life and its influence on the development of cerebrovascular pathology.

Metabolic Syndrome has been linked to dementia [4], fronto-subcortical symptoms and poorer cognitive performance associated with aging [5,6]. It has also been related to white matter lesions [7-10] as a sign of small vessel disease. Our previous findings provide evidence that Metabolic Syndrome is related to subtle white matter alterations and a specific neuropsychological profile [11-13] suggesting that it has an influence on the CNS probably due to the chronic state of vascular dysregulation in the brain. Metabolic Syndrome may be a prodromal state of vascular cognitive impairment.

In summary, this chapter reviews the effects of vascular risk factors in the brain and cognition, and their influence in unsuccessful aging.

1. Introduction

The increase of sedentary habits and lifestyle changes in modern societies, as well as the progressive aging of the world population involves an increase in the prevalence of vascular risk factors in middle-age and old adults. The influence of vascular risk factors is related to a higher prevalence of cardiovascular disease. Moreover, the presence of vascular risk factors is associated to brain and cognition alterations. This situation entails an increase in cerebrovascular pathology among the elderly and the development of unsuccessful aging.

Over the last years,control of the risk of vascular pathology has focused on detection and treatment of isolated vascular risk factors, among the most common are hypertension, diabetes and dyslipidemia. Recently, clinical guidelines recommended the study of vascular risk factors from a broader perspective, and proposed a holistic approach with a global vascular risk assessment to determine treatment options and prevention. It has been observed that a limited percentage of the population suffered vascular risk factors in isolation, though the most common situation is to suffer more than one vascular risk factor, and usually these factors are related. Indeed, it is proposed that the presence of concomitant vascular risk factors could cause the increase of the individual effect of each one. In this way, and to optimize treatment to people suffering from vascular risk factors, total vascular risk should be considered in each patient [14].

Several measures have been developed to estimate the total risk of suffering a cardiovascular event; such measures usually inform the patient about this risk in the following 10 years of life. Among the index most widely used in European population are the Framingham coronary risk profile [15], the Framingham risk profile stroke [16], and the Systematic Coronary Risk Evaluation (SCORE) [17]. These measures help to estimate the global vascular risk. The global vascular risk is high in the groups of patients who have previously suffered a vascular event, in those patients suffering from vascular risk factors that carry a high risk for themselves (type I diabetes, type II diabetes), or in patients with vascular risk factors presented jointly, such as the Metabolic Syndrome (MetSd) (Table 1). All these patients can be considered an overall high vascular risk and intervention is necessary [14,18].

The influence of vascular risk factors in the CNS has been studied in several ways, and the obtained results are still controverted. Here, in congruence with the ideas proposed in the previous paragraphs, we present a revision about the effect of the most common vascular risk factors in the CNS, as well as the recent evidences about the effect of MetSd as a cluster of vascular risk factors on brain structure and cognition, especially in the aging.

Table 1. Metabolic Syndrome criteria

Clinical Measure	WHO (1998)	EGIR (2001)	NCEP ATP III (2001)	AACE (2003)	IDF (2005)	NCEP ATP III AHA/NHLBI (2005)	ESC/ESH (2007)	IDF, NHLBI, AHA,WHF, IAS, IASO (2009)
Insulin resistance	IGT, IFG, T2DM or 2 insulin sensitivity plus any two of the following	Plasmatic insulinPc >75 plus any two of the following	None But 3 of the following criteria	IGT or IFG plus any of the following criteria	None	None But 3 of the followig criteria	None But 3 of the followig criteria	None But 3 of the followig criteria
Body weight	Males: waist to hip ratio >0.90; females: waist to hip ratio >0.85 and/or BMI >30 kg/m2	WC ≥94 cm in men or ≥80 cm in women	WC ≥102 cm in men or ≥88 cm in women	BMI ≥25 kg/m2	Increased WC (population specific) plus any 2 of the following	WC ≥102 cm in men or ≥88 cm in women	WC ≥102 cm in men or ≥88 cm in women	Elevated waist circumference, specific population and country definition.
Lipid	TG ≥150 mg/dL and/or HDL-C <35 g/dL in men or <39 mg/dL in women	TG ≥150 mg/dL and/or HDL-C <39 mg/dL in men or women	TG ≥150 mg/dL HDL-C <40 mg/dL in men or <50 mg/dL in women	TG ≥150 mg/dL and HDL-C <40 mg/dL in men or <50 mg/dL in women	TG ≥150 mg/dL or on TG Rx HDL-C <40 mg/dL in men or <50 mg/dL in women or on HDL-C Rx	TG ≥150 mg/dL HDL-C <40 mg/dL in men or <50 mg/dL in women or on Rx	TG≥150mg/dL HDL-C < 46 mg/dL D < 40 mg/dL H	TG ≥150 mg/dL HDL-C <40 mg/dL in men or <50 mg/dL in women or on Rx
Blood preassure	≥140/90 mm Hg	≥140/90 mm Hg or on Rx	≥130/85 mm Hg	≥130/85 mm Hg	≥130/ 85 mm Hg or on Rx	≥130/ 85 mm Hg or on Rx	≥130/ 85 mm Hg or	≥130/ 85 mm Hg or on Rx
Glucosa	IGT, IFG, or T2DM	IGT or IFG (but not diabetes)	>110 mg/dL (includes diabetes)	IGT or IFG (but not diabetes)	≥100 mg/dL (includes diabetes)	>100 mg/dL (includes diabetes) or on Rx	>100 mg/dL	>100 mg/dL (includes diabetes) or on Rx
Other factors	Microalbuminuria			Other features of insulin resistance				

WHO: World Heath Organization, EGIR: European Group for Study of Insulin Resistance,NCEP: National Cholesterol Education Program Adult Treatment Panel III; AACE: American Association of Clinical Endocrinologists, IDF: International Diabetes Federation; ESC/ESH:European Society of Cardiology and the European Society of Hypertension, NHLBI-AHA: National Heart, Lung, and Blood Institute American Heart Association, WHF: World Heard Federation, IAS: International Atherosclerosis Society, IASO: International Association for the Study of Obesity.

IGT: impaired glucose intolerance, IFG: impaired fasting glucose, T2DM: type 2 diabetes mellitus; WC; waist circumference; BMI: body mass index; TG: triglycerides, HDL-C: HDL cholesterol, Rx: medical prescription.

2. Brain at Vascular Risk, the Concept of Vascular Cognitive Impairment

2.1. Concept of Vascular Cognitive Impairment

Vascular Cognitive Impairment (VCI) is a broad concept that encompasses patients across the entire continuum of cognitive impairment resulting from cerebrovascular disease (CVD), ranging from high-risk patients with no frank cognitive deficit (the brain-at-riskstage) through severe dementia (vascular dementia(VD))[19]. VCI covers individuals who have cognitive impairment related to stroke, multiple cortical infarcts, multiple subcortical infarcts, or both, silent infarcts, strategic infarcts, small-vessels disease with white matter lesion, and lacunes. Vascular cognitive impairment also plays an important role in patients with AD pathology who have coexisting vascular lesions [20].

The VCI concept emerges from the redefinition of VD proposed by Hachinski in 1994 [19].VD is the second cause of dementia associated to aging [21] and nowadays it has higher probabilities to be prevented than other dementia types [22]. VD can be related to both isquemic and hemorragic pathology or to ischemic damage produced by blood hipoperfusion, as a result of cardiovascular or/and circulation alteration.

Classical diagnostic criteria for dementia include the presence of progressive and irreversible memory deficits, as well as the functional affectation of daily life activities. These criteria are included in current versions of the Diagnostic and Statistical Manual of Mental Disorders (DSM IV-TR, 2000) [23] and the National Institute of Neurologic Disorders and Stroke - Association Internationale pour la Recherche et l'Enseignement en Neurosciences (NINDS-AIREN)[24]. In some of these definitions the experts also include the presence of brain damage detected by neuroimaging techniques [25].

At the beginning of the 1990's, Hachinski and Bowler proposed the new concept VCI to emphasize the differences between VD and Alzheimer disease (AD)[26]. The authors recognized the association between these two pathological conditions, and sometimes their interaction. However, they highlight the importance of identifying these cases of VCI in which there are noassociations to AD. The authors also remark that memory deficits arenot essential criteria to VCI and cognitive deficit is not a constant in patients with cognitive impairment related to cerebrovascular disease. On the other hand, the authors argued that alterations in daily life activities are restricted criteria and entails the detection of advanced cases in which the possibilities of intervention are reduced or limited. Finally, in cerebrovascular disease patients, brain damage is not easily detected using conventional neuroimaging techniques and the application of this criterion limited the detection of the very initial phase of VCI.

In summary, the authors proposed the new VCI concept as an umbrella term, which allowsfordetection of all kinds of cognitive impairment secondary to cerebrovascular pathology. Nowadays VCI is moreprevalent in clinic population and it involves a high investment of economic resources [27]. VCI has been related to an adverse outcome, more dependence, and serious cognitive impairment [28].The study of vascular risk factor, as well as the prevention and control of VCI could be an effective option from an economical perspective[29].

Since the beginning, Hachinski stressed the necessity of formal criteria for defining the VCI. The authors believed that future studies should introduce more data on neuropsychology and neuroimaging of each etiologic VCI subgroup and it may help to define specific criteria [26]. Other authors agreed with the criticism and highlight the relationship between VCI and typical manifestations of normal aging, as well as the absence of objective criteria, which guarantees the work with clinical research [30]. For some years, the VCI concept has been used with little accuracy and this has led to confusion regarding the results of the studies. Recently this situation has changed, and although no strict criteria for the diagnosis of VCI existed, a group of experts has drawn up recommendations to address the clinical and research of VCI in a systematic way [2]. Thus they recommend the collection of demographic and clinical data and detailed concrete neuropsychological and neuroimaging protocols. Specifically, they suggested neuropsychological examination of these patients to obtain data regarding the performance of executive tasks and speed of information processing, because these alterations are sensitive to fronto-subcortical damage typically detected in the VCI. Furthermore, neuroimaging protocols specify the use of MR images (1.0 Tesla minimum power) compared to other techniques (Computed Tomography). Among the MR sequences is recommended the use of 3D T1-weighted, T2-weighted, Fluid-Attenuated Investment Recovery, and Gradient echo. Finally, the use of diffusion sequences was also emphasized, because these sequences are very sensitive to white matter damages, which are typically observed in these patients.

2.2. Subcortical Vascular Cognitive Impairment

The VCI concept includes a wide group of clinical syndromes related to different vascular mechanisms and brain changes. The damage associated to VCI is not limited to the focal area of vascular lesion [21]. The brain damage caused by cerebral ischaemia also includes the perilesional area and distant regions, functionally connected to the focal lesion area (diaschisis).

From the different types of VCI, subcortical ischaemic vascular disease represents from 36 to 67% of all the cases [31]. According to clinical studies, this subtype showed homogeneity in its characteristics and foreseeable outcome. White matter alteration and lacunar infarction are the most common brain changes observed in subcortical VCI, these changes are related to small vessel disease aetiology [21]. Small vessel lesions are associated to lumen occlusion, producing a complete lacunar infarction, or multiple small vessels stenosis causing hipoperfusion and incomplete infarction. Complete infarctions are related to acute and sever ischaemia, as a consequence we can detect necrosis and cerebral tissue lost. Incomplete infarcts involve chronic ischaemia, less severe affectation and a progressive and selective alteration of most vulnerable tissue types[31]. Small vessel disease is also related to arteriosclerotic changes; it produces alteration in little arteries and capillaries, which irrigate deep white matter and basal ganglia. The affectation of these vessels reduces its autoregulation capability in front of blood pressure changes. On the other hand, loss in the white matter (WM) integrity linked to subcortical cerebrovascular pathology is related to the blood brain barrier (BBB). In chronic hypertension conditions, BBB could increase its permeability allowing the transport of toxic particles through the cerebral parenchyma [32].

Finally, the cerebral changes observed in subcortical VCI could be related to cerebral amyloid angiopathy. This phenomenon is characterized by the extracellular deposition of amyloid in the vessels, typically in the small or medium arteries but also in small capillariesand in the veins. Amyloid angiopathy principally affects deep grey matter structures, subcortical white matter and the basal brain. This process affects the vascular system during the aging process but is more frequently associated to cerebral haemorrhages and Alzheimer disease [32,33]. Indeed, the white matter lesions often detected in Alzheimer disease patients could be related to artherosclerotic and amyloid changes combined. White matter lesions observed in subcortical VCI included araiosis, axonal loss, changes in oligodendrocytes and glial cells, as well as noncavitated and cavitated small infarcts [34].

2.2.1.Structural Brain Changes and Subcortical VCI

The alteration typically associated to subcortical vascular pathology involves periventricular and deep WM lesions (WML). This alteration affects specially the genu of corpus callosum, the internal capsule, the anterior corona radiata and the anterior semioval center. The complete lacunar infarcts are often located in basal ganglia, talamus, internal capsule, corona radiata and frontal WM. [21]. Several studies showed structural changes in VCI patients, among the most relevant aspects authors detected: cortical atrophy, temporal lobe changes and WM alteration [35].

The hippocampal alteration has been reported in VD patients in comparison to a control group [36]. In subcortical VCI patients bilateral atrophy of parahipocampal gyrus and hipocampal atrophy in the right hemisphere have been detected [37]. Recently, Jokinen et al., (2009)[38] observed high temporal lobe atrophy in patients with subcortical vascular pathology. In a review [35], the authors concluded that the hippocampal atrophy pathogenesis in subcortical VCI patients is variable and it could reflect the combination of ischaemic and degenerative pathology.

WML are the most common lesions associated to subcortical VCI. Different types of WML have been related to subcortical VCI, the most commonly reported are the periventricular and deep WML. The exact cause and consequences of these lesions are not completely known. However, the presence of deep WML has been typically linked to the presence of vascular risk factors while the periventricular WM hypertensities have been related to the ageing process [39]. Periventricular WM hypertensities are situated in anterior and posterior ventricular horns. The deep WM lesions affect specially the fronto-subcortical loops, and these are related to cognitive and conductual alterations associated to small vessel disease [20,21]. Several authors proposed that WML affect preferably long associating fibers (for instance cingulum, superior longitudinal fasciculum and fronto-occipital fasciculum) that involve connection deficits and executive function alterations. Both proposals are not mutually excluding and both mechanisms could affect the cognition of subcortical VCI patients [40].

Throught the use of magnetic resonance imaging techniques it is possible to quantify the WM lesions burden. This measure allowsthe establishment of several relations between the WM lesions presence and cognition [41,42]. However, these studies are not widely consistent. For the last 10 years, the use of new imaging techniques, such as diffusion tensor imaging (DTI), has improved the evidences about microstructural changes related to VCI. This technique has shown higher sensitivity in the detection of WM damage in comparison to conventional sequences [43]. At present, DTI studies allow the detection of WM changes in

apparently normal WM [44] as well as to establish correlations between WM state indicators and cognitive performance, for example the relation between executive function performance and WM in patients with leucoaraiosi [45]. In a recent study with 35 patients, executive function performance correlated to DTI measures while neither cerebral volume values nor the number of WM lesions or the number of lacunar infarcts showed significant results. A 27 subject subsample wasfollowed-up for 1 year. The authors found changes in DTI measures but they did not find any significant change in cognition or magnetic resonance imaging (MRI) measures [46]. Finally, Correia et al., 2008 studied subcortical VCI patients and found significant differences in DTI measures, these changes were detected in corpus callosum fibers and were related to cognitive changes, concretely to the slower processing speed performance [47].

2.2.2.Cognitive Changes and Subcortical VCI

The cognitive profile associated with VCI varies depending on the subject and the point where it is in the VCI continuum. For instance, the cognitive profile of VD is characterized depending on specific subtypes of VD and these show specific cognitive and behavioral alterations (post-myocardial infarction, multi-infarct, strategic infarct, intracerebral hemorrhage, AD with cerebrovascular disease or subcortical VD). Generally there is initial involvement of attention and executive functions, as well as decrease in speed of information processing and psychomotor speed. Episodic memory is relatively preserved compared with other types of memory, and language production (verbal fluency) may be slightly altered while other aspects of language tend to be preserved [48]. Other cognitive functions may be affected depending on the involvement of anatomic substrates in each case. Psychiatric symptoms are common in these patients; changes in mood states, depressive symptomatology, emotional lability and apathy are typical manifestations of VD[49].

Regarding the less severe VCI phases, although they do not meet the criteria of classic dementia (vascular cognitive impairment no dementia (VCI-ND)), several studies have found a similar neuropsychological profile of VD characterized by executive dysfunction (abstraction, flexibility and working memory), psychomotor slowing and in some cases mild deficits in immediate memory [50,51].

Mild cognitive impairment (MCI) is a concept that implies a state of cognitive impairment without dementia. Amnesic MCI type was the first subtype of MCI described by Petersen in 1999 and is now widely accepted as a stage of AD risk (52). During the following years, several subtypes of MCI have been proposed, including amnesic, not-amnestic and multidomain MCI. These subtypes include the involvement of different cognitive functions apart from memory, all in an early stage of deterioration. These new subtypes were not associated exclusively with one type of dementia but some authors suggest that multi-domain MCI subtype could be a stage of preclinical VD [53]. Zanetti et al. (2006) conducted a 3 year follow-up study and observed that 65% of subjects with amnesic MCI in the baseline remained stable, while 35% expressed the AD at the follow-up[54]. Similarly, 74% of patients with multi-domain MCI remained stable while 26% are diagnosed with VD. In this study the diagnosis of multi-domain MCI was associated with an increased presence of vascular risk factors such as hypertension, and with more presence of subcortical vascular damage detected with neuroimaging. Among the neuropsychological tests, multi-domain MCI patients showed poor performance in attention, mental flexibility and speed of information processing. Other studies show that MCI-associated with vascular disease differs from MCI-

not associated with vascular pathology in tests of attention, speed, executive and visoespacial functions. In addition, most patients included in the MCI-associated with vascular disease category are classified as multi-domain MCI [55].

Over the last years, the “brain at vascular risk” stage (defined by Hachinski as the beginning of the VCI continuum) has been related to several factors. Some health conditions and lifestyles have been associated with higher predisposition to suffer VCI. The lack of control of vascular risk factors such as hypertension, diabetes, dyslipidemia and obesity is associated with increased susceptibility of VCI. Furthermore, the intake of a well-balanced diet [56], and the control of the consumption of toxic substances such as alcohol or tobacco [57,58] have recently been associated with less cognitive impairment.

Especially interesting are the studies of several authors, which evaluated the cognitive state of preclinical VCI. Garrett et al., (2004) assessed the difference between VD, VCI-no dementia and subjects with "brain in a state of vascular risk"[50]. The authors found a better execution of the latter group in comparison to both clinical conditions. Subjects classified as "brain vascular risk" did not obtain clinically abnormal scores for the neuropsychological tests. However, these authors did not compare the performance of the latter group with a completely healthy sample; the authors compared the scores with normative data. Other authors studied subjects without cognitive impairment who progress to VCI after 5 years of follow up. They showed a worse performance at baseline in some neuropsychological tests of memory, abstract reasoning, speed, and executive functions [59]. This study found subtle differences in the cognitive function of patients in prodromic states of VCI, years before a clinical diagnosis. Recently, patients with 3 or more vascular risk factors (brain with high vascular risk) have been characterized by alteration of executive and psychomotor functions, as well as by the preservation memory [60]. Accordingly, some studies suggest the effect of some isolated vascular risk factors on cognition, while others believe the cumulative and even synergistic effect of these factors, as has been proposed in the case of SdMet [61].

3. Vascular Risk Factors and Central Nervous System

The concept VCI allows identifying different phases of cognitive impairment in cerebrovascular disease patients, as well as the detection of pre-pathological phases, when the brain is at vascular risk.

Bowler and Hachinski (2002) suggested that either people who suffered vascular risk factors or cerebrovascular disease patients should be considered as possible VCI patients [26]. The detection of early phases of VCI allowed studying new treatment and prevention programs to avoid future impairment in these patients.

3.1.Obesity

3.1.1.Obesity and Cognitive Impairment

Some studies have related the presence of AD to obesity in the aging [62], while other authors showed that risk of AD in aging was related to obesity through adulthood [63,64].

Van den Berg et al., (2009) in a review, conclude that studies about obesity in adulthood reported more consistent results than those that study the obesity effect in the aging. However, both show evidences about an inverse relation between obesity and cognition [65].

A recent meta-analysissuggested that obesity is an independent risk factor to developing dementia, but the authors pointed out that the body mass index (BMI) follows an inverse U pattern in relation to that risk [66]. West et al (2009) performed a cohort study of older persons which found that BMI at baseline was inversely associated with rate of dementia/CIND (dementia/ "cognitive impairment but not demented" number of cases) during the follow-up period (67). In contrast, large waist circumference at baseline was associated with an increased rate of dementia/ CIND. Abdominal fat accumulation in late life could be increased risk for dementia, but whole body obesity appears to be protective. These authors suggested that the effects of generalized obesity and central obesity in late life on rate of cognitive impairment may be masked without complete adjustment for body size and stature.

Abdominal obesity has been related to poor general cognitive performance [68] and executive dysfunction [69-71]. According to Beydoun et al., (2008)(66), other authors suggested the accumulative effect of obesity over the life, as well as underweight, in the cognitive performance [72].

Kuo et al., (2006) [73] compared normal weighted, overweight and obese samples. The authors showed an inverse U pattern among the groups in relation to memory, reasoning and processing speed. Han et al (2009) [74] showed a curvilinear association between the waist circumference (WC) and the change in cognitive function over time (assessed by CERAD). This indicates that the predicted changes in cognitive function over time are higher at both the lower and upper ends of the WC measurements. When the authors studied the body mass index (BMI), WC and waist-hip ratio (WHR) according to gender, for men, increased obesity parameters over time when obese at baseline assessment (BMI, WHR, WC) were associated with a positive change in cognitive function. For women, decreased obesity parameters over time when obese at baseline assessment (WHR) and decreased obesity parameters over time when not obese at baseline assessment (WC) were both associated with cognitive decline. Finally, the authors stressed that cognitive status in the baseline assessment was linearly associated with changes in cognitive function in the elderly without cognitive dysfunction in the baseline. However, there were no associations between any adiposity parameter and changes in cognitive function in the elderly with mild cognitive impairment.

The performance in learning and memory tasks has been related to obesity. In a follow-up study, the BMI is related to learning, attention and processing speed scores in the baseline. Moreover, higher BMI values in the baseline are associated with changes in verbal learning in the follow-up [75]. Elias et al., (2005)[76] showed that obese men, not women, obtained worse results in global cognitive scores, visual reproduction tests and forward digits subtests.

Nilsson et al, (2009)[77] in a cohort study, found an association between body weight and cognition (episodic memory, semantic memory and spatial ability). The authors reported that the effect of weight is not a main effect, but there was an interaction. For episodic memory and spatial ability, being overweight interacts with age, whereas for semantic memory, there is an interaction between being overweight and sex that is most prevalent. For episodic memory the differences between normal-weight and overweight people existed in the old-old group (> 70 years old) showing a better cognition for overweight subjects. For spatial ability the pattern is similar for the old-old group, whilst the inverse pattern is shown in the middle-

age group. Finally, for semantic memory, the normal-weight group is better than overweight group, only for females. The authors highlight that some of the effect on cognition is due to the relatedness of being overweight to diseases like hypertension, stroke, and diabetes. When the effect of these diseases on cognition is controlled, for the effect of being overweight is strongly reduced.

3.1.1.Obesity and Structural Brain Changes

In relation to the structural alteration in CNS related to obesity, previous studies reported a relationship between obesity and total brain volume [78,79] and changes in the grey matter (GM) [80], concretely a reduction in frontal lobe areas [81]. Walther et al (2010)[82] found an inverse correlation between BMI and GM using a voxel-based approach. Specifically, the authors reported significant negative correlation between BMI and GM, after controlling the possible effect of hypertension, in the left orbitofrontal gyrus, the right inferior and precentral frontal cortex, the right posterior cortex extending from the parahippocampal gyrus to the fusiform and lingual gyri, and the right posterior and lateral cerebellar gray matter. Similarly, Raji et al (2009)[83] studying normal elderly adults found that higher BMI was significantly correlated with lower GM and WM volumes throughout the brain. Areas of strongest negative correlation were found in the orbital frontal cortex, and subcortical areas. In a comparison between groups, the authors found that the overweight group (BMI= 25–30) had lower brain volumes than those with normal BMI in the basal ganglia, corona radiata, and parietal lobe. Obese persons (BMI >30) had lower GM and WM volumes in the frontal lobes, anterior cingulate gyrus, hippocampus and basal ganglia in comparison to normal weight subjects. There were no statistically significant differences in GM and WM between the obese and overweight groups.

Ho et al (2010a)[84] studying a large sample of healthy elderly subjects scanned with brain MRIs to identify structural brain changes related to fat mass and obesity associated (FTO) gene. FTO risk allele is associated with obesity and carried by 46% of Western Europeans. The authors showed that those subjects carrying at least one copy of the risk allele (C allele for rs1421085 and the G allele for rs17817449), showed brain tissue deficits in the frontal and the occipital lobes. Carriers of the obesity-associated risk allele showed an average 8% deficit in brain tissue versus noncarriers in the bilateral frontal lobe and an average 12% deficit in the bilateral occipital lobe. The authors also reported that subjects with higher BMI also showed brain volume deficits in frontal, temporal, parietal, and occipital lobe regions and in the brainstem and cerebellum.

Some authors proposed that the relationship between obesity and brain volume reduction is not limited to the aging period (Gunstad et al., 2008)[85].

Regarding the effect of obesity in pathological aging, Ho et al (2010b)[86] studied two separate cohorts and showed that higher body tissue adiposity is correlated with brain atrophy in patients diagnosed with MCI and AD. These correlations are still found within diagnostic groups, and they persist after adjusting for other factors, such as age, sex, and education.

On the other hand, obesity is associated to WM changes; Jagust et al., (2005)[87] related antrophomorphic measures and age with presence of white matter hyperintensities. Anan et al (2009)[88] studied a type II diabetic patients sample, comparing a subsample of patients with WM lesions to a subsample without WM lesions, the authors performed a multivariate logistic analysis and showed that the high visceral fat accumulation levels and insulin resistance were independent risk factors for the presence of WM lesions (WML) in type 2

diabetic patients. Alkan et al., (2008)[89] showed an increment of diffusion apparent coefficient in areas linked to reward circuits (orbitofrontal cortex and amygdala); it suggested microstructural damage in this regions.

To test the hypothesis that brain changes in obesity are linked to white matter deterioration, Ho et al (2010) [84], studied the effect of the FTO gene in brain structure. The authors wanted to test if the brain volume deficit in FTO carriers observed in their study was related to white matter deficit. The authors found that greater white matter burden (WMB) (logarithm transformation of white matter hyperintensities measure) was also associated with a 10% brain volume deficit in the frontal lobes and precuneus. However the effect of the FTO risk allele on brain atrophy was not explained by an increase in the WMB, this measure was not statistically higher in FTO risk allele carriers than in noncarriers.

Analysis of spectroscopy by resonance imaging reported congruent findings to previous results. In a recent study, Gazdzinski et al., (2008)[90] showed a correlation between low BMI indexes and low levels of N-acetyl aspartate and choline in gray and white matter of frontal lobe.

Contrary to the previous results, some authors have linked obesity to an increase in white matter [91] and gray matter volume [81]. The results of Haltia et al., (2007) [91]showed a correlation between BMI in obese samples and different white matter regions; this correlation effect is lost when the subjects are treated by diet restriction. The authors proposed that the abnormal lipidic metabolism in obese people could affect brain tissue. Recently Anan et al (2009)[88], studying a sample of type II diabetic patients, showed that visceral fat accumulation levels were an independent predictor of hipocampal volume indicators scores. Elevated levels of visceral fat accumulation were characterized by an increase in hippocampus volume. The specific mechanism that links the visceral fat accumulation level and hippocampus volume remains to be elucidated and the authors suggested that this finding should be studied more deeply in the future. Finally, Walther et al (2010)[82] found a positive correlation between BMI and white matter in areas of whole brain; this result is maintained after the control of hypertension effect.

3.2.Hypertension

3.2.1.Hypertension and Cognitive Impairment

In the study of the influence of hypertension in cognitive functions, some authors reported a relationship between the presence of hypertension and the general cognitive impairment [92-95], mild cognitive impairment (specially non-amnesic mild cognitive impairment) [96] and risk of dementia [97,98]. Other authors stressed the influence of hypertension in cognition only when it appears concomitant with other vascular risk factors [99]. Conversely, Posner et al (2002)[100] in a longitudinal study found that hypertension in middle agewas associated neither to higher risk of AD nor to cognitive changes. Hypertension was associated to higher risk of VD, particularly in the presence of other vascular risk factors. Similarly, Johnson et al 2008[101] studied a postmenopausal women sample and found that hypertensive women at baseline appeared to be at greater risk for probable dementia or mild cognitive impairment, although when potential confounders were accounted for, this association was no longer significant. Oveishgaran et al (2010)[102] showed that hypertension did not result in cognitive deterioration across a cohort. However, there was

increased progression to dementia among subjects with hypertension whose cognitive impairment was associated with executive dysfunction but not memory dysfunction. Knech et al., (2009)[103] found that systolic blood pressure explains an 8% variance in global cognition in the age group 44–65 years (midlife) but 0.1% in late-life (age group 65–82 years). The same study reported that a compound of several vascular risk factors (age, smoking pack years, BMI, serum cholesterol, HbA1c, hsCRP) also explain similar percentages of the variance.

Moreover, in patients with a current diagnosis of dementia, hypertension has been related to worse cognitive outcome over time. In AD, Bellew et al., (2004)[104] found that the risk of increased cognitive decline was approximately 1.5 times greater for AD patients with hypertension than for AD patients with normal blood pressure; other authors also found that hypertension was a predictor of worse cognitive performance in AD patients [105].

The cognitive profile of hypertensive patients has not been determined yet, but several authors proposed the alteration of some cognitive domains. Concretely, in a studyahypertensive patient sampleof 65-80 years old were compared to normotensive control group, authors found that hypertensive group presented a decrease in attention, processing speed, memory and executive functions [106]. Saxby et al.,(2003)[107] studied a sample aged between 70-89 years and compared the group of hypertensive subjects to normotensive participants. The authors found that hypertensives performed less well than normotensives in speed of cognition, executive function, episodic memory, and working memory. There was no significant difference in continuity of attention. The executive deficit hasalso been reported in middle age samples of hypertensive patients, [108] and patients older than 65 years [109]. Specifically, Bucur et al., (2010)[110] found that high blood pressure patients have a significant effect in executive function for older adults whilst this effect did not exist in younger samples;theeffect remained when the results are controlled by processing speed performance. On the contrary, other authors only find a negative effect of blood pressure in perceptual motor processing tasks [111].

Suhr et al., (2004)[112] studied a young sample of healthy subjects, and found that after controlling for demographic variables and resting blood pressure, the interaction of systolic blood pressure by age was a significant predictor of performance on the test of attention. Gao et al., (2009)[113] found an association between hypertension and cognitive decline, specifically in the learning and recall on the CERAD word list. Finally, Van den Berg et al., (2009)[65] in a revision concluded that blood pressure has been associated with memory alteration, processing speed, attention and visoconstructive functions.

Transactional studies showed controversial results when studyingthe relationship between blood pressure measures and cognition. Some of these studies found positive association while others did not find significant results [114] or an inverted U pattern [115]. As for longitudinal studies, the results are more consistent, and the vast majority linked a high level of blood pressure to cognitive impairment [116]. There are little differences between those studies that assessed the effect of hypertension in middle-aged samples in comparison to those studies that assessed this effect in elderlypeople. Indeed, the effect detected between hypertension and cognition in studies that used elderly samples was not very strong (van den Berg et al., 2009)[65]. In accordance, Li et al., (2007)[117] found high SBP was associated with greater risk of dementia in the young elderly (<75) but not in older subjects.

3.2.2.Hypertension and Structural Brain Changes

In the last few years, hypertension has been related to structural changes detected by MRI, for instance deep lesions observed in hypertensive patients with lacunar infarcts [118], WM [119] and GM changes [120,121], increase in WM lesions volume [122], increase in lateral ventricle volume [123], as well as changes in specific brain structures. For instance, Harris et al., (2008)[124] reported that increased atrophy in posterior regions of the corpus callosum were significantly associated with increases in blood pressure among elderly subjects;contrarily Leritz et al., (2010)[125] found that increased mean arterial blood pressure was associated with decreased FA in the genu; this effect was more strong in the nonmedicated group.

In a longitudinal study, authors showed that risk of WML lesions increase in hypertensive patients who suffer from long evolution hypertension(more than 20 years)[126]. In accordance, an increase in blood pressure for a period of 5 years has been related to brain atrophy and an increase subcortical lesions [127]. Similar to MRI studies, a recent histopathological study found that high sistolic blood preassure measured at baseline (7.7 years on average before death) was associated with higher occurrence of cerebral microinfarcts (more than two microinfarcts) in younger participants (65–80 at enrollment) but not with the presence of other pathological changes (cystic macroinfarcts, AD neuropathological changes, or neocortical Lewy bodies). This higher risk for microinfarcts was particularly strong in participants who did not control the blood pressure with medications [128].

Nitkunan et al., (2008a)[129] related hypertension to axonal dysfunction and axonal loss. The authors observed an increase in mean diffusivity and a decrease in fractional anisotropy (FA) obtained by DTI in a subgroup of hypertensive patients. These measures correlated with N-Acetil aspartate, a neuronal integrity marker obtained using magnetic resonance spectroscopy. Recent studies showed similar changes in DTI measures in hypertensive patients, both in anterior and posterior cerebral areas; these results emphasize the role of vascular risk factors as modifiers in the effect of aging in WM [130]. Burgmans et al., (2010)[131] studied a sample of patients between 50 and 77 years old and revealed significant effects of hypertension on the WM volumes,WM hyperintensitites (WMH) and FA. In addition, they observed a significant interaction effect on fractional anisotropy between hypertension and age. The authors suggested that FA is more sensitive than classic measures of WM alteration such as WM volumes or WMH.

Cognitive alterations related to hypertension are also associated with structural brain changes. Changes in GM and WM in these patients are associated with poor performance in processing speed, short-term memory[120], attention [119] and executive function [132].

3.3.Dyslipidemia

3.3.1.Dyslipidemia and Cognitive Impairment

Dyslipidemia, as an independent vascular risk factor, has not been widely studied and the results of the studies are controversial. We include in the dyslipidemia definition an abnormal or atypical lipidic profile detected by blood tests. Specifically, in this review we discuss the results about the effect of high level of triglycerides, low level of high-density lipoproteins (HDL), and levels of total cholesterol.

The effect of triglyceride levels in cognitive function has been reported in previous studies, showing an influence in reaction time, mental control and verbal fluency tasks [133]. In a 10-year follow-up study, triglyceride level in baseline was related to verbal knowledge tasks performance; this association was stronger in the apolipoproteine allele ε4 carriers [134]. In type II diabetes patients, there is an inverse relationship between high levels of triglycerides and the performance of processing speed and reaction time tasks. The authors suggested that high level of triglycerides in these patients contribute to the observed cognitive impairment, independently to the effect of chronic control of glucose levels [135].

High cholesterol levels in the adulthood [136] as well as a reduction of this level in posterior periods [137] have been related to a high risk of mild cognitive impairment development during aging. Hypercholesterolemia has been related to poor global cognitive performance in postmenopausic women [138], and Muldoon et al., (1997) [139] related high cholesterol levels in middle age to low levels in verbal knowledge performance. Similarly, in an18-year follow-up study, Mielke et al., (2005)[140] reported that non-smoker subjects with high total cholesterol levels in the last studied years have less risk of dementia. Familiar hypercholesterolemia studies showed that these patients (with high total cholesterol levels, high levels of low densitity lipoproteines (LDL) cholesterol and high triglycerides levels) showed poor neuropsychological profiles in comparison to the control group. Moreover, the patient group developed mild cognitive impairment more likely than the controls. Specifically, the familiar hypercholesterolemia patients showed worse scores on the Mini Mental State Examination (MMSE) and memory tests. Cholesterol level was independently associated only with worse scores in the Trail Making Test Part B [141]. Finally, Carlsoon et al., 2010 [142], in a population-based cohort study, found that participants in the highest quartile of non-HDL (total minus HDL) cholesterol had an increased likelihood of general cognition impairment compared to those in the lowest quartile (cognition was evaluated by screening methods using MMSE). Statin use was associated with better cognition but this relationship was not significant after adjusting for other factors. In relation with this last aspect, van Vliet et al., (2009)[143] reported that statin use is associated with better cognitive function in cross-sectional and short follow-up studies, whilst randomized controlled trials and observational studies show that statin use is not associated with the risk of cognitive impairment.

In relation to HDL cholesterol, among the different effects linked to this type of cholesterol, some studies reported its protective effect to prevent mild cognitive impairment development. In a sample of elderlypeople, only the low level of HDL cholesterol and high blood pressure measures were significant factors to explain the cognitive impairment and the subcortical alterations (Geroldi et al., 2003)[144]. Atzmon et al., (2002) [145] found a positive correlation between HDL cholesterol level and general cognitive performance assessed by MMSE in 95 year old people. On the other hand, some follow-up studies found that neither total cholesterol levels nor HDL-cholesterol levels during aging are related to posterior development of AD [146]. Other authors did not find any relationship between the lipidic profile and cognitive functions such as language, memory and visuospatial skill [147]. Teunissen et al., (2003) [148] did not find any relationship between total cholesterol levels and cognition in a follow up study, even though they found a significant relationship between cholesterol precursor (lathosterol and lanosterol) and memory task in baseline and follow-up. In summary, cross-sectional studies, which included old subjects, showed an association between high levels of HDL cholesterol and less risk of dementia or better cognitive

performance. But most follow-up studies reported no association between HDL cholesterol levels and cognition. Therefore, to date there are no conclusive evidences for beneficial effect of HDL cholesterol levels and the risk of dementia or cognitive decline in late-life [149].

Contrary to the previous reported results, Henderson et al., (2003)[150] observed that high levels of LDL cholesterol, and increase of total cholesterol and LDL cholesterol in the last 8 years were associated with better memory performance. Accordingly, other authors showed that high levels of total cholesterol are related to better execution in different cognitive functions such as visoconstructive skills [139] and reaction time [151]. Zhang et al., (2004)[152] studied the relationship between total cholesterol concentration and the results in neuropsychological tasks such as immediate memory, visuomotor speed, and codification speed in people between 20-59 years old. The results showed that low total cholesterol level is associated with a decrease in processing speed, specifically in middle-aged men. This association remains independent to the effect of other sociodemographic variables or vascular risks factors.

The observed controversy can be explained by different reasons. On the one hand, in previous studies the lipidic profile is defined using different variables. The total cholesterol measure is the combination of different kinds of cholesterol;the association between total cholesterol level and cognitive functions reported by some of the studies could be the consequence of both an increase or decrease of HDL or LDL, or maybe the combination effect of both. Some of the reviewed studies did not specify the lipidic profile of their samples, and therefore the obtained results could be interpreted in a different way. On the other hand, the age effect is an important variable to take into account in the revision of these studies' results. The lipidic profile can affect differentially to cognition depending onthe life period assessed. Accordingly, Reynolds et al., (2010)[153] found that the effect of lipids and lipoproteins on cognition is most prominent before age 65, specifically in women. Low triglycerides, low apolipoproteineB (apoB), and high HDL-C values were beneficial to maintaining cognitive abilities, particularly for verbal ability and perceptual speed. Lipid values were less predictive of cognitive trajectories in men. The same study analysed the differences in the lipidic profile of twenty-one pairs of discordant twins for incident dementia.The authors concluded that the affected twin had significantly higher values of total cholesterol and apoB than the unaffected cotwin in the baseline assessment. Van Vliet et al., (2009)[149] performed a complete review about this topic and summarized that, when measured in midlife, high total serum cholesterol levels are associated with an increased risk of mild cognitive impairment and dementia. On the contrary, the relation between high total serum cholesterol level and cognitive impairment measured in late-life is unclear.

Finally, the results of recent studies suggested that the effect of lipidic profiles on cognition depends of other concomitant factors, such as the presence of inflammation. In accordance with this, Van den Kommer et al., (2010)[154] in a longitudinal study, reported associations between HDL and LDL cholesterol, triglycerides, and cognitive functioning over 6 years of follow-up. They focused on the modifying effect of inflammation on these associations in persons aged 65 years and older. The authors showed an association between low level of LDL and high levels of triglycerides with cognition, modified by the effect of levels of inflammation (measured by inflammation marker C-reactive protein, α-antichymotrypsin). A negative additive effect of low LDL cholesterol and high inflammation was found on general cognition and memory performance. Also, high triglycerides were associated with lower memory performance in those with high inflammation.

3.3.2.Dyslipidemia and Structural Brain Changes

Hypercholesterolemia is a development mechanism of atherosclerosis [155]. Moreover, high level of cholesterol is related to high risk ofVD [156] and the presence of silent infarction [157]. Specifically, low HDL levels have been associated with high risk of brain infarct and atherosclerosis in carotid artery [158]. Recently, Crisby et al., (2010)[159] found that low HDL levels were associated with a higher severity of WM changes on MRI, although the presence of the Apo E4 allele was higher in the group of subjects with severe changes;the authors did not find a statistically significant group difference in severity of WML between carriers and noncarriers of Apo E4 allele.

Some neuroimaging studies related both the lipid profile and high blood pressure to the presence of multifocal lesions observed in T2 weighted gradient echo images. The authors found a relation between the low levels of total cholesterol and high severity levels of these lesions [160]. Other authors just found a tendency regarding the association between HDL cholesterol and silent brain infarcts, but they reported that total cholesterol, LDL cholesterol and non-HDL cholesterol were significantly associated to these lesions after adjustment for age, smoking status, serum triglycerides, maximal-intima-media thickness, obesity, hypertension, diabetes mellitus, hyperuricemia, coronary heart disease and lipid-lowering agent use. The author suggested that these results could be influenced by the small numbers of participants in the study with silent brain infarcts [161]. Similarly, total cholesterol and LDL cholesterol correlated positively to cerebrospinal fluid volume, and negatively to total gray matter volume in old subjects without dementia [162].

Familiar hypercholesterolemia studies showed that these patients, under treatment, did not show WML in comparison to healthy people [163,164]. Even though other studies have related the HDL cholesterol levels in these patients to an increase in preclinic carotid atherosclerosis [165].

Other authors found a relationship between deep WML and plasmatic cholesterol levels jointly to the effect of other vascular risk factors. This relationship is not observed in the periventricular WML[166]. However, Amarenco et al., (2006)[167] showed a relationship between all cerebral infarction types and lipid profiles, especially with total cholesterol levels and LDL cholesterol level.

3.4.Insulin Resistance and Type II Diabetes

3.4.1.Insulin Resistance, Type II Diabetes and Cognitive Impairment

Some authors suggested two relevant life periods in which diabetes or insulin resistance can affect cognition; first during childhood and later after 65 years old [168]. However, other authors proposed that diabetes and insulin resistance could affect cognition throughout life, and could produce cognitive and structural changes, affecting aging.

High fasting glucose levels have been related to VD and AD [169], diabetes has been linked to high risk of vascular cognitive impairment (170) and the risk of progression to mild cognitive impairment to dementia[171]

Glucose intolerance and fasting glucose abnormalities are insulin resistance manifestations. Longitudinal studies related glucose intolerance and cognition; the authors associated this condition with low general cognitive scores (MMSE) and poor short-termmemory performance in a three-year follow-up period [172]. Another work showed that

glucose intolerance is related to verbal fluency [173]. These studies emphasize that cognitive impairment is found when glucose intolerance is constantly present in the follow-up period. Transectional studies found that fasting glucose abnormalities were related to processing speed, attention, verbal fluency and memory in diabetic women [174], as well as frontal lobe tasks performance in healthy people [175]. On the contrary, other studies did not agree with that proposal, and did not find evidences about the relationship between glucose intolerance and cognition [176,177]. In accordance with these negative results, Euser et al., (2010)[178] found that in participants without a history of diabetes, a rise in fasting glucose levels in the nondiabetes range was not associated with impairment in cognitive function for any of the cognitive tests. However, at baseline moment, participants with a history of diabetes had worse cognitive performance compared with participants without a history of diabetes.

General cognitive impairment measured with screening tests [99,179] as well as cognitive alterations in specific cognitive domains has been reported in diabetic patients [180]. Moreover, diabetes is an independent predictor of cognitive decline in the elderly population [181].

Regarding the specific cognitive decline related to diabetes, processing speed alterations havebeen shown in middle aged adults [182] and elderlypeople (more than 60 years old) [183] with type II diabetes. A similar effect is detected between glicosilate haemoglobin levels and processing speed in type I diabetes patients [184]. In addition to processing speed deficit, some studies showed executive functions [185,186], working memory [187] and declarative [188,189] deficits in different samples of diabetic adult patients. A recent study showed significant differences in executive functions and speed in diabetic patients. The authors observed a tendency for worse performance in older diabetic subjects but the effect was not strong. They proposed that the effect could be constant throughout the years, at least in the range of severity and duration of study [190].

Other studies found that diabetic patients older than 85 years old showed a less evident cognitive decline [185]. However, future studies could elucidate the possible ceiling effects, selective survival or even protective effects of high glucose levels in the most elderly. Several authors suggested that a bell-shaped relationship could exist between ages, diabetes and cognitive function with the greatest effect in the 65– 75 age range and declines thereafter [191].

3.4.2.Insulin Resistance, Type II Diabetes and Structural Brain Changes

In relation to structural damage in prediabetic and type II diabetic patients, several studies have observed WM and GM abnormalities, as well as a relationship between these structural changes and the cognitive profile previously reported.

In a longitudinal study, the presence of diabetes and prediabetes in baseline was related to ventricle volume increase in the follow-up. The authors proposed the hypothesis that diabetes and/or other concomitant vascular risk factors could be the cause of microvascular lesions, and consequently the brain volume alterations and, with time, cognitive impairment [191]. In accordance with these results, Kumar et al., (2008a)[192] observed more atrophy and higher cerebrospinal fluid volume in diabetic patients in comparison to healthy subjects, even though in this study the authors did not observe significant differences in GM or WM volume or number of WML. Tiehuis et al., (2008)[193] studied patients with symptomatic arterial disease (cerebrovascular, coronary, or peripheral artery disease), and found that patients with type II diabetes showed more brain atrophy and larger WMH volumes than identical patients

without type II diabetes. Other recent studies reported negative results; Raji et al., (2009)[83] studied a sample of healthy elderly subjects and found that higher fasting plasma insulin and type II diabetes was associated with lower regional brain volumes in both GM and WM. Specifically fasting plasma insulin was associated with reduced volume in the frontal lobes, hippocampus, and the splenium of the corpus callosum. In Type II diabetes patients, the authors detected lower volume in multiple brain regions including the frontal lobes, prefrontal cortex, genu and splenium of the corpus callosum, middle cingulate gyrus, superior parietal lobule, the occipital lobes, and the cerebellum and basal ganglia. However, in the multiple regression models, BMI was the only variable that was significantly linked with brain atrophy in GM and WM, and there were no independent associations between fasting plasma insulin andtype II diabetes once BMI was accounted for, with the degree of brain atrophy.

Other authors showed that diabetes is related to WM changes, especially in structures involved in memory, language [194,195] and frontal lobe functions [183]. Indeed, Korf et al., (2006) [196], studied a Japanese-American cohort and showed that subjects with type 2 diabetes had a moderately elevated risk for lacunes and hippocampal atrophy, even though they did not find significant results for impaired glucose tolerance subjects.

Several studies reported structural brain alterations in type II diabetes patients groups. For instance, type II diabetic patients have more deep WM alterations than controls [180], as well as more cortical and subcortical atrophy [180,197] and hippocampal volume reduction [189]. These structural alterations have been related to low performance in attention, executive functions [180], processing speed [180,198] and memory [180,199]. Several of these studies highlight the concomitant effect of other vascular risk factors, for instance Bruehl et al., (2009) [189] showed that cortisol control and dislipemia were associated with declarative memory performance, as well as an inverse correlation between hipocamppal volume and obesity in diabetic patients.

In a comparison among type I, type II diabetic patients and healthy subjects [200] both patient groups had a similar cognitive profile, but the type II diabetic patients showed more WML and cortical atrophy. The authors concluded that these structural abnormalities could be associated with the presence of other vascular risk factors, commonly related to type II diabetes diagnostic.

Over the last few years, DTI has been used to study diabetes effects in the brain. The first studies found that type I diabetic patients showed microstructural WM abnormalities. This study showed a FA reduction in corona radiata and optic radiation in diabetic patients. Moreover, the DTI measures correlated to cognitive performance in Rey's Complex Figure and Grooved Pegboard test [201]. Recently, Yau et al., (2010)[202] studied type II diabetic patients using this technique, and showed a reduction in FA in patients in comparison to a control group, with the largest cluster in the temporal lobe. Moreover, the measures of declarative emotional and neutral memory correlated positively with temporal FA measures.

Finally, even though all the evidences from cross-sectional studies, a recent longitudinal study about brain changes in type II diabetic patients reported that both groups, patients and controls, showed a significant change over time. Patients with type II diabetes had a greater increase in lateral ventricular volume than control participants. The authors suggested that cerebral atrophy in patients with type II diabetes progresses only slightly faster relative to control participants over the course of the years [203].

In conclusion, diabetes and hypertension and, to a lesser extent, obesity and dyslipidemia are associated with mild to moderate decrements in cognitive functioning in demented and

non-demented subjects. The results are still controverted and more studies are required to specify the profile of cognitive decrements, however, we could summarize that the most consistent results showed deficits in memory, processing speed and executive functions associated with the presence of these vascular risk factors.

4. Metabolic Syndrome: The Role in Brain and Cognition

4.1.Concept of Metabolic Syndrome: Definition and Prevalence

The MetSd has been defined as a cluster of vascular risk factors including: obesity, insulin resistance (IR), low HDL cholesterol, high levels of triglycerides (TG) and hypertension (HTA). Although the exact mechanisms of its development have not been fully defined, its aetiology includes genetic factors and modifiable environmental factors, such as eating habits and a sedentary lifestyle.

In the early twentieth century, Kylin described a syndrome that involved hypertension, hyperglycemia and hyperuricemia. Subsequently, Vague (1940) explained that the distribution of abdominal fat could influence the development of diabetes and related illnesses. Avogardo and Crepaldi in 1965, during the European Association for Study of Diabetes Annual Meeting [204], referred to a syndrome involving hypertension, hyperglycemia and obesity. The definition of syndrome progressed in 1988 with the intervention of Reaven who introduced the term Syndrome X or insulin resistance syndrome [205]. Subsequently, this syndrome has received other names: hypertriglyceridemic waist (hypertriglyceridemic waist) [206], The Deadly Quartet [207], dismetabolic cardiovascular syndrome [208] or MetSd.

MetSd is the most commonly used name at present; the definition criteria have been revised by different organizations. Definitions proposed by these organizations do not differ too much and show different positions in relation to the predominant cause of MetSd [209] (Table 1).

In the definition proposed by the World Health Organization (WHO) in 1998 the fundamental criteria for diagnosing was the presence of insulin resistance. Similarly, a year later the European Group for Study of Insulin Resistance (EGIR) proposed the term syndrome of insulin resistance and gave more importance to this symptom. In 2001 the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) generated less stringent and more practical criteria for prevention and clinical practice. The NCEP ATP III criteria did not require the presence of a specific risk factor. The diagnosis is positive ifthe case showed 3 of the 5 following risk factors: obesity, hypertriglyceridemia, reduced HDL cholesterol levels, hypertension and elevated glucose levels (or insulin resistance in type II diabetes). In 2003 the American Association of Clinical Endocrinologists (AACE) proposed a hybrid between the criteria of NCEP-ATP III and WHO, demanding insulin resistance for diagnosis of MetSd. In 2005 the International Diabetes Foundation (IDF) highlighted obesity as a fundamental requirement for the diagnosis [210]. The same year 2005, the American Heart Association / National Heart, Lung, and Blood Institute Scientific Statement (AHA / NHLBI) considered keeping the NCEP-ATP-III definition except for minor modifications;

these criteria were simple to implement in clinic practice and allowed avoidance of emphasizing a single cause of the syndrome. Finally, in 2007 The International Consensus of the European Society of Cardiology and the European Society of Hypertension (ESC / ESH) reformulated the criteria proposed by NCEP-ATP III in 2001, changed the thresholds for the measurement of HDL cholesterol, and excluded the treatment of hyperglycemia and diabetes from the MetSd criteria[14]. Currently, most studies continue using the NCEP ATP-III criteria (2001) or the NCEP-ATP III, AHA / NHLBI (2005). Recently, Alberti et al., (2009)[211] in a joint interim statement of the International Diabetes Federation task force on epidemiology and prevention, National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society and International Association for the Study of Obesity discussed the SdMet definition and proposed unified criteria. The experts based their work in the NCEP-ATP III AHA/NHLBI (2005) definition, with minor modifications; concretely they pointed out the use of specific cut-off points according to the population and country to determinate the waist circumference criterion, similarly to previous IDF suggestions (see Alberti et al., 2009[211] to see current recommended waist circumference thresholds for abdominal obesity).

The prevalence of MetSd in the world is high and increases progressively. This increase is closely related to the growing worldwide aging population. Recent reviews use the concept of pandemic due to the Metabolic syndrome presence being between 20% and 30% of the world's population [3].

In the United States, 23.7% of the population over 20 years suffers MetSd and the percentage increases with age, reaching 43.5% of the population of 60-69 year olds[212]. In Europe approximately 25% of adults meet the MetSd criteria, although these values vary by age, geographic location or the characteristics of the study population, as well as by the defining criteria used for the study. Regarding the criteria used, the Diabetes Epidemiology: Collaborative Analysis of Diagnostic Criteria in Europe (DECODE) [213] determined the prevalence of MetSd in European cohorts according to the use of various definitions. The results were that IDF criteria ranged between 27 and 35.9% for males and 19.7 and 34.1% for females;these results are higher than those obtained with other criteria. This is because the definition of the IDF provided a lower threshold criterion for waist circumference.

MetSd has been related to pathological processes: glucotoxicity, lipotoxicity and inflammation which increase the risk to suffer different pathologies [214], especially cardiovascular disease. Until now, there is little evidence regarding the fact that MetSd is associated with CNS damage. One of the hypotheses is that molecular alterations, which occur typically in aging [215], could be modified by MetSd, and it could produce a quantitative modification of these processes affecting the CNS. The presence of vascular risk factors has been associated with the alteration of brain circulation regulation. It could affect brain protection mechanisms, which typically prevent cerebrovascular damage [216]. Recently, some authors proposed that MetSd is related to cerebrovascular alterations and cognitive impairment, mainly during aging.

Table 2. Metabolic syndrome and cognitive impairment

Studies	Sample (n)	MetSd Criteria	Cognitive assessment	Main results
Kalmijn et al., 2000	3734	NS	CASI, IQCODE, DSM-IV	Higher risk of dementia associated with MetSd
Yaffe et al., 2004	2632	NCEP ATP-III	3MS	Worse cognitive performance at follow-up
Roriz-Cruz et al., 2006/ 2007	422/420	NCEP ATP-III (2005)/WHO	MMSE	Higher risk of cognitive impairment associated with MetSd
Ho et al., 2007	1352	IDF	MMSE	Higher risk of cognitive impairment associated with MetSd
Yaffe et al., 2007	1624	NCEP ATP-III	3MS, delayed Word recall	Worse cognitive performance associated with MetSd
Komulainen et al., 2007	101	NCEP ATP-III	MMSE, Stroop Letter-Digit Substitution Word recall	Higher risk of low performance in memory tests associated with MetSd.
Dik et al., 2007	1183	NCEP ATP-III	MMSE Verbal learning test Codification task Raven matrices	Worse cognitive performance associated with MetSd
Razay et al., 2007	125	NCEP ATP-III	DSM-IV NINCDS/ADRDA	Association between Alzheimer's disease and MetSd
Van den Berg et al., 2007	599	NCEP ATP-III	MMSE Stroop Digit Coding Test 12 Word Learning test	Decelerated cognitive decline in MetSd patients older than 85 years.
Muller et al, 2007	2476	NCEP ATP-III/ EGIR	Neuropsychological history, Medical history, functional assessment, neurological assessment, CDR DSM-IV NINCDS/ADRDA	The authors did not find higher risk of dementia associated with MetSd.
Luadisio et al., 2008	353	NCEP ATP-III	AMT	Sdmet is associated with better cognitive performance in women older than 80 years.
Van den Berg et al., 2008	247	NCEP ATP-III	Raven matrices Digits WAIS-III Corsi Block Tapping Task AVLT Spatial learning The Rey–Osterrieth Complex Figure Test Stroop test TMT A i B Brixton Spatial anticipation test Digit symbol WAIS-III Token test Verbal fluency	DM-II group and MetSd group showed processing speed deficits. The DM-II group also showed low performance in attention and executive functions.
Gatto et al., 2009	856	NCEP-ATP-III	SDMT TMT B JLO BNT Letter and Numbers WMS-III Verbal fluency Blocks WAIS-III Faces I-II WMS-III Logical memory WMS-III CVLT	SdMet adults showed a trend to worse performance in semantic memory tasks

Table 2. (Continued)

Studies	Sample (n)	MetSd Criteria	Cognitive assessment	Main results
Gatto et al., 2009	856	NCEP-ATP-III	SDMT TMT B JLO BNT Letter and Numbers WMS-III Verbal fluency Blocks WAIS-III Faces I-II WMS-III Logical memory WMS-III CVLT	SdMet adults showed a trend to worse performance in semantic memory tasks
Lee et al., 2009	2944	NCEP-III	K-MMSE	In people older than 60 years, MetSd was not associated to k-MMSE scores.
Yaffe et al., 2009	4895	NCEP-III	Short Blessed Test TMT A and B Verbal fluency Word list recall	Higher percentage of cognitive impairment in the MetSd group.
Roberts et al., 2009	1969	NCEP-III	Logical memory WMS-R Visual reproduction WMS-R AVLT Verbal fluency Simbol digits WAIS-III Picture Completion Blocks (WAIS-R) BNT TMT A and B	MetSd and hgh levels of inflamation were associated with non-mnesic MCI.
Fergen baum et al., 2009	190	NS	Clock drawing test TMT A and B	Obesity and metabolic syndrome were associated with low cognitive performance (assessed by TMT)
Segura et al., 2009	90 (50 Metsd patients)	NCEP-APT III	RAVLT Faces I WMS-III Digit WAIS-III Arithmetic WAIS III Benton Facial Recognition Test (short version) Blocks WAIS-III WCST (short version) TMT A and B Verbal fluency Stroop test SDMT GPT Reaction time in CPT-II BNT	There were differences between groups in speed of processing and some executive functions after controlling for the influences of education and gender.
Raffitin et al., 2009	7087	NCEP-APT III	MMSE Benton Visual Retention Test Isaac's Set Test DMS-IV	Subjects with metabolic syndrome at baseline had a significantly increased risk of vascular dementia over 4 years but had a non significant increased risk of all-cause dementia.

NS: not specified. CASI: Cognitive Abilities Screening Instrument. IQCODE: Informant Questionnaire on Cognitive Decline in the Elderly. DSM-IV: Diagnostic and Statistical Manual of Mental Disorders, 4th Edition. NCEP-ATP-III: The National Cholesterol Education Program (NCEP)-Adult Treatment Panel III (ATP-III) 3MS: Modified Mini Mental State Examination. WHO: World Health Organization. Modif: Modified. MMSE: Mini Mental State Examination. IDF: International Diabetes Foundation. NINCDS/ADRDA: National Institute of Neurological Communicative Disorders and Stroke and the Alzheimer Disease and Related Disorders Association. DSM-III-R: Diagnostic and Statistical Manual of Mental Disorders, 3th Edition review. CDR: Clinical Dementia Rating score. AMT: the Hodkinson Abbreviated Mental Test. AVLT: Auditive verbal learning test. TMT: Trail Making Test.SDMT: Symbol Digit Modalities Test. JLO: Judgment of Line Orientation. WAIS- III: Wechsler Adult Intelligence Scale 3rd edition. WMS-III: Wechsler Memory Scale 3rd edition. BNT: Boston Naming Test. CVLT-II: California Verbal Learning Test 2nd edition. WCST: Wisconsin Card Sorting Test. GPT: Groove Pegboard test. WMS-R: Wechsler Memory Scale Revised WAIS-R: Wechsler Adult Intelligence Scale Revised.

4.2.Metabolic Syndrome and Cognitive Impairment

Only few authors studied the hypothesis of MetSd effect on cognition (Table 2). In 2000 appeared a longitudinal study, prospective from 1965 to 1991, which correlates the presence of vascular risk factors MetSd of the baseline (when subjects were 55.8 ± 4.7 years) with presence of dementia 25 years later (average age was 77.8 ± 4.6 years).

The diagnosis of dementia in old age, especially in the diagnosis of VD, correlated positively with the presence of vascular risk factors of MetSd [217]. Subsequent studies have shown that elderly subjects diagnosed with MetSd have greater cognitive impairment, measured by the Modified Mini-Mental State Examination (3 MS) than subjects with no diagnosis [218]. The association between MMSE decreased and the MetSd is observed after controlling the effect of other vascular risk factors [219].

Apart from general cognitive impairment evidences, several studies reported concrete cognitive deficits related to MetSd. In a longitudinal study with a 3-year follow-up, patients with MetSd also show changes in recognition memory tasks. A prospective study of women over 60 years showed a higher percentage of subjects with cognitive impairment among people diagnosed MetSd compared with a group of people without these criteria [220]. Other studies reported that the overall risk of cognitive impairment increases 23%, age adjusted. Moreover, a study found a decrease in the semantic memory scores and learning tasks for each added component of MetSd [221].

In a 12 year follow-up study, a sample of women who were between 60-70 years old was studied, showing that women who met MetSd criteria at baseline had a 4.7 times higher risk to worse memory performance assessed by word memory test at the follow-up time. The risk model was obtained adjusting variables such as age and education. Contrary to what might be hypothesized, the authors did not find an association between MetSd and general cognitive performance assessed by MMSE, and neither a relationship between MetSd and processing speed performance [6]. In another study with a sample of 1,183 elderly, the authors found a relationship between MetSd and processing speed, as well as other functions such as immediate memory, fluid intelligence and general cognitive level (MMSE) [5].

Other studies have related MetSd to slowing processing speed and executive functions deficits [222]. Thus, van den Berg et al., (2008)[222] studied two groups of patients, one with type II diabetes and the other with a prediabetes state (with MetSd diagnostic criteria);they found that both groups performed worse on attention, executive functions and processing speed tasks in comparison to a control group. Accordingly, in a study of Fergenbaum et al., (2009) [223] obese subjects and subjects having MetSd were more likely to have low Trail Making Test composite score (TMTB-TMTA/TMTA) compared to those who were not obese and who did not have MetSd. The MetSd has also been associated with fronto-subcortical alterations during aging [224], greater dependency in activities of daily living, depression, cognitive impairment and worse perception of quality of life in individuals over 60 years [225].

To our knowledge, most studies of MetSd are cross-sectional and use regression analysis to evaluate the association between the syndrome and cognitive decline. Only a few of them include an extensive neuropsychological assessment of MetSd patients. For that reason, we recently did a case-control study in which we compare the neuropsychological performance of a MetSd group (50-80 years old, without other pathology) and a control group (without any of the vascular risk factors of MetSd) matched by age, gender and educational level. In this

study, we performed an extended neuropsychological assessment of different cognitive functions. Similarly to previous works, we detected poor performance of MetSd subjects in comparison to controls. Specifically, we detected a reduction in processing speed and poor performance in some executive functions (see Table 3). After controlling for the effect of education and gender, the effect was maintained for several neuropsychological tests, concretely for the Grooved Pegboard test dominant and non-dominant hand, retroactive interference of Rey Auditory verbal learnig test, semantic fluency and arithmetic subtest of WAIS-III. On the contrary, we did not find significant differences in verbal learning and long term recall, visuoperceptual and visuospatial abilities, and high-level executive functions. Our results showed a specific neuropsychological profile in MetSd patients characterized by a reduction in processing speed and mild executive dysfunctions. Our results suggested that MetSd presence mightindicate a prodromal state for vascular cognitive impairment [11].

Table 3. Neuropsychological results of metabolic syndrome and control groups. Raw scores (Mean±SD, range and number of subjects)

Neurospychological Test	Metabolic Syndrome				Control				t/U	p	ESr
	M	SD	Range	n	M	SD	Range	n			
RAVLT Total	46.07	8.99	28-66	55	48.23	9.64	33-69	35	-1.08†	NS	-
RAVLT Del	9.51	2.40	5-15	55	10.34	2.00	6-15	35	-1.71†	NS	-
RAVLT RI	3.37	1.89	0-8	54	2.35	1.77	0-7	34	2.52†	0.014	0.27
Faces I	35.42	4.95	23-48	55	37.74	5.00	26-46	35	-2.16†	0.033	0.24
Forward D.	6.96	1.81	4-13	55	8.09	1.88	4-12	34	-2.81†	0.006	0.29
Semantic F	18.55	4.80	10-31	55	21.43	4.95	12-31	35	-2.75†	0.007	0.28
Phonetic F	12.67	4.80	4-22	55	14.09	4.54	7-25	55	-1.39†	NS	-
WCST Persev	16.38	14.16	2-63	55	11.14	9.48	1-34	55	1.92†	NS	-
WCST Cat	1.85	1.08	0-3	55	2.23	0.97	0-3	35	-1.67†	NS	-
TMT B	131.70	72.25	50-385	43	112.87	57.51	39-55	31	1.20†	NS	-
Stroop test	2.54	8.52	(-18)-22	53	4.78	8.45	(-12)-26	35	-1.21†	NS	-
Arithmetic	9.44	3.44	5-22	39	11.88	4.13	5-18	26	325††	0.014	0.32
SDMT oral	33.67	16.19	9-76	55	38.29	14.51	15-74	35	-2.81†	0.006	0.15
GPT DH	89.87	27.72	59-221	55	74.03	15.39	62-233	34	3.05†	0.003	0.33
GPT NDH	101.02	32.40	62-233	54	82.29	20.26	55-170	34	528††	0.001	0.36
CPT-II RT	453.23	63.30	305-557	42	414.57	49.79	322-487	26	334††	0.007	0.33
TMT A	52.73	22.64	24-126	52	46.45	22.98	22-146	33	1.24†	NS	-
Facial Benton	47.18	4.05	39-55	55	48.00	3.75	40-54	34	-0.95†	NS	-
Block	27.22	11.74	10-54	55	35.09	11.45	12-59	35	3.13†	0.002	0.32
BNT	12.60	1.38	8-15	55	12.83	1.12	10-15	35	-0.82†	NS	-

RAVLT, Rey Auditory Verbal Learning Test; Del, Delayed; RI, retroactive interference; Faces I: Faces I subtest of Wechsler Memory scale III; Forward D, Forward Digits subtestWechsler Adult Intelligence Scale III; F, Fluency;WCST, Wisconsin Card Sorting Test; Perserv, perseverative responses; Cat, number of Categories; TMT B, Trail Making Test Part B; Backward D, Backward Digits subtest ofWechsler Adult Intelligence Scale III; Arithmetic, Arithmetic subtest ofWechsler Adult Intelligence Scale III; SDMT, Symbol Digit Modalities Test; GPT, Grooved Pegboard Test; DH, Dominant Hand, NDH, Non Dominant Hand; CPT-II RT, Continuous Performance Test Reaction time; TMT A, Trail Making Test Part A; Block, Block subtest ofWechsler Adult Intelligence Scale III; BNT, Boston Naming Test; ES r, effect size r absolute value; NS, non-significant values.

† t d' Student's test.

†† U Mann–Whitney test.

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In recent years, the first studies about the relationship between MetSd and AD appeared[226]. Comparing a sample of patients with AD and healthy controls, a study showed that MetSd triples the risk of the disease. Moreover, in a longitudinal study [4] in which the presence of vascular risk factors was assessed three and a half years before the completion of an evaluation of dementia, AD was more frequent in subjects with MetSd and the prevalence was higher in women. Contrarily, Raffaitin et al., (2009) [227] studied the influence of MetSd at baseline in the risk of dementia over 4 years. The authors did not find significant increased risk for all-cause dementia over 4 years. High triglyceride level was significantly associated with the incidence of all-cause dementia. Diabetes, but not impaired fasting glycemia, was also significantly associated with all-cause dementia. When the authors studied the risk of dementia subtypes, they found that MetSd at baseline increased the risk of VD over time. Regarding AD, the presence of MetSd was not a significant factor to increase the AD risk; in fact among the MetSd criterion only high waist circumference at baseline was significantly associated with risk of AD at the follow-up. The authors suggested that MetSd is not a valuable variable to detect dementia risk (all dementia subtypes) in the studied population. Solfrizzi et al., (2010)[228] in a 3.5-year follow up study found similar results according the association between MetSd and VD. The authors reported that MetSd patients had threefold increased risk of VD but not for other dementia subtypes. This risk was even higher in the subjects with high levels of inflammation. The authors, opposite toRaffaitin et al (2009) suggestions, verified the synergic effect of MetSd in comparison to the individual effect of its components.

Recently, the association between MetSd and mild cognitive impairment was studied. Roberts et al. (2009) [229] found that high levels of inflammation and MetSd were associated with non-amnesic mild cognitive impairment. Soffizi et al., (2009)[230] did not find a significant association between MetSd and incident mild cognitive impairment (defined by Petersen et al., 1999[52] criteria), but among the mild cognitive impairment subjects, patients with MetSd were at higher risk of progression to dementia.

The association between cognitive impairment and MetSd has been described above in the population younger than 75 years old. Conversely, in samples of higher age (over 85 years) van den Berg et al. (2007) [231] described the presence of MetSd associated with decelerated cognitive decline. In relation to this conclusion, Forti et al., (2010) [232] found a borderline significant effect between the presence of MetSd and lower risk of AD in an older group of subjects (>75 years). Other studies described the absence of cognitive deficit [220,233] or differences did not reach statistical significance [234]. Lee et al. (2009)[220] showed that MetSd was not related to MMSE (Korean version) scores, adjusting the analysis for the effect of age, education and gender. In addition the authors examined the effect of genotype of Apoε for the diagnosis of MetSd, its components, and cognitive impairment in aging. Among the MetSd componentsa significant interaction between TG, HDL cholesterol and Apo (ε4), which affects cognition was observed. Finally, a multiethnic study of 2,476 people (over 65 years old) found that MetSd is not associated with an increased risk of dementia, while diabetes and hyperinsulinemia are major risk factors, indicators of AD. The authors explain the negative results of these studies as a survival effect, a protective effect of vascular risk factors in elderly (over 65), and the influence of ethnic heterogeneity of the samples.

4.3.Metabolic Syndrome and Structural Brain Changes

During the last decade, evidences about the fact that MetSd is related to high risk of cerebrovascular disease increased (Table 4). The presence of MetSd is associated with risk of stroke in middle-aged people (235), independently of the presence of other vascular risk factors [236] and the MetSd diagnostic criteria used [237]. Boden-Albala et al., (2008)[238] showed an association between MetSd and risk of stroke, and vascular events (ischemic stroke, myocardial infarction, or vascular death) after controlling the analysis for sociodemographic and risk factors. The stroke risk was higher in women and Hispanic people in comparison to Afro-American and Caucasian people.

The relationship between MetSd and stroke has been studied in a stroke patient sample using MRI angiography. Patients with intracraneal atherosclerosis were compared to a group of patients without atherosclerosis or with extracranial atherosclerosis. The presence of MetSd was higher in intracranial artherosclerosis patients than in other groups. In this group, WM and GM alterations were more present, and these lesions were more prevalent [239].

The presence of MetSd is an independent risk factor of silent brain infarction (SBI) in healthy people. In a study of healthy people between 20 and 86 years old, the presence of silent infarction was higher in people who fulfill MetSd criteria, and the prevalence of lesions was higher in people who suffered a higher number of MetSd criteria. In this study the silent infarctions were identified by MRI, and defined as focal lesions of $\geq$ 3mm in diameter (in T2 and FLAIR sequences)[10]. Similar results have been reported in middle-aged samples [8]. Other studies reported that MetSd has been associated to different brain lesions such as SBI, periventricular hyperintensities and subcortical WML. In spite of the fact that this association was influenced by the effect of other factors such as age, gender, and smoking habit, a multivariate logistic analysis showed that MetSd is an independent risk factor to the three types of lesions. Moreover, the prevalence of SBI is associated to the number of MetSd components [9]. Choi et al., (2009) [240] found that cerebral WMH were more frequently observed in subjects with MetSd compared to those without; its effect was maintained after the adjustment for age and gender. However, the authors also reported that among the components of MetSd, hypertension showed significant association with WMH.

Leukoaraiosis is defined as a subcortical WM alteration observed by computerized tomography (CT) or MRI techniques. This alteration has also been related to MetSd presence. Park et al., (2007) [7] studied a healthy adult sample (from 28 to 78 years old) and assessed the presence of leukoaraiosis using the rating scale of the Atherosclerosis Risk in Communities study on MRI. The authors detected higher MetSd prevalence in people with leukoaraiosis, as well as significant association between the syndrome and every level of leukoaraiosis, including minimal level.

Recently, we performed a DTI (applying a voxel based approach) comparing two groups, a MetSd group and a control group, matched by age, gender and education level. We found that patients with MetSd showed an anterior–posterior pattern of deterioration in WM with reduced FA and increased in apparent diffusion coefficient (ADC) values compared with controls. WM changes were not related to any isolated vascular risk factor. Specifically, we found differences in FA in the frontal lobe bilaterally and higher ADC values in WM of temporal and frontal lobes bilaterally in patients group (Figure 1 and Tables 5-6). This deterioration followed an anterior-posterior pattern previously reported in aging studies. Although the specific mechanism underlying these results is not clear, we suggested that our

results could be related to mild small-vessel disease, due to the chronic state of vascular disregulation in the brain mostly affecting WM [12]

Table 4. Metabolic syndrome and brain structure

Studies	Sample (n)	MetSd Criteria	Neuroimaging technique	Main Results
Kwon et al., 2006	1588	NCEP-III	SBI (focal lesion ≥3mm diameter in T2/FLAIR MRI)	MetSd was associated to SBI
Bokura et al., 2008	11 51	NCEP-III	SBI (focal lesion ≥3mm diameter in T2/FLAIR MRI) PVH (0 to 4) ASBS (Fazekas scale)	MetSd was associated with all types of lesions.
Park et al., 2007	10 30	NCEP-III	WMH (focal lesion ≥3mm diameter in T2/FLAIR MRI). Grades of LA (*Atherosclerosis Risk in Communities study scale*)	Higher prevalence of LA in MetSd group. MetSd is associated to all LA severity grades.
Park et al., 2008	20 76	NCEP-III	SBI (focal lesion ≥3mm diameter in T2/FLAIR MRI)	MetSd was associated to SBI
Heikkilä et al., 2008	18	IDF	MRI spectroscopy (NAA, Cho, tCr, mI, Glc and H2O) T1, T2 and FLAIR.	In the Metsd group, there was higher tCR in the thalamus. The tCr increase correlated to plasmatic glucose concentration and ml levels.
Choi et al., 2009	54 98	IDF	WMH (focal lesion ≥3mm diameter in T2/FLAIR MRI)	Cerebral white matter hyperintensities was more frequently observed in subjects with MetSd.
Segura et al., 2009	38	NCEP-III	Diffusion tensor imaging. VBM	Patients with MetSd showed an anterior–posterior pattern of deterioration in WM with reduced FA and increased ADC.
Segura et al., 2009	38	NCEP-III	Diffusion tensor imaging. TBSS.	**In the MetSd group, FA values correlated with processing speed measures in anterior and posterior parts of the corpus callosum**

NCEP-ATP-III: The National Cholesterol Education Program (NCEP)-Adult Treatment Panel III (ATP-III). SBI: Silent brain infarction.MRI: Magnetic Resonance Imaging. FLAIR : Fluid-Attenuated Inversion Recovery. PVH: PeriVentricular Hyperintensities. SWMA: Subcortical White Matter Alteration. LA: LeucoAraiosis. NAA: N Acetyl Aspartate, Cho: Cholina, tCr:Creatine Total, mI:myo-inositol Glc:glucose. VBM: Voxel Based Morphometry. FA: Fractional Anisotropy. ADC: Apparent Diffusion Coefficient. TBSS: Tract-Based Spatial Statistics.

Table 5. Between groups differences in FA (Patients < Controls)

Anatomical region	Lobe	p value	Size	MNI coordenates			t	FA	FA	r
			(corr)	(mm^3)	x	y	z		Cont.	SdMet.
R Ant. C. Callosum	F	0.001	5195	11	25	−17	4.51	0,33	0.28	0.60
R Ant. C. Callosum	F			17	45	-16	4.00	0.33	0.28	0.55
R Uncinate fas		F		29	27	-2	3.81	0.35	0.32	0.54
L Ant. C. Callosum	F	0.043	2886	-10	26	-18	4.37	0.32	0.27	0.59
L Uncinate fas		Sub		-27	19	-9	4.34	0.34	0.30	0.59
L Ant. C. Callosum	Lb			-10	34	-9	3.59	0.32	0.29	0.51

R: Right. L: Left. Ant: Anterior. C. Callosum: Corpus Callosum. fas: fasciculus. F: Frontal lobe. Sub: Sub-lobar. Lb: Limbic lobe. Size: cluster size. t: t-student test values. FA Cont: FA mean values in the control group. FA SdMet: FA mean values in the patients group. r: Effect size. Reprinted from Neurology, 11, 73 (6). Segura et al. Microstructural white matter changes in metabolic syndrome: a diffusion tensor imaging study.. pp438–444. 2009 with permission from Wolters Kluwer Health (12).

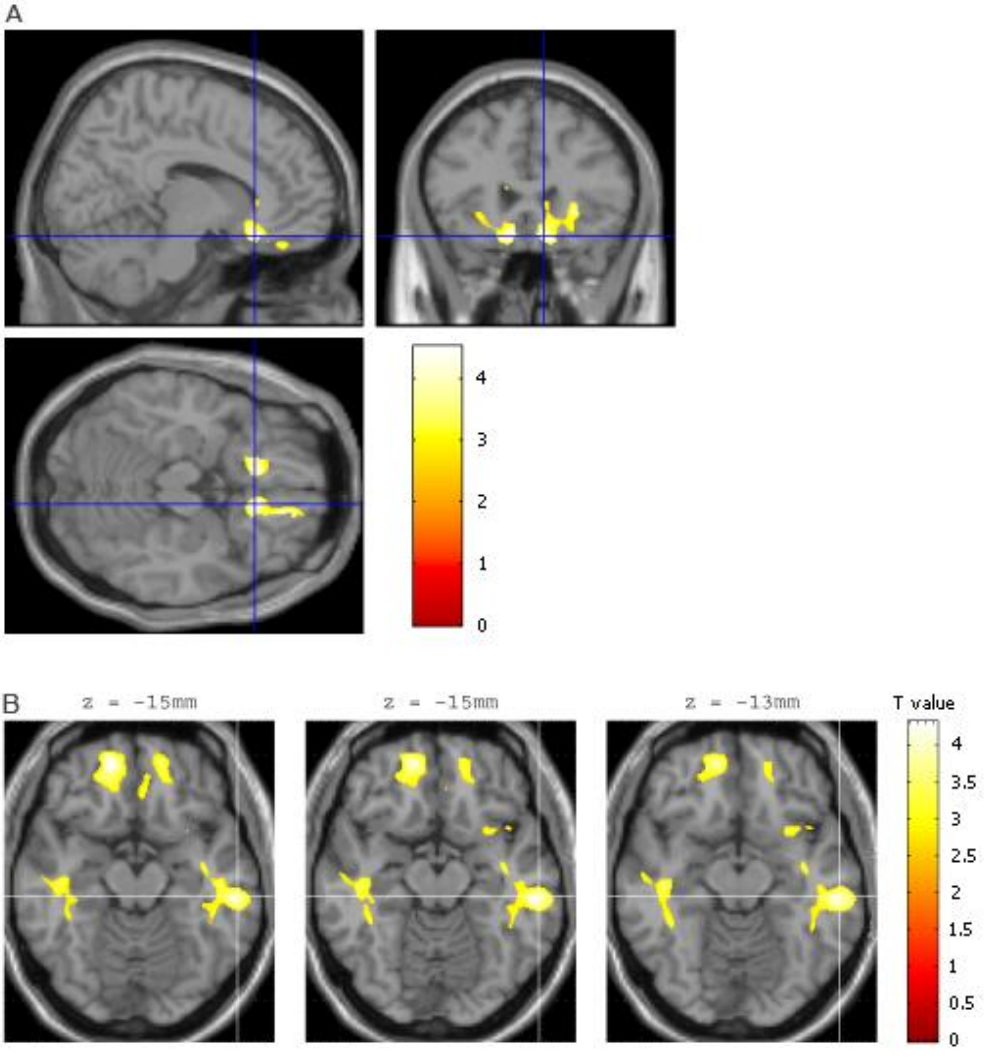
Table 6. Between groups differences in ADC (Patients > Control)

Anatomical region	Lobe	p value	Size	MNI coordenates			t	ADC‡	ADC‡	r
		(corr)	(mm^3)	x	y	z		Cont.	SdMet.	
L Inf. longitudinal fas	T	0.000	22673	-54	-28	-14	4.31	79.48	86.39	0.58
L Inf. longitudinal fas	T			-43	-8	-29	4.22	81.13	88.61	0.58
L Inf. longitudinal fas	T			-36	1	-38	4.20	82.27	87.09	0.57
R Inf. longitudinal fas	T	0.009	4732	44	-25	-7	4.12	85.07	90.79	0.57
R Inf. longitudinal fas	T			49	-34	-6	3.86	81.90	87.60	0.54
R Inf. longitudinal fas	T			46	-21	-32	3.41	82.27	93.94	0.47
L Ant. C. Callosum	F	0.013	4480	-18	14	40	3.99	74.88	80.29	0.55
L Ant. C. Callosum	Lb			-8	13	44	3.21	92.51	102.25	0.47
L Sup. longitudinal fas	F			-37	3	20	3.19	77.24	81.54	0.47
R Ant. C. Callosum	F	0.013	4452	17	51	-17	4.61	89.64	107.51	0.61
R Ant. C. Callosum	F			14	36	-22	4.11	95.15	110.86	0.57
R Ant. C. Callosum	F			9	40	-35	2.91	94.57	116.83	0.44

R: Right. L: Left. Inf: Inferior. Ant: Anterior. Sup: Superior. C. Callosum: Corpus Callosum. fas: fasciculus. F: Frontal lobe. T: Temporal lobe. Lb: Limbic lobe. Size: cluster size. t: t-student test values. ADC Cont: ADC means values in the control group. ADC SdMet: ADC means values in the patients group. r: Effect size. ‡ADC values (mm2/s x10-6). Reprinted from Neurology, 11, 73 (6). Segura et al. Microstructural white matter changes in metabolic syndrome: a diffusion tensor imaging study.. pp438–444. 2009 with permission from Wolters Kluwer Health (12).

Moreover, the presence of MetSd has been associated with brain metabolism alterations. A study showed significant differences in total creatinine in thalamus between a MetSd risk group and a control group. This result is related to an increase in the plasmatic glucose levels in the risk group, and suggested a relationship between a chronic glucose level alteration and the thalamus metabolism in these subjects [241].

Finally, in our last study, we reported a relationship between cognition and brain imaging measures in MetSd patients. According to previous studies MetSd patients showed deficits in processing speed.



Reprinted from BMC Neurology, 10-64. Segura et al. White matter fractional anisotropy is related to processing speed in metabolic syndrome patients: a case-control study. 2010. Open access. [13].

Figure 2. Correlation Results between FA Values and Processing Speed Variables.Figure 2.A, 2.B and 2.C show positive correlation (warm) between FA values and SDMT performance. Figure 2.B shows negative (cool) correlation between FA and RT CPT-II performance. Images are represented according to radiological convention (left corresponding to the right hemisphere). R: right, L: left, A: anterior, P: posterior, I: inferior; S: superior.

Recent studies in the elderly corroborate our neuropsychological and DTI findings, showing associations between WM degeneration and slower processing speed and executive deficits, specifically in the frontal lobe [242]. Our results highlight the importance of differentiating between healthy aging (for instance, our control group) from normal and pathological aging. We found a correlation between FA values and processing speed in MetSd patients but not in our healthy sample. Successful aging is a wide term initially proposed to explain the heterogeneity within the elderly [243]. The healthy ageing goes beyond avoidance of disease and disability. It involves freedom from cognitive and physical impairment, and also a high social functioning during aging. The healthy aging, especially the maintaining of cognitive functioning, is related to healthy life habits (physical activity), the absence of pathological chronic conditions and subjective health during all the life. In conclusion, the results reported above could indicate that although vascular risk factors are very common in aging, when they are present in MetSd may predispose to pathological processes and produce a very early state of deterioration, which makes successful aging more difficult.

In a case-control study, we compared a MetSd group and a control group without vascular risk factors and we found a larger proportion of slow subjects in the patient group (cut-off points of normative population) (Table 7). Moreover, FA values correlated with processing speed measures in anterior and posterior parts of the corpus callosum only in the patient group (Table 8 and Figure 2). The fact that our sample was composed ofnot very elderly people, mean age of sixty years, emphasized the importance of MetSd control also during adulthood [13].

In summary, MetSd patients show a specific cognitive profile that is related to structural brain damage, especially in the aging. The detection of its specific manifestations could be the next step in preventing cognitive decline. Therefore, MetSd should be included as a variable in research studies on cognitive impairment associated with ageing, in order to study its role in the differences between normal and successful aging.

Table 7. Cognitive test results

	Metabolic Syndrome			**Control**				
Cognitive Test	Mean(SD)	Range	Pc<25	Mean(SD)	Range	Pc<25	Chi^2	p value
SDMT	42.63 (15.90)	19-61	6	49.58 (11.57)	31-68	0	7.135	0.008
GPT DH	88.53 (37.88)	59-221	7	72.16 (12.81)	59-110	3	2.171	0.141
GPT NDH	99.74 (41.63)	62-233	8	81.95 (24.22)	55-170	2	4.886	0.027
CPT-II RT	440.53 (71.33)	329-557	8	422.01 (55.23)	322-487	7	0.110	0.740

SDMT = **Symbol Digit Modalities Test;** GPT = Grooved Pegboard Test, DH = Dominant Hand, NDH = Non Dominant Hand; CPT-II RT = Continuous Performance Test Reaction time; Pc= Percentile; Chi2= Chi square test. Reprinted from BMC Neurology, 10-64. Segura et al. White matter fractional anisotropy is related to processing speed in metabolic syndrome patients: a case-control study. 2010. Open access. (13)

Table 8. Anatomical regions where FA correlated with cognitive performance in the MetSd group

Cognitive test	Corpus Callosum region	Lobe	Size	MNI coordinates			p value	r
				x	y	z	(corr)	
SDMT	**Right Anterior**	**F**	**8419**	**21**	**29**	**28**	**0.016**	**0.8**
	Left Posterior	**0-T**	**11331**	**-33**	**-44**	**9**	**0.015**	**0.7**
CPT-II RT								
	Left Anterior	**F**	**25171**	**-17**	**26**	**22**	**0.003**	**0.7**

SDMT = Symbol Digit Modalities Test;CPT-II = Continuous Performance Test II; RT =Reaction time; F= Frontal; O= Occipital; T=Temporal; Size= Cluster size at p 0.05; corr=Corrected; r= Pearson coefficient. Reprinted from BMC Neurology, 10-64. Segura et al. White matter fractional anisotropy is related to processing speed in metabolic syndrome patients: a case-control study. 2010. Open access. [13].

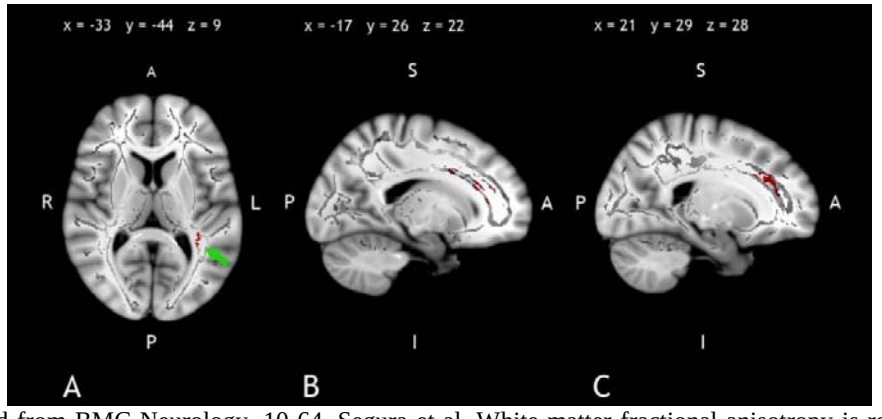


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Figure 2. Correlation Results between FA Values and Processing Speed Variables. Figure 2.A, 2.B and 2.C show positive correlation (warm) between FA values and SDMT performance. Figure 2.B shows negative (cool) correlation between FA and RT CPT-II performance. Images are represented according to radiological convention (left corresponding to the right hemisphere). R: right, L: left, A: anterior, P: posterior, I: inferior; S: superior.

Conclusion

Nowadays, the increased lifestyle changes and the acquisition of sedentary habits involve a rise in the prevalence of vascular risk factors. The effect of vascular risk factors' presence throughout life in the CNSis evident. However future studies should do an effort to discern specific cognitive profiles, as well as specific patterns of structural brain changes in order to detect preclinical stages of deterioration. Current contributions have highlighted the effect of the concomitant vascular risk factors, suggesting an empowerment of the individual effect of each individual factor. In that context, recent studies have stressed the influence of metabolic syndrome, as a cluster of vascular risk factors, in brain and cognition. The prevention and

treatment of these conditions could decrease the risk of CNS deterioration in aging and promote successful aging in modern society, in which there is a progressive increase of older people.

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Chapter II

Oxidative Stress Induced Vascular Hypoperfusion, Mitochondrial Failure Are Missing Links for the Development of Alzheimer Disease

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Abstract

Mitochondrial dysfunction may be a principal underlying event in aging, including age-associated brain degeneration. Mitochondria provide energy for basic metabolic processes. Their decay with age impairs cellular metabolism and leads to a decline of cellular function. Alzheimer disease (AD) and cerebrovascular accidents (CVAs) are two

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leading causes of age-related dementia. Increasing evidence strongly supports the theory that oxidative stress, largely due to reactive oxygen species (ROS), induces mitochondrial damage, which arises from chronic hypoperfusion and is primarily responsible for the pathogenesis that underlies both disease processes. Mitochondrial membrane potential, respiratory control ratios and cellular oxygen consumption decline with age and correlate with increased oxidant production. The sustained hypoperfusion and oxidative stress in brain tissues can stimulate the expression of nitric oxide synthases (NOSs) and brain endothelium probably increase the accumulation of oxidative stress products, which therefore contributes to blood brain barrier (BBB) breakdown and brain parenchymal cell damage. Determining the mechanisms behind these imbalances may provide crucial information in the development of new, more effective therapies for stroke and AD patients in the near future.

Keywords*:* Oxidative Stress; Vascular Dementia; Alzheimer Disease; Antioxidants; Hypometabolism, Mitochondria; Metabolism; Neurodegeneration

1. Introduction

Alzheimer disease (AD) and cerebrovascular accidents (CVAs) are two leading causes of age-related dementia. Increasing evidence supports the notion that chronic hypoperfusion is primarily responsible for the pathogenesis that underlies both disease processes. In this regard, hypoperfusion appears to induce oxidative stress, which is largely due to the formation of reactive oxygen species (ROS). Oxidative imbalance is also associated with other age-related degenerative disorders such as atherosclerosis, ischemia/reperfusion, and rheumatic disorders.

We have found that a chronic injury stimulus induces the hypoperfusion seen in the microcirculation of vulnerable brain regions. This leads to energy failure, which is manifested by damaged mitochondrial ultrastructure, the formation of a large number of non-mature or ***"young"*** electron dense ***"hypoxic"*** mitochondria and by the overproduction of mitochondrial DNA (mtDNA) deletions. Moreover, these mitochondrial abnormalities coexist with increased redox metal activity, lipid peroxidation and RNA oxidation. This oxidative stress occurs within various cellular compartments, in various parenchymal cells in the brain and most notably in the vascular endothelium, and in mitochondria found therein, which is associated with atherosclerotic damage. Further, the associated pathology is accompanied by neuronal and glial damage, known to be a part of the development of AD pathology. In addition, vascular wall cell pathology in the AD brain correlates linearly with the degree of neuronal and glial cell damage. Mitochondrial lesions in all of these cellular compartments show the same pattern, namely DNA deletions, the overexpression of oxidative stress and appear strongly to be the central target for brain damage in AD, due to high energy demand and susceptibility to oxidation. The result is manifested as energy failure and results in cognitive impairment and memory decline. In this review we outline recent evidence, as well as our own experimental and clinical data, indicating that chronic injury–stimulus induces hypoperfusion in the microcirculation of vulnerable brain regions, which leads to energy failure.

2. Vascular Changes and their Influence in the Pathology Seen in AD

Recent findings demonstrate that there is a similarity between the ultrastructural features of both vascular lesions and mitochondria in brain vascular wall cells from human AD brain biopsies, human short postmortem brain tissues, yeast artificial chromosome (YAC R140) and C57B6/SJL Tg+ mice overexpressing amyloid ß precursor protein (AßPP) [1,2]. Performing *in situ* hybridization using mtDNA probes for human wild type, 5kb deleted and mouse mtDNA, and immunocytochemistry using antibodies against APP, 8-hydroxyl-2'-guanosine (8OHG) and cytochrome c oxidase subunit 1 (COX) provide congruent ultrastructural localization [1,2]. A higher degree of amyloid deposition in the vascular walls of the human AD, YAC and C57B6/SJL Tg (+) mice exists compared to age-matched controls [1]. Severely damaged vessels exhibit immunopositive staining for APP. More mitochondrial abnormalities are present in human AD, YAC and C57B6/SJL Tg (+) mouse microvessels where lesions occur [1,2]. Undamaged regions of human AD tissues, YAC and C57B6/SJL Tg (+) mouse tissues and in age-matched control subjects lack these features, while damaged vessels manifest cells possessing clusters of wild and deleted mtDNA–containing positive probes [1,2]. Our observations demonstrate that vascular wall cells, especially their mitochondria, appear to be central targets for oxidative stress-induced damage before the development of AD pathology [1,2]. On the other hand, the positive correlation between AD and cholesterol levels suggests that antioxidant therapy and cholesterol–lowering drugs could delay the occurrence of AD [1]. However, despite their frequencies, the pathophysiological and morphological changes in brain microcirculation that accompany AD remain poorly understood, and the specific factors controlling vascular tone in AD remain unknown [2].

3. Features that Influence the Development and Prognosis of AD during the Interactions between Cerebrovascular Diseases and Dementia

ROS are generated at sites of injury and/or inflammation. The vascular endothelium, which regulates the passage of macromolecules and circulating cells from blood to tissue, is a major target of oxidative stress and plays a critical role in the pathophysiology of vascular diseases. In addition, the vascular endothelium, neurons, and glia can synthesize, store, and release ROS and vasoactive substances in response to certain stimuli, especially to chronic hypoxia/hypoperfusion. Their contribution to the pathophysiology of stroke, cerebrovascular disease and AD is extremely important. Moreover, the role of hypoperfusion as a key factor for vascular lesions that causes oxidative stress, appears to be widely accepted as an initiator of AD [1,2]. This idea is based on a positive correlation between AD and cardiovascular diseases [1,3–6].

Specifically, accumulated oxidative stress increases vascular endothelial permeability and promotes leukocyte adhesions, which is coupled with alterations in endothelial signal transduction and redox–regulated transcription factors [for the references and review

see: [[1,3,4]]. We hypothesize that the cellular and molecular mechanisms by which hypoperfusion-induced ROS accumulation results in the development of AD is through impairing endothelial barrier function, promoting leukocyte adhesion and altering normal vascular function. The sustained hypoperfusion and oxidative stress of brain tissues also could stimulate the secondary overexpression of iNOS and nNOS and endothelin–1 (ET-1) in brain cells [7]. It is likely that the increased accumulation of oxidative stress products probably contributes to damage to brain parenchymal cells and the decompensation of the blood brain barrier (BBB), which normally prevents permeability of large molecules from passing to the cerebrospinal fluid. Therefore, determining the mechanisms behind these disturbances in experimental animals may provide crucial information in the development of new, more effective therapies for the treatment of cerebrovascular and neurodegenerative diseases, including AD.

Many common underlying risk factors play key roles in the development of cardiovascular, cerebrovascular and neurodegenerative diseases [4,8-10]. Cigarette smoking causes chronic hypoxic conditions and the formation of a large amount of free oxygen radicals that appear to be key factors in the development of these diseases. Latest evidence indicates that continuous exposure to free oxygen radicals induce cellular damage and decreases antioxidant defenses [11].

Several recent studies indicate that cigarette smoking is a cofactor in the initiation of AD via its effect on the vasculature, as previously discussed. Nicotine, via nicotinic receptor activation may counter these effects in part. Vascular insufficiency/hypoperfusion has been considered as a pathogenetic factor in the development of AD, and the positive relationship between cerebrovascular diseases such as stroke and especially cerebrovascular atherosclerosis indicates the latter may also be linked to the pathogenesis of AD [4]. However, the role of tobacco smoking in the pathogenesis of AD is still unclear and controversial.

4. The Influence of Oxidative Stress on the Function of Brain Microvessels in AD

ROS can function as signaling intermediates at low levels and regulate fundamental cell activities including growth and adaptive responses [11]. However, at higher concentrations, ROS can cause cell injury and death. Vascular endothelium modulates the passage of macromolecules and circulating cells from blood to tissue and is a major target of oxidant stress [12]. Specifically, oxidative stress increases vascular endothelial permeability and promotes leukocyte adhesions, which are coupled with alterations in endothelial signal transduction and redox-regulated transcription factors [12]. Based on these recent findings, we hypothesize that impairing endothelial barrier function and promoting leukocyte adhesion also induce alterations in normal vascular endothelial cell (EC) function, resulting in AD progression [12].

Compared to other organs or tissues, the brain is more vulnerable to ROS-induced damage due to its high rate of oxygen consumption, high polyunsaturated lipid content, and relative paucity of classic antioxidant enzymes [13]. The AD brain contains increased regional levels of oxidative stress markers [14–20]. Several studies demonstrate a decline in

polyunsaturated fatty acids (PUFA) [21– 23], increased levels of lipid peroxidation markers [19, 21], as well as protein oxidation [24, 25], DNA oxidation [26–28] and RNA oxidation [1, 29–31] during AD. Additionally, the presence of advanced glycation end products (AGE), glycoxidative end products such as N–ε–carboxy–methyl–lysine and lipid peroxidation adducts are present in both neurofibrillary tangles (NFT) and senile plaques (SP) in AD [1,15,18,19,24,25,29,30,31–34] as well as in post–ischemic tissues [35–39].

Vascular aging correlates with both structural and functional changes that can take place at the level of the endothelium, vascular smooth muscle cells (vSMC) and the extracellular matrix of blood vessels. In the endothelium, reduced vasodilatation in response to agonists occurs in large conduit arteries, as well as in resistance arteries as a result of aging [40]. Furthermore, enhanced oxidative stress by hypoperfusion contributes significantly to the deleterious effects of aging on the endothelium by means of NO breakdown due to ROS. The relative contribution of the above phenomenon to age-related endothelial dysfunction is highly dependent on the species and the type of vascular bed involved [9,40–42].

Cortical, subcortical, meningeal gray matter and blood vessels (congophilic angiopathy) all contain Aβ deposits and are prominent features of AD [5,6,9,40–42]. *In vitro* experimental evidence shows that these Aβ deposits induce cerebrovascular dysfunction in the rat brain [43], and that the beta amyloid (Aβ) peptide produces endothelial dysfunction in cerebral microvessels via ROS. This occurs when the ROS superoxide–scavenging enzyme, superoxide dismutase, prevents acetylcholine–induced endothelium–dependent vasodilation [43]. In addition, accumulating evidence supports the idea that the Aß peptide is responsible for the cerebrovascular effects of the upstream molecule amyloid beta precursor protein (AßPP) and its overexpression [44,45].

A study by Iadecola and coworkers shows how transgenic (Tg) mice overexpressing AßPP have a profound and selective impairment in endothelium–dependent regulation of neocortical microcirculation [39]. Moreover, peptides derived from AßPP processing may contribute to the alterations in cerebral blood flow (CBF) and neuronal dysfunction during AD [44]. The study confirmed that Aß1-40 did not influence the increasing CBF produced by the endothelium–independent vasodilators [40]. In contrast, Aß1-42 did not reduce resting CBF or the increasing CBF produced by endothelium–dependent vasodilators. The superoxide scavengers, SOD and MnTBAP, reversed the cerebrovascular effects of Aß1-40 [40–41]. These data strongly suggests that soluble amyloid beta protein (Aß1-40), but not amyloid aggregate (Aß1-42), produces the cerebrovascular alterations seen in transgenic AßPP mouse, and thus, Aß1-40 could play a role in the cerebrovascular alterations observed in AD [6,45]. This study supports recent evidence that microvessels isolated from the AD brain kill neurons *in vitro* [46].

The growing body of evidence suggests that AD shares many common underlying etiologies with other neurodegenerative disorders. For example, multiple sclerosis (MS) is a relatively common disease with no cure. It is the leading cause of neurological disability in young adults, affecting over two million people worldwide (reviewed in reference [47]). Traditionally, MS has been considered a chronic, inflammatory disorder of the central white matter in which ensuing demyelination results in physical disability. Recently, MS has become increasingly viewed as a neurodegenerative disorder in which axonal injury, neuronal loss, and atrophy of the central nervous system leads to permanent neurological and clinical disability. The latest developments on MS research, includes etiology, pathology, genetic

association, EAE animal models, mechanisms of neuronal injury and axonal transport, and therapeutics [47]. Moreover, the mechanisms of mitochondrial dysfunction that are involved in MS, including mitochondrial DNA defects and mitochondrial structural/functional changes that accompanies this devastative disease have been able to open new and much more effective treatment strategies [47]. However, despite all the research on the effects of unknown etiology as well as Aß, the source of the potential ROS *in vivo* and its link to mitochondrial dependent hypoperfusion is not completely understood.

5. The Role of Mitochondrial Abnormalities during the Development of AD

In aerobic cells 90–95% of the total amount of adenosine triphosphate (ATP) production requires oxygen. The synthesis of ATP via the mitochondrial respiratory chain is the result of electron transport across the electron transport chain coupled to oxidative phosphorylation [48]. The main radical produced by mitochondria is superoxide anion. Intramitochondrial antioxidant systems scavenge this radical to avoid oxidative damage, which can lead to impaired ATP production [49–52]. During aging and some neurodegenerative diseases, including AD, damaged mitochondria are unable to maintain the energy demands of the cell [53,54]. This can lead to an increased production of free radicals, inducing the interruption of oxidative phosphorylation, and resulting in decreased levels of ATP [51]. Both processes, defective ATP production and increased oxygen radicals, may induce mitochondria–dependent cell death [51].

Animal studies using mitochondrial toxins provide the association between neurodegeneration with mitochondrial dysfunction and oxidative damage [2,51]. These consequences are implicated in the pathogenesis of human as well as animal models of neurodegenerative diseases [55–58] and in particular AD [1,49,50,53,59–62].

After long–term ischemia/reperfusion the mitochondria ultrastructure disintegrates *in vivo* and *in vitro* [9,38,39]. Apoptosis of degenerating neurons occurs in association with the accumulation of perikaryal mitochondria and oxidative damage to the nucleus [63]. This same pattern of mitochondrial lesions is observed in human AD brain biopsy samples [53,59]. The reduced expression of both mtDNA and nuclear DNA encoded genes is consistent with a physiological down-regulation of the mitochondria respiratory chain in response to declining neuronal activity [49–58,61,62,64]. However, the role of somatic cells and mtDNA mutations in the pathogenesis of mitochondria failure during AD is still controversial [50, 58, 61, 62].

The deleted mtDNA increases at least 3-fold in AD cases as compared to controls in humans [53]. Moreover, mtDNA isolated from the brains of AD patients includes oxidative modifications containing 8-hydroxy–2'–deoxyguanosine (8OHdG) [26–28]. Studies using *in situ* markers for 8OHdG and 8OHG showed that RNA oxidation is a prominent feature of damaged neurons in AD [29–31]. Quantitative analysis revealed a strong positive correlation between mtDNA deletions and 8OHG ($r = 0.934$) [53]. However, given that mtDNA (even DNA containing the 5kb deletion) is spared relative to 8OHG, we suspect that mitochondrial abnormalities correlate, but do not directly produce ROS. Therefore, it is important to recognize that 8OHG is formed by the direct attack of OH^o. These OH^o radicals have only a 2

nm sphere of diffusion and thus are unable to diffuse through the mitochondrial membrane [53].

More recently polarographic studies by Cormier and coworkers describe the effects of nicotine on respiratory chain in rat brain mitochondria [65]. The measurements of oxygen consumption show significant concentration–dependent inhibition by nicotine. Nicotine binds to complex I of the respiratory chain, inhibits NADH-Ubiquinone reductase activity and competes with NADH for complex I [65]. Furthermore, nornicotine, but not cotinine, the main nicotine metabolite, inhibits mitochondrial respiration. Complex I generates superoxide anion, nicotine, and was able to inhibit this ROS generation [65]. This may explain a part of the beneficial and protective effects of nicotine in a few neurodegenerative diseases, as suggested by many epidemiological studies [65]. However, future studies should focus on elucidating the effect of nicotine on the mitochondria functions as well as DNA overexpression and/or deletion during the development of neurodegenerative disorders including AD. The exact cellular mechanisms behind vascular lesions and their relation to oxidative stress markers identified by RNA oxidation, lipid peroxidation, or mtDNA deletion remain unknown [66]. Futures studies comparing the spectrum of oxidative stress-induced damage during reperfusion injury or, more importantly, during hypoxia/hypoperfusion, with AD damage are warranted [67].

6. Cofactors for Oxidative Stress-Induced Cerebrovascular Lesions

6.1. Hypoperfusion-Induced Oxidative Stress as a Key Factor for the Development of AD

Hypoperfusion-induced oxidative stress in vascular abnormalities coincides with the pathogenesis of AD [62]. Several studies conclude that chronic cerebral hypoperfusion in AD is secondary to oxygen reduction [10,68-71]. However, recent evidence reveals that a greater fraction of oxygen is removed from the vasculature in AD patients as compared to non–AD controls [72]. This suggests that low vascular blood flow is a prominent feature of the brain during chronic hypoxia/hypoperfusion and may be a main initiating factor during the development of AD [4,73,74]. An impairment of energy metabolism characterizes the AD brain [60]. Positron emission tomography (PET) reveals a decline in the cerebral metabolic rate of the parietal and temporal lobes during AD [25,75]. These metabolic defects are present before AD symptoms develop in ApoE ε4 homozygote patients [25]. De la Torre [73,76] proposes that advanced aging with a comorbid condition, such as a vascular risk factor that further decreases cerebral perfusion, promotes a critically attained threshold of cerebral hypoperfusion(CATCH). With time, CATCH induces brain capillary degeneration and suboptimal delivery of energy substrates to neuronal tissue [73,76]. Because glucose is the main fuel of brain cells, its impaired delivery, together with a deficient delivery of oxygen, compromise neuronal stability because the substrates for aerobic glycolysis fail to meet brain tissue demand. The outcome of CATCH is a metabolic cascade that involves, among other things, mitochondrial dysfunction, oxidative stress, decreased ATP production and increased calcium entry, abnormal protein synthesis, cell ionic pump deficiency, signal transduction

defects, and neurotransmission failure. These events contribute to the characteristic progressive cognitive decline of patients with AD, as well as regional anatomic pathology, consisting of synaptic loss, senile plaque (SP), NFT, tissue atrophy, and neurodegeneration. CATCH characterizes the clinical heterogenic pattern of AD and provides compelling evidence that a multitude of etiopathophysiologic vascular risk factors, in the presence of advanced aging, can lead to AD [2,3,4,10,73,76–79]. Therefore, we hypothesize that taken together with vascular EC and SMC atrophy, hypoperfusion is a key factor in the development of AD.

6.2. Cerebrovascular Lesions Observed during Ischemia/Reperfusion Induced Oxidative Stress

The risk for Alzheimer dementia and stroke are known to increase at comparable rates with age. Recent advances suggest that vascular risk factors linked to cerebrovascular disease and stroke in the elderly significantly increase this risk [6]. Although some vascular lesions such as cerebral amyloid angiopathy, endothelial degeneration, and periventricular white matter lesions are evident in most AD cases, one third will exhibit cerebral infarction. Longitudinal clinical studies suggest that stroke and AD occur in tandem more often than randomly [80]. Strokes often occur in patients with AD and have been linked to the pathogenesis of dementia [6]. Nevertheless, the nature of this relationship remains unexplored. Cerebral ischemia is a possible causal factor for AD. Irrespective of the ultimate pathogenic mechanism, these findings suggest that managing vascular disease is important in the treatment and prevention of AD [8,10] or mixed dementia [6].

Chronic hypoxia can alter cerebral microvessels ultrastructure, but this effect is heterogeneous and in some cases capillaries can respond to hypoxia independently of the arteriole [81]. Exposure to three weeks of hypobaric hypoxia results in increased capillary density in rat models [82]. Capillary segment elongation plays a role in this increase in the deeper layers of the cerebral cortex [82]. Therefore, prolonged hypoxia results in structural and functional adaptive responses that improve tissue oxygen delivery [83]. Mitochondria of brain capillary EC maintain normal density in hypoxia, but the number of mitochondria in the surrounding neuropil decreases significantly about 30% [84]. Moreover, exposure to hypobaric hypoxia yields an increase in basic fibroblastic growth factor (bFGF) mRNA in brain tissue [85]. During moderate hypobaric hypoxia, increased brain vasculature is associated with increased density of the brain capillary glucose transporter (Glut-1). However, this change is reversible and dependent on hypoxia exposure time [86]. This same pattern has been observed in the microvascular system of the human AD brain [1, 87,88,89]**.** Based on these findings, the relationship between oxidative stress markers and extracellular matrix binding ligands in the hypoxic brain during stroke and AD deserves further investigation. In addition, the injury induced by reperfusion after chronic hypoxia is important to note because the oxidative products that accumulate during hypoxia induce more tissue and cellular damage than the hypoxia itself.

Ischemia/reperfusion is a systemic process affecting the whole organ or tissue. Different types of blood cells may contribute to the pathogenesis of ischemia/reperfusion, including platelets, monocytes, neutrophils and others [35]. According to Bednar and coworkers [90] neutrophils might be important contributors to ischemia–induced brain injury whereas the

role of platelets is more nebulous. In fact, systemic depletion of neutrophils reduced the volume of cerebral infarct after transient middle cerebral artery occlusion in the rat [91]**.** EC affected by ischemia during the early stages is completely reversible and dependent upon reperfusion. Eventually, however, injured tissue passes a *"point of no–return"* and the damage becomes irreversible [92]. Initially, cells strive to increase their surface area for gas and nutrient exchange by expressing cytoplasmic microvilli [9,35,36,38,39] or by extending membrane protrusions into the vessel lumen [9,35,36,38,39,93]. The appearance of these microvascular changes corresponds to the duration of the ischemia and may be an adaptive EC response to altered hemodynamic conditions [9,93]. The functional significance of microvilli, microblebs and other morphological changes is not clear, but they may have a role in the production of delayed, post–ischemic hypoperfusion by increasing vascular resistance [9,93]**.** The extent of EC injury depends on the duration of ischemia and on the metabolic needs of the affected vascular system. The duration of experimental ischemia or acute anoxia required to cause damage varies for different organs. It takes approximately 10–15 minutes for irreversible damage to occur in brain [93,94,95]. After long–term ischemia and the following reperfusion, the decreased number of active capillary vessels is proportional to the ultrastructural lesions in ischemic vessels and underlying tissues and cells [9,35,36,38,39]**.** Cada and coworkers demonstrate that decreased CBF in aging rats produces deficits in visuospatial behavior after permanent surgical occlusion of both carotid arteries [96]. This deficit is coupled with metabolic abnormalities of the brain as visualized by quantitative COX histochemical mapping [96]. These results suggest that deficits in visuospatial learning are not exclusively the result of hippocampal dysfunction, but may be directly involved with altered oxidative energy metabolism in other integrative visuomotor regions identified in this study. They also suggest that chronic cerebrovascular ischemia in this aged rat model produces neurometabolic and behavioral alterations that may be relevant risk factors for the development of AD [96]**.**

7. The Potential Role of Vasoactive Substances in the Endothelial Content during Ischemia/ Reperfusion

The synthesis and release of vasoactive substances, such as the endothelium–derived vasodilator NO and vasoconstrictor ET-1, regulate EC's role in controlling vascular tone [65,97–99]. Aside from vascular tone NO regulates platelet aggregation, leukocyte adhesion, SMC proliferation, synaptic neurotransmission and cytotoxic/cytostatic actions of macrophages [84,99–106]. This labile molecule may carry out important biological roles both within the cell in which it is synthesized, and by interacting with nearby cells and molecules [107,108]. Three distinct isoforms of NOS derived from different genes generate NO: nNOS, iNOS, and endothelial NOS (eNOS) [97–111]. These isoforms are similar in structure and function [97,108,110,111]. eNOS was first purified and cloned from vascular endothelium, but is found in cardiac myocytes, blood platelets, brain cells [98,99,104,106,110,112] and in cellular compartments such as mitochondria [113,114]. The activity of eNOS is a major determinant of vascular tone and blood pressure. It is altered

in diseases such as hypertension, diabetes, atherosclerosis, ischemia/ reper-fusion [41,87,104,105,115] and AD [73,89].

Excess NO is produced during excitotoxicity, inflammation and ischemia/reperfusion injury [116], and the oxidation products of NO, namely peroxynitrite and peroxynitrate. Also, ONOO– can generate the highly reactive hydroxyl radical, a more powerful oxidant than NO itself [17,102,116]. The increased nitrotyrosine immunoreactivity in AD is present in the neuronal cytoplasm of the cerebral cortex within regions of neurodegeneration, yet it is undetectable in corresponding control regions [17]. This distribution is essentially identical to that of free carbonyls [15]. The widespread occurrence of nitrotyrosine immunoreactivity in neurons [17] suggests that chronic oxidative damage is not restricted to long–lived polymers such as NFTs, but instead, reflects a generalized oxidative stress contributing to the pathogenesis of AD.

NOS positive neurons are present in subgroups throughout many regions of the brain [102]. Immunostaining for reduced NADPH-diaphorase, as well as nNOS and eNOS, reveals their presence in dendritic and axonal terminals that closely interact with the middle cerebral artery and cerebral microvessels [101–103,115]. The presence of L–arginine in astrocytes in vivo suggests that glia may store this chemical for NO production in brain [101,115,117]. Moreover, glial cells exhibit an inflammatory response during infection or ischemic disease. They also release pro–inflammatory cytokines and synthesize and release NO [117]. The large amount of NO that is released from glial cells via the expression of iNOS after their stimulation is neurotoxic, because it induces oxidative stress, mitochondrial dysfunction and excitotoxicity [101,106,118]. Hypoxic brain injury (acute or chronic) is associated with the formation of both NO [102,117,119,120–122] and the superoxide anion, which may react to form free radicals [17, 106] and cause neurotoxicity [101–103,106, 119,120,123,124]. Further investigations into determining the exact ultrastructural localization of the different NOS isoforms in the brain vascular tree, neurons and glia in post–hypoxic and AD brain are warranted.

7.1. eNOS Involvement in the Cerebrovascular Tone

A dynamic balance of relaxing and constricting factors regulates cerebrovascular tone. Constitutively produced NO normally influences basal cerebral vascular tone, and mediates vascular responses to diverse stimuli [125] and cerebral vasodilation [117]**.** Vasorelaxation of brain microvessels is a feature of some diseases including chronic hypertension, diabetes, hypercholesterolemia, subarachnoid hemorrhage (SAH), and ischemia [41,117,125]. NO is also involved in regulating the cerebral circulation during hypercapnia [126,127] and focal [115,128,129,130] or global brain ischemia [127,131,132,133,134,135]. Furthermore, arginine–derived NO mediates the powerful effects of CO_2 on cerebral circulation. NO synthesized by the action of eNOS participates in regulating basal CBF and is the major contributor to the hypercapnic CBF response [136]**.** Chronic inhibition of constitutive NO production increases EC permeability during various vascular diseases [8,87,95,98,99,110]. Due to its vascular effect, NO might improve tissue perfusion and exert a protective action. Overproduction, either by activation of nNOS by excitatory amino acids [137]**,** or by induction of iNOS in glial, vascular, or blood cells [132,133,134] during the ischemic episodes, might be deleterious. Mice with eNOS gene knockout exhibit a decrease in vascular

relaxation. Thus, NO synthesized by eNOS protects against ischemic damage by increasing CBF, whereas NO produced by nNOS contributes to lesions [138,139]**.** The inhibition of NO synthesis by EC leads to increased intracellular oxidative stress, which induces neutrophil-EC interactions [9,37] and may promote the development and progression of vascular diseases such as atherosclerosis [41,105] and ischemia/reperfusion injury [9,35,6,117,125,140].

7.2. nNOS Expression and Regulation

Modification of nNOS expression in the entorhinal cortex and hippocampus occurs during AD [141]. Tissues containing the constitutive forms of NOS, like brain, kidney, and vascular endothelium express dimethylargininase [142,143,144]. It regulates NO production by hydrolyzing free methylated arginine derivatives (effective endogenous inhibitors of NOS) [145]. The expression of dimethylargininase dramatically increases during AD [20]. Dimethylargininase abnormalities in the AD are the result of elevated levels of nitration from effective oxidants peroxynitrite or peroxynitrate [17,119,146]. However, the ultrastructural localization of dimethylargininase immunoreactivity in different cellular compartments of the AD brain or in Tg animal models of AD has yet to be described.

7.3. iNOS as a Mediator of Oxidation during AD

A variety of cells express iNOS in response to lipopolysaccharides, certain cytokines and ROS generators [8,97,99,101–104,112,115,117]**.** iNOS may be an important mediator of cytotoxicity in the brain because it produces much greater amounts of NO than either eNOS or nNOS [107]**.** Thorns and collaborators suggest that iNOS plays a role in the formation of NFT [141]. Iadecola and coworkers propose that iNOS contributes to ischemic brain damage [133]. The catalytic activities of iNOS enzymes or mRNA expression are evident in brain tissue after 2 hours of transient focal ischemia or 1–2 days after permanent focal ischemia [132,134]**.**

8. Subcellular Mechanisms Involved in the Development and Maturation of Human AD

Ultrastructural features of the brain biopsy from the age-matched control (Figure 1 A) and AD (Figure 1 B–D) patients are characterized by heterogeneous morphology. The EC in intact microvessels did not show visible changes. Mitochondria in EC are intact (Figure 1 A).

Contrary to this observation, short postmortem (<2 h) brain tissues from AD patients, show microvessels with severe damage, which characterizes the presence of clusters of mitochondria derived lysosomes and necrotic changes in their ultrastructure (Figure 1 B). The capillary endothelium shows the presence of a cluster of damaged mitochondria containing positive mitochondrial DNA (mtDNA) signals visualized by using in situ hybridization following indirect 17 nm colloidal gold decorations (Figure 1 C). In addition, in AD brain microvessels EC occupied only the small part of the vessel wall. Perivascular cells show the

presence of large mitochondria derived vacuoles in their matrix. Adhesion of the activated platelets (PLT) to damaged endothelium appeared to be hallmark of these microvessels (Figure 1 D). However, undamaged microvessel endothelium did not show any particular changes in their ultrastructure. Mitochondria also were intact (Figure 2 A). However, the perivascular spaces contained large vacuolar structures (see Figure 2 A). At the same time often the microvessel EC shows the presence of degenerative mitochondria (Figure 2 B). The presence of *"electron-dense"* hypoxic mitochondria coexists with the formation of mitochondria derived lysosomal structure in the cytoplasmic matrix of EC and perivascular cells (Figure 2 C).

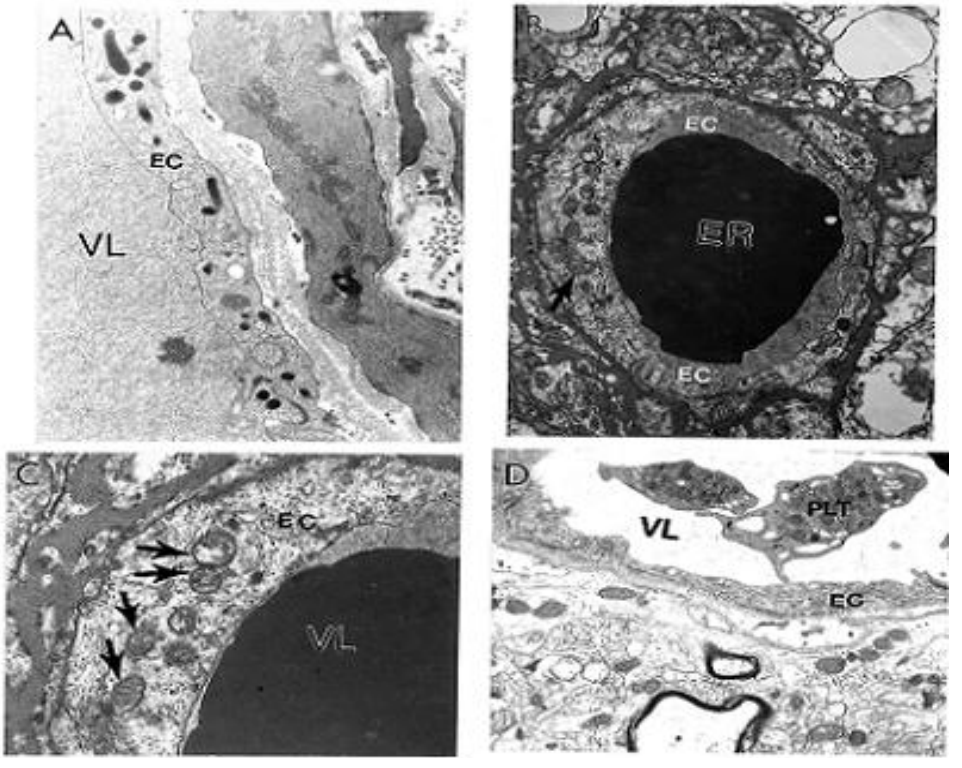

Figure 1. Ultrastructural features of the brain biopsy from the age-matched control (A) and AD (B–D) patients are characterized by heterogeneous morphology: A. An intact microvessel shows the absence of any particular abnormalities in the ultrastructure of endothelial cells (EC) and perivascular cells. Mitochondria in EC are intact. Original magnification: X 13,900. B. Short postmortem (<2 h) brain tissue from AD patients shows microvessels with severe damage such as the presence of clusters of mitochondria derived lysosomes (single arrow) and necrotic changes in the ultrastructure of the EC and perivascular cells. Original magnification: X 8,300. C. Capillary endothelium (from Figure 1–B), under higher magnification, shows the presence of a cluster of damaged mitochondria (single arrows) containing positive mitochondrial DNA (mtDNA) signals visualized by using in situ hybridization following indirect colloidal gold decoration (17 nm gold particles). Original magnification: X 46,000. D. AD brain biopsy. EC occupied only the small part of the vessel wall. Perivascular cells show the presence of large mitochondria derived vacuoles in their matrix. Adhesion of the activated platelets (PLT) to damaged endothelium. Original magnification: 8,300. Abbreviations used in figures: EC–Endothelial cells; ER–Erythrocyte; PLT–Platelets; VL–Vessel lumen (reprinted from [19 and 172] with permission).

The mitochondria abnormality appeared to be a permanent feature of vascular endothelium and perivascular cells where damage became visible which characterizes the presence of a hypoxic and completely damaged mitochondria (Figure 2 D).

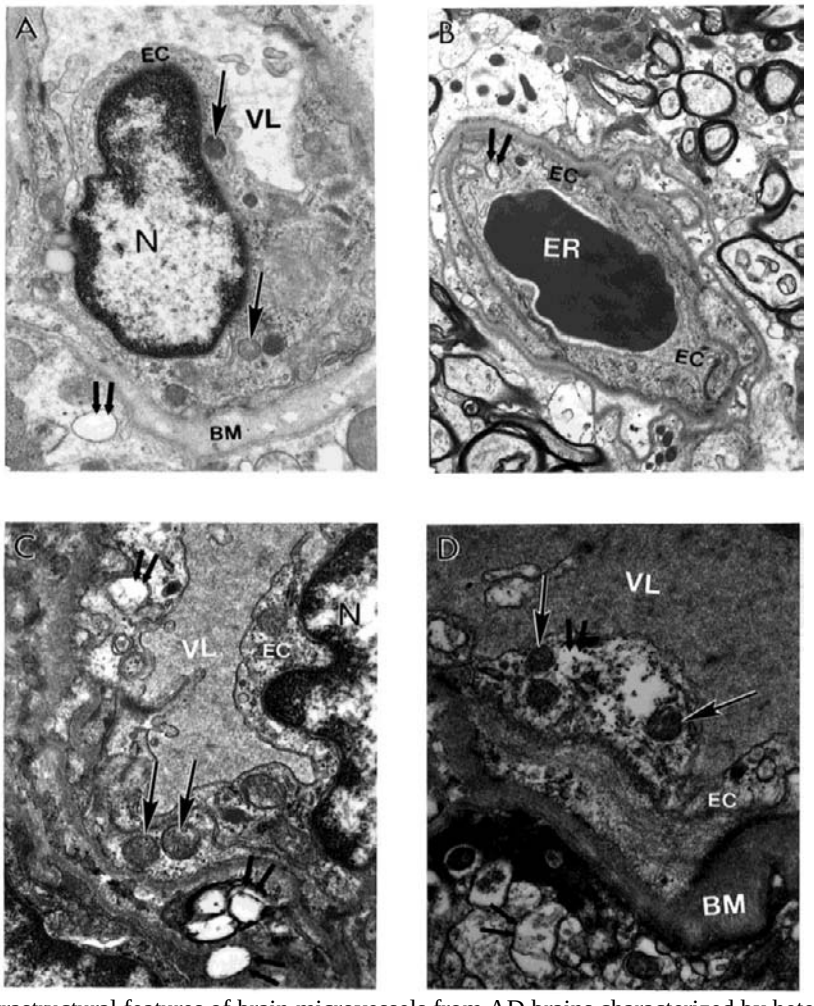

Figure 2. Ultrastructural features of brain microvessels from AD brains characterized by heterogeneous mitochondrial morphology. A. Undamaged microvessel endothelium did not show any particular changes in their ultrastructure. Mitochondria also were intact (single arrows). However, the perivascular spaces contained large vacuolar structures (double arrow). Original magnification X 13,000. B. Vascular EC shows the presence of degenerative mitochondria (double arrow). Original magnification X 6,600. C. The presence of "electron-dense" hypoxic mitochondria (single arrows) coexists with the formation of mitochondria derived lysosomal structure in the cytoplasmic matrix of EC and perivascular cells (indicated by double arrow). Original magnification X 20,000. D. The permanent feature of vascular endothelium and perivascular cells where damage became visible (single and double arrows indicate hypoxic and completely damaged mitochondria, respectively). Original magnification X 20,000. Abbreviations used in figures: BM–basal membrane of endothelium; EC–endothelial cell; ER–erythrocyte; VL–vessel lumen (reprinted from [19 and 87] with permission).

We demonstrate in recent work that cortical neurons from AD brain biopsies have selective localization of mitochondria abnormalities in the cell body [1,3,53,59,87,89]. The

majority of the neurons, which closely associate with the lesioned vessels, possess differing degrees of ultrastructural abnormality. The ultrastructural characteristics of neuronal mitochondria damage from AD brain biopsies show the presence of neurons with differing degrees of ultrastructural lesions (Figure 3). In the neuronal cell body partially and completely damaged mitochondria appeared to be hallmark of these neurons (Figures 3 A and 3 C). The lesioned mitochondria appeared to be a major substrate for the lipofuscin formation. The electron dense hypoxic mitochondria are seen throughout the cell body and characterize the abnormal mitochondrial cristae (in Figures 3 B and 3 D).

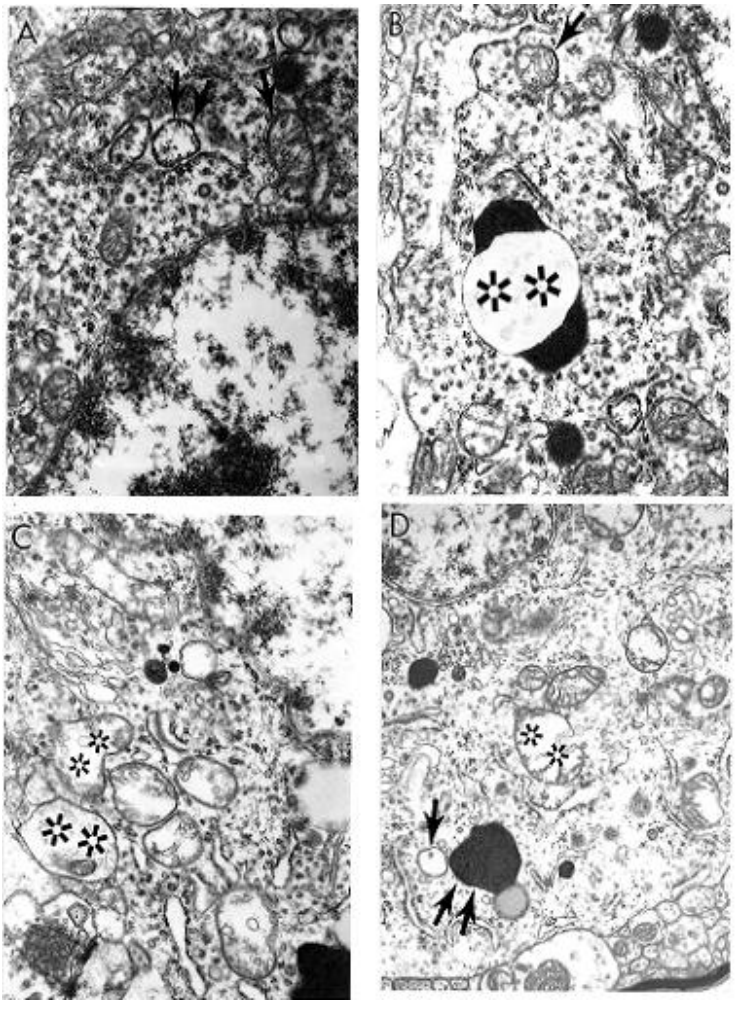

Figure 3. The ultrastructural characteristics of neuronal mitochondria damage from AD brain biopsy. Neurons with different degree of ultrastructural lesions. In the neuronal cell body partially and completely damaged mitochondria (indicated by single arrows and double asterisk respectively in A and C). The lesioned mitochondria appeared to be a major substrate for the lipofuscin formation (double arrow). The electron dense hypoxic mitochondria are seen throughout the cell body and characterize the abnormal mitochondrial cristae. Original magnification: A and B X20,000 respectively. C and D X 16,000 respectively (reprinted from [19 and 172] with permission).

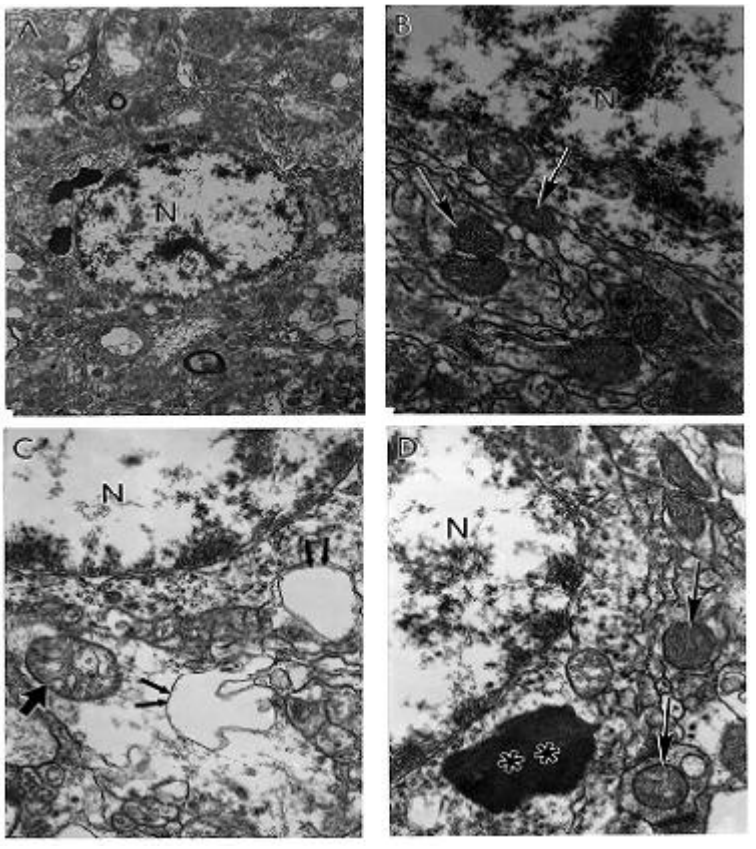

Figure 4. The ultrastructural characteristics of the neuronal mitochondria from AD brain biopsy. A. Neurons with different degrees of ultrastructural lesions. Partially and completely damaged mitochondria are mostly located in the neuronal cell body and coexist with lipofuscin formation. Original magnification X 5,000. B. Large numbers of electron dense hypoxic mitochondria (indicated by single arrows) were present throughout the cell body and characterized the abnormal mitochondrial cristae. Original magnification: X 20,000. C. Partially (indicated by single arrow) and completely damaged (double arrow) mitochondria are typically located in the neuronal cell body. Original magnification X 20,000. D. The neuronal cell body shows the presence of hypoxic mitochondria (indicated by single arrows) close to lipofuscin (double asterisk). Original magnification X 20,000. Abbreviations used in figure: N– neuronal nucleus (reprinted from [19 and 87] with permission).

Neurons with different degrees of ultrastructural lesions were seen throughout cortex (Figure 4 A). Partially and completely damaged mitochondria are mostly located in the neuronal cell body and coexist with lipofuscin formation (Figure 4 A). Moreover, another feature of these neurons appears to be the presence of large numbers of electron-dense hypoxic mitochondria, which were present throughout the cell body and characterized the abnormal mitochondrial cristae (Figure 4B). In addition, these abnormalities coexist with the presence of clusters of the partially and completely damaged mitochondria (Figure 4 C). The neuronal cell body always shows the presence of hypoxic mitochondria close to lipofuscin (Figure 4 D).

The features of wild type mitochondrial DNA (mtDNA) and 8-OHG staining in the hippocampus of short postmortem (<2 h) human AD brain shows that wild type mtDNA (17 nm gold) is associated with severely damaged mitochondria and mitochondria derived lysosomes (Figure 5 A). However, the area containing lipofuscin did not show any mtDNA containing positive gold particles (Figure 5 A). Features of 8-OHG staining in postmortem

AD brain shows that the 8-OHG containing positive signals (17 nm gold particles) was seen throughout neuronal cell body and within in the matrix of damaged mitochondria (Figures 5 B–D). However, non–damaged mitochondria (in Figure 5 C) and lipofuscin (in Figures 5 B and 5 D) do not contain 8-OHG positive gold particles.

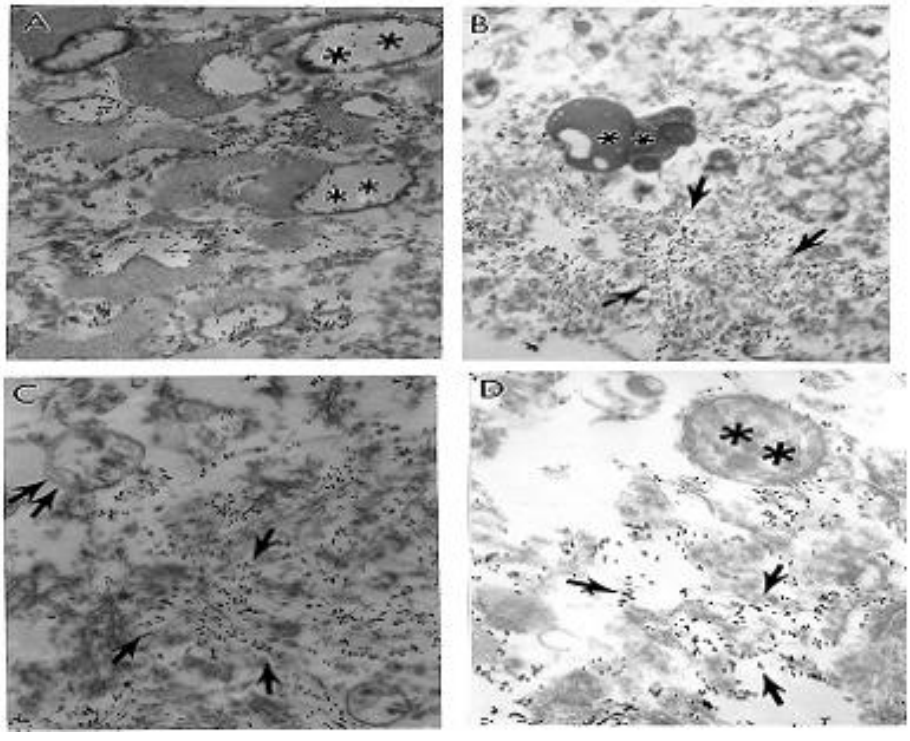

Figure 5. The features of wild type (A) mitochondrial DNA (mtDNA) and 8-OHG (B–D) staining in the hippocampus of short postmortem (<2 h) human AD brain. Postmortem AD hippocampus shows that wild type mtDNA (17 nm gold) is associated with severely damaged mitochondria and mitochondria derived lysosomes (double asterisk). Any area containing lipofuscin did not show mtDNA containing positive gold particles. Original magnification X 26,000. B–D: Features of 8-OHG staining in postmortem AD brain. 8-OHG containing positive signals (17 nm gold particles) was seen throughout neuronal cell body and within in the matrix of damaged mitochondria (single arrow). Non–damaged mitochondria (indicated by double arrows in Figure.5C) and lipofuscin (double asterisk in Figures. B and D) do not contain 8-OHG positive gold particles. Original magnification: X16,000, 26,000 and 33,000 respectively B, C and D (reprinted from [19 and 172] with permission).

The features of wild type (in Figures. 6 A–B), 5kb deleted mtDNA (in Figure. 6C), and COX immunoreactivity (in Figure. 6D) in the hippocampus of a postmortem human AD case shows wild type mtDNA containing positive signals (17 nm colloidal gold) detection were seen in the completely damaged mitochondria or mitochondria derived lysosomes. Any areas containing lipofuscin did not show mtDNA–containing positive signals (in Figures. 6A–B). In addition, the 5kb deleted mtDNA containing gold particles (17 nm) were mostly located in mitochondria–derived lysosomes (in Figure. 6C). Damaged, abnormal mitochondria shows COX positive containing gold particles in their matrix (in Figure. 6D).

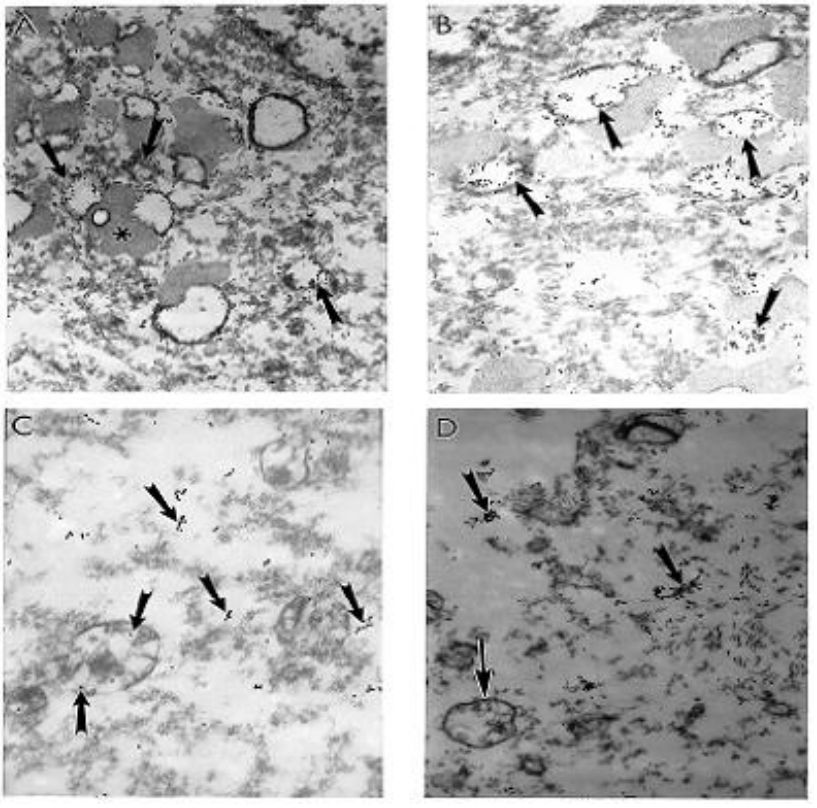

Figure 6. The features of wild, 5kb deleted mitochondria DNA (mtDNA), and COX immunoreactivity in the hippocampus of a post–mortem human AD case A and B. Hippocampal neuron shows wild type mtDNA containing positive signals (17 nm colloidal gold) detection were seen in the completely damaged mitochondria or mitochondria derived lysosomes (single arrows). Areas containing lipofuscin (asterisk) did not show any mtDNA containing positive signals. Magnification X 26,000 and X 20,000, respectively A and B. C. 5kb deleted mtDNA containing gold particles (17 nm) were mostly located in mitochondria derived lysosomes (single arrows). Original magnification X 33,000. D. Damaged, abnormal mitochondria shows COX positive containing gold particles in the matrix (single arrows, colloidal gold 17 nm). Original magnification X 26,000 (reprinted from [19 and 172] with permission).

Mitochondrial lesions and lipofuscinogenesis are also present in other cellular compartments of the brain parenchyma. Glial cells at the damaged area, also characterized by the accumulation of lipofuscin and mitochondria derived lysosomes appear to be a major component and source for these substrates (data not shown). In addition, glial cells also show the intracellular accumulation of different sized amyloid deposits, and they are accompanied by the presence of giant sized lipid–laden vacuoles and mitochondria derived lysosomes**.**

Quantitative morphometric measurements of the percentage of the different types of mitochondria (normal, partially damaged and completely damaged) indicate that age-matched control groups have a significantly higher percentage of normal mitochondria, compared to completely damaged mitochondria from AD cases [2,3,51,56]. No significant differences between partially damaged mitochondria are seen in both groups, indicating that aging induces damage to mitochondria. However, the main differences between the age-matched controls and AD cases appear to be significant differences in the percentage of the normal and completely damaged mitochondria [2, 3,51,56].

9. Antioxidant Application for the Treatment of AD

AD treatment has yet to yield a successful therapy that addresses the cause of injury found in AD brain biopsies [11]. Of the various theories proposed for AD etiology, ROS generation is cited as a common factor based on several cellular, molecular, and animal model studies of AD. During aging, ROS may play a crucial role in cell death, an important factor responsible for disease progression [66]. Efforts to reduce the pathology associated with ROS via antioxidants seem to offer new hope to patients suffering from this devastating disease [11].

Mitochondria has been considered a primary target in the search for age-related cognitively impaired conditions to restore cognitive function including treatments for dementia [11,147–154]. This is because the brain, which is characterized by a high energy metabolism and abundance of oxidizable materials such as polyunsaturated fatty acids and neuropeptides, is exceedingly susceptible to oxidative damage [155], which is known to cause mitochondrial dysfunction [154]. Furthermore, Aβ is reported to promote an excess accumulation of intracellular Ca^{2+} into mitochondria, inducing mitochondrial permeability pores to open, damaging mitochondrial structures, which in return increase the production of defective mitochondria, decrease mitochondrial trafficking, and alter mitochondrial dynamics in neurons affected by AD [156]. The association of mitochondrial activity with the antioxidant capacity of certain micronutrients such as alpha-Lipoic acid (LA), a coenzyme essential for the maintenance of energy homeostasis in mitochondria, has been shown to influence cognitive function in an different animal species [147–154].

Previous studies have demonstrated the potential protective effects of selective mitochondrial antioxidant treatments on brain mitochondria from aged rats [147–150,152,157]. When treating aged rats with selective mitochondrial antioxidants, such as acetyl-L-Carnitine (ALCAR) and LA, they were able to reduce OS and restore cognitive function and mitochondrial structural abnormalities in all parenchymal cells [147,151,157,158]. It is important to note that the oxidative damage is associated with mitochondria early in AD progression [159]. In addition, to study mitochondrial decay and oxidative damage resulting from aging, an examination into the activities and kinetics of the mitochondrial complexes (a hallmark of the mitochondrial ability to produce energy) was performed, and showed that mitochondrial complexes can be restored by selective mitochondrial antioxidant treatment [158]. This established that in the brain mitochondria of old rats, when compared with that of young rats, there were significantly decreased endogenous antioxidants and less superoxide dismutase activity; more oxidative damage to lipids and proteins; and decreased activities of the mitochondrial complex I, IV and V [158]. In regards to this, mutant proteins associated with AD are reported to block the transport of nuclear–encoded mitochondrial proteins to mitochondria, interact with mitochondrial proteins and disrupt the electron transport chain, induce free radicals, cause mitochondrial dysfunction, and, ultimately, damage neurons [160]. Moreover, the mitochondrial complex I showed a decrease in binding affinity (increase in K(m)) for substrates. Feeding ALCAR+ LA to old rats partially restored age-associated mitochondrial dysfunction compared to young rats. These results indicate that oxidative mitochondrial decay plays an important role in brain aging, inducing the generation of free radicals that leads to oxidative damage in postmortem

brain neurons from AD patients and in brain neurons from cell models and transgenic mouse models of AD [161], and that a combination of nutrients targeting mitochondria, such as ALCAR+LA, could ameliorate mitochondrial decay through preventing mitochondrial oxidative damage [158]. In a recent study [147] we were able to demonstrate that the integrity of mitochondrial ultrastructure, which is dependent on aging, could be improvement in old rat brain mitochondria when compared to the control group by using antioxidant treatments [147]. In contrast, neurons obtained from aged control groups showed a series of mitochondrial abnormalities, such as the presence of giant mitochondria and mitochondria with partial or complete damaged cristae. Targeting mitochondrial OS improved the overall cognitive ability of aged rats [148-150,152,157,162] and aged dogs [153].

Reid and colleagues [163] noted that there is epidemiological evidence that links vascular diseases, such as hypercholesterolemia, with an increased incidence of AD. While no theory has yielded a satisfactory explanation for the pathological changes that lead to neurodegeneration and cognitive dysfunction [163], vascular risk factors seem to offer the most interesting results [2,164]. The relationship between hypercholesterolemia and AD arose in great extent from ApoE4, a known risk factor for AD and a major carrier of cholesterol in the CNS. The detrimental processes of ApoE4 have been shown to influence AD pathological processes, including lipid homeostasis and NFT formation [165], which suggests that brain vascular alternations play a key role in the progression of AD [163]. ApoE4 mechanisms that contribute to the neurodegeneration of the brain could offer strong insights into AD susceptibility. For example, it was shown in a rat model that ApoE levels would increase as a response to peripheral nerve injury [165], implicating the role of ApoE as a repair mechanism. If the delivery of lipophilic antioxidants is impaired due to ApoE4, this could lead to OS [166]. It has been proven that ALCAR can improve memory deficits in animal models of AD and reduce cognitive deficit in AD patients [155]. The contribution of fatty acids in these cerebrovascular processes, and their effect on AD pathogenesis is still uncertain, but the suggestion that they can elicit neuronal overexcitation and synaptic depression as contributor factors to AD is suggested [167].

Several therapeutic strategies have been developed to treat AD, including anti-inflammatory, antioxidant, and herbal treatment approaches. These have been tested in animal and cellular models of AD and in clinical trials with AD subjects. In AD animal models and cell models, herbal extracts appear to have fewer adverse effects than beneficial effects on cognitive functions because of their antioxidant, anti–inflammatory properties [168]. We analyzed the effect of mitochondrial antioxidants ALCAR+LA as a treatment model for AD on ApoE4 transgenic mice [154]. The decrease in cerebrovascular oxygen levels seen in AD patients led to the hypothesis that hypoperfusion in the CBF, which over time causes OS and mitochondrial damage, was the main cause of ApoE–related cognitive deficits was seen in AD patients with ApoE4 overexpression [169–171]. Our study demonstrated for the first time, that ApoE4 caused brain hypoperfusion by gradually reducing CBF when compared to a control group. Structural damage of vascular wall cells, especially in mitochondria, seems to play a key role in the generation of ROS, resulting in oxidative damage to the neuron and inducing pathological factors associated with AD [154]. Therefore, we believe that expanding the focus of study in AD towards mitochondrial pathobiology as a treatment strategy will be able to open new and more successful effective treatment strategies for this devastating disease [62,67,147,154,164,172].

Conclusions

In this chapter we indicate that chronic vascular hypoperfusion is a part of the common underlying mechanisms involved in the initiation and development of neurodegenerative disorders, stroke and arteriosclerosis. In this regard, it appears that the central initiating factor for vascular abnormality is mitochondrial damage and a sum of elucidators for the imbalance in the activity of NOS isoforms, ET-1, oxidative stress markers, mtDNA and mitochondrial enzymes in the vascular wall and in brain parenchymal cells. This is believed to be due to their predominance in CVAs and AD. We hypothesize that an imbalance between the NOS species and the endothelium, along with antioxidant system deficiencies, are predominant brain features of stroke and AD patients. Elevated chronic hypoperfusion and physical distortion of tissue are likely to contribute to the collapse of post–ischemic/hypoxic or AD vessels. We theorize that future eliminating mitochondrial abnormalities can be considered as a new and more effective treatment strategies for this devastative disease in the near future.

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Chapter III

GRK2 Overexpression is a Primary Hallmark of Vascular Hypoperfusion and Mitochondrial Lesions during Early Alzheimer Disease: New Target for Drug Treatment?

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Abstract

Increasing evidence points to vascular damage as an early contributor to the development of two leading causes of age-associated dementia, namely Alzheimer disease (AD) and AD-like pathology such as stroke. This review focuses on the role of G protein-coupled receptor kinases, particularly GRK2 as they relate to dementia and how the cardiovasculature is involved in cerebrovascular pathogenesis. Any possible involvement of GRKs in AD pathogenesis is an interesting notion, whose exploration may help bridge the gap in our understanding of the heart-brain connection in relation to neurovisceral damage and vascular complications in AD. The a priori basis for this inquiry stems from the fact that kinases of this family regulate numerous receptor functions in the brain, the myocardium and elsewhere. The aim of this review is to

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discuss the finding of GRK2 overexpression in the context of the early AD pathogenesis, since increased levels of GRK2 immunoreactivity were found in vulnerable neurons from AD patients and from a two-vessel occlusion (2-VO) mammalian model of cerebral ischemia. Also, we consider the consequences for this overexpression as a loss of G-protein coupled receptor (GPCR) regulation, as well as suggest a potential role for GPCRs and GRKs in a unifying theory of AD pathogenesis and cerebrovascular disease. We therefore synthesize this newer information in an attempt to put it into context with GRKs as regulators of diverse physiological cellular functions. The complex mechanisms, which underlie regulation of GRK expression, degradation and function now are being elucidated and the levels of these kinases have been described to be altered in several pathological situations, such as cardiac failure, hypertension, inflammation and cancer. We suggest that GRKs may contribute to the development of pathology, making these proteins potential diagnostic and therapeutic targets for future pharmacological intervention.

Keywords*:* GRK2, Alzheimer Disease, Cerebrovascular disease, Mitochondria, Vascular Hypoperfusion, Oxidative Stress

Introduction

G protein-coupled receptor kinases (GRKs), such as GRK2, are cytosolic proteins that contribute to the adaptation of the heptahelical G protein-coupled receptors (GPCRs) and to the regulation of their downstream signals. GPCRs mediate the action of messengers that are key modulators of cardiac and vascular cell function [1]. A family of seven mammalian serine/threonine protein GRKs have been identified. GRK2 and 3 form the second subfamily, namely, β-adrenergic receptor kinase (βARK), whose members can phosphorylate and regulate agonist-occupied or constitutively active GPCRs [2]. When recruited to the cell membrane, homology domains of GRK2 modulate the simultaneous inhibition of signaling by G-alpha, G-beta and G-gamma subunits. Recent studies suggest that GRKs, particularly GRK2, may have more diverse protein/protein cellular interactions because of a consensus caveolin binding motif within the pleckstrin homology domain of GRK2 [3].

Normal aging and sporadic late-onset AD have many features in common. AD-like symptoms only appear to manifest when certain quantitative levels of damage occur [4,5]. This damage includes metabolic and oxidative stress, as well as those factors associated with impaired cerebral perfusion, which all are attributed to risk. Examples of impaired perfusion include cardiovascular and cerebrovascular diseases, hypo/hypertension and stroke, which impair the body's ability to adequately cope with further insults [5,6]. We speculate that an imbalance in the activity of nitric oxide synthase (NOS) isoforms, endothelin-1 (ET-1), and oxidative stress, as evidenced by several biomarkers of this damage, along with mitochondrial DNA (mtDNA) aberrations and mitochondrial enzyme imbalance in vascular wall cells and neurons, leads to inadequate antioxidant response [4]. An adequate antioxidant reserve capacity is needed to sufficiently abate metabolic and oxidative insults, which are two key initial features in the brains of stroke and AD patients [7]. We further hypothesize that GRK2 plays a role in these deleterious processes [8].

Under conditions associated with advanced aging, any imbalance in the activity of NOS isoforms, ET-1, and oxidative stress can lead to a potential and very destructive positive

feedback loop [9-11], in which increased levels of reactive oxygen species (ROS), interfere with NO function, lead to endothelial relaxation by reducing its bioavailability (through ROS scavenging), increase the amount of oxidative stress levels through the production of peroxynitrite, impair endothelial barrier function, promote leukocyte adhesion and induce alterations in normal vascular function, which further decreases cerebral blood flow (CBF) [11]. It appears that transient GRK2 activity correlates with compensatory changes to oxidative stress and arterial occlusion, including changes in endothelin-1 (ET-1) expression [4,10]. Although we are aware that correlation does not necessarily imply causation, we are equally cognizant of the axiom necessitating correlation in order for causation to be supported. With this in mind, we investigate what role GRK2 may play in the early pathogenesis of dementia and determined the cellular, subcellular and ultrastructural distribution and localization of GRK2 immunoreactivity in cases of human AD as well as in a mammalian model of chronic brain hypoperfusion (CBH), as first seen with light microscopy and confirmed by western blott [8].

Additionally, the involvement of CBH and physical distortion of the surrounding tissue exacerbate this imbalance and more than likely contribute to the collapse of post-ischemic/hypoxic vessels. Sustained hypoperfusion and oxidative stress, both primary features of aged brain tissue during the prodromal stages of AD [6,12,13] also may stimulate the expression of various NOS species and subsequent ET-1 in brain cells and probably increase the accumulation of oxidative stress products, thereby contributing to blood-brain barrier (BBB) breakdown, increased NO and peroxynitrite production and result in brain parenchymal cell damage (For a more in-depth discussion regarding the interactions of each of these factors please see our previous work [10,14]). These findings raise questions regarding the direct relationship between oxidative stress, energy failure (e.g. mitochondrial lesions) or metabolic insufficiency, neuronal and vascular damage, BBB breakdown, and Aβ deposition during the maturation of AD-like pathology [4,10].

Increasing evidence for the roles of GRKs and angiotensin 1 and 2 (AT_1 and AT_2) in hypertension, stroke, and heart disease with association between these receptors/ligands in heart disease and AD, [15] as well as early amyloid-β (Aβ) accumulation *in vitro* [16] and our *in vivo* work with models of hypoperfusion [8] prompts further consideration of AD and AD-like pathology in terms of possible inclusion and classification as disorders of the cerebrovasculature, because they involve common receptor types. Our *in vivo* findings demonstrated the early involvement of this kinase in both cerebrovascular ischemia and in AD [8]. During ischemic injury and in the vulnerable neurons of AD patients, we found increased GRK2 immunoreactivity. Therefore, cellular and subcellular investigations into the mechanisms preceding Aβ deposition and progression, as well as the possible accelerating effects of environmental factors such as chronic hypoxia/reperfusion, were crucial to understanding events that precede amyloid deposition and which may lead to insights into new pharmacological treatments of AD [8,17].

General Features of GRKs

GRK function and interaction is complicated and its importance compels increasing research interest. GRK function to regulate receptor trafficking of GPCRs and, thus, dictates

the appropriate signal magnitude and specificity for the downstream signal events from diverse extracellular stimuli, controlling a vast number of physiological processes. GRKs regulate numerous receptor functions in both the brain and myocardium [18] and its actions are more functionally diverse than previously thought. General features of GRK interaction with GPCRs involves complex regulatory mechanisms that modulate receptor responsiveness and underlie signal integration and desensitization [19]. GRKs are members of a multigene family, which are classified into three subfamilies. GRK2 and 3 form the second subfamily [beta-adrenergic receptor kinase (beta ARK) subfamily], which phosphorylate and regulate agonist-occupied or constitutively active GPCRs. Beta ARK1 (also known as GRK2) is the most abundant GRK in the heart, and it is increased in several cardiovascular diseases associated with impaired cardiac signaling and function, suggesting that this protein could have pathophysiological relevance in the setting of heart failure.

GRKs phosphorylate PAK, MEKK/Raf, MEK, MAPK, SAP/JNK, p38, Arrestins, beta2-adrenergic receptor and other proteins. GRKs phosphorylate and recruit Beta arrestin to the receptors in order to attenuate receptor desensitization and initiate internalization of G protein-coupled receptors by phosphorylating serine and threonine residues and trigger arrestin binding, which can then be sorted to endosomes and lysosomes. Further, covalent modification of GPCRs with ubiquitin serves as a signal for internalization as well. Failures in ubiquitin-mediated proteolysis, may explain the accumulation of GRKs in AD and hypoperfusion, where posttranslational modification of critical lysine residues may impair proteolysis. Many receptors for neurotransmitters and hormones rely upon members of the Gq-alpha family of heterotrimeric G proteins because they directly link receptors to the activation of PLC-beta isoforms, which, in turn, stimulate inositol lipid, calcium and PKC signaling. Hubbard and Hepler demonstrated that Gq-alpha, G11alpha, G14alpha and G15/16alpha regulate both overlapping and distinct signaling pathways independent of inositol lipid signaling, while also showing the inhibition of Gq-alpha-dependent PLC-beta1 activity by PKG and PKA when mediated by stimulatory phosphorylation of RGS4 and GRK2 [20].

GRKs critically regulate beta-arrestin signaling via receptor phosphorylation and the triggering of desensitization. The beta-arrestins play a crucial role in regulating the responsiveness of multiple GPCRs [19]. The molecular mechanisms of desensitization are quite complex and have been investigated largely with the beta2-adrenergic receptor (beta2AR) used as the main model system. Recent data from Mayor and colleagues indicate that, besides the uncoupling function, GRK2 and beta-arrestin also directly participate in beta2AR sequestration, thus providing the trigger for its resensitization. This is followed by binding of uncoupling proteins (arrestins) and transient receptor internalization, which plays a key role in resensitizing GPCR by allowing its dephosphorylation and recycling [19]. A detailed knowledge of the role of GRKs and arrestins in betaAR internalization would make their physiologic role in the modulation of cellular responses to messengers better understood and is much too complex to address in this review. It has been shown that stimulated β1-AR can recruit GRK to the membrane. Either β-AR subtype's interaction with GRKs is via the intracellular loops and at the c-terminus of the receptors.

Recent work has revealed potential phosphorylation-independent regulation of GPCRs by GRK2 and GRK3 [21]. Further, GRKs themselves may be regulated by caveolin [3]. Nevertheless, reduced expression of GRK and beta-arrestins leads to supersensitization of GPCRs and increase the response to neuropeptides, neurotransmitters, chemokines, and many

other molecules. Thus, overexpression of these GRKs could serve a protective or compensatory response to these stressors and chronic stress conditions involving excitotoxicity. Further, GRKs together with A-kinase anchoring proteins (AKAPs) assemble several cAMP effectors, including target protein kinase A (PKA) for cAMP signaling to the cytoplasmic surface of mitochondria and stimulate PKA-dependent phosphorylation of the proapoptotic protein BAD, inhibits release of cytochrome c from mitochondria, and protects cells from apoptosis [22].

Expression and Localization of GRK's

The various GRK subtypes differ in their localization, regulation and mode of action. Many GRKs are highly expressed in the heart, brain and other tissues as shown in the rat and hamster [23], where they regulate numerous receptor functions in the brain and myocardium [24]. Desensitization and resensitization of a wide variety of GPCRs are processes involved in numerous brain functions and GRK2 expression is increased in the developing rat brain, which is consistent with an involvement in brain maturation processes [25]. The expression in the developing brain and in AD is another hallmark of AD, which is characterized by ectopic expression of a multitude of cell cycle markers and proteins that are involved in cell division and development, which we propose is an apparent recapitulation of ontogeny in this disease [26]. These same analogies have been considered in the parallels observed between AD and cancer and we propose they are examples of embryological parallelism in both diseases [27]. In the rat brain, mRNA expression pattern of GRKs family of proteins (GRK2, GRK3, GRK4 and GRK6) was widely distributed and had nearly the same expression pattern, although GRK3 generally was expressed more weakly than GRK2 in most tissues.

GRK2 has been well characterized in the heart, where the onset of congestive heart failure (CHF) is associated with characteristic changes in myocardial expression of GRK2 and is known to significantly contribute to myocardial regulation and function in the failing heart [28]. Signaling through cardiac β adrenergic receptors (βARs) is significantly impaired in many cardiovascular disorders, including CHF. Chronic heart failure leads to upregulation of GRK2, both in cardiac myocytes and in adrenal chromaffin cells, which results in increased phosphorylation and desensitization of betaARs. Further, elevated levels of GRK2 mRNA and GRK2 activity have been reported in human left ventricle explants from heart failure patients [29]. In the heart, βARs control numerous trophic responses to the catecholamine neurotransmitters, norepinephrine and epinephrine. Heart failure onset is characterized by reduced responsiveness to β-adrenoreceptor in cardiac tissues [30] and by changes in the expression of GRK2 or β-adrenoreceptor kinase1 (bARK1) and signaling of these receptors through the Gs protein-AC-PKA signaling pathway [31]. When β-adrenoreceptor responsiveness was examined in a completely developed reperfused myocardial infarction model, higher levels of tissue catecholamines and GRK2 were observed in the ischemic epicardium, leading to sympathetic overstimulation of the failing heart [32]. It was found that the density of the β-adrenoreceptor in the viable ischemic regions can be modified by GRK2 and catecholamines. Conversely, cardiopulmonary intervention was found to decrease GRK expression [33]. In chronic heart failure, the adrenal chromaffin cell results in increased GRK2 levels, which in turn results in increased phosphorylation and

desensitization of alpha2ARs and subsequent increased catecholamine secretion and thus circulating catecholamines. The exposure to high levels of circulating catecholamines has been reported to be toxic to cardiac myocytes [34,35] and perhaps may adversely affect the brain as well. Simultaneous inhibition of GRK2 in the heart, the adrenal gland and brain with an appropriate pharmacological inhibitor, may have a positive use in treating chronic heart failure and perhaps AD.

G protein-coupled receptor desensitization is emerging as an important feature of several cardiovascular diseases. GRK2 plays a key role in the regulation of a variety of these receptors and cardiac muscle expression is altered in pathological situations at the promoter level such as in CHF [36], portal hypertension [37] and in other tissues and cells, such as lymphocytes [38] in these conditions. GRK-dependent receptor desensitization and regulation of βAR or other GPCRs, is a rapid process that appears to involve agonist-promoted receptor phosphorylation by GRKs. GRK-mediated receptor phosphorylation promotes the binding of arrestin proteins, such as β-arrestin [39]. β-arrestin binding uncouples G protein-coupled receptors from their respective G proteins by sterically blocking receptor coupling to G proteins. These same regulatory proteins also modulate GPCR endocytosis and the processes of transient receptor internalization, intracellular trafficking and resensitization [40]. Further, the internalization process leads to ERK activation, as is the case for beta2AR and lysophosphatidic acid receptor [41] and ERK activation, known as a prominent feature of AD. Consequently, the β-arrestins play a crucial role in regulating the responsiveness of many GPCRs. GRK2, along with β-arrestin, plays a key role in resensitizing GPCRs by allowing its dephosphorylation and recycling. Data by Mayor and colleagues indicate that besides the uncoupling function of β-arrestin, which together with GRK, directly participates in beta2AR sequestration and may provide one trigger for resensitization [19]. GRK2 levels in myocardium and lymphocytes may be associated with β-AR dysfunction and heart failure severity.

Signaling through cardiac betaARs is significantly impaired in many cardiovascular disorders, including CHF. Recent studies in several different mouse models have demonstrated that betaARK1 plays a key role not only in the regulation of myocardial signaling, but also in cardiac function and development [24]. Moreover, studies have shown that targeting the activity of GRKs, especially betaARK1, appears to be a novel therapeutic strategy for the treatment of the failing heart. The development of small molecule inhibitors of betaARK1 and GRK activity may advance therapeutic options for heart disease [42], which may be useful for AD as well, perhaps under conditions where excitotoxicity is evident.

GRK, ET-1 and Insulin Signaling

Diabetes and AD have merged as a comorbidity factor and failures of energy homeostasis are involved in both disease processes, as diabetic individuals have a 30 to 65 percent higher risk of developing AD compared to non-diabetic individuals [43,44]. Hyperglycemia increases DAG activates PKC-β and-δ, which can decrease eNOS and increase ET-1 and effect blood flow. It also activates VEGF, which affects vascular permeability, angiogenesis and NADPH oxidases, which increase oxidative stress. GRK2 receptor regulating signaling at the G protein level by interacting with Gαq, Gα11, and Gα14, but not Gαs, Gαi, Gα12/13 or

Gα16 [45,46]. The phosphorylation-independent binding of the N-terminal domain of GRK2 to Gαq/11 can attenuate Gαq/11-mediated receptor signaling of angiotensin II, endothelin receptor, thromboxane A_2 receptor, thyrotropin receptors and mGluR1a/5. This process depends on the GRK2 RGS homology domain [47,48]. Importantly, direct activation of AMP-activated protein kinase stimulates NOS in human aortic endothelial cells (EC). Phosphoinositide 3-kinase-dependent activation of the 5'-AMP-activated kinase (AMPK) has been demonstrated through peroxynitrite ($ONOO^-$) and hypoxia-reoxygenation in cultured EC. Exposure of aortic EC to $ONOO^-$ increased the phosphorylation of AMPK and its downstream enzyme endothelial specific NOS (eNOS), and is accompanied by increased phosphorylation of protein kinase Czeta (PKCzeta) [49].

In regard to energy metabolism and homeostasis, Gαs can activate adenylyl cyclases, converting ATP into cAMP and initiating PKA activation [50,51]. Conversely, adenylyl cyclases are inhibited by Gαi and the Gαq subunits activate phospholipase C (PLC)β, which hydrolyses phospho inositol phosphate (PIP2) into diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3), which then activate PKC and the release of intracellular calcium. Adiponectin and its receptors are integral players in mechanisms of energy homeostasis, which stimulate glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase [52], which regulates energy homeostasis and glucose and lipid metabolism in adipocytes found in the brain. Adiponectin is relevant, because it and its receptors have recently been localized in the human pituitary gland, hypothalamus and other brain regions and hormone producing cells, such as FSH, GH, LH, TSH and ACTH [53].

When GRK2 was overexpressed *in vitro*, binding to PI3 kinase was found to promote PI3 kinase recruitment to the plasma membrane, increasing receptor endocytosis [54]. PI3 kinases, with catalytic and regulatory subunits, generate lipid products, which activate Akt. Class IB of PI3 kinases sole member (PI3 kinase-γ) is composed of the regulatory p101 subunit and the catalytic p110γ subunit. Upon GPCR stimulation, Gβγ subunits directly bind to p110γ in the activation of PI3 kinase-γ [55]. In addition, activated Gβγ subunits control the activation of several effectors, including K^+ channels, small G proteins, phosphoinositide-3 kinases (PI3 kinases) and PLCβ [56].

GRK2 interacts with components of the PI3 kinase-Akt and MAPK signaling cascades with consequences for receptor signaling and desensitization. It has also been reported that an interaction between GRK2 and Akt inhibits the kinase activity of Akt in sinusoidal endothelial cells (EC) from portal hypertensive rats [37]. PKC, PKA and PLCβ can mediate upstream activation of MAPKKK by G protein interaction. The MAPK cascades are MAPK kinase (MAPKKK), which phosphorylates and activates a MAPK kinase (MAPKK) and, in turn, activates one of the MAPKs, whose members are ERK1/2, JNK1-3, p38 MAPKα/β/γ and the more recently identified ERK5 [57]. All Gα and Gβγ subunits have been described to trigger activation of the different MAPK pathways, with some Gα subunits exerting inhibitory effects on MAPKs [58,59]. For example, phosphorylation of p38MAPK by GRK2 uncovers a novel mechanism of inhibition of the association of p38 with some of its partners [60].

Since we have hypothesized the existence of an imbalance between the NOS species and ET-1, we now suggest a putative role of GRK2 in chronic ET-1-induced insulin resistance in the brain and vascular wall cells, as it has been found for other cells and tissues, which also may contribute to consequences for Alzheimer and stroke patients by similar mechanisms. In that regard, GRKs, which are classical serine/threonine kinases that desensitize agonist-occupied GPCRs, have been found to regulate other receptors such as the insulin receptor

(IR), which is a tyrosine kinase receptor. GRK2 was found to negatively regulate glycogen synthesis in mouse liver FL83B cells [61]. This group demonstrated that the IR also couples to G-proteins, specifically GRK2, and utilizes downstream signaling components to negatively regulate IR signaling in those cells. In other tissues and cells, GRK2 can function as a negative regulator of insulin action by interfering with G protein-q/11 alpha-subunit (Galphaq/11) signaling [47], causing decreased glucose transporter 4 (GLUT4) translocation [48]. This same group reported that chronic ET-1 treatment leads to heterologous desensitization of insulin signaling with decreased tyrosine phosphorylation of insulin receptor substrate (IRS)-1 and Galphaq/11, and decreased insulin-stimulated glucose transport in 3T3-L1 adipocytes. It is GRK2, which mediates ET-1 induced insulin resistance via the inhibition of both Galphaq/11 and insulin receptor substrate-1 pathways in these cells. Another mechanism is one where GRK2 functions as a negative regulator of insulin action through cdc42-associated phosphatidylinositol 3-kinase activity and the phosphorylation of IRS-1 and IRS-1 protein degradation. Taken together, the importance of GRK2 in AD, vascular dementia and other metabolic diseases, such as diabetes should not be underestimated. GRK2 deficiency was found to increase the basal and insulin-stimulated phosphorylation of Ser(21) in glycogen synthase kinase-3alpha. Insulin-induced tyrosine phosphorylation of the IR was similar in control and GRK2-deficient cells [61]. Of interest here is the finding that GRK2 mediates Et-1 induced insulin resistance via the inhibition of both Galphaq/11 and insulin receptor substrate-1 pathways in 3T3-L1 adipocytes [48]. Further, the same group showed that chronic ET-1 treatment leads to heterologous desensitization of insulin signaling with decreased tyrosine phosphorylation of insulin receptor substrate (IRS)-1 and Galphaq/11, and decreased insulin-stimulated glucose transport in 3T3-L1 adipocytes.

Recent data suggest possible alternate roles for GRK2 other than as a kinase. In that regard, when the role of phosphorylation of the endothelin B receptor (ETBR) in agonist-induced desensitization was investigated, using a mutant lacking C-terminal 40 amino acids (delta 40 ETBR). In cells expressing the wild type or delta 40 ETBR, ET-1 caused rapid desensitization of calcium responses [62]. These investigators found the wild type ETBR was phosphorylated by ET-1, and the phosphorylation was markedly enhanced when coexpressed with GRK2. However, delta 40 ETBR was not phosphorylated regardless of coexpression with GRK2. Phosphatidylinositol 3 formation was ET-1-induced in these cells and was decreased by coexpression with GRK2 or kinase-dead GRK2 by a similar mechanism, by which the authors suggest the presence of phosphorylation-independent desensitization mechanism in delta 40 ETBR as a possible alternate role for GRK2, other than those that are kinase-related in the strict sense.

Of importance in these GRK2 AD studies is the cellular response to certain stimuli from the vascular endothelium, neurons and glia, which all are able to synthesize, store and release ROS, NO, and ET-1, a vasoactive peptide. In that regard, elevated circulating glucose concentration leads to many forms of pathology including increasing superoxide formation that can form hydrogen peroxide (H_2O_2), leading to hydroxyl radical production, which can interact with NO to form peroxynitrite. GRK2 is impaired by H_2O_2 [63]. All of these reactive species can damage nucleic acids, protein and lipids, leading to oxidative stress through loss of reducing equivalents, DNA strand breaks, poly-ADP-ribose polymerase activation and consumption of NAD^+, which ultimately impairs energy homeostasis. In support of this notion, it is important to consider PARP activation in diabetes, which is linked of increased

oxidative stress and leads to NAD^+ depletion [64]. Their contribution to the pathophysiology of stroke or stroke-like conditions and AD cannot be understated. ET-1 is produced by multiple cells and is differentially coupled to G-proteins [45] in response to hypertrophic stimuli *in vitro* and in the development of heart failure *in vivo* [65,66]. Nevertheless, the endothelin A and B receptors (ET_A-R and ET_B-R) undergo desensitization, most likely also through GRK2 [67]. For example, ET-1 can elicit several responses; it activates eNOS via G-protein beta/gamma subunits signaling through protein kinase B/Akt [68] as well as prolonged physiologic responses, including mitogen-activated protein kinase (MAPK) activation [69] and c-Jun NH2-terminal kinase (JNK) and extracellular signal regulated kinase (ERK) in cultured animal cells and *in vivo* [70]. MAP kinases have been long-associated with AD and ERK activation may be another important early event, perhaps downstream from GRK2 activation [71]. Interestingly, these pathways also have been implicated in cell cycle dysregulation in human AD cases [72,73]. Recently we have demonstrated that since successful dysregulation of the cell cycle is also the hallmark of a neoplastic changes, early cell-cycle pathophysiology in AD may recruit oncogenic signal transduction mechanisms and, hence, could be viewed as pseudo-neoplastic transformation, which is eventually aborted [74]. Further, it has been shown that phosphorylation of GRK2 by MAPK also triggers a turnover of GRK2 degradation through the proteasome pathway, in which GRK2 is targeted for proteolysis by β-arrestin function and Src-mediated phosphorylation [75]. Therefore, GRK2 may play a very important role in AD pathogenesis mechanisms through oxidative stress and mitochondrial dysfunction.

GRK Studies in AD and CBH

Studies of the details and consequences of GRK's mechanisms have focused heavily on the original beta-adrenoreceptor kinase (beta-ARK) family (GRK2 and GRK3) and, in particular, on phosphorylation-dependent recruitment of adaptor proteins such as the beta-arrestins. Several lines of evidence implicate GRK and beta-arrestin expression in AD and after cerebral hypoxia/ischemia (HI) [18] and the differential GRK2 expression in compensated hypertrophy and heart failure after myocardial infarction in the rat. Moreover, G protein-coupled receptor kinases regulate metabotropic glutamate receptor 5 function and expression [76], which has also been implicated in AD pathogenesis and GRKs may offer a mechanism for desensitization of this receptor isoforms.

The main experimental goal of our previous study was to investigate and better clarify the relationship between GRK2, vascular lesions and the development of pathology in a CBH model and AD, at the cellular and subcellular level [8]. In that regard, we examined a connection between vascular damage and predisposing factors for AD, where we explored the changes in brain distribution of GRK2 in microvessel wall cells and neurons using a CBH model and in AD cases. Our previous studies and those of others have reported that CBH will result in a 22%–30% reduction of hippocampal blood flow that will stabilize after several weeks without further reduction [77-79]. This model is relevant to examining the physiopathology of AD and stroke and enables exploration of the relationship between vascular events and AD.

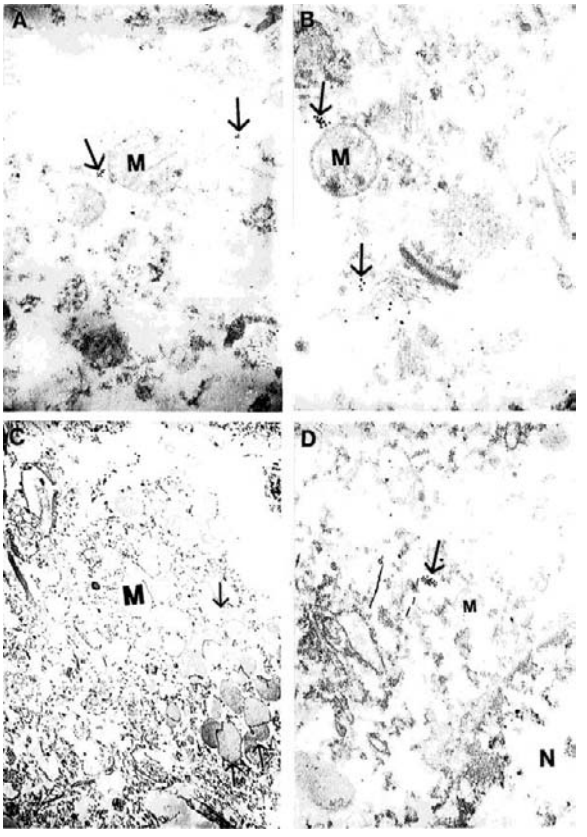

Figure 1. Subcellular localization of GRK2 immunoreactivity detected by using pre-embedding immunogold decoration in hippocampus of aged matched control (A, B) and AD brain (C, D). A and B – The neuronal cell body from the age-matched control brain hippocampal tissue shows the presence of GRK2 containing positive gold particles (arrows) attached to the external membrane of partially damaged mitochondria. GRK2 immunopositive gold particles localized in the matrix of damaged mitochondria and Golgi cistern. X 30,000 and X 40,000, respectively, A and B. C – Hippocampal tissue from the AD brain shows that the neuronal cell body is characterized by the presence of large number of mitochondria derived lysosomes (M), and disperse distribution of GRK2 positive gold particles (arrows). X 6,000. D - Glial cell body from the AD brain tissue shows clusters of GRK2-immunoposotive gold particles in the matrix of mitochondria derived lysosomes (single arrow), X 20,000. Abbreviations: M-Mitochondria, N-Cell Nucleus (reprinted from [11] with permission).

Our study was the first where ultrastructural localization demonstrated that the overexpression of GRK2 occurs during the early stages of damage in aged human and AD cases (see Figures 1 and 2), and also in a our 2-VO model of CBH (see Figures 3-5). This overexpression is an early event, occurring at prodromal stages, before and up to a point when the damage is reversible.

Based on our observations, we were able to detect the subcellular localization of GRK2 immunoreactivity in the neuronal cell body of the age-matched control brain hippocampal tissues. We achieved this by using pre-embedding immunogold decoration in the hippocampus of age-matched control (Fig. 1A-B) and AD brains (Fig. 1C-D). This brains showed the presence of GRK2 containing gold particles attached to the external membrane of partially damaged mitochondria. In addition, GRK2 immunopositive gold particles localized in the matrix of damaged mitochondria and Golgi cistern (Fig. 1A-B). However this

observations were seen in the case when aged-matched damage occurred in the hippocampal neurons. Contrary to this observation, hippocampal tissues from the AD brain showed that the neuronal cell body was characterized by the presence of large number of mitochondria derived lysosomes and disperse distribution of GRK2 positive gold particles (Fig. 1C). Moreover, glial cell body from the AD brain tissue also shows clusters of GRK2-immunoreactivity in the matrix of mitochondria derived lysosomes (Figure 1D). In our AD and hypoperfusion models, we observed less positive signals for GRK in control cases, generally. Mostly GRK positive gold label in electron microscopic studies was observed bound to the residues of the different cellular compartments, such as damaged mitochondria, distorted perivascular cells. Some positive signals were observed in perivascular cells associated with damaged vessels as well as in cellular compartments with lesions. Nevertheless, there are pathological cellular structures, such as neurofibrillary tangles (NFT)-like and/or vacuolar degenerative structures (GVD), which colocalized with GRK2 [8]. In some cases GRK positive signals were bound to degenerated vacuolar structures. That data was the first known *in vivo* evidence demonstrating GRK2 activation in early cerebrovascular disease, including AD, and thus, GRK2 could serve as a new target for treatment approaches to AD, cerebrovascular dementia or stroke [80].

Usually, GRK2 immunoreactivity was found to be associated with damaged cellular compartments, especially mitochondria and/or mitochondria-derived lysosomes or granular/vacuolar degenerative structures (see Figures 1 and 2). The immunopositive reactivity was observed in damaged vessel wall cells and their subcellular compartments (Figure 2). We have found that neurons that contain NFT show abundant GRK2 immunopositive reactivity (Figure 2). The intensity of the reaction varied from cell to cell and within cellular compartments as well (Figures 1 and 2). However, cellular lipofuscin was not associated with any GRK2 immunoreactivity. Nevertheless, there are pathological hallmarks of AD present in harvested neurons, e.g. neuronal inclusions, or those neurons containing structures such as NFTs, GVD, as well as in microvascular wall cells, which show a highly intense immunopositive reaction. While late stages of damage reveal scarce GRK2-immunorectivity in areas that were previously abundant, suggest that overexpression of GRK2 can be lost or reduced, which was confirmed by western blotting [4]. Thus, this protein can serve as an earlier marker of the brain damage that typifies cerebrovascular and/or mild cognitive impairment, human AD and damage in an animal model that mimics AD. In addition, the overexpression of GRK2 immunoreactivity complements our earlier observation that oxidative stress induced damage is observed in mitochondria and or other cellular compartments before any amyloid deposition occurs [4.67]. One mechanism of regulation of GRK2 stability includes Mdm2 and the PI3K/Akt axis, orchestrated in a stimulus, cell type, or context specific way, as well as the functional consequences of altering GRK2 expression/functionality in specific cell types and experimental models.

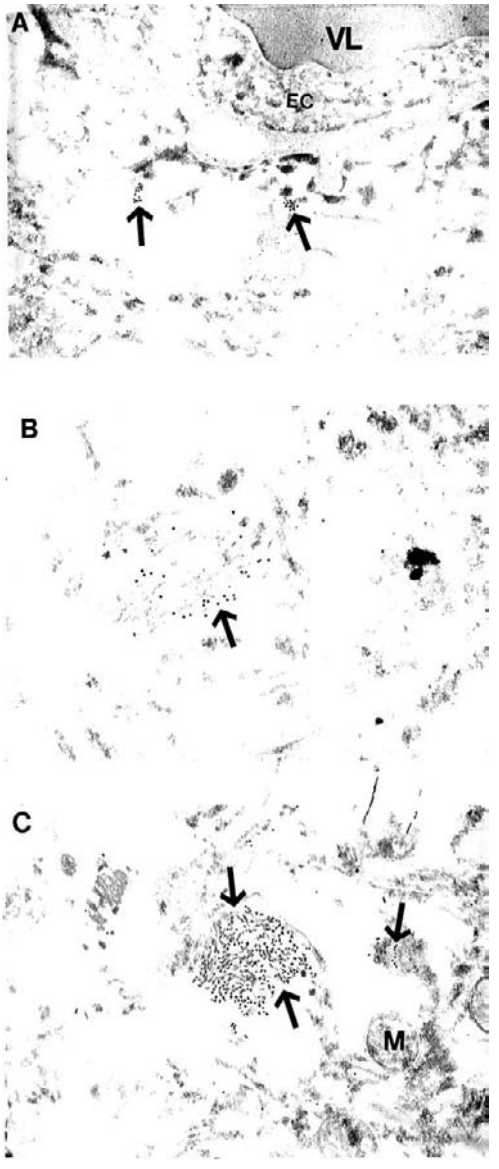

Figure 2. The ultrastructural localization of GRK2 immunopositive gold particles in postmortem human AD (A) and age-matched control brain (B, C) tissues. A- The GRK2 immunopositive containing gold particles in the matrix of perivascular pericytes (indicated by single thick arrows) but not in the cytoplasmic matrix of severely damaged vascular endothelium (EC), X 40,000. B and C - The neurons close to perivascular regions show the presence of GRK2 containing gold particles in their matrix, where most gold particles were associated with the NFT-like structures (arrows). However, the intact mitochondria (M) were free from GRK2-immunopositive gold particles, X 40,000, respectively, B and C (reprinted from [11] with permission).

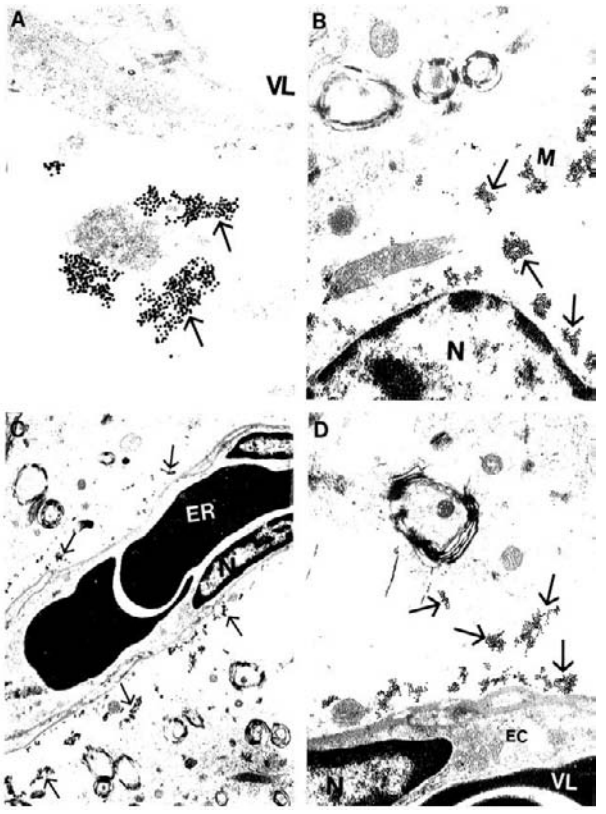

Figure 3. Ultrastructural characteristics of GRK2 immunopositive gold particles from the rat brain in control (same-operated: A, B) and 2 vessel occlusion (2-VO; respectively C, D) experiments. A – Clusters of GRK2 immunopositive gold particles in the cytoplasmic matrix of perivascular pericytes (arrows) but not in the vascular EC, X 20,000. B- The presence of GRK2 immunopositive gold particles associated with the edematous portion of the perivascular pericytes cytoplasmic matrix (arrows). Intact, but not giant mitochondria (M), are free from any GRK2-immunopositive positive gold particles, X 30,000. C – The GRK2 containing positive gold particles was seen in the hippocampal tissues from rat exposed to 2-VO. The presence of GRK2-immunopositive gold particles was seen throughout the matrix of damaged perivascular pericytes (arrows), X 8,000. D – Perivascular regions of this area from the figure C under higher magnification display the presence of islands of GRK2-containing positive gold particles which are associates with the damaged regions of the cytoplasmic matrix (arrows). Nucleus (N) and intact mitochondria are from the GRK2-immunoreactivity, X 30,000 (reprinted from [11] with permission).

A parallel study reported abnormal GRKs *in vitro* for early stages of AD, which is associated with early amyloid beta (Aβ) accumulation *in vitro* and showed that subthreshold Aβ pretreatment disrupts binding of GRKs to activated GPCRs [16]. This led to reduced membrane GRK2/5, which subsequently led to retarded GPCR desensitization, prolonged GPCR signaling, and cellular supersensitivity to GPCR agonists [16]. The same group went on to report in a transgenic mouse model of AD, where the double mutant form of APP695 is overexpressed under the regulation of a prion promoter, the overexpression of GRK2, and to a lesser extent GRK5, occurred in the cytosolic vs. membrane fractions from hippocampal and cortical brain homogenates with increasing age and plaque deposition. While the *in vitro* observation is quite likely to occur within microglia, the increase in the overexpression of GRK2 and GRK5 in the cytosol of neurons was not differentiated in this study. Nevertheless,

we report the subcellular localization of GRK2 in neurons and the earlier involvement of vascular lesions *in vivo* as a key event in this process and, thus, in the development of human AD and AD-like pathology. Data to support this notion has been explored in various rat models [77,81]. In this regard, we have demonstrated that abnormal mitochondria (mitochondria with electron dense matrix and mitochondrial-derived lysosomes) and lipofuscin appear to be features of damaged hippocampal neurons in aged Tg (+) mice and human AD, suggesting a direct relationship between vascular abnormalities, BBB breakdown, neuronal loss and amyloid deposition [4,10,12,14,82].

Our *in vivo* data discussed in light of another study involving amyloid beta precursor protein overexpression [16], our model shows a similar effect, but attributes the overexpression of GRK2 to oxidative stress and events prior to Aβ deposition. While the effect observed in the Sou study involved subthreshold levels of the protein, which may reflect an early event as well, the use of total homogenates from the transgenic model of Aβ overexpression does not indicate which cells are affected, failing to control for glia or other immunologic cells that may be involved. However, since Aβ deposition is a later hallmark lesion in AD, we suspect that the appearance of Aβ along with the loss of GRK2 immunoreactivity may be linked somehow, but the role of Aβ on GRK2 translocation may be cell-specific and has not been characterized. Therefore, the appearance Aβ is unlikely to be the primary predisposing factor for GRK2 overexpression, as Aβ deposition occurs much later in the disease process. Any cytotoxicity may emanate from mechanisms other than amyloid directly as earlier events seem to be more crucial in the disease pathogenesis. Perhaps early cytotoxicity resulting from non-amyloid-mediated mechanisms may be more crucial in the etiopathology and suggest Aβ is less likely to be the primary predisposing factor for GRK2 overexpression [12,83].

Our studies demonstrate an increase in GRK2 localization to the cytosol, but in particular to subcellular components and only those components with damage and/or pathology evident (see Figures 1-5). In our study we used perfusion fixation for the 2-VO model and for this reason preservation of the tissue in 2-VO exposed animals was much better than when compared to human postmortem AD brain tissues, even after a very short postmortem time period (<2-4 hours). However, in the general distribution and the density of the GRK-2 containing gold particles, it was very similar in the human AD and 2-VO animal models. Recruitment of GRK to the cell membrane is followed by inhibition of signaling. Therefore, the sequestration of GRKs to subcellular locations may indicate a compensatory adaptation to AD. However, other studies suggest that GRKs have more diverse protein/protein cellular interactions, and that β-arrestins together with GRKs play a crucial role in regulating the responsiveness of many GPCRs. Further, GRK2 levels in myocardium and lymphocytes may be associated with β-AR dysfunction as well, which is one area that should be addressed in AD. One explanation for the subsequent loss of GRK2 may lie in the ability of Aβ to act as a bioflocculant [84] and a possible role in the sequestration of GRK2, thereby limiting downstream phosphorylation events as well, or leading to translocation of GRK2 to the cytosol. Regardless, the reduced availability of GRK2 and beta-arrestins to regulate GPRC signaling most likely would lead to a state of GPCR supersensitization, thereby increasing response to neuropeptides, neurotransmitters, chemokines, and many other molecules, all of which could have deleterious consequences. Conversely, it may be plausible that increased GRK2 expression, and particularly localization an expression of modulation, and this would

impart a compensatory or survival response to excitotoxicity, which is a claim made for Aβ as well [85]. Finally, neurodegeneration can have numerous overlapping features, and GRK2 along with the action of specific phosphatases has been implicated in other neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) [86]. Thus, there are numerous parallels that can be drawn between the neurodegenerative and cerebrovascular disorders with heart disease and systemic vascular disorders, which drives home the important connection the role GRK can have in all disorders, particularly AD [87].

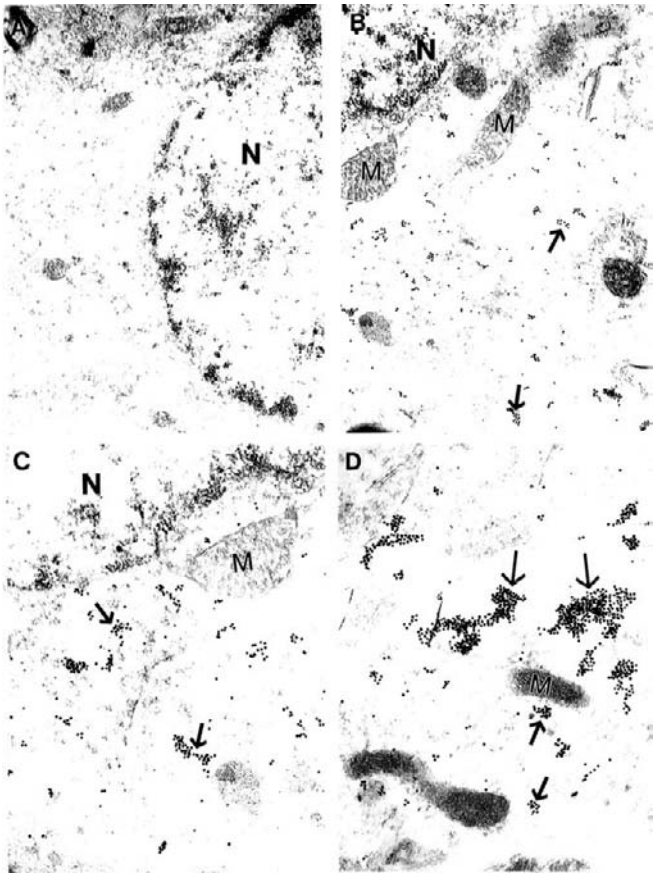

Figure 4. The subcellular features of the GRK2-immunoreactivity in the hippocampus of the rat subjected to 2-vessel occlusion. A – Intact neurons show absence any GRK2 immunopositive gold particles in their cytoplasmic matrix, X 15,000. B – Neuron with the effect of chronic cellular hypoperfusion demonstrate the presence of a GRK2 overexpression (arrows) throughout the cell body, however the intact mitochondria (M) were free from any GRK2 immunopositive gold particles, X 30,000. C – *"Hypoperfusion"* affected neuronal cell body shows the presence of islands of GRK2 positive immunodecoration in the external membrane and in the matrix of damaged mitochondria and mitochondria-derived lysosomal structures (arrows), X 40,000. D - Neurons with severe damage shows the presence of islands of GRK2 containing immunopositive gold particles that associated with the completely damaged (mitochondria-derived lysosomal structures) (arrows), but not with non-damaged mitochondria (intact and giant), X 40,000 (reprinted from [11] with permission).

In comparison to controls, ultrastructure in AD and animal models are predominated by abnormal mitochondria. Studies examining deleted mtDNA and mitochondrial-derived

lysosomes in regions closely associated with lipofuscin, suggest that proliferation, deletion and duplication of mtDNA occur in mitochondria in human AD and transgenic mouse models of neurodegeneration [4,10,12,14,82]. *In situ* hybridization with a chimeric mouse and human mitochondrial cDNA probes for the 5kb common deletion indicate that the deletion is increased at least 3 fold in AD cases as compared to controls and in yeast artificial chromosome (YAC) APP mouse hippocampus [4,10,14,88], which is strongly positively correlated (r=0.934) with the marker of DNA oxidation, 8-OH deoxyguanosine. These findings indicate that the mitochondrial DNA overproliferation and/or deletion are key initiating factors for disruption of the BBB and the development of pathology and GRK2 immunoreactivity overexpression would be coincident with these processes.

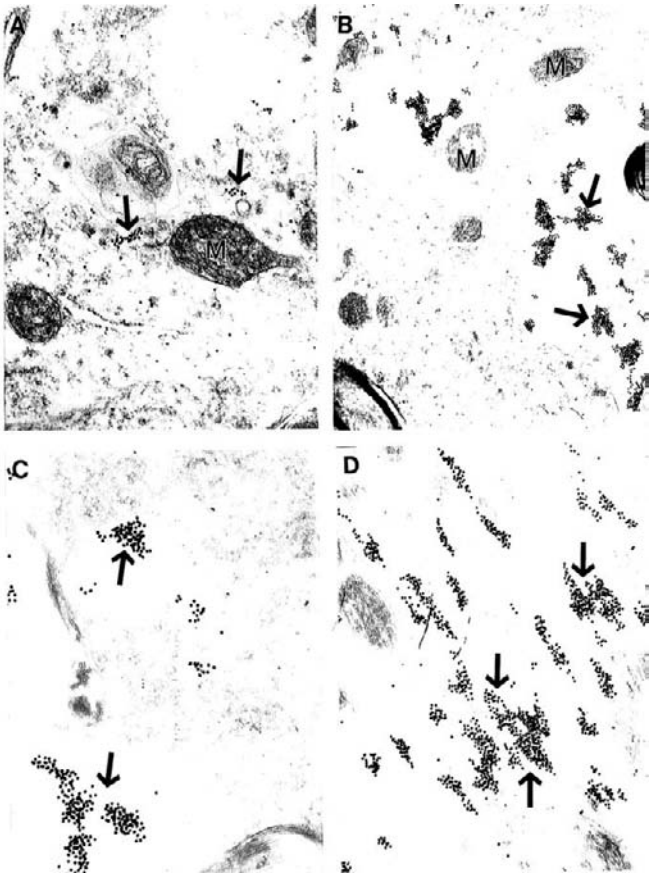

Figure 5. The GRK2-immunoreactivity in rat brain hippocampal tissues exposed to 2-vessel occlusion and determined by using pre-embedding immunogold cytochemistry techniques. A Subcellular determination of GRK2 in the neuronal cell body shows the presence of GRK2 immunopositive gold particles (arrows), which associates with the external membrane and the matrix of damaged but not intact mitochondria (M), X 40,000. B – Neurons containing granular vacuolar degenerative structures shows island of GRK2 immunopositive gold particles (single arrow), X 30,000. C - The glial cell body shows overexpression of GRK2-immunoreactivity in the matrix of granular vacuolar degenerative structures (single arrow), X 50,000. D – Neurofilament from the damaged neurons shows the presence of GRK2 immunopositive gold particles (single arrows), X 40,000 (reprinted from [11] with permission).

Earlier in a 2-VO model, we reported that ultrastructural examination of hippocampal CA1 capillaries in rats revealed a smaller size EC containing damaged mitochondria, characterized by transformation of lysosomal structures within the EC and in the perivascular area [83]. Along with mitochondrial abnormalities these changes appeared to be associated with amyloid deposition found surrounding the capillary vessel wall. Electron microscopic immunoassaying showed sparse eNOS-containing positive gold particles in the matrix of the vascular endothelium, in contrast to substantial labeling in the cytoplasmic matrix of perivascular cells and electron-dense mitochondria, indicative of a hypoxic insult [83]. Immunoreactive eNOS-containing positive gold particles were found markedly expressed in hippocampal neurons and in glial cells, when compared to non-occluded controls [83]. Of interest is the comparison between the eNOS overexpression pattern to that of GRK2 [37], which has the same pattern as our previous observation of eNOS.

The EM findings in rat hippocampus after CBH also support the general hypothesis that chronic oxidative stress caused the EC structural changes and the mitochondrial and immunoreactive eNOS changes, since such changes were observed only in 2-VO rats. In addition, previously we have demonstrated that oxidative damage is the earlier event in AD [89]. These findings support our working hypothesis that oxidative stress-induced vascular changes, such as an abnormality in vascular NO level, is an important molecule in spatial memory functions, at least in this CBH model. Further, this damage coexists with overexpression of GRK2 immunoreactivity and in rats, hypoxia/ischemia modulated GRK2 and beta-arrestin-1 levels [90].

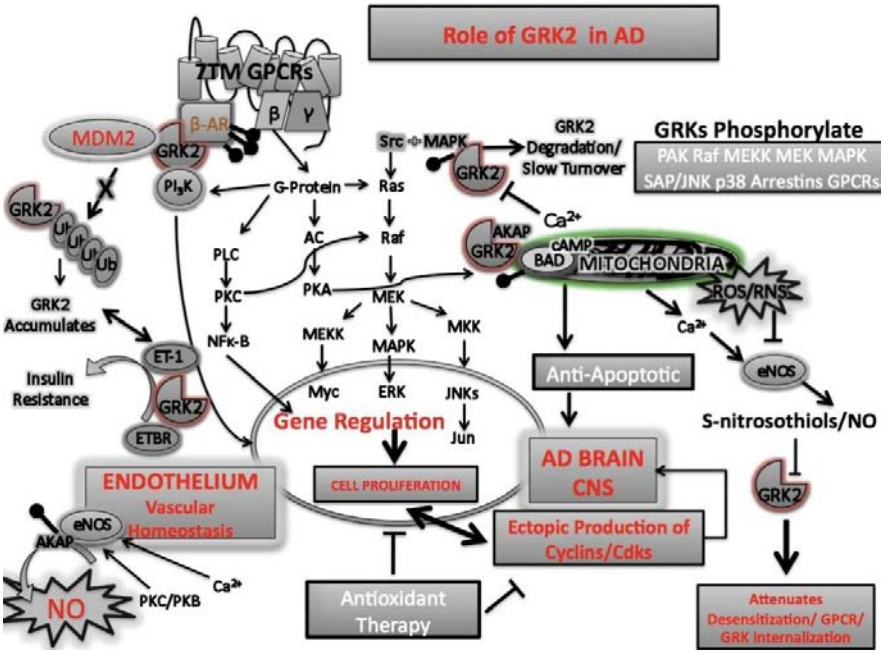

Figure 6. The role of GRK2 in AD showing the activation pathways and consequences of increased expression of GRK2 with the endothelium, brain and mitochondria (reprinted from [11] with permission).

Therefore, chronic oxidative stress-mediated inhibition of eNOS may coexist with the early overexpression of GRK2 immunoreactivity and would appear to support a compensatory role or reaction in brain tissue to potentially mitigate chronic injury stimuli such as oxygen depletion and nutrient deficiency or imbalance in metabolic homeostasis found in 2-VO conditions [83]. This data supports the present observation that it is chronic injury stimuli that not only initiates damage and compensatory changes, but accelerates brain damage in tissues, which can contribute to some types of mental retardation and cognition deficits involving the consequence of Aβ accumulation in the brain and in some types of mental retardation, such as Down Syndrome [91]. The connection to a cerebrovascular component to AD is further borne out in other rat studies, where differentially expressed cardiac GRK2 expression and activity has been found. Here, GRK2 expression has been reported to be inhibited in animals with cardiac hypertrophy without heart failure, whereas animals with heart failure had elevated GRK2 [18]. The same can be said for failing human hearts, which are reported to have elevated levels of GRK2 [92]. This expression pattern indicates differential regulation in hypertrophic non-failing and hypertrophic failing hearts. Nevertheless, it is now a commonly held belief that GRKs may likely become effective therapeutic targets for heart disease [42] and should be considered for AD or related neuropathology as well (Figure 6).

Conclusions

Our growing understanding of GRK2 and its cognate regulatory proteins provides support for a unifying hypothesis of AD where these proteins play a pivotal role by linking the many phenomenological observations into a conceptual framework that contributes to a growing body of evidence favoring the reclassification of AD as, primarily, a cerebrovascular disorder. For example, one clue also may lie in the finding that GRK2 is a microtubule-associated protein, and tubulin was identified as a novel GRK2 substrate [93]. These results suggest that tubulin is most likely phosphorylated *in situ* by GRK2 and that the phosphorylation may affect the interaction of microtubules with microtubule-associated proteins (MAPs) [94]. Phosphorylation by GRKs may have downstream consequences for neuronal cell death and perhaps contribute to the hyperphosphorylated state of tau protein, as seen in AD or in earlier events as well, perhaps one that would predispose to neuronal toxicity via NFT formation. However, recent work has revealed potential phosphorylation-independent regulation of GPCRs by GRK2 and GRK3 [21] and GRK2 was not found to phosphorylate MAPs under conditions where MAPs were already well-phosphorylated by endogenous kinases, which copurified with tubulin [95]. Nevertheless, the role of this kinase in early phosphorylation of tau cannot be discounted. Therefore, GRK2-mediated desensitization, may involve many diverse mechanisms. However, the role of GRKs, may be a pivotal one in AD pathology, as GRK-mediated desensitization, in the absence of phosphorylation and arrestin binding, has been reported for metabotropic glutamate receptor 1 (mGluR1), the gamma-aminobutyric acid B receptors [96] and in regulation of metabotropic glutamate receptor 5 function and expression [97]. Both of these receptors have been implicated in AD pathogenesis as well [98,99]. Therefore, GRKs may hold hope as therapeutic targets for AD and related pathologies. Taken together, this line of evidence

strongly supports our findings of a role for GRK2 as an earlier marker in AD pathogenesis and may couple the contribution of oxidative stress, NO, eNOS and ET-1 to the pathobiology of AD (Figure 6).

Our findings also suggest a role for GRK2 as a GPCR signal transducer, which may mediate the effects of GPCR activation on cytoskeletal structure and function in AD [8]. Our study is the first to demonstrate the cellular and subcellular localization and offer *in vivo* evidence for GRK2 activation as an early sign of cerebrovascular aging complications in age-associated diseases involving cerebrovascular abnormalities, neurodegeneration and cognitive impairment before any amyloid deposition can be seen. GRKs as physiological regulators could become an appropriate target for future pharmacological intervention. Moreover, determining the mechanisms of the damage, or potential protective nature of GRK2 receptor antagonist, may provide crucial information in the development of new and more effective therapies for stroke and AD patients. Further, research in this direction may enable GRKs to serve as a new target for treatment approaches to AD, stroke, mild cognitive impairment or related cerebrovascular disorders.

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Chapter IV

Mitochondrion Selective Antioxidants as Drug Treatments for Alzheimer Disease

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Abstract

Age-related dementias such as Alzheimer disease (AD) have been linked to vascular disorders like hypertension, diabetes and atherosclerosis. These risk factors are known to cause ischemia, inflammation, oxidative damage and consequently reperfusion, which is largely due to reactive oxygen species (ROS) that are believed to induce mitochondrial damage. At higher concentrations, ROS can cause cell injury and death which occurs during the aging process, where oxidative stress is incremented due to an accelerated generation of ROS and a gradual decline in cellular antioxidant defense mechanisms.

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Neuronal mitochondria are especially vulnerable to oxidative stress due to their role in energy supply and use, causing a cascade of debilitating factors such as the production of giant and/or vulnerable young mitochondrion who's DNA has been compromised. Therefore, mitochondria selective antioxidants such as acetyl-l-Carnitine (ALCAR) and R-alpha-Lipoic acid (LA) seem to be potential treatments for AD as they target the factors that damage mitochondria and reverse its effect, thus eliminating the imbalance seen in energy production and amyloid beta oxidation, making these antioxidants very powerful alternate strategies for the treatment of AD.

Keywords*:* Alzheimer disease, Antioxidants, Mitochondria, Oxidative Stress, Reactive Oxygen Species. Acetyl-L-Carnitine, Lipoic Acid

Introduction

Oxidative stress in brain microvessels and/or parenchymal cells results in an accumulation of ROS, thus promoting leukocyte adhesion and increasing endothelial permeability. In this regard, chronic injury results in progressive cellular hypometabolism, which is responsible for AD and cerebrovascular accidents (CVAs). Progressive cellular hypometabolism is a central initiating factor for vascular abnormality, mitochondrial damage and an imbalance in the activity of vasoactive substances; such as variable isoforms of nitric oxide synthase (NOS), endothelin-1 (ET-1), oxidative stress markers, mtDNA and mitochondrial enzymes in both the vascular wall and brain parenchymal cells.

The ultrastructural pathology in the neurovascular region coexists with neuronal and glial damage, known to be important in the development of AD. Vulnerable neurons and glial cells show mtDNA deletions and overexpression of oxidative stress markers in regions which associate closely with damaged vessels. This evidence strongly suggests that chronic hypoperfusion induces the accumulation of oxidative stress products. Moreover, the degree of vascular wall cell lesions in AD brains is proportional to the degree of neuronal and glial cell damage. Mitochondrial lesions such as DNA deletions and over-expression of oxidative stress markers are consistent in all of these cellular compartments; therefore chronic hypoperfusion is a key initiator of oxidative stress on different brain parenchymal cells, especially mitochondria, and appears to be the central target for brain damage in AD. With increased oxidative stress caused by vascular disorders, the brain attempts to maintain an energy balance by enlarging its mitochondria in order to compensate for the high energy demand. As injury stimulus gradually increases, functional changes begin to emerge. Changes include an increasing production of young, oxidation sensitive mitochondria that accelerate structural alterations and mitochondrial DNA mutations.

We hypothesize that continuous accumulation of oxidative stress products, such as peroxynitrite and large amounts of nitric oxide (NO), appear to be secondary accelerating factors for damage and compromising the blood brain barrier (BBB) in hypoxia/hypoperfusion or AD. NO is generated by the overexpression of the inducible and/or neuronal NO synthase (iNOS and nNOS, respectively). Pharmacological intervention targeted towards correcting chronic hypoperfusion may change the natural history of the dementing neurodegeneration.

The Association Between Health Risk Factors Caused by Aging and AD

The association of amyloid beta (Aβ) with cerebral vessels is an intriguing feature of AD. While some degree of cerebral Aβ angiopathy involving the leptomeninges and intraparenchymal vessels occurs in almost all cases of AD, Aβ deposits within the neocortical region is unknown [1]. In addition, the mechanisms behind the effects of several vascular factors and peripheral vascular pathophysiology might promote a late-onset of AD [1-4]. Apolipoprotein E (ApoE), a major risk factor for atherosclerosis [5-7] and AD [8], may be linked to AD via its effects on vasculature and vascular NO [9-12].

In clinical and pathology confirmed AD cases studied by Thomas and coworkers, cerebral microvessels in the temporal cortex and parahippocampal gyrus associate with the predominant Aβ1-42 form of the Aβ peptide which may affect microvascular function [1]. Surprisingly, double immunostaining methods reveal that at least 40% of the microvessels in the two brain regions contain Aβ1-42 deposits but no correlation of such localization with the ApoE genotype [1]. However, ε4 homozygote's display a greater Aβ1-40 burden. Moreover, the levels of total serum, low density lipoproteins (LDL), and ApoB correlate with an increased deposition of Aβ in demented individuals with neuropathologically confirmed AD [13]. These findings also indicate a key role for vascular abnormalities in the pathogenesis of AD. Given that chronic hypoxia/hypoperfusion, Aβ depositions, and AD are maladies with similarities to atherosclerosis, we would expect them to share risk factors [9, 10, 14]. We would then expect that utilizing the same preventive measures against these shared risk factors would alleviate patients symptoms [13, 14].

The correlation between health risk factors that emerge during the aging process and early unset AD are key elements to both the understanding and development of drug treatments. The AD brain, affected at the vascular level by oxidative stress (especially in cerebral microvessels), shows abnormalities arise from cerebrovascular accidents. Addressing oxidative damage is the best way to assess possible treatments.

Pathological Features of Brain Cerebrovascular Lesions and AD

Several morphometric features of BBB dysfunction are present in pathologically confirmed AD patients [15]. Biopsy samples from an AD brain showing an accumulation of Aβ deposits around blood vessels could indicate progressive damage of the BBB during AD [14-16]. Our findings [14] demonstrate that structural or physiologic abnormalities of the BBB itself may represent a seminal pathogenic event during AD development which leads to vascular amyloid deposition in the brain [15, 17, 18]. The heterogeneous pathology of AD arises from the variability in nature and severity of vascular lesions. Additionally, Aβ co-exists with cerebrovascular diseases such as cerebrovascular arteriosclerosis (CVA) [12]. Significantly higher densities of Aβ immunoreactive plaques being present in AD+CVA as compared to AD alone is an example of this co-existence [12].

The Aβ deposits in senile plaques (SP) and cerebrovascular angiopathy are derived from Aß precursor proteins (PP) expressed in neurons and in a variety of non-neuronal cells (outside the central nervous system) [19-23]. Perivascular Aβ deposition may be a risk factor for reduced regional CBF [24]. The age-related loss of mechanisms/cells that are capable of removing Aβ deposits involve subtle molecular alterations in the components that bind Aβ and protect the basement membrane from cellular degradation [25]. The activation of non-neuronal cells, including microglia, further contributes to neuronal damage [26]. Factors that may ameliorate AD either improve CBF or prevent CBF decline [24]. Ultrastructural studies revealing widespread penetration of Aβ deposits by degenerating microvessels reflect direct relationship between vascular changes in brain and the AD pathology [3, 27].

According to numerous morphometric studies endothelial cells (EC) contact with the vast majority of SP occurs randomly. It is clear that a certain subpopulation of senile plaque (SP) show an intimate relationship with the vasculature [28, 29]. It is likely that SP has more than one origin [30], and that vessels are one of their direct targets. In over 90% of AD cases, Aβ can be detected in at least some vessels [31]. The source of this Aβ is likely vascular EC and smooth muscle cells (SMC) rather than neurons, since EC and SMC show abundant amyloid beta precursor protein (AβPP) immunoreactivity [14, 29, 32, 33]. Ultrastructural studies on blood vessels with Aβ deposits indicate their intermittent associations with membrane abnormalities of SMC [30]. In AD cases with a clinical history of cerebral bleeding, Aβ deposits completely replace the muscle layers [29, 32, 33]. These findings suggest that alterations in the vascular cell wall, such as EC damage and muscle cell atrophy, may occur in AD. These alterations may occur in the absence of visible Aβ depositions thus indicating the vascular system may be a primary target for the development of this disease.

Ultrastructural features of brain microvessels from AD brain biopsy indicate that EC and perivascular cell from non-damaged vessels shows absence pathological change in their ultrastructure. Mitochondria in EC are intact (Figure 1-A). Contrary to this observation, microvessels with severe damage such as the presence of clusters of mitochondria derived lysosomes and necrotic changes in the ultrastructure of the perivascular cells appeared to be permanent features of this vessel (Figure 1-B). Very often, the capillary endothelium shows the presence of giant sized lipid vacuoles in their matrix. Mostly mitochondria are already transformed to mitochondria-derived lysosomes (Figure 1-C). In addition, non-reversible damage of the endothelium occupied only the small part of the vessel wall. Perivascular cells show the presence of large sized mitochondria-derived vacuoles which coexist with the presence of lipid laden vacuolar degenerative structure (Figure 1-D). Addressing factors that damage the vascular system would be suitable steps in the development of treatment strategies for this devastating disease.

ET-1 Role as a Vasodilator in AD Brains

ET-1 appears to be a vasodilator at physiologically relevant concentrations, and a potent vasoconstrictor in several pathologies associated with a rise in ET-1 plasma and tissue levels [34-38]**.** Several immunoreactivity studies identify augmented ET-1 levels in human atherosclerotic vessel wall cells [5, 35-37, 39], post-ischemic vascular lesions [34]**,** aged transgenic mice [40]. Augmented levels are also identified in diseases such as metastatic

adenocarcinoma of the prostate [41], human colorectal liver metastases [42, 43]**,** and thoracic EC from human and animal models of atherosclerotic vessels; whose expression increase ET-1 production [5, 35, 37, 39]**.** This increase correlates inversely with the depression of eNOS immunoreactivity [5, 40, 42, 44, 45]**.** An imbalance between endothelium-derived vasorelaxant and vasoconstrictor substances may play a key role in the development of chronic brain hypoxia and in the adaptive response of the brain to oxidative stress in ischemia and AD [40].

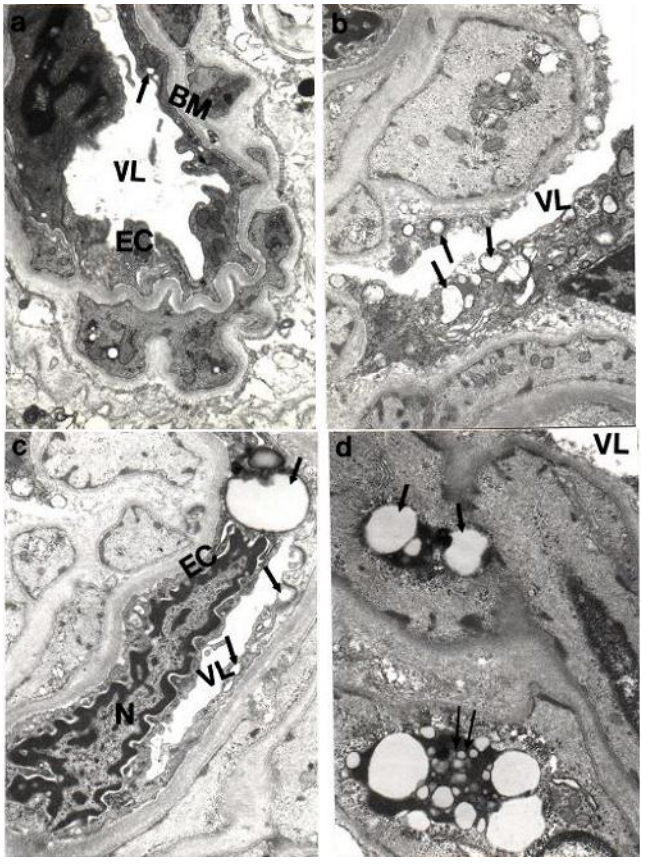

Figure 1. Ultrastructural features of brain microvessels from AD brain biopsy. A-Endothelial cells (EC) and perivascular cell from non-damaged vessels show absence pathological change in their ultrastructure. Mitochondria in EC are intact. Magnification: 8,300. B- Microvessels with severe damage such as the presence of clusters of mitochondria derived lysosomes (single arrow) and necrotic changes in the ultrastructure of the perivascular cells appeared to be permanent features of this vessel. Magnification: 10,000. C- Capillary endothelium shows the presence of giant sized lipid vacuoles in their matrix. Mostly mitochondria already transformed to mitochondria-derived lysosomes (single arrow). Magnification: 10,000. D- Non-reversible damage of the endothelium occupied only the small part of the vessel wall. Perivascular cells show the presence of large sized mitochondria-derived vacuoles (single arrow) which coexist with the presence of lipid laden vacuolar degenerative structure (indicate by double arrow). Magnification: 10,000. Abbreviations: BM-basal membrane; EC-endothelial cell; N- cell nucleus; VL-vessel lumen. Reprinted from [30] with permission.

Transgenic Animals as Models of Study for Cerebrovascular and Neuronal Lesions in AD

Developing an animal model is crucial for investigating the molecular and cellular etiology of AD [19-21, 40, 46-49]. There are a number of transgenic (Tg) animals that overexpress normal AβPP or AβPP with familial AD (FAD) mutations or fragments of AßPP [19-21, 46-49]. Heterogeneous genetic and environmental factors cause clinical and neuropathological phenotypes of AD. Several identified genes appear to be responsible for most familial forms of the disease. Conversely, the ε4 allele of ApoE is a significant risk factor for late onset forms of AD [10, 14, 19-21, 46-50]. Impairment of spatial memory in mice overexpressing wild type AβPP751 or wild type AβPP 695 and the neuropathology in mice expressing Aβ1-42 are documented [51]. Expression of AβPP in FAD mutant mice results in deposition of Aβ, while mice expressing the carboxyl terminus 100 or 104 amino acids of AβPP demonstrate both neurodegeneration and specific impairment of spatial memory [52].

Calhoun et al. [53] link the formation of amyloid beta (Aß) plaques leading to region-specific loss of neurons in a Tg mouse. In addition, mice overexpressing the human mutant APP (hAPP) show learning deficits, but the apparent lack of a relationship between these deficits and the progressive Aß plaque formation that the hAPP mice display is puzzling [54, 55]. Using a new watermaze training protocol, that PDAPP mice also exhibit a separate age-related deficit in learning a series of spatial locations [54]**.**

This learning impairment correlates with Aß plaque burden and is evident in both cross-sectional and longitudinal experimental designs. These findings indicate that Aß overexpression and/or Aß plaques parallel with the disturbed cognitive function. They also suggest that some but not all forms of learning and memory are suitable behavioral assays of the progressive cognitive deficits associated with AD-type pathologies [54]**.** Later studies demonstrate that AßPP expression also occurs in different pathological conditions such as after the global ischemia, even without the presence of a genetic abnormality [56]. This finding indicates a central and crucial role of the chronic injury stimuli (e.g. ischemia, hypoxia, virus, toxins, etc.) in the pathogenesis of AD**.**

Cerebral amyloid (CA), thought to be produced in the lysosomes of EC [49], was first proposed as the cause of BBB breakdown, allowing neurotoxic serum proteins access to neuronal cells and beginning the cascade of neurodegeneration. We demonstrate that the C57B6/SJL Tg mouse model, which overexpresses AβPP [57] with FAD mutations, contains an Aβ deposition patterns similar to those seen in cases of AD. In addition, the C57B6/SJL Tg mouse possesses a beta fibroblast growth factor (bFGF) binding pattern similar to that seen in AD. Immunohistological assays from respective mice tissues reveal that the cores of amyloid plaques exhibit intense staining for the antibody against Aβ (4G8). Additionally, bFGF binding was greatly diminished by heparinase pretreatment [58, 59].

Brain Aβ deposition is associated with normal aging and with physiological changes underlying age-related brain Aβ accumulation [60]. In a clinical study, aged AβPP Tg mice exhibiting memory deficits were analyzed. The mice were treated with the antioxidants N-acetyl-L-cysteine and Tempol to recover cerebrovascular function, and Pioglitazone to normalize impaired CBF. It was shown that the compounds were able to restore the

cerebrovascular reactivity of isolated cerebral arteries concurrently with changes in proteins regulating oxidative stress without reducing brain Aβ levels and/or Aβ plaque load [61].

Vascular aging is associated with both structural and functional changes that can take place at the level of the endothelium, the vascular SMC, and the extracellular matrix of blood vessels [62, 63]. In the endothelium, reduced vasodilatation in response to agonists occurs in large conduit arteries and in resistance arteries as a result of aging [64]. Furthermore, enhanced oxidative stress by hypoperfusion contributes significantly to the deleterious effects of aging on the endothelium by means of NO breakdown caused by ROS. The relative contribution of the above phenomenon to age-related endothelial dysfunction is highly dependent on the species and the type of vascular bed involved [5, 6, 64, 65].

Mitochondria Abnormalities as an Initiator for AD

Ultrastructural characteristics of neuronal mitochondria damage from AD brain biopsy shows the neurons with different degrees of ultrastructural damage (Figure 2A-D). In the neuronal cell body, partially and completely damaged mitochondria co-exist with lipofuscin formation and mitochondria derived vacuolar structures which appear to be a major substrate for their formation (Figures 2A, B and D). Clusters of electron dense (ED) *"hypoxic"* mitochondria were seen throughout neuronal cell body and characterized by the abnormal mitochondrial cristae (Figure 2-C). We furthered studied ApoE4 [66], whose purpose is essential for the normal catabolism of triglyceride-rich lipoprotein constituents, but due to its isoform it was found to be dysfunctional. There has been an increasing amount of evidence relating AD with this cardiovascular protein. ApoE4 Tg mice were associated with cerebral hypoperfusion and their brains analyzed by transmission electron microscopy (TEM). It was shown that the structural damage of cerebral microvessels (Figure 3A-D) extended to the cytoplasm of the perivascular cells and hippocampal neurons [66]. These abnormalities were correlated with mitochondrial structural alterations such as mtDNA mutations and mitochondrial enlargement (Figure 4A-D). When these mice were given mitochondrial antioxidants such as acetyl-L-Carnitine (ALCAR) and R-alpha-Lipoic acid (LA), their spatial and temporal memory was improved [66, 91]. Ultrastructural feature of age-associated neuronal mitochondrial change in ApoE4 mice characterizes the presence of a mitochondria-derived lysosomes association with lipofuscin appears to be the main feature of mitochondrial damage (Figure 4A-D).

Mitochondrial abnormalities such as ED mitochondria, mitochondrial-derived lysosomes, and lipofuscin appear to be features of damaged neurons in aged C57B6/SJL Tg (+) mice [67]. The main characteristic of cortical and hippocampal neuronal damage in C57B6/SJL Tg mouse brain appeared to be the transformation of completely damaged mitochondria (mitochondria without any residues of mitochondrial cristae) into the lipofuscin granules, characterized by their cluster type localization in the neuronal cell body (Figure 5A-D).

Pathological conditions can be adverted in perivascular and EC in non-damaged cells from AD brain (see Figure 1A). This indicates that the vascular abnormalities correspond with the selective damage of cortical neurons, raising questions about the relationships between vascular abnormalities, blood brain barrier (BBB) breakdown, neuronal loss and amyloid deposition during the maturation of human AD and AD-like pathology in this Tg

mice [14, 67]. However, no serum amyloid protein (SAP) immunoreactivity is found in the Tg mouse brain [59]. Since only peripheral organs synthesize SAP, its presence in the AD brain suggests impairment of the BBB [68]. These results suggest that the pathogenesis of BBB impairment in this mouse model differ from that in AD [59].

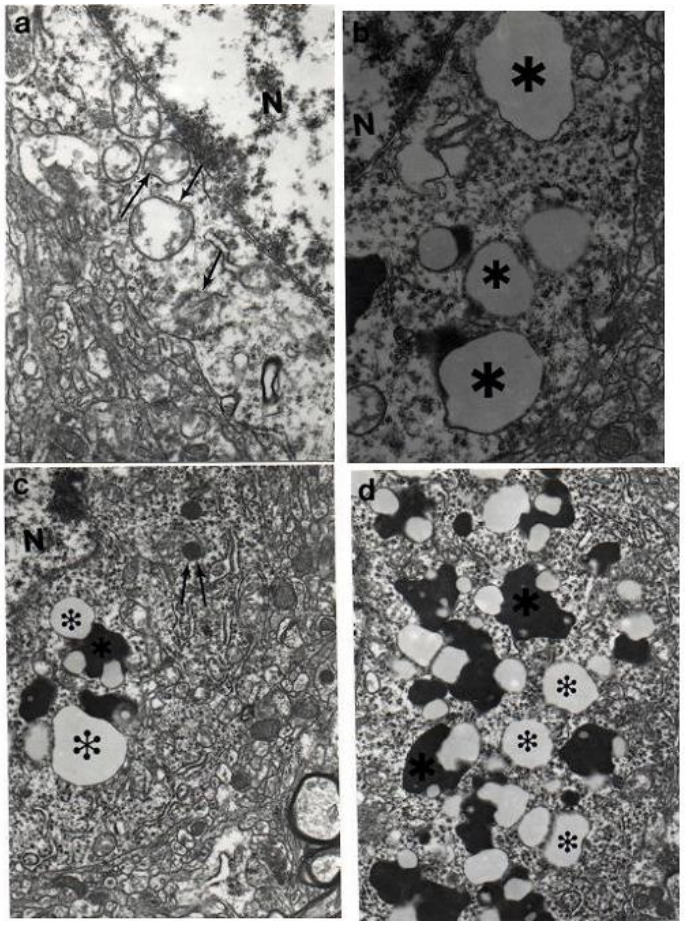

Figure 2. Ultrastructural characteristics of neuronal mitochondria damage from AD brain biopsy. Neurons with different degrees of ultrastructural damage. In the neuronal cell body, partially and completely damaged mitochondria (indicated by single arrows and asterisk respectively) coexist with lipofuscin formation and mitochondria derived vacuolar structures which appear to be a major substrate for their formation. Clusters of electron dense "hypoxic" mitochondria were seen throughout neuronal cell body and characterized by the abnormal mitochondrial cristae (indicates by double arrow in figure c). Magnification: a and b X 16,000 respectively. c and d X 10,000 respectively. Abbreviations: N-neuronal cell nucleus. Reprinted from [30] with permission.

Recently, a yeast artificial chromosome (YAC) Tg mouse model that overexpresses Aβ was developed [19-21, 46-48, 68]. The YACs contain the entire ~400kbp human gene encoding AβPP. The gene harbors one of the following: a) asparagine for lysine and leucine for methionine FAD substitution at the 670/671 codons ($APP_{K670N/M671L}$), b) isoleucine for valine FAD substitution at 717 codon (APP_{V7171}), or c) both substitutions combined [19-21, 46-48, 68]. Lowered levels of α-secretase-generated soluble AβPP derivatives are observed in

these mice [19-21, 46, 47]. Moreover, there are elevated levels of extended Aβ peptides (species terminating at amino acids 42/43) in YAC Tg mice that express human $A\beta PP_{V7171}$, suggesting that these mice should be appropriate for detailed analysis on the *in vivo* effects of AβPP metabolism and Aβ production. YAC Tg approaches avoid problems regarding regional and temporal specificity of molecular pathogenesis and may therefore, provide unique insights into the mechanisms behind the progression of AD in humans [14].

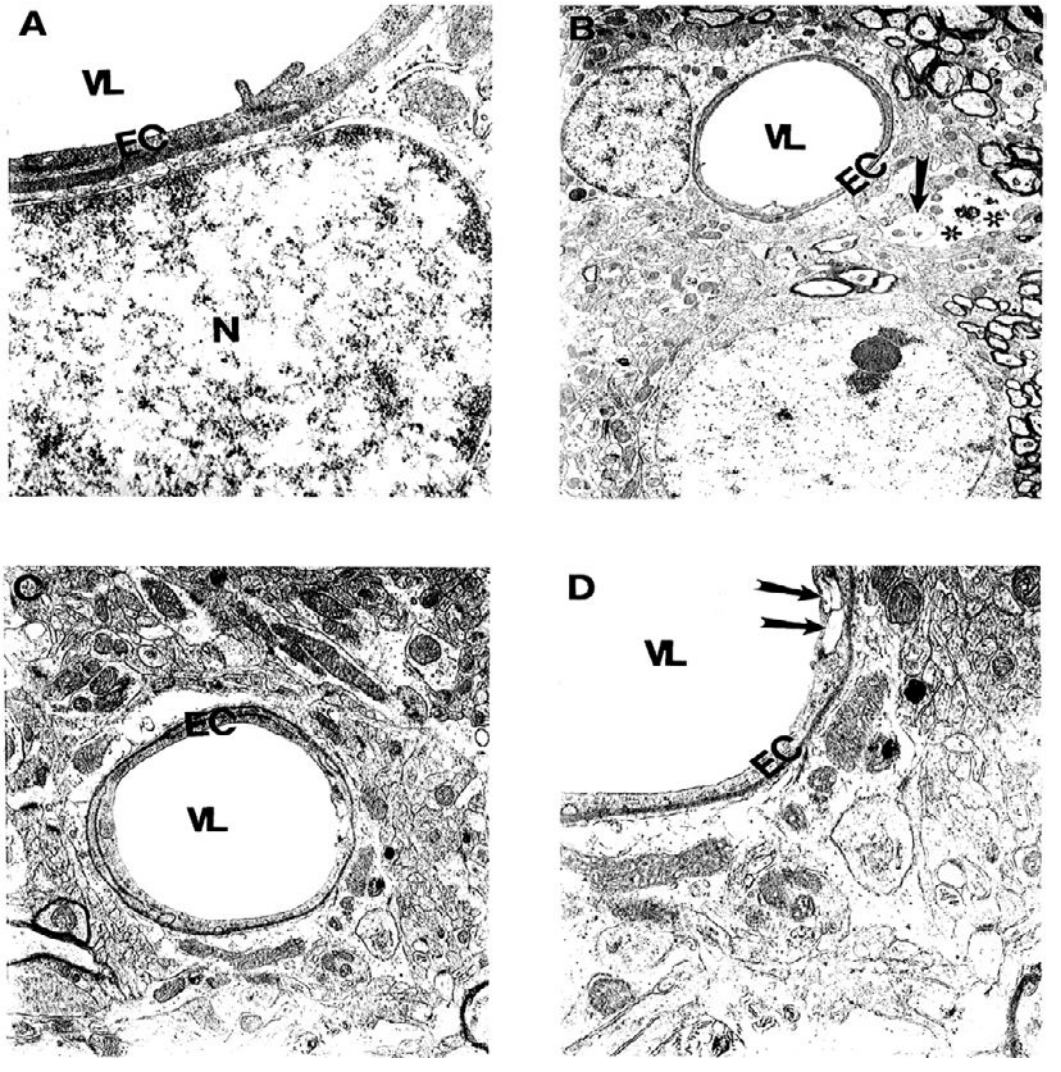

Figure 3. Microvessels from young (A-B) and aged (C-D) ApoE 4 mice show the stress reaction of vascular endothelium. Destruction was also seen in the matrix of perivascular nerve terminals (arrow) and perivascular cells (indicated by double asterisk). Magnification: A) 25,000; B) 5,000; C) 10,000; D) 20,000. Abbreviations: EC- endothelial cell; N- cell nucleus; VL-vessel lumen. Reprinted from [30] with permission.

The changes of the vascular wall in YAC AβPP mice, but not age-matched controls, reveal a different degree of amyloid deposition present in vascular wall cells [14]. These vessels also exhibit immunopositive staining for AβPP and are characterized by the presence of a large number of lipid-laden vacuoles in the matrix of endothelial and perivascular cells [14, 58]. Mitochondria observed a transformed of their mitochondria into lysosomes. Neuronal cell bodies of parietal cortical neurons from aged YAC AβPP mice include clusters of AβPP-containing immunopositive gold particles [58]. Aß deposits around the microvessels are commonly present within the ultrastructural abnormalities in vascular wall cells and neurons [14, 58]. This data indicates that disruption of BBB function in vascular EC may be a major factor in lipid accumulation and amyloid deposition during the development of AD-like pathology in YAC AβPP mice [14].

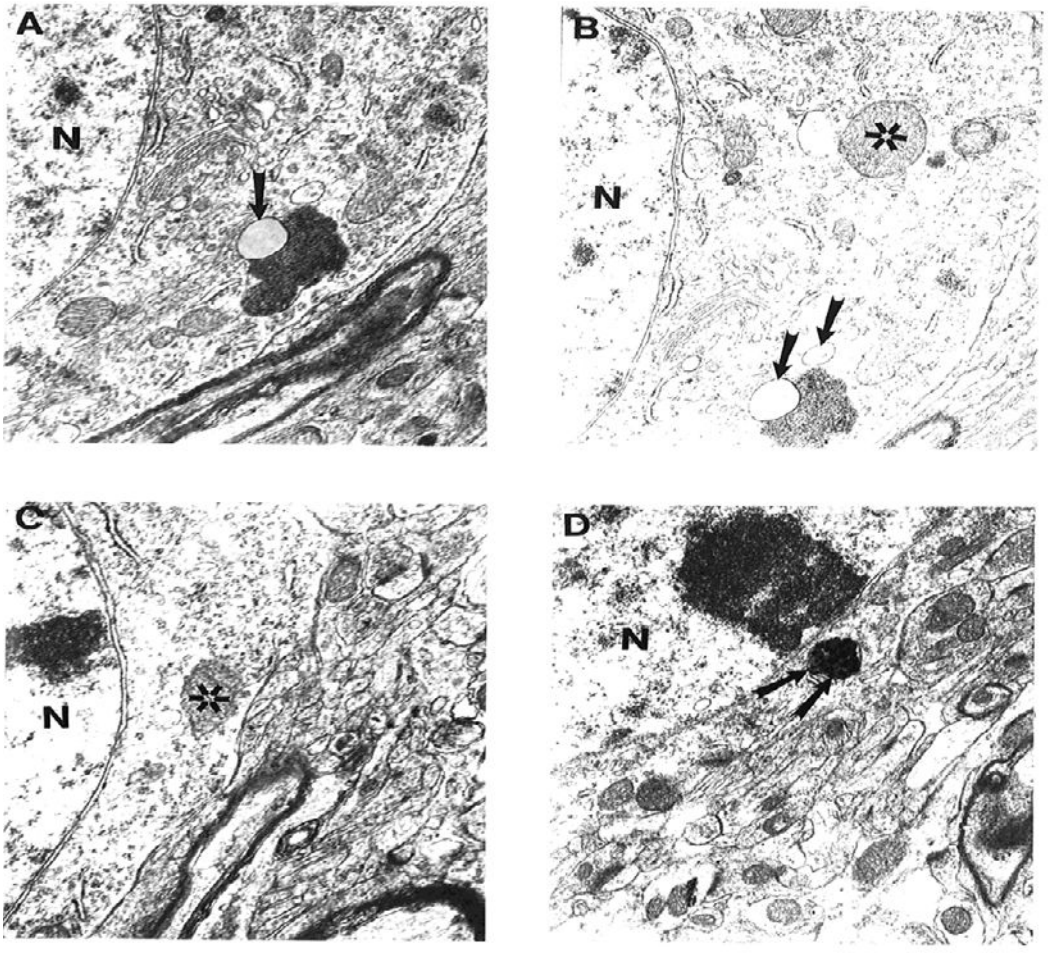

Figure 4. Ultrastructural feature of age-associated neuronal mitochondrial change in ApoE4 mice. Mitochondria-derived lysosomes association with lipofuscin appears to be the main feature of mitochondrial damage. Asterisk: normal mitochondria. Arrowhead: mitochondria-derived lysosomes. Double Arrowhead: *"hypoxic"* mitochondria. Magnification: A and B) 20,000; C) 25,000; D) 15,000. Abbreviation: N- neuronal cell nucleus. Reprinted from [30] with permission.

Furthermore, damage to the vascular endothelium induced by chronic hypoperfusion acts as a primary key factor for oxidative stress and contributes to potential neuronal lesions. Non-reversible changes in neurons that induce AβPP overexpression and Aβ depositions are also consequences and permanent features of AD [14, 39, 58]. These findings raise questions regarding the direct relationship between vascular abnormalities, BBB breakdown, neuronal loss, mitochondrial lesions and Aβ deposition during the maturation of AD-like pathology [14, 39, 58]. Therefore, cellular and subcellular investigations into both the mechanisms behind Aβ deposition development and the possible accelerating effects of environmental factors (such as chronic hypoxia/reperfusion) may open the door to new pharmacological treatments of AD; especially in the avenue of antioxidant drug research.

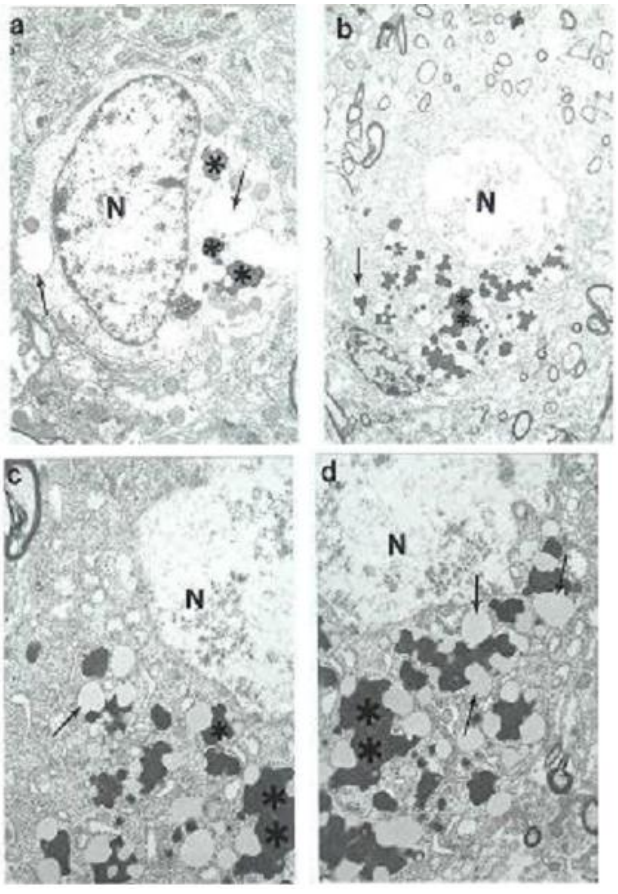

Figure 5. Ultrastructural characteristics of cortical neurons in C57B6/SJL transgenic (Tg +) mouse brain. The main characteristic of neuronal damage appeared to be the transformation of completely damaged mitochondria (mitochondria without any residues of mitochondrial cristae; indicated by single arrows) into the lipofuscin granules, characterized by their cluster type localization in the neuronal cell body. Original magnification: A and B X 5,000; C and D X 20,000, respectively. Abbreviation: N-neuronal cell nucleus. Reprinted from [30] with permission.

Tg Mouse Model as a Tool for Comparing the Mechanisms Involved in AD Pathology

Recently we applied the C57B6/SJL Tg mouse model overexpressing Aβ to assess the binding of bFGF and SAP to Aβ as a measure of BBB integrity. Adjacent sections of brain were stained with 4G8, a monoclonal antibody to amyloid, with bFGF binding followed by 48.1, a monoclonal antibody against bFGF. The binding of bFGF in this model is similar to that of AD cases in which bFGF binds specifically to Aβ neuritic plaques and the basement membrane (BM) of cerebral microvessels [59]. In addition, the cores of amyloid plaques are intensely stained with the 4G8, and bFGF binding is colocalized with Aβ immunoreactivity as visualized by polyclonal antiserum to amyloid.

Our ultrastructural study indicates that bFGF immunostaining in aged C57B6/SJL mice appear to be that of immunopositive peroxidase-anti-peroxidase (PAP) products or gold particles that bind with damaged, but not normal neurons [14, 67]. Moreover, this corresponds with the different degree of Aβ immunostaining in the neuronal cell body and vascular wall cells [14]. In addition, we have found the degree of mitochondrial abnormality, such as electron dense (ED), mitochondria-derived lysosomes and lipofuscin. Together, they appear to be features of damaged neurons in aged (24 m old) C57B6/SJL Tg (+) but not age-matched control mice neuronal damage appeared to be the transformation of completely damaged mitochondria (mitochondria without any residues of mitochondrial cristae) into the lipofuscin granules, characterized by their cluster type localization in the neuronal cell body (Figure 5A-D). Consequently the selective damage of cortical neurons manifests the degree of vascular abnormality. The direct relationships between vascular abnormality, BBB breakdown, neuronal loss and amyloid depositions during the maturation of AD like pathology remain fully elucidated [14].

The ultrastructural features of vascular lesions and mitochondrial changes in neuronal cell bodies in aged YAC AßPP and non-Tg age-matched control mice are analyzed following perfusion fixation [69]. EM immunocytochemistry, using a monoclonal antibody, revealed different sizes of fibrils and extracellular types of amyloid deposits of brain tissues in YAC AßPP Tg mice [14, 67]. In addition, the amyloid depositions support the formation of parietal helical filamental (PHF) structures, which is a permanent feature of neuronal lesions in AD brains [67]. These vessels also manifest immunopositive staining for AβPP and the presence of a large size of lipid-laden vacuoles in the matrix of EC and perivascular cells [14]. These changes reflect those similar to cortical microvessels in AD [14]. The ultrastructural abnormalities of vascular wall cells depend on the presence of Aß deposits around the microvessels [14]. In contrary to these observations, brain vessels of age-matched control mice did not show any particular changes in the ultrastructure of vascular EC at different levels of microcirculation. A *"minute"* amount of lipid droplets appear in the matrix of perivascular cells [14]. These data clearly indicate that disruption of BBB function in vascular EC may be major factor in lipid accumulation and amyloid deposition during the development of AD-like pathology in YAC AßPP Tg (+) mice without cholesterol feeding [14, 58].

Different degrees of ultrastructural alterations in mitochondrial structures characterize the similar cortical neuronal cell bodies in YAC AßPP mice as AD [16, 67]. *Giant* and ED mitochondria are permanent features of the neuronal abnormality as a result of AβPP

overexpression [67]. We also observe the similarity in AD brains during different stages of mitochondrial lesions (see Figure 2 and 7). The cytoskeleton appears to correlate with the absence of microtubule and lipofuscin formation [70]. Conversely, the neuronal cell body from partially and completely damaged mitochondria would coexist with lipofuscin formation (Figure 2). Age-matched human and control mice did not show any particular changes in their neuronal ultrastructure [67]. *In situ* hybridization analysis with mouse and human mtDNA probes detected and abundance of deleted mtDNA in human AD and YAC AßPP compared to age-matched controls [14, 67] (Figure 6). The majority of mtDNA deletion localizes in mitochondria-derived lysosomes (Figure 6 A-D). These regions correspond to lipofuscin deposits, thereby suggesting that proliferation, deletion and duplication of mtDNA occurs in mitochondria (Figure 6C-D). Many of these mitochondria fuse with lysosomes, in human AD and in YAC AßPP mice [67].

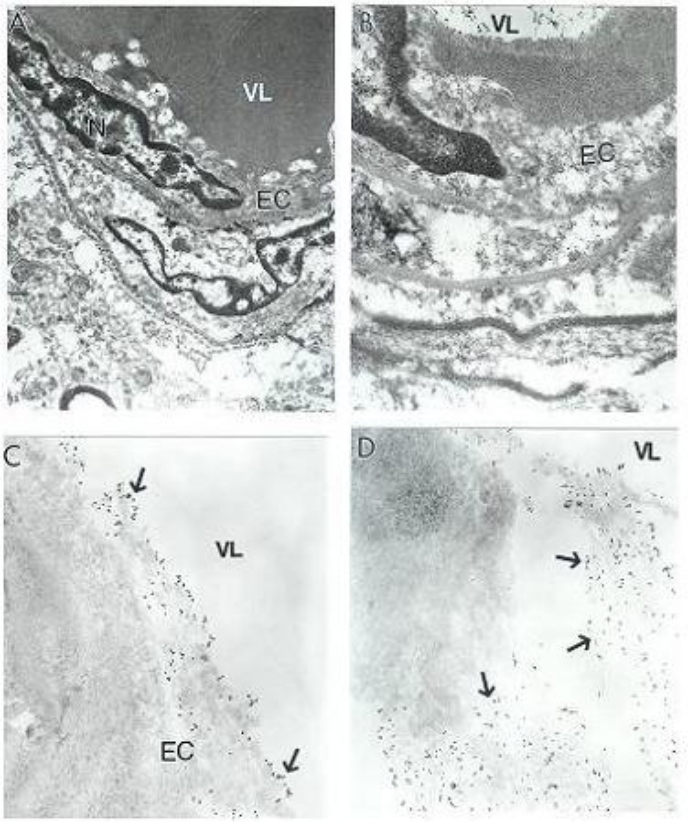

Figure 6. Electron microscopic determination of mtDNA signals visualized by using wild type and chimeric 5 kb deleted mtDNA probes in a human postmortem AD brain (A–B) and 24-month old AβPP-YAC transgenic mouse brain (C–D). A and B: AD brain microvessels endothelium and perivascular cells show clusters of wild type mtDNA containing positive signals visualized by indirect colloidal gold techniques (indicated by the dark dots). Original magnification: A and B X 10,000 and X 25,000, respectively. C–D: AβPP-YAC transgenic mice show the presence of clusters of chimeric 5 kb deleted mtDNA positive signals throughout the matrices of vascular and perivascular cells (single arrows). Original magnification: C and D X 30,000 and X20, 000, respectively. Abbreviations: BM-basal membrane; EC- endothelial cell; N- cell nucleus; VL- vessel lumen. Reprinted from [30] with permission.

Neurons in samples from AD are significantly dominated by abnormal mitochondria as compared to the control group [71] (Figure 7). *In situ* hybridization analyses, with a chimeric cDNA probe to the 5kb common deletion [71], showed deleted mtDNA increased at least 3 fold for the AD cases as compared to the controls. Quantitative analysis of the mtDNA deletion and 8OHG, in the same cases, showed a strong positive correlation (r = 0.934; Figure 8). Ultrastructural localization of mtDNA using *in situ* hybridization with colloidal gold manifests the location of deleted mtDNA within abnormal mitochondria (those lacking cristae, swollen, and in many cases, fused with lipofuscin) [71]. These findings suggest that the mtDNA *in situ* hybridization detected mtDNA proliferation, deletion and duplication in abnormal mitochondria, many of which fused with lysosomes, thus indicating turn over of such mitochondria.

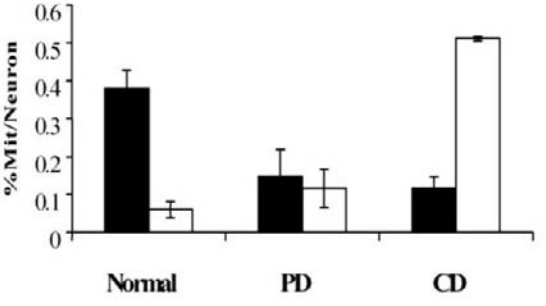

Figure 7. The percentage of different types of mitochondria with intact cristae (normal) and with partial (PD) or complete (CD) cristae destruction in brain biopsy samples from control (n = 6) and AD (n = 8) patients. Reprinted from [30] with permission.

We speculate that the oxidative stress markers seen in the AD brain selectively affect the population of vulnerable neurons, vascular EC and perivascular cells. In this regard, the microvessels observed destruction in their endothelium and perivascular cells in young and aged ApoE 4, C57B6/SJL Tg (+) and YAC AβPP mice when affected by chronic oxidative stress. These observations suggest that hypoperfusion-induced oxidative stress plays a key role in the pathogenesis of vascular and non-vascular cell lesions during the maturation of AD. In fact, age-associated neuronal mitochondrial changes in ApoE4 as well as in C57B6/SJL Tg (+) and YAC AβPP mice revealed mitochondria-derived lysosomes were associated with lipofuscin during damage (see, Figure 4). The detailed analysis of 8-OHG immunostaining demonstrated that only vulnerable neurons elicit immunopositive staining for 8-OHG in AD, but not in age-matched controls (see, Figure 8). By using ultrastructural analysis we discover that 8-OHG immunostaining is selectively present in vulnerable neurons and microvessels of the AD brain [14, 71]. The 8-OHG immunogold labeling (17 nm) is seen throughout the cytoplasm, including the damaged mitochondria or ED abnormal mitochondria [14, 71]. However, we did not find 8-OHG in normal mitochondria or in lipofuscin. The capillary EC and perivascular pericytes show the high intensity of 8OHG immunostaining [14].

Detailed immunocytochemical analyses using colloidal gold probes indicate that the vascular wall in YAC AßPP Tg mice possesses atherosclerotic lesions, while control and non-damaged vessels from YAC AßPP mice do not show AβPP immunopositivity [14]. Very often the clusters of AβPP positive immunoreactivity were observed in the neuronal cell bodies of parietal cortical neurons from aged YAC AßPP Tg mice [14, 67]. *In situ*

hybridization using wild and deleted mtDNA probes (human and mice specific) reveal mtDNA containing gold particles in YAC AßPP, but not in control mouse hippocampus (Figure 6) [67.] The main source of the mtDNA probes is located in damaged mitochondria and mitochondria-derived lysosomes, but not in lipofuscin (Figure 6 C-D). We have found that wild and chimeric mtDNA were also detectable in YAC AßPP Tg (+) mouse microvessels but not in control age-matched brain tissue [14]. In addition, vessels with atherosclerotic lesions show that endothelium and perivascular cells contain clusters of wild and deleted mtDNA containing positive signals [14]. These observations suggest that the key role of hypoperfusion, mitochondrial abnormality and oxidative stress in the pathogenesis of vascular and non-vascular cells lesions during the development of AD-like pathology in YAC AßPP mice [14] and at the many point overlap with the neuropathology of human AD.

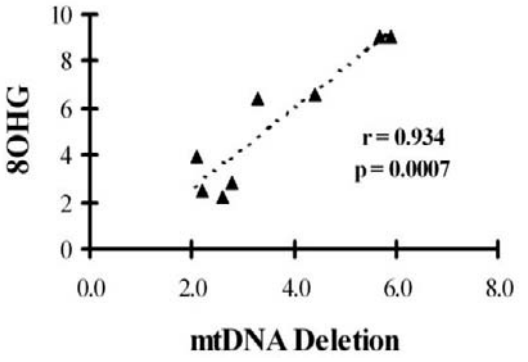

Figure 8. The extent of oxidative damage (8OHG) in AD brain is highly dependent on the degree of mitochondrial abnormalities, in arbitrary units. Reprinted from [30] with permission.

Antioxidant Therapies as a Alternate Treatments for AD

AD treatment has yet to yield a successful therapy that addresses the source of damage found in AD brains [72]. Of the various theories proposed for AD etiology, ROS generation is cited as a common factor. Efforts to reduce the pathology associated with ROS via antioxidants seem to offer new hope to patients suffering from this devastating disease [72].

Mitochondria have been considered a primary objective in the search for age-related cognitively impaired conditions to restore cognitive function including treatments for dementia [63, 66, 72-78]. Microvessels from young and aged ApoE 4 mice show the stress reaction of vascular endothelium (Figures 3A-B and 3C-D, respectively). Destruction was also seen in the matrix of perivascular nerve terminals and perivascular cells (Figure 3B). This is because the brain, characterized by high energy metabolism and an abundance of oxidizable materials (such as polyunsaturated fatty acids and neuropeptides), is exceedingly susceptible to oxidative damage [79]. Oxidative damage is known to cause mitochondrial dysfunction [66]. The association of mitochondrial activity with the antioxidant capacity of certain micronutrients such as LA, a coenzyme essential for the maintenance of energy homeostasis in mitochondria, has been shown to influence cognitive function in a different animal species [63, 66, 73-78].

Previous studies have demonstrated the potential protective effects of selective mitochondrial antioxidant treatments on brain mitochondria from aged rats [63, 73-75, 77, 80]. When aged rats were treated with selective mitochondrial antioxidants (ALCAR+LA), oxidative stress (OS) was reduced, cognitive function was restored, and mitochondrial structural abnormalities were reduced in parenchymal cells [63, 76, 80, 81]. In addition, to study mitochondrial decay and oxidative damage resulting from aging, an examination into the activities and kinetics of the mitochondrial complexes (a hallmark of the mitochondrial ability to produce energy) was performed. Results show that mitochondrial complexes can be restored by selective mitochondrial antioxidant treatment [81]. This established that in the brain mitochondria of old rats, when compared with that of young rats, there were significantly decreased endogenous antioxidants and less superoxide dismutase activity; more oxidative damage to lipids and proteins; and decreased activities of the mitochondrial complex I, IV and V [81]. Moreover, the mitochondrial complex I showed a decrease in binding affinity (increase in K(m)) for substrates. Feeding ALCAR+LA to old rats partially restored age-associated mitochondrial dysfunction compared to non-treated old rats. These results indicate that oxidative mitochondrial decay plays an important role in brain aging and that a combination of nutrients targeting mitochondria, such as ALCAR+LA, could ameliorate mitochondrial decay through preventing mitochondrial oxidative damage [81]. In a recent study [63] we were able to demonstrate that the integrity of mitochondrial ultrastructure, which is dependent on aging, could be improvement in old rat brain mitochondria when compared to the control group by using ALCAR+LA antioxidant treatments [63]. In contrast, neurons obtained from aged control groups showed a series of mitochondrial abnormalities, such as the presence of giant mitochondria and mitochondria with partially or completely damaged cristae. Targeting mitochondrial OS improved the overall cognitive ability of aged rats [73-75, 77, 80, 82] and aged dogs [78]. In this regard, the percentage of control and AD patient's mitochondria per neuron cristae revealed that their complete destruction in AD patients compared to the control, whereas normal and partial mitochondria per neuron percentage had an inverse relation (Figure 8).

Reid and colleagues [83] noted that there is epidemiological evidence that links vascular diseases, such as hypercholesterolemia, with an increased incidence of AD. While no theory has yielded a satisfactory explanation for the pathological changes that lead to neurodegeneration and cognitive dysfunction [83], vascular risk factors seem to offer the most interesting results [84, 85]. The relationship between hypercholesterolemia and AD arose in great extent from ApoE4, a known risk factor for AD and a major carrier of cholesterol in the CNS. The detrimental processes of ApoE4 have been shown to influence AD pathological processes, including lipid homeostasis and neurofibrillary tangles (NFT) formation [86], which suggests that brain vascular alternations play a key role in the progression of AD [83]. ApoE4 mechanisms that contribute to the neurodegeneration of the brain could offer strong insights into AD susceptibility. For example, it was shown in a rat model that ApoE levels would increase as a response to peripheral nerve injury [86], implicating the role of ApoE as a repair mechanism. If the delivery of lipophilic antioxidants is impaired due to ApoE4, this could lead to OS [87]. It has been proven that ALCAR can improve memory deficits in animal models of AD and reduce cognitive deficit in AD patients [79]. The contribution of fatty acids in these cerebrovascular processes, and their effect on AD pathogenesis is still uncertain, but the suggestion that they can elicit neuronal over-excitation and synaptic depression as contributor factors to AD is suggested [88].

We demonstrated the therapeutic effects of mitochondrial antioxidants (ALCAR+LA) as a treatment model for AD on ApoE4 transgenic mice [66]. The decrease in cerebrovascular oxygen levels seen in AD patients led to the hypothesis that hypoperfusion in the CBF, which over time causes OS and mitochondrial damage, was the main cause of ApoE-related cognitive deficits was seen in AD patients with ApoE4 overexpression [62, 89, 90]. Our study demonstrated for the first time, that ApoE4 caused brain hypoperfusion by gradually reducing CBF when compared to a control group [91]. Structural damage of vascular wall cells, especially in mitochondria, seems to play a key role in the generation of ROS, resulting in oxidative damage to the neuron and inducing pathological factors associated with AD [66]. Therefore, we believe that expanding the focus of study in AD towards mitochondrial pathobiology as a method of treatment will create new and more effective treatment strategies for this devastating disease [63, 66, 85, 92-94].

Conclusion

Based on published literature and evidence presented in this review, we propose that the hypothetical time line of damage in neuronal structure and relationships to lipid peroxidation, nitration, and oxidative stress during the maturation of AD is a very complex dynamic of physiological pathways and functions. With the onset of AD, normal neurons develop numerous forms of oxidative damage that include nitration (nitrotyrosine) and lipid peroxide adducts (lipid peroxidation). Nucleic acid oxidation also occurs prior to the formation of pre-NFT, which induces non-reversible damage to the neurons. Consequently, failure of neurotransmission occurs as a final outcome in the AD brain.

We theorize that the evidence garnered from this review will direct future investigations and provide us with a clearer understanding of the relationship between a number of age-related disorders; atherosclerosis, ischemia/reperfusion, stroke, and neurodegenerative diseases such as AD, to name a few. In order to have a better understanding of how antioxidants therapy play a role in dementia, it is necessary to study the major factors altering and/or controlling CBF during accumulation of chronic hypoperfusion and/or the development of atherosclerotic changes in brain microvessels. This is especially important because the roles of vasoactive substances (namely NO and ET-1) during the development of these changes are not well understood and arise the question *"does chronic hypoperfusion with concomitant oxidative stress accelerate the vascular and neuronal lesions (including the mtDNA deletions) during normal aging and/or when the brain is exposed to chronic hypoxia?"* Resolving these issues will allow for novel therapeutic approaches that will modify the natural history of these chronic age onset disorders.

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Chapter V

What are the Roles of Vitamins in Vascular Dementia?

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Abstract

Several vitamin deficiencies have been repeatedly reported to be associated with cognitive impairment, though there is not a consensus about all of these associations. Some vitamins, like vitamin A, vitamin C and vitamin E were investigated for their prevention from neuronal death as being antioxidant agents. Deficiencies of several B vitamins have been reported to be associated with cognitive dysfunction in many studies. More recently, deficiencies of folate (vitamin B9) and cobalamine (vitamin B12) have been studied in relation to their causing to hyperhomocysteinemia. As homocysteine is a proatherogenic and protrombotic factor, it is natural the deficiencies of these vitamins to cause vascular events in brain and resultant vascular dementia. The most recent reported vitamins related to cognitive functioning are vitamin D and vitamin K. Vitamin D has vasculoprotective effects through various mechanisms and a vitamin K dependent receptor can protect neurons against apoptosis. Also, there is a possibility that vitamin K may decrease brain damage produced by cerebrovascular disease.

Most of the evidence about the association of vitamin deficiencies and vascular dementia is based on cross-sectional studies, which can not prove whether a nutrional deficit is the cause or the result of an impaired cognive status. In fact, cognitive impairment can also determine changes in dietary habits and and consequently cause vitamin deficiencies. In this section, the relations between vascular dementia and vitamin deficiencies have been discussed under the light of literature knowledge and potential of vitamin supplementation to prevent or treat vascular dementia has been tried to be evaluated. It has been realized that though there are promising, positive results about vitamin supplementation; well designed studies with larger number of participants are needed to clarify the subject.

Introduction

A vitamin is an organic compound required as a nutrient in tiny amounts by an organism. Vitamins are classified by their biological and chemical activity, not their structure. Thus each vitamin refers to a number of vitamer compounds that all show the biological activity associated with a particular vitamin. Such a set of chemicals are grouped under an alphabetized vitamin generic descriptor title, such as vitamin A, which includes the compounds retinal, retinol and four known carotenoids. Vitamers by definition are convertible to the active form of the vitamin in the body, and are sometimes inter-convertible to one another, as well (Wikipedia from internet).

The role of vitamins in vascular dementia should be discussed from many different aspects. The deficiencies of vitamins can cause or increase vascular disease or vascular dementia via different mechanisms. Cognitive impairment can also determine changes in dietary habits and consequently cause vitamin deficiencies. Most of the evidence about the association of vitamin deficiencies and vascular dementia is based on cross-sectional studies, which can not prove whether a nutrional deficit is the cause or the result of an impaired cognitive status.

Until the 1900s, vitamins were obtained solely through food intake, and changes in diet can alter the types and amounts of vitamins ingested. Vitamins have been produced as commodity chemicals and made widely available as inexpensive pills for several decades, allowing supplementation of dietary intake (Wikipedia from internet).

We will try to overview the roles of antioxidant vitamins, B vitamins and lastly vitamin D and K in vascular dementia in this section.

Antioxidant Vitamins

Pathophysiological Mechanisms

Brain tissue is particularly vulnerable to free-radical damage due to the high rate of oxygen consumption, subsequent damage to cells and low level of endogenous antioxidants (Reiter RJ, 1995). The link between oxidative stress, especially its long term effects, and cognitive impairment may be a direct result of selective neuronal damage as well as the indirect result of atherogenic factors (Parigi AD, 2006). In fact, antioxidants such as β-carotene (Jama JW , 1996), vitamin C (Gale CR, 1996) and vitamin E (Sano M, 1997) have been shown to be protective factors against both atherosclerosis and dementia. Additionally, it seems that antioxidant treatment improved neuronal function through maintaining mitochondrial homeostasis. In a canine model of human aging, it was shown that aged canine mitochondria showed significant increases in reactive oxygen species production and a reduction in NADH-linked respiration. Mitochondrial function was improved selectively in aged dogs treated with antioxidant diet (Head E, 2009). The vitamins also have other protective effects for the molecular integrity of tissues in addition to their antioxidant activity. Vitamin E may modulate signal transduction pathways and participate in the synthesis pathways of neurotransmitters (Azzi A, 1992; Martin A, 1997; Meydani SN, 1997).

Large geographical variations in vascular diseases clearly relate atherosclerosis of coronary and brain vessels to environmental factors. The impressive decline in stroke and cardiovascular disease (Biesalski HK, 1997) over the last three decades is in part related to active treatment of risk factors, but probably even more so to improved nutrition, particularly the intake of antioxidant micronutrients (Walter P, 2001). The striking 4-fold higher stroke mortality in subjects with simultaneously low plasma concentration of vitamin C and carotene (<quartile 1) demonstrates that concentrations already at plasma levels in the low normal range increase the risk for vascular diseases (Stahelin HB 1997).

Cross-Sectional Studies

Some epidemiological studies have indicated a relationship between blood concentrations of antioxidants and cognitive impairment. But it must be considered that some results are confounded by the fact that blood samples were not always drawn in fasting conditions. Goodwin et al (Goodwin JS, 1983) found a correlation between memory test scores and plasma levels of vitamin C (and other vitamins) in 60 years and older healthy individuals. The SENECA study reported a positive, although weak, correlation between plasma concentrations of lycopene, α-carotene, β-carotene, total carotenes, β-cryptoxanthin, α-tocopherol and Mini Mental State Examination (MMSE) scores (Haller J, 1996). In the elderly population studied by Ortega et al., dietary intake of vitamin C, β-carotene, and vitamin E were associated with a better cognitive function (Ortega RM, 2002). The Austrian Stroke Prevention Study reported a lower plasma concentration of α-tocopherol in individuals with a poor cognitive function as compared to control subjects (Schmidt R, 1998). In the multiethnic elderly sample of the Third National Health and Nutrition Examination Survey, low serum levels of vitamin E, but not of other oxidants, were associated with poor memory performence (Perkins AJ, 1999). In this study, blood concentrations were not measured after a standart period of fast, hence these results must be considered with caution. In the InCHIANTI study (Cherubini A, 2005), which was a population-based cohort study investigating whether vitamin E plasma levels were related to the presence of dementia and cognitive impairment, participants with plasma vitamin E levels in the bottom tertile had a significantly higher probability and also suffering from cognitive impairment compared to those in the highest vitamin E tertile after adjustments for the confounding factors. In another study studying plasma tocopherols and the risk of cognitive impairment in an elderly population (Ravaglia G, 2008), plasma concentrations of some non-alpha-tocopherol forms of vitamin E were associated with cognitive impairment. However, the associations depended on concurrent cholesterol concentration. Tocopherols were analysed as plasma absolute values divided by serum total cholesterol because lipids affected their blood availability.

Prospective Longitudinal Studies

Prospective studies investigating the effect of the intake of antioxidant vitamins on the risk of developing cognitive impairment reported controversial results. The inconsistency of the results of the prospective studies may depend on the differences in the durations of follow up.

Perrig WJ et al showed that higher plasma ascorbic acid and β-carotene concentrations were associated with better memory performance in older people, both cross-sectionally and longitudinally over a 22 year period (Perrig WJ, 1997). Vitamin E (from food and supplements) and vitamin C (from supplements) protected against cognitive decline, respectively, in a 3.2 (Morris MC, 2002) and a 4 year (Paleologos M, 1998) follow up studies. Masaki et al found that supplementations with vitamin C and E were associated with reduced prevalance of vascular dementia in their population based prospective study with a follow up period of 3-5 years on 3385 Japanese-American men. One of the limitations of this study was that no measures of cognitive function were collected at baseline, when the vitamin consumption was determined. If cognitive decline has already begun, it might have caused the subjects to stop taking vitamins (Masaki KH, 2000). Maxwell CJ et al examined longitudinal data from the Canadian Study of Health and Aging, a population based, prospective 5-year investigation of the epidemiology of dementia among Canadians aged older than 65 years. After adjusting for potential confounding factors, vitamin E and C supplements or any antioxidant vitamin supplements were significantly less likely to experience significant vascular cognitive decline during a 5-year follow- up period, but a reduced risk for incident dementia was not observed. They concluded that a possible protective effect for antioxidant vitamins in relation to cognitive decline (Maxwell CJ, 2005). On the contrary, after a 3- year follow-up, Kalmijn S et al did not find any association between intakes of antioxidant vitamins and cognitive decline (Kalmijn S, 1997). Gray SL found that the use of supplemental vitamin E and C, alone or in combination, did not reduce risk of dementia over 5.5 years of follow up (Gray SL, 2008). In another longitudinal study, Fillenbaum GG et al studied on 616 old persons selected from the population in southeastern US where vitamin supplement use was low (%8 of the subjects in this study), use of vitamins C and/or E did not delay the incidence of dementia in 13-14 years follow up period (Fillenbaum GG, 2005).

Interventional Studies

The most convincing evidence that the intake of antioxidant vitamins has useful for cognitive functions is provided by interventional studies. In fact, it has been documented that rats given dietary supplements of fruit and vegetable extracts for 8 months, beginning at 6 months of age, slowed age-related declines in neuronal and cognitive functions (Joseph JA, 1998). More importantly, these rats were able to reverse age-related deficits in several neuronal and behavioral parameters when administration was started at 19 months of age (Joseph JA, 1999). In another study investigating the effects of acute, short-and long- term pre- training administration ascorbic acid on passive avoidance learning and memory in rats, it was concluded that short- and long-term supplementation with ascorbic acid (Vitamin C) had facilitatory effects on acquisition and retrieval processes of passive avoidance learning and memory in rats (Shadidi S, 2008). In humans, a 1-year randomized, double blind, placebo controlled intervention study (n=86) reported that supplementation with antioxidants enhanced cognitive function (except for long term memory recall) in elderly individuals. However, no significant correlations were observed between circulating concentrations of single micronutrients and cognitive performance (Chandra RK, 2001).

Conclusion

In conclusion, although there are positive results about the supplementation of antioxidant vitamins on prevention and treatment of cognitive impairment, new studies must be performed on the subject. In the studies, attention must be paid to the content of supplementation. In the last years, it seems that the researches on vitamin E and C have dominated. It can be due to the relation of these vitamins to the structure and physiology of brain. However, vitamin A is also good candidate for more future studies. The intake of vitamins through food or supplementation forms and the number or quantity of different vitamins in the supplementation forms can change the effect and benefit. In diet, the interaction of antioxidant vitamins with other antioxidants like flavonoids and other chemicals present in fruits and vegetables can also be the determining factor of the beneficial effects.

B Vitamins

Pathophysiological Mechanisms

Deficiencies of several B vitamins, including thiamine (B1), riboflavin (B2), niasin (B3), pyridoxine (B6), folate (B9) and cobalamin (B12), have been related with cognitive function in many observational study (Riedel WJ, 1998). In some studies, pathophysiological models have been formulated, including the association of B vitamin deficiencies with metabolic disturbances in the structural constituents of cerebral tissue, such as phospholipids and myelin, as well as in signaling molecules, such as neurotransmitters (Rampersaud GC, 2003). In particular, thiamine deficiency has been associated with lactic acid accumulation, reduction in oxygen uptake, decrease in transketolase activity, and an impairment in cholinergic activity, leading to the loss of memory and other cognitive functions (Micheau J, 1985). Cobalamin is essential for neuronal generation and its deficiency can cause degeneration of the nervous system (Herrmann W, 2007). Various cobalamines were shown to have intracellular antioxidant activity in vitro. The compounds inhibited intracellular peroxide production, maintained intracellular glutathione levels, and prevented apoptotic and necrotic cell death (Birch CS, 2009). Folic acid plays an important role in neuroplasticity and in the maintenance of neuronal integrity (Kronenberg G, 2009). It enhances the plasma concentrations of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). EPA, DHA, and arachidonic acid are of benefit in dementia by up-regulating gene expression concerned with neurogenesis, neurotransmission and connectivity, improving endothelial nitric oxide (eNO) generation, enhancing brain acetycholine levels, suppressing the production of proinflammatory cytokines and precursing to anti-inflammatory compounds that protect neurons from the cytotoxic action of various noxious stimuli, oxidative stres and neuronal apoptosis (Das UN, 2008).

Recently, the association between the deficiency of B vitamins, particularly folate and cobalamin, and cognitive impairment has been investigated in relation to hyperhomocysteinemia (hHcy). Several epidemiological studies have also suggested that it may play a role in the cognitive performance (Prins ND, 2002) and pathophysiology of

dementia in older people (Bell IR, 1992; Nilsson K, 1996; Wahlin A, 1996), possibly as the metabolic link between micro-vascular disease and old-age dementia (Morris MS, 2001; Parnetti L, 1997). B vitamin deficiency in mice have caused hHcy and vascular cognitive impairment due to microvascular changes (Troen AM, 2008). hHcy associated with low levels of folic acid and vitamin B12 have been found to be biochemical risk factors of vascular dementia after lacunar strokes and after multiinfarcts in strategic areas (Graban A, 2009).

Homocysteine (Hcy) is an aminoacid entirely derived from the body's intermediary metabolism (Fekkes D, 1998; Pietrzik K, 1997), which can be converted to either methionine or cysteine. Both folate and cobalamin participate in the methylation of homocysteine to methionine and in the remethylation and synthesis of S-adenosylmethionine (Bottiglieri T, 1996; Parnetti, 1997). The other metabolic pathway, which converts Hcy to cysteine requires the active form of vitamin B6 (pyridoxal phosphate) (Pietrzik K, 1997). The most common cause of hHcy is accepted to be a deficiency of folate or cobalamin (Selhub J, 2000). Although the catabolic rate of Hcy results from the interaction between genetic make-up and B vitamin status, it is generally accepted that elevated plasma Hcy concentrations are a sensitive marker for folate and cobalamin tissue deficiency (Bottiglieri T, 1996; Joosten E, 1993; Lokk J, 2003; McCaddon A, 1998; Nilsson K, 1996; Nilsson K, 1999; Parnetti L, 1997). Folate and vitamin B12 are essential cofactors for the methionine/Hcy cycle in the brain. These vitamins mediate the remethylation of Hcy, which affects the production of the universal methyl donor, S-adenosylmethionine, in the brain among other organs. Hypomethylation, caused by low B-vitamin and hHcy, is linked to key pathomechanisms of dementia (Obeid R, 2007). Also, Hcy is recognised to be proatherogenic and protrombotic (Hassan A, 2004) and accepted to be an independent risk for developing occlusive arterial diseases (Refsum H, 1998; Ueland PM, 1989). In a population based study on 1779 subjects, hHcy has been reported to be an independent risk factor for dementia and cognitive impairment without dementia (Haan MN, 2007). hHcy exerts an inhibitory effect on adult mouse brain neurogenesis (Rabaneda LG, 2008). Low folate status and elevated Hcy increase the generation of reactive oxygen species and contribute to excitotoxicity and mitochondrial dysfunction which may lead to apoptosis (Kronenberg G, 2009). hHcy is also proposed to be one of the effects of the oxidation of vitamin B12, as a result of oxidative stres (McCaddon A, 2002). Furthermore, experimental studies in cell cultures have shown that Hcy is neurotoxic, possibly by activating N-methyl-D-aspartate receptors (Lipton SA, 1997) or DNA damage and consequent apoptosis (Kruman II, 2000).

Depending on the used marker, 3-60 % of the elderly are classified as vitamin B12 deficient and about 29 % as folate deficient. Predominantly, the high prevalance of poor cobalamin status is caused by the increasing prevalance of atrophic gastritis type B, which occurs with a frequency of approximately 20-50% in elderly subjects (Wolters M, 2004). Atrophic gastritis results in declining gastric acid and pepsinogen secretion, and hence decreasing intestinal digestion and absorption of both B vitamins. Folic acid intake among elderly subjects is generally well below the recommended dietary reference values (Wolters M, 2004). So, folic acid deficiency is primarily caused by dietary deficiency. Meanwhile, vitamin B12 deficiency is due to two main causes, food cobalamin malabsorption and pernicious anemia (Andres E, 2004). Almost two-thirds of the prevalance of high Hcy is attributable to low vitamin B status or intake (Selhub J, 2008).

Cross-Sectional Studies

Apart from the epidemiological studies suggesting that hHcy may play a role in the cognitive performance and pathophysiology of dementia, more than 77 cross-sectional studies including more than 34 000 subjects (Clarke R, 2008) have reported a significant association between low blood levels of folate and vitamin B12 (or high levels of Hcy) and prevalent dementia (Malaguarnera M, 2004; Köseoglu E, 2007; Quadri P, 2004; Koike T, 2008). This association was described for different types of cognitive impairment including vascular dementia. On the contrary, several cross-sectional studies did not report a significant association between plasma levels of vitamin B or homocysteine and dementia (Arıoğul S, 2005; Ravaglia G, 2000). These cross sectional or case control studies are unable to exclude the possibility that such associations of hHcy or vitamin B deficiencies are rather a result than a cause of the disease.

Prospective Longitudinal Studies

More than 33 longitudinal studies (including more than 12 000 subjects) have analysed, with significant results, the associations between low vitamin B12 and folate blood levels (or hHcy) and incident dementia, incident cognitive impairment without dementia or cognitive decline (Vogel T, 2009; Clarke R, 2008; Seshadri S, 2002). However, not all studies confirmed the link between vitamin B, Hcy and cognition, in terms of dementia, cognitive test scores or cognitive decline (Luchsinger JA, 2004; Mooijaart SP, 2005). In addition, it is not clear whether the observed associations between Hcy and cognition are causal or whether they are caused by Hcy, independent actions of the vitamin B, or both.

A 3-year longitudinal study (Veterans Affairs Normative Aging Study) documented an independent contribution of serum folate levels on cognitive scores (after adjustment for Hcy and other vitamin B) and of dietary folate on cognitive functions (after adjustment for dietary vitamin B). These findings suggested that folate may have cognitive effects by mechanisms other than the elevation of Hcy (Tucker KL, 2005).

Concerning Hcy, six prospective longitudinal studies (Haan MN, 2007; Ravaglia G, 2005; Seshadri S, 2002; Dufouil C, 2003; McCaddon A, 2001; Tucker KL, 2005) reported that increased serum Hcy was associated with an increased risk of dementia or cognitive decline, whereas four studies (Luchsinger JA, 2004; Kalmijn S, 1999; Mooijaart SP, 2005; Teunissen CE, 2003; Clarke R, 2007) did not describe any association between Hcy and cognition, which further fuels the debate (Vogel T, 2009).

Interventional Studies

Despite potential benefits of vitamin B supplementation for lowering Hcy (Jacques, 1999; Naurath HJ, 1995), the positive contribution of this supplementation to cognitive function among demented and non demented patients remains debatable. The results of a multicenter, prospective, randomised, double blind, placebo controlled clinical trial performed on 8000 subjects with recent stroke or TIA will find out the effect of addition of B-vitamin

supplements to reduce serious vascular events including stroke, other major atherothromboembolic vascular events and dementia (VITATOPS Trial Study Group, 2007)

There was a large heterogeneity among finished vitamin B interventional studies with cognitive assessments in terms of dosage, routes of intervention (for vitamin B12), age and cognitive function assessments. Some randomised controlled trials, including patients with normal cognitive function, cognitive impairment and dementia, evaluated the effect of folate supplementation on cognitive function. Among cognitively impaired subjects (n=30) with low folate serum levels, Fioravanti et al (Fioravanti M, 1998) observed a significant improvement of some scores of the Randt Memory Test in the folate treated group compared with the placebo group after 60 days of treatment. In another trial using a mixed factorial design in normal subjects (n=211), the authors observed that folate-treated older women's cognitive test scores (Rey Auditory-Verbal Learning Test) improved (Bryan J, 2002). Controversially, in another small study including 7 subjects with dementia reported no statistically significant differences between the supplemented group and the control group and noted a negative trend in specific test scores of the supplemented group (Sommer BJ, 2003). Because of the small number of subjects, study results needs to be interpreted cautiously. Finally, the 3-year randomised controlled FACIT trial included 818 older subjects (older than 60 years) with augmented plasma total Hcy and normal serum vitamin B12 levels. The effect of folic acid supplementation on cognition was the secondary end point. The 3-year change in memory, information processing speed and sensorimotor speed were significantly improved in the folic acid group in comparison to the placebo group (Durga J, 2007). In addition to these randomised controlled trials, a 17 week uncontrolled cohort study including demented subjects reported a significantly improved performance in different cognitive tests after folate supplementation (Rapin JR, 1988).

Some other randomised controlled studies assessed the effect of vitamin B12 intervention on cognitive functions in humans. There is a large heterogeneity among trials regarding the cognitive status of participants, the doses and administration routes of vitamin B12, the duration of supplementation and the cognitive function assessment instruments used. Sample sizes ranged from 18 to 78 subjects receiving vitamin B12, and the duration of supplementation ranged from 4 weeks to 6 months. For most cognitive tests, there was no significant improvement in vitamin B12 supplemented patients as compared with the placebo group (Stoot DJ, 2008; Eussen SJ, 2006; Hvas AM, 2004). However, Bryan J et al found that healthy younger, middle-aged and older women (n=211) who took vitamin B12 (or either of folate and vitamin B6) for 35 days showed better performance on some measures of memory performance compared to placebo (Bryan J, 2002). Interestingly, a statistically significant worsening of cognitive tests was reported in two studies. In 195 vitamin B12 deficient subjects of normal and impaired cognition, Eussen et al (Eussen SJ, 2006) observed that improvement of the cognitive test score in the placebo group was significantly more marked than that of the vitamin B12 group. Similarly, another study reported a significant worsening of the '12 words learning test' score in a vitamin B12 treated population of 140 old patients with cognitive impairment and methylmalonic acidemia compared with the placebo group (Hvas AM, 2004). For reasons of heterogeneity of these controlled trials, no reasonable conclusion can be drawn regarding the effects of vitamin B12 on cognition. In addition, several uncontrolled cohort studies assessed the effects of vitamin B12 intervention on cognitive function in humans with conflicting results.

A few studies (Lewerin C, 2005; McMahon JA, 2006; Stott DJ, 2005; van Uffelen JG, 2007) reported data of combined B vitamin intervention on cognition, in subjects with normal cognition, dementia or vascular disease (17-409 participants). Trial durations ranged from 12 weeks to 2 years. One study found a significant improvement in one of eight cognitive tests (Reitan trail-making test, part B) (McMahon JA, 2006).

Conclusion

Most studies reporting associations between cognitive function and Hcy or B vitamins have used a cross-sectional or case-control design and have been unable to exclude the possibility that such associations are a result of the disease rather than being causal. The Hcy hypothesis of dementia has attracted considerable interest, as Hcy can be easily lowered by folic acid and vitamin B12, raising the prospect that B-vitamin supplementation could lower the risk of dementia (Clarke R, 2008). While some trials assessing effects on cognitive function have used folic acid alone, vitamin B12 alone or a combination, few trials have included a sufficient number of participants to provide reliable evidence. Among these studies, FACIT Trial (Durga J, 2007) is an outstanding one. This large, randomised and controlled trial have showed that folic acid supplementation improves several cognitive domains that tend to decline with advancing age. Therefore, folate supplementation may be an interesting approach to prevent cognitive decline in elderly people. New trials with larger number of participants will more accurately test the importance of vitamin B supplementation either in alone or combined form.

Vitamin D

Vascular-related brain damage may result from an influx of excitatory amino acids, inflammatory responses, and changes in cellular polarity, which result in excessive calcium entry. In concert with these changes is an increase in intracellular nitric oxide production and increased oxidative stres (Buell JS, 2008). Vitamin D may help ameliorate vascular-related brain disease by mediating deleterious effects of inflammation, calcium dysregulation, and increased oxidative stres. During transient ischemic events, transforming growth factor and glial cell line derived neurotrophic factor (GDNF) are upregulated in hippocampal cells to promote survival (Garcion E, 1999). Vitamin D augments innate antioxidative defences by increasing glutathione and GDNF (Wion D, 1991; Naveilhan P, 1996).These particular changes were shown to attenuate ischemic brain damage in rodents (Wang JY, 2001). In in-vitro and animal models of cerebral ischemia, vitamin D inhibits antigen presenting cell maturation (Carthy EP, 1989), down regulates NF-κB activity (Kong XF, 1999), and stimulates anti-inflammatory cytokine production (Timms PM, 2002). Epidemiological studies show an inverse association between vitamin D and C-reactive protein levels, a marker of inflammation (Timms PM, 2002).

In addition to its neuroprotective activity, observational evidences exist that low serum 25-hydroxyvitamin D has been associated with increased risk for cardiovascular diseases, diabetes mellitus, depression, dental caries, osteoporosis, and periodontal disease, all of which

are either considered risk factors for dementia or have preceded incidence of dementia (Grant WB, 2009). It is plausible that vitamin D may influence vascular dementia via the indirect mechanisms of blood pressure irregularities, cardiac hypertrophy, congestive heart failure, irregularities of activity causing cardiovascular diseases (Buell JS, 2008). A recent study from the Framingham Heart study revealed that vitamin D insufficiency is associated with incident cardiovascular disease (Wang TJ, 2008). In a retrospective study, Przybelski RJ et al have reviewed the findings of older adults presenting to a university-affiliated clinic providing consultative assessments for memory problems. They have found that serum 25-hydroxyvitamin D concentration correlated with mini-mental state examination score, suggesting a potential role for vitamin D in cognitive function in older adults (Przybelski RJ, 2007).

Grand WB proposed vitamin D deficiency as the cause of dementia in his new hypothesis. He supported the hypothesis presenting the evidence from the previous observational and laboratory studies. Nevertheless, there do not appear to be observational studies of incidence of dementia with respect to prediagnostic serum 25-hydroxyvitamin D or vit D supplementation. Such studies now appear to be warranted (Grant WB, 2009).

Vitamin K

Vitamin K is necessary for liver functioning. Vitamin K dependent γ-carboxylation of glutamate takes part in formation of the coagulation factors 2, 7, 9 and 10. More recently, it has been established that vitamin K dependent γ-carboxylation of glutamate occurs also in extrahepatic sites and modifies proteins with other functions. One of these sites is brain. Allison proposed a possible role of vitamin K deficiency in the pathogenesis of Alzheimer disease and in augmenting brain damage associated with cerebrovascular disease (Allison AC, 2001). In a review, Tsaioun (Tsaioun K, 1999) proposed the vitamin K-dependent processes contribute to age related changes in central nervous system. She concludes that 'the study of the effects of the dietary vitamin K on the central nervous system functioning in populations of different age, gender and disease states will facilitate the development of a concept for optimal nutrient intake for specific population groups with relation to vitamin K nutrition'.

Vitamin K is required for normal brain development and function (Allison AC, 2001). The maternal exposure to coumarin derivatives is associated with abnormalities of the fetal central nervous system (Pauli R, 1993). Vitamin K deficiency is associated with decreased sulfation in the brain. Keratan sulfate is dramatically decreased in the cerebral cortex of Alzheimer disease patients (Lindahl B, 1996). Considering keratan sulfate proteoglycan being the major protein of synaptic vesicles (Scranton TW, 1993), one manifestation of decreased sulfation can be abnormal structure and function of the major protein of synaptic vesicles (Allison AC, 2001). Likewise, addition of vitamin K to the chick embriyo increases tyrosine phosphorylation in the brain adhesion and cytoskeletal proteins (Saxena SP, 1997), suggesting that vitamin K plays an important role in the development of the central nervous system. Another vitamin K dependent protein in the brain is Gas 6, a product of growth arrest specific gene 6. Both Gas 6 and its tyrosine kinase receptor are widely distributed throughout

the central nervous system (Prieto AL, 1999). Interaction of these plays an important role in preventing neurons from apoptosis (Allen MP, 1999).

Considering that a relative deficiency of vitamin K, affecting the extrahepatic functions of the vitamin, is common in aging men and women (Allison AC, 2001); it is obviously useful to do experimental animal and case controlled human studies in the first step to clarify the role of vitamin K in the pathogenesis of dementia.

Multivitamin Supplementation

Two randomized controlled trials (Wolters M, 2005; McNeill G, 2007) found no effects of multivitamin supplementation on cognitive performance. One study (Wolters M, 2005) was performed on 220 healthy, free living women older than 60 years of age. After taking multivitamins (containing vitamin C, magnesium, vitamin E, pantothenic acid, beta carotene, pyridoxine, riboflavin, thiamine, folic acid, biotin, selenium, cobalamin) for 6 months daily, no change on cognitive performance has been observed as compared to placebo. This intervention period of only 6 months may be too short for improving cognitive performance in well-educated elderly women without dementia. The other study (McNeill G, 2007) was performed on 910 healthy men and women aged 65 years and over. Four hundred -fifty six of them were on active daily treatment with 11 vitamins and 5 minerals (containing vitamin A, vitamin C, vitamin D, vitamin E, thiamin, riboflavin, niacin, pantothenic acid, pyridoxine, vitamin B12, folic acid, iron, iodine, copper, zinc and manganese) for 12 months, while the remaining ones took placebo. Cognitive function tests were conducted at the start and end of the intervention period. For digit span forward test, there was no evidence of an effect of supplements in all participants or in sub-groups defined by age or risk of deficiency, assessed by a simple risk of questionnaire at baseline. For verbal fluency test, there was no evidence of a beneficial effect in the whole study population but there was weak evidence for a beneficial effect of supplementation in the two pre-specified subgroups: in those aged 75 years and over and, in those at increased risk of micronutrient deficiency assessed by the risk questionnaire. The possibility of beneficial effects of daily multivitamin and multimineral supplements in older people and those at greater risk of nutritional deficiency deserves further attention.

Comments

There is a relationship of levels of antioxidant vitamins and B vitamins to vascular dementia. Nevertheless, whether this relationship is based on causality, it is not so clear after performed longitudinal and interventional studies. New trials with larger number of participants will be more clarifying. Recently, vitamin D deficiency has been found to be related with vascular dementia. Longitudinal and interventional studies will be informative about its role. Additionally, vitamin K is thought to have a possible role in the pathogenesis of dementia.

The subject has multiple aspects. The roles of vitamin intake through food or supplementation forms should be evaluated. Nutritional strategies followed up with nutritional screening tools and combined supplementation of vitamins should be assessed.

Also, investigations on different subgroups of participants, based on age, nutritional status or vitamin level, may be useful.

Hopefully, there are positive results about the use of vitamins in prevention and treatment of dementia and vascular diseases. Thinking the possible useful effects of vitamins in dementia at an individual and at the public level, it will be worthwhile to concentrate on the related researches.

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Chapter VI

Vascular Factors on Alzheimer's Disease

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Abstract

Vascular risk factors (VaRF) have increasingly being recognized as important contributors for late-onset Alzheimer´s disease (AD). There is growing evidence implying neurovascular dysfunction as an integral part of AD. Both AD and vascular dementia show similar microvascular and cerebral amyloid angiopathic pathological changes, suggesting that they are closely related entities. VaRF may lower the threshold for clinically manifested AD/mixed type dementia by decreasing cognitive reserve. VaRF may increase the risk of AD by increasing both β-amyloid and hyperphosphorized tau burden in the brain through several mechanisms, such as high levels of oxidative stress and impairment to the blood-brain barrier. A chronic ischemic brain state also seems an uncontrolled and desperate attempt to increase neuroplasticity. However, this sustained state may favor amyloid oligomerization and trigger tau hyperphosphorylati to induce neuroplastic failure, as increased β-amyloid expression on the brain might be on, promoting neurodegeneration. Several VaRF have already being associated with increased risk for AD. *High cholesterol levels may also induce β-amyloid oligomerization and deposition.* Hypertension produces vascular abnormalities *contributing to arteriolar lipohyalinosis and WMLs.* Insulin resistance and hyperinsulinism promote endothelial proliferation, microvascular disease, and chronic neuronal oligemia. Sustained hyperglycemia increases the formation of Advanced Glycated Endproducts (AGEs), which, in turn, also promotes β-amyloid misfolding and tau hyperphosporilation. Obesity itself may increase oxidative stress and cerebral biological aging, contributing to accelerate AD pathology. *All of these risk factors may contribute to amyloid angiopathy and microbleeding, further decreasing cognitive reserve.* For these reasons, AD-vascular dementia has being considered by many authors as the most common dementia subtype.

Introduction

The number of demented elderly people is expected to increase up to 70% until 2040 in the world[1]. Alzheimer's disease (AD) is the leading cause of dementia, followed by vascular dementia (VaD). It is traditionally considered a neurodegenerative disease of unknown cause where amyloid deposition and tau pathology play pivot roles. However, there is growing evidence implying neurovascular dysfunction as an integral part of AD[2]. Along with the classical pathological finding of senile plaques and neurofibrillary tangles (NFT), there are profound changes in brain capillaries, such as microvascular degeneration, cerebral amyloid angiopathy (CAA), cerebral infarcts and even hemorrhage in AD[3]. Conversely, many patients with VaD show concurrent AD pathology changes[3]. This overlap between AD and VaD suggests that both are rather closely related other than two distinct entities.

Several epidemiological studies have demonstrated association between vascular risk factors and AD. The Nun Study[4] demonstrated that a few small brain infarcts in strategic regions would be sufficient to diminish the threshold for dementia, requiring a lower burden of amyloid to develop the disease. Also, those with brain infarcts had poorer cognitive function. Data from the largest population-based study, the Rotterdam Study, support that there are a number of risk factors for AD that are also linked to vascular disease. Further data supporting this hypothesis comes from other epidemiological studies, such as the Kungsholmen project, EURODEM, and the Honolulu-Asia study (table 1).

As numerous evidence indicates that AD is a vascular disorder, this disease may be considered a vasocognopathy, a term that describes its etiology and its primary effect on cognition[5].

Table 1. Reported risk factors for AD[9]

Aging	ApoE genotype
Atherosclerosis	High/low blood pressure
Diabetes	Microvessel pathology
Smoking	Adiposity
Dyslipidemia	Cardiac disease

Microvascular Changes

It is well determined that there are profound changes in cerebral microvessels in AD, more prevalent in the hippocampus [6-9], region where the NFT formations are early deposited[9]. It has been demonstrated abnormalities in various cellular elements of these vessels, as well as in capillaries related to blood-brain barrier (BBB)[3], such as basement membrane thickening, endothelial compression, luminal "buckling" and narrowing, pericyte degeneration[9], loss of tight junctions[10], reduced total microvascular density and occasional swelling of astrocytic end feet[11]. It has been suggested that there is an extensive degeneration of the endothelium during disease progression[11,13]. Moreover, the low expression of the mesenchyme homeobox gene 2 (MEOX-2) determines events that results in brain hypoperfusion [11,12] and amyloid-beta (Aβ) accumulation[11]. The length and density of degenerated capillaries are related to amyloid plaque burden around the vessels, but not to

neuritic tau pathology[14]. Although these changes occur along with Aβ deposition in the vessels, this peptide does not appear to be the targeting phenomenon[11]. There is no correlation between the distortions in the cerebral microvessels and the stage of the disease (Braak I-VI), suggesting that these aberrant formations are not consequence of AD pathology[15].

Cerebral Amyloid Angiopathy

Vascular amyloid deposits are biochemically similar to those comprising senile plaques in AD, in which the primary constituent is Aβ pepitide, a fragment of the amyloid precursor protein (APP)[16]. These pepitides are deposited within the walls of the leptomeninges and parenchymal arteries, arterioles, and capillaries[2], and can involve the surface wall to complete infiltration of meningeal and intracortical vessels throughout all cortical lobes[3]. With the thickening of their walls and degeneration of smooth muscle cells, there is a predisposition to the formation of microaneuryms and consequent hemorrhage and white matter lesions[17].

CAA seems to be an important contributor to cognitive impairment and dementia in both VaD and AD[11,18],. Even though it is still unclear wether it is an independent, primary cause of dementia or not, it is assumed that is has an aggravating effect in both VaD and AD, lowering the threshold for AD to manifest[17]. CAA is present in 70 to 97.6% of all AD subjects, but its incidence increases with age to almost 100% after 80 years[19].

The origin of the Aβ deposited within blood vessel walls is unclear, and several theories have been proposed to explain its deposition. Because most AD cases are sporadic and overproduction of Aβ is not the primary mechanism[2], inefficient clearance of this peptide is possibly the major mechanism for its accumulation[20]. There is some evidence that the majority of the Aβ-peptide is cleared outside the brain, through the BBB via the low-density lipoprotein receptor related protein 1 (LRP1), the major efflux transporter of Aβ[11,21-23]. Thus, considering the neurons as the main source of Aβ-peptide, a dysfunction in the specialized transporters responsible for Aβ clearance would lead to its accumulation in wall vessel, determining degeneration and death of endothelial cells and obliteration of the capillary lumen[20,24]. Also, it has been reported that the expression of the receptor for advanced glycation products (RAGE), responsible for the influx of AB through the BBB, is increased in AD[2,25,26]. Other hypothesis suggests that the Aβ-peptide is originated, at least partly, either in the systemic circulation[17,27,28], or in the smooth muscle cells within the vessel walls and/or pericytes [17,27,29].

Oxidative Stress and Endothelial Dysfunction

Oxidative stress is defined as an imbalance between the process of production and removal of reactive oxygen species (ROS), with consequent accumulation, leading to a critical failure of biological function[30-32]. During normal aging, the nervous system is particularly susceptible to this ROS's insults, both for its high consumption of oxygen, and low antioxidant systems compared to other tissues[33].

Recent studies are linking oxidative stress to the pathogenesis of several neurodegenerative diseases[33]. Many of the risk factors that play key roles in AD are associated with vascular oxidative stress[2], and, although yet unclear whether this oxidative stress is the initiating event or a secondary effect, there are evidence suggesting that it exacerbates the neurodegenerative process, playing an important role in the pathogenesis of AD[2,33].

It has been established that there are increased levels of oxidative stress in AD[34-36]. Findings such as diminished levels of polyunsaturaded fatty acids and increased of lipid peroxidation markers, protein, DNA and RNA oxidation, and ultrastructural features of vascular and mitochondrial lesions in brain vascular walls are all suggestive of oxidative damage[36]. Also, patients with mild cognitive impairment (MCI) and AD have similar levels of two antioxidant enzymes, which suggest that MCI subjects that further developed AD may have an antioxidant enzymatic activity inadequate to counteract the hyperproduction of free radicals during a recently established condition of oxidative stress[33].

It is suggested that this oxidative damage may also play a role in Aβ deposition, as it causes aggregation of Aβ peptides and contributes to aggregation of tau[33,37,38]. Reciprocally, Aβ can induce a sequence of events that leads to intracellular accumulation of ROS, cell lysis, and indirect generation of an oxidative microenvironment via induction of a local immune response[33,39].

The vascular endothelium is a major target of oxidant stress[26,40,41], notably interfering in nitric oxide (NO) function, which is converted to peroxynitrite, a powerful oxidant, responsible for cellular toxicity. Because of its diminished bioavailability, vascular homeostasis may be compromised, with impairment of platelet aggregation and increased vascular inflammation and atherogenesis[42].

Pathogenic Pathways: BBB Impairment and the Role of Hypoperfusion

Recent studies using AD animal models suggest that BBB dysfunction might be a common, if not a constant, event in AD, even more than previously thought, as there is an increase in its permeability months before the disease onset and plaque deposition[2,43]. There are several models, summarized by Zlokovic 2008[44], proposing a pathogenic pathway for AD in which a BBB impairment would play a major role.

The low expression of MEOX-2 in capillary endothelium mediates an abnormal angiogenic response, leading to neuronal death, brain capillary regression and, consequently, to hypoperfusion. Moreover, changes in the expression of this gene determine a reduced LRP1 expression, and, thus, accumulation of Aβ, which is a potent vasoconstrictor in cerebral circulation[45]. The overexpression of serum response factor and myocardin leads to a hypercontractile state of the vascular smooth muscle cells, reducing the resting blood flow and diminishing functional hyperemia.

The hypoperfusional state generated by these abnormal gene expressions and the changes in the cerebral microvessel characteristics of AD compromise several BBB functions. Initially, the inefficient clearance of Aβ leads to accumulation of Aβ oligomers, which are extremely toxic to neuronal cells. Along with focal reductions of cerebral blood flow (CBF),

there is a synaptic dysfunction, neuronal injury, recruitment of microglia and, later, secretion by the endothelium of proinflammatory cytokines and CBF suppressors, which will eventually lead to a more pronounced hypoperfusion, generating a vicious circle, culminating with BBB breakdown. With the loss in the Aβ clearing capability, there is amyloid formation on the outer side of the capillary membrane, and increased number of NFT and activated microglia and astrocytes.

Besides impairment of the BBB, cerebral hypoperfusion generates other regional metabolic changes. Under certain stimuli, especially chronic hypoperfusion, vascular endothelium neurons and glia are able to synthesize, store and release ROS[36], with consequent oxidative stress, which is known to play a role in AD[33,36]. Moreover, mitochondria almost always show signs of damage during ischemia and have an elevated susceptibility to oxidative stress[33]; therefore, it is likely that chronic hypoperfusion will trigger mitochondrial damage/dysfunction in vascular cells which, in turn, will enhance the production of ROS[36].

Hypoperfusion

Whether this above-mentioned hypoperfusional state is the initial trigger to the neurodegenerative changes is still debatable, although a number of collective evidence supports this theory. Large cerebral arteries in AD subjects are frequently affected by atherosclerosis[44,46,47], which might reduce brain perfusion and precipitate a chronic ischemic condition[44]. The severity of atherosclerosis correlates with the chance of developing AD, as well as the presence of plaques in the common carotid arteries, known to cause brain hypoperfusion[48]. In several animal experimental models, the induction of chronic hypoperfusion generated brain capillary degeneration in CA1 hippocampus and neuronal damage very similar to those observed in AD individual's brains[49]. Metabolic changes were observed before any neuronal damage and, although no brain microinfarcts, hemorrhage, or white matter lesions occurred, the CBF was reduced[9,50,51]. Advanced aging in the presence of a vascular risk factor can converge to create a critically attained threshold of cerebral hypoperfusion that triggers regional brain microcirculatory disturbances and impars optimal delivery of energy substrates needed for normal brain cell function[49]. Moreover, neuroimaging studies with MCI patients who later developed AD suggests that hypoperfusion is a very early feature during the development of AD[36].

The Amyloid-β Peptide Paradox

Most theories support that Aβ is the central mediator of the pathogenesis of AD. Nevertheless, some theories propose that rather than being the initiator of the pathogenic cascade, its accumulation would be the consequence of a chronic ischemic neuronal milieu.

Soluble α-APP is known to have neuroplastic properties, and it is released by neurons in response to neuronal injury[52]. However, its fragments Aβ 1-40 and specially 1-42 peptides display a neurotoxic properties when oligomerized[53,54] It is suggested that these oligomer forms may impair neuronal plasticity by inducing long-term synaptic depression due to

disruption of glutamate transport mechanisms[55,56]. With the accumulation up to cytotoxic levels, it would contribute to progressive neuronal loss in AD[56]. Therefore, in the presence of a chronic injury, such as oligemia, there may an overproduction of Aβ in a desperate attempt to increase neuroplasticity, with consequent neuronal damage. Also, a chronic cerebral oligemic state may increase the expression and phosphorylation of tau, potentially promoting the polymerization of tau into NFT[57-59].

Chronic ischemic injury to neurons may also precipitate an increased level of oxidative stress, which is known to play a role in AD pathogenesis, both by increasing Aβ deposition and promoting direct vascular and neuronal damage[32,33,36], causing a vicious circle. A chronic oligemic brain state also decreases β-amyloid removal by the BBB, further increasing Aβ levels in the brain[44].

Interestingly, some studies suggest that Aβ peptide may even function as a primary line of antioxidant defense to the brain[60,61], thus diminishing brain cells injury by ROS. This finding is in line with the rationale described in the above paragraph – Aβ peptide may function initially both as an antioxidant and neuroplastic peptide but an uncontrolled positive feedback mechanism may lead to its overproduction and aggregation, leading to neurodegeneration.

Based on these evidences, we would be facing an amyloid-β paradox – the same mediator that, initially, defends and repairs neuronal function may also play a role in sustaining the disease process. This possibility is in accord with Mesulam´s theory, by which AD represents a failure of brain´s neuroplasticity[59].

Vascular Risk Factors

There are multiple factors involved in AD pathogenesis, including environmental and genetic vascular risk factors. It is possible that they interact altogether to the development of AD.

1. Apoliprotein E (Apoe)

The ApoE protein plays a vital function in the transport of cholesterol and other lipids through the cells, and its gene occurs in three common alleles (ε2, ε3 and ε4). The mechanisms by which ApoE affects AD patients brains is unclear, but it is suggested that they influence amyloid aggregation, mediate tau hyperphosphorylation, and cause a direct isoform specific toxicity[62].

The ApoE ε4 allele is not only associated with higher levels of total cholesterol[63], but also with sporadic and familial AD[64]. It is as well related to younger age onset in a dose-dependent manner[65]. It has also being associated with a faster rate of cognitive decline, most significantly in the earliest stages of AD[66]. Moreover, the occurrence of NFT and amyloid plaques in the hippocampus and cortex of diabetic ApoE ε4 allele carriers seems to be higher than in non-carriers [67,68]. There is an association between ApoE and vascular disease [69]; however, the results on serum ApoE levels in AD are controversial, as the level differences found in AD patients are due to distribution of ApoE genotypes [62].

2. Blood Pressure

Elevated blood pressure is a well-known risk factor for vascular disease. It is associated with endothelial dysfunction due to an impaired response to agents affecting endothelial-dependent relaxation, and with arterial stiffness, which shows a strong correlation with cognitive impairment [42]. Nevertheless, there are conflicting data surrounding this issue, with several longitudinal studies confirming an association between elevated blood pressure and AD development or cognitive impairment, whereas other concluded to exist no correlation between them [2,70-72]. There appears to be an age-dependent relationship between the occurrence of hypertension and the risk of developing dementia, as midlife hypertension is associated to an increased risk of developing both AD and VaD, whereas higher blood pressure in the very elderly would be protective [72].

At least in part, the reasons for this controversy are most possibly due to discrepancy in methodologies [72] and potential source of bias. Most variables frequently associated with hypertension, such as cholesterol and glucose levels, may also play a role in the pathogenesis of AD [42]. Also, there is a strong correlation between hypertension, cardiovascular disease and stroke, which are known to be important factors leading to the onset of dementia [2,73]. Moreover, some studies used an incomplete approach for the assessment of dementia, either lacking a formal neuropsychological approach [42] or, conversely, relying solely on a diminished score on cognitive tests [72]. Although collective data from epidemiological studies are conflicting, some studies have found associations between hypertension and brain pathology, such as hyppocampal atrophy, lower brain weight, and higher number of NFTs and amyloid plaques in hippocampus [2,74,75].

Elevated blood pressure causes vascular alterations, such as arteriosclerosis and lipohyalinosis of small cerebral vessels that could lead to lacunar infarcts and/or chronic hypoperfusion [71]. Degeneration of microvasculare can lead to impairment of BBB, and along with an increased Aβ production due to direct adverse effect on neuronal health, there is an accumulation of Aβ peptide [2,71,75,76]. Also, hypertension and Aβ act on endothelial cells increasing the production of ROS [71], which is known to play a role on the pathogenesis of AD [32,33,36]. It is likely, thus, that these multiple factors interact contributing to the development of AD.

A low blood pressure may also be a risk factor for development of dementia. Several studies have shown a correlation between hypotension and dementia[2,71]. However, it is unclear whether low blood pressure is a cause or consequence of dementia pathology[77]. Vasculature abnormalities, such as loss of autoregulatory capabilities[72] and Aβ deposition, may precipitate hypoxic insults, and it is possible that LPB may predispose or accelerate the development of dementia, as it decrease cerebral blood flow[77].

3. Diabetes

It is widely known the long-term complications of uncontrolled DM. Along with cerebrovascular disease, there is growing evidence[68,78] suggesting that DM is implicated in the development of other neurological co-morbidities, such as cognitive dysfunction and dementia[79]. However, most studies lack an adjustment for confounding factors, as DM is strongly associated with other systemic co-morbid conditions that may also play a role in

cognitive decline, such as hypertension, dyslipidemia, and ApoE genotype[71]. The exact pathophysiology of cognitive dysfunction in DM is uncertain, but it is likely to involve a mixed pathway, where both hyper- and hypoglycemia, vascular disease and insulin resistance would play a role in this process[79].

Several lines of evidence suggest that high levels of glucose have a toxic effect on brain, leading to progressive functional and structural abnormalities. Chronic hyperglycaemia can affect brain tissue by two main related yet separated mechanisms: (1) by fostering protein glycation and (2) by promoting widespread microvascular damage, causing microinfarcts and white-matter degeneration[68]. It has been demonstrated that leukoaraiosis is an early finding in type 2 DM patients[80].

The mechanisms that lead to these alterations, such as oxidative stress, accumulation of advanced glycation end-products (AGEs) and microvascular pathology are also implicated in the normal aging of brain, and the pattern of brain atrophy in diabetic patients mimics certain aspects of physiological brain ageing. Therefore, the effects of high glucose levels in brain can be referred as "accelerated brain ageing"[68]. Indeed, these patients have higher hyppocampal and amygdale atrophy, structures that are responsible memory and behavior and are atrophied in AD patients[81].

It has also been suggested that Aβ metabolism is affected in DM. Particularly in type-2 DM, insulin resistance generates a compensatory hyperinsulinemia, which is independently associated with cognitive impairment and dementia[82-87]. Insulin degrading enzyme is the responsible for both Aβ and insulin degradation in brain. As it is more selective for insulin, it is likely that Aβ degradation is impaired in hyperinsulinaemia states, with consequent accumulation of this peptide[88]. Amyloid metabolism may also be disturbed by insulin resistance due to the formation of AGEs and ROS, leading to Aβ deposition and tau phosporylation, which are the pathological hallmarks of AD[89].

There is growing evidence supporting an association between DM and AD. Recent data show diminished insulin levels in CSF[90,91], as well as lower concentration of insulin mRNA in AD subjects. Because of this marked reduction in CNS insulin in AD brains[92] some authors suggested that AD could be a type 3 DM[79].

4. Cholesterol

Several studies reported that, similarly to hypertension, high levels of cholesterol in midlife is a risk factor for AD, while in late life it is an indicator of better health status[62]. It is suggested that the time elapsed between cholesterol measurements and the onset of dementia would be a possible explanation for these heterogeneous results[62].

There is growing evidence that cholesterol is critically involved in Aβ generation[93], as its metabolism modulates Aβ production[94]. Increased levels of low density lipoproteins cholesterol (LDL-C) in AD patients correlate with brain Aβ levels, suggesting that it influences the expression of AD-related pathology[95]. Nevertheless, the mechanism by which cholesterol influences AD is unclear. It seems that high levels of LDL-c causes a disorganization in the structure of the lipid layer where enzymes responsible for the metabolism of APP and Aβ are located, resulting, thereby, in activation of the amyloid cascade, with diminished levels of soluble APP and increased Aβ[71,96]. This may lead to

neuronal damage because soluble APP is neuroprotective, while oligomeric Aβ forms are known to be neurotoxic[2,59,96,97].

The relationship between cholesterol and AD varies and appears to be age-dependent, as midlife high cholesterol seems to increase the chance of develop dementia. There is a well-established benefit of statins in secondary prevention of vascular disease. However, its role in prevention of dementia has not been fully elucidated[71].

5. Adiposity and the Metabolic Syndrome

Metabolic syndrome is a common metabolic disorder that results from the increasing prevalence of obesity, in which the constellation of metabolic abnormalities includes glucose intolerance, insulin resistance, central obesity, dyslipidemia and hypertension[98]. Several studies have found associations between the individual components of the syndrome and the risk for developing dementia, although a few ones have considered the components of metabolic syndrome as a whole[99]. However, recent evidences are suggesting that metabolic syndrome is associated with accelerated cognitive decline[99,100,101], even after adjustment for possible confounders[100]. It is also an independent risk factor for silent brain infarctions[102]. Several possible mechanisms may explain an association between metabolic syndrome and cognitive decline, including vascular disorders, inflammation, adiposity, and insulin resistance[99]. It is suggested that levels of inflammation may serve as a marker of active pathologic process, which, in the setting of the metabolic syndrome, might help identify those at high risk for cognitive decline and dementia[100].

Obesity is associated to cardiovascular disease, vasculature abnormalities, and alterations in blood pressure and lipids, which all may play a role in the development of dementia. However, an elevated body mass index (BMI) is independently associated with dementia, even after adjustment for multiple vascular factors[101,103]. As overweight and obesity increases the risk for vascular disorders, theses states may be an initial trigger eventually leading to AD and vascular forms of dementia[101].

Studies evaluating the role of obesity and development of dementia show conflicting results. Because of the different methods of approaching the diagnosis of dementia or assessing obesity (abdominal circumference vs BMI), and, most importantly, different follow-up periods, a comparison between then is difficult[101,103]. However, it seems that the relationship between obesity and dementia is age-dependent. In fact, the cross-sectional association between dementia and low body weight is most certainly explainable through reverse causality[102,103]. Conversely, midlife obesity seems to be a true risk factor for the development of dementia at old age[103].

Conclusion

Although yet controversial, many epidemiological data and laboratorial evidence implicate vascular disorder as an integral part of AD pathogenesis. Both AD and VaD share the same risk factors for vascular disease, which impairs cerebral blood flow and leads to a chronic brain hypoperfusion state which seems to produce a oxidative stress milieu that, in

turn, triggers the amyloidogenic cascade and *tau* hyperphosphorylation, leading ultimately to neurodegenerative changes typical of AD[9]. Furthermore, the increased AD pathology among those with overt cerebrovascular disease strongly suggests a vascular role in AD.

Numerous evidences linking several vascular risk factors to the development of AD suggests that it is a lifetime disorder rather than a late life disease. Therefore, the recognition of vascular risk factors in AD's pathology is of great importance, as the proper management of them may help reduce the incidence of dementia, giving the elderly a better life quality.

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Chapter VII

New Concepts on Vascular Dementia

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Mild Cognitive Impairment of Vascular Origin

The concept of Mild Cognitive Impairment (MCI) refers to a group of patients with a degree of cognitive decline that cannot yet characterize dementia because it still does not interfere with the Activities of Daily Living. Virtually, MCI may be caused by all types of dementia, including the reversible ones. In this sense, it may assume from the beginning (sub)clinical features of the subjacent pathophysiological process. The diagnosis of vascular MCI (vMCI) is made when the patient with MCI has higher degree cerebrovascular ischemic lesions than it would be expected from normal aging only. This is especially true for small-vessel disease vMCI.

MCI may be classified into four basic subtypes, according to type and number of altered cognitive domains: single-domain amnestic MCI (saMCI), single-domain non-amnestic MCI (snMCI), multi-domain amnestic MCI (maMCI), and multi-domain non-amnestic MCI (mnMCI). saMCI is typical of Alzheimer´s disease and it is not a common presentation in vMCI. All other three forms may be related to vMCI, depending on the site and number of ischemic lesions.

A dysexecutive syndrome is the most common initial presentation of snMCI in vMCI. It may be clinically differentiated from preclinical stages of frontotemporal dementia by the lack of disinhibition and/or language problems of cortical subtype in vMCI. Besides, vMCI is often accompanied by pyramidal and extrapyramidal signs, including small-stepped gait. In most advanced vMCI stages, both mnMCI and maMCI get more common, but this means that the physician should start considering the diagnosis of Vascular Dementia (VaD).

1 Background: Specialist in both Neurology and Geriatrics. Ex-Fellow in Geriatric Neurology – University of Kyoto, Japan. PhD in Neuroscience of Aging – Kyoto University, Japan.

The diagnosis of post-stroke MCI does not poses diagnostic doubts. The most difficult scenario is when the onset of vMCI is insidious and depression coexists. Depression may be considered a reversible cause of MCI. In another hand, vascular depression is often associated with vMCI, since both are often caused by lesions in similar frontostriatal networks, specially the dorsomesial circuit. Even though apathy is often a manifestation of v-MCI, coexistent vascular depression should be identified not only in order to alleviate suffering, but also because it may improve cognition itself and help clarifying differential diagnosis.

vMCI is not reversible, but progression to VaD may be. Appropriately identifying and treating vascular risk factors in vMCI may decelerate or even avoid progression to VaD.

The Microvascular Frontal-Subcortical Geriatric Syndrome

As a group, humans show a steeper decline in both cognitive and functional performances from the seventh decade on. Cerebral Small-Vessel Disease (CSVD) might be one of the pathological hallmarkers of this transition. However, rates of cerebral degenerative and cognitive/functional changes differ widely from one person to another. This difference has been shown to be related to CSVD.

About ten percent of community-dwelling elderly presents a clinical syndrome characterized by (1) Mild Cognitive Impairment, (2) Late-onset Vascular Depression, (3) small-stepped Gait Disorder, and (4) Urge-type incontinence, all of which occurring in the presence of at least one frontal-release sign: hand grasping, palmomental, snout, and/or inextinguible glabellar reflex. All of these signs/symptoms are caused by disruptions of the frontal-subcortical network which, in turn, occurs as a consequence of CSVD. Because the most profound regions of the frontal lobe are irrigated by the brain's deepest arterioles, this region is more subjected to multiple ischemic insults. Therefore, CSVD may ultimately promote frontal-subcortical disconnection and atrophy. Because circuits that control cognitive, affective, executive, and motor functions are in close proximity, multiple small vascular lesions may simultaneously cause dysfunction in the entire circuit.

To the coexistence of most of the above clinical manifestations the name Frontal-Subcortical (Geriatric) Syndrome" (FSCS) has been applied. The vascular hypothesis for the FSCS is supported by: (1) the high rate of occurrence of FSCS and its individual components in patients with hypertension, diabetes, coronary disease, and Met.S; (2) the high rate of components of the syndrome in patients with CSVD; (3) the high prevalence of an advanced degree of leukoaraiosis and lacunas in patients with FSCS. Therefore, the extreme manifestation of CSVD would lead to FSCS, but the elderly who do not experiences much CSVD would decline much slower.

Our group was the first to propose a clinical criterion for this syndrome, based on the presence of at least one frontal-release sign plus three or more of the four central clinical features above numbered. Frank dementia and Parkinson's disease are criteria of exclusion. Our proposed criteria for FSCS can easily be accessed in a neurogeriatric consultation by performing a simple neurological exam, by conducting a simple MMSE test, diagnosing vascular depression, evaluating the presence of small-step gait disorder, and diagnosing urge-incontinence.

Risk factors for cerebrovascular disease may be the main modifiable determinants of pathological neuroaging. FSCS may be a key element in explaining the concomitant and interrelated decline in cognitive, affective, executive and neuromotor functions among the elderly. Features of the FSCS are often inadvertently attributed to normal aging and, therefore, not considered to be amenable to intervention. Recognizing this syndrome as an age-associated disease that, like Alzheimer's disease, does dramatically increase in prevalence with age, but does not necessarily affect all elderly (and therefore is not 'normal') is, hence, the first step in improving medical care for this large group of elderly people.

Neuroimaging Vascular Dementia

Vascular dementia (VaD) is a heterogeneous entity with several clinicopathological forms. Among all dementing subtypes, VaD is where neuroimaging plays the most important role in confirming or excluding clinical diagnosis. Both computed tomography (CT) and magnetic resonance imaging (MRI) are very useful tools in evaluating ischemic lesions in VaD. They are both less important, however, in excluding other causes of neurological diseases leading to dementia, such as Alzheimer´s disease and Lewy-body dementia.

Ischemic lesions are usually hyperintense in T2 and Flair when they are related to gliosis and hyperintense in T2 and hypointense in Flair when they are related to brain malacia. Dilatate perivascular (Virchow-Robin) spaces (état criblé) may be considered a consequence of white matter (WM) atrophy

If the clinical suspicion of VaD is strong enough, there is no reason to request a MRI brain scan instead of a CT scan. In addition, there is no need in requesting a brain MRI when CT already confirms VaD. When there is no clinical history of stroke, however, small-vessel VaD should be differentiated from other causes of dementias that may present with a similar clinical picture. This includes other subcortical dementias, Lewy-body dementia, and tauopathies. In this specific case, it may be worthy directly requesting a MRI instead of beginning with CT scan.

Dementia often follows a single large territorial infarct (single-stroke VaD). In this case, diagnosis is obvious. Pure Biswanger´s disease is caused by chronic periventricular ischemia caused by cerebral microvascular disease, leading to extensive WM lesions. Dementia may also be caused by multiple lacunar infarcts (état lacunaire), in which case the term multiinfarct dementia is often used.

In clinical practice, lacunes often coexists with some degree of periventricular WM lesions. In no other situation the term small-vessel VaD can be better used than in these patients, since this expression involves both arterioles and capillaries. This is the most common form of VaD.

An important issue in VaD is how much ischemic lesions are needed in order to develop dementia. The answer is, depends on the localization. A strategic localized small stroke on the striatum or the thalamus might be enough to cause a dysexecutive syndrome that is important enough to impair functionality and cause dementia (strategic stroke VaD).

Another related issue is up to which extend WMLs can be attributed to normal aging? This is a very important point, but difficult to obtain consensus. In this case, the concept of abnormality depends on curves elaborated from normal aging subjects and, again, one may

question to what extend even a small degree of WML can be considered normal aging. One operational alternative is simply estimating the absolute amount of WML in VaD patients by utilizing magnetization transfer MRI. It estimated that at least 20% of brain´s white matter should be damaged in order to cause VaD.

Atherosclerosis, Blood Pressure Control, and Cerebral Perfusion in Vascular Dementia

Patients with Vascular dementia (VaD) are rarely included on clinical trials involving the control risk factors and, therefore, results originated from non-dementing subjects should be interpreted with caution. VaD is associated with extra- and intracranial atherosclerosis. Besides, VaD may be caused by cerebral microangiopathy, or small vessel disease. The level of systemic blood pressure (BP) necessary to irrigate the anterior cerebral circulation is directly related with the degree of carotid stenosis.

Cases with carotid stenosis and vascular mild cognitive impairment (vMCI), or yet mild VaD, should be treated in the same way that non-demented subjects. Carotid endarterectomy is usually recommended for severe stenosis (70% to 99%) in symptomatic stroke cases (Level A evidence), but it may be also recommended for symptomatic patients with 50% to 69% stenosis (level B). Asymptomatic patients with 60% to 99% stenosis have a smaller benefit-to-risk ratio than do symptomatic patients, requiring individual decision-making on a case-by-case basis.

The issue of BP control in patients with severe carotid stenosis is controversial. A study has shown that, in the presence of severe bilateral carotid stenosis, mean BP level is inversely related to the risk of stroke and mortality, reflecting decrease cerebral perfusion. Mean systolic BP associated with lowest adverse outcomes was 150 mmHg in the case of unilateral severe stenosis.

BP control in moderate-to-advanced VaD is controversial and involves ethical as well as clinical issues. When VaD is associated with extra- and/or intracranial stenosis, it requires higher cerebral perfusion pressures in order to maintain best perfusion. Conversely, higher BP levels would accelerate endothelial damage, leading to both arteriolosclerosis and small-vessel disease. The HYVET study showed that treating hypertensive people aged 80 and older to attain a systolic BP equals to 150 mmHg was associated with decreased mortality. However, this study included only relatively health individuals, and results cannot be generalized to other populations, including VaD subjects.

Non-Cognitive Symptoms in Vascular Dementia

Dementia may be a late manifestation of cerebral small-vessel disease (CSVD). Non-cognitive symptoms of Vascular Dementia (VaD) might precede the diagnosis of dementia in years. However, unnoticed cognitive changes in executive function domains commonly precede vascular mnemonic problems. In fact, executive dysfunction, ´marche à petit pas´ (small-stepped gait), urge-type urinary incontinence and vascular depression may be the first manifestations of an ongoing vascular dementing process. Indeed, the all these manifestations

share a same pathophysiological process, namely fronto-subcortical dysfunction due to disruptions in frontostriatal circuits, especially the frontal dorsomesial circuit. The periventricular and anterior cingulum gyrus corresponds to an area of watershed between the anterior- and middle-cerebral arteries, at one side, and between the pial arteries and the lenticulostriate arteries, at another side. Because these regions are irrigated by long penetrating arteries, they are especially vulnerable to processes causing arteriolosclerosis and capillary bed dysfunction, such as hypertension and diabetes mellitus. Because, if vascular risk factors are left untreated, above symptoms seem to inexorably progress to vascular dementia, we propose that the term 'vascular dementia' be changed to 'vascular frontostriatal dysconnective syndrome´ with dementia. However longer, this term has two main advantages. First, it is based on the pathophysiology of the disease. Second, it makes easier to medical students and physicians in general to understand the non-mnemonic manifestations of the syndrome. This fact might have important implications for the early diagnosis of the syndrome and the appropriate control of its risk factors. Since VaD remains the most common preventable dementia, the importance of its early diagnosis cannot be overemphasized.

Vascular Parkinsonism and Dementia: Subtypes and Shared Pathophysiology

Vascular dementia (VDe) and vascular parkinsonism (VPa) share a similar pathophysiology and may even be subclassified into similar subtypes. Yet, very few authors have attempted to study both neurologic manifestations under the same theoretical framework. Both VDe and VPa may be caused by a single large stroke or a few territorial strokes of any size, a strategically localized subcortical single lacune, multiple lacunes in the basal ganglia, white matter microvascular disease/Biswanger´s disease, or yet, most commonly, by any combination of these subtypes. Both VDe and VPa are typically caused by lesions that involve the basal ganglia and/or surrounding white matter. These lesions disrupt frontostriatal circuits involved in cognitive, executive, affective, and praxis functions. Because both Parkinson´s Disease (PD) with parkinsonian dementia and Lewy-body dementia are important differential diagnoses of VDe with VPa, knowing the different presentations of VPa is important in diagnosing this complex. Moreover, since coincidental vascular lesions in idiopathic PD are common, the mere presence of these lesions on brain imaging is not diagnostic of VPa. Thus, the importance of history and physical examination in elaborating the differential diagnosis cannot be overemphasized. In VPa, symptoms vary in accord to the type of lesion. Extensive periventricular white matter disease typically cause slowly progressive symmetrical bradykinesia and rigidity, predominantly affecting lower extremities, without association with pill-rolling type resting tremor. Differential diagnosis should be made with rigid-acinetic form of Parkinson´s Disease (PD). Besides, in this subgroup, paratonia may be even more important than rigidity. Strategic infarcts localized in the basal ganglia, however, may present asymmetrically and with tremor. In the cases with presentation more similar to Parkinson´s disease, however, a detailed interview may reveal a stroke between 1 week and 3 months the onset of symptoms. In such cases, a brain scan may reveal a lacune in the basal ganglia or thalamus, and limb dystonia associated with tremor is common, though it may happen also in sporadic PD. Like in VDe, additional features, such as

pyramidal and pseudobulbar signs, and urge-type urinary incontinence, also frequently accompany VPa. VPa is generally considered to be poorly to L-dopa therapy, another feature that may help differentiate it from PD. In the coexistence of dementia, knowing the forms of presentations of VPa is important in differentiating VDe/VPa complex from other diseases such as Lewy body dementia, PD with parkinsonian dementia, and atypical forms of parkinsonism presenting with dementia.

Mixed-Type Alzheimer´s/Vascular Dementia: The Most Common Dementia Subtype

There is a positive association between the Alzheimer's disease (AD)-related amyloid burden and the amount of cerebrovascular ischemic lesions in the brains of people with dementia.

Mixed Alzheimer's/vascular dementia seem to be the most common dementing illness among the elderly. In fact, whereas the most ´pure cases´ of Alzheimer's disease (AD) can be seen in presenile cases, it is more difficult to find an elderly person with ´pure AD´, even if memory impairment is the dominant complain. Instead, vascular dementia often presents initially as a dysexecutive syndrome associated with any degree of gait disturbance, such as small-stepped gait, and urge-type urinary incontinence. These symptoms have in common a same pathophysiological process, namely, ischemic frontostriatal disconnection.

Clinical suspicion of mixed AD/VaD etiology in a patient with a dominant mnemonic subtype cognitive deficit should not begin with the demonstration of ischemic lesions by CT or MRI brain scan. In fact, mixed AD/VaD cases often presents initially with important deficits on both mnemonic and fronto-striatal domains.

Since the Nun study we know that increasing number of ischemic lesions decreases the threshold of AD-related neurodegeneration necessary to cause dementia (AD/VaD mixed-type). Besides, the etiology of both diseases may be related. Chronic ischemia induces APP expression and amyloid oligomerization, besides triggering tau protein hyperphosphorilazation. On the other hand, amyloid angiopathy is the most common cause of cerebral microbleeding. High cholesterol levels have also been related to amyloid oligomerization and deposition. Hyperinsulinism stimulates capillary endothelial proliferation and small-vessel disease, which, in turn, causes chronic ischemia and triggers the amyloidogenic pathway.

Correctly identifying mixed type AD/VaD is very important, since controlling vascular risk factors are essential in decelerating the dementing process. Besides, AD/VaD mixed cases are less likely to respond to both anticholinesterasics and memantine.

Neurogenetics of Vascular Dementia

Vascular dementia (VaD) is not a single entity, but a syndrome that includes conditions characterized by different etiopathogeny and presenting variable morphological and clinical findings. Unraveling the genetic factors that may play a role in VaD is a very important step into trying to prevent he disease. VaD, like coronary disease, is a multifactorial disorder and, as such, a complex array of environmental and polymorphic genetic risk factors interacts in

other to determine the risk of the disease. Polygenic diseases, as opposed to monogenic ones, refers to diseases in which multiple genes play a role simultaneously in its causation process by interacting in both ways with the environment. Many common risk factors for VaD themselves, like hypertension and diabetes, are polygenetic conditions. Therefore, multiple genetic loci contribute to different extend to the VaD phenotype. Although most of the genetic risk factors for VaD are complex and polygenic, there are few well known entities responsible for monogenic forms of stroke and VaD. Cerebral autosomal-dominant arteriopathy with subcortical infarcts (CADASIL), amyloid angiopathies, Fabry disease, familial Moyamoya disorder, Neurofibromatosis type 1, Ehlers-Danlos syndrome type IV, and Marfan syndrome are some examples of these monogenic syndromes. Even representing less than 1% of all VaD patients, the identification of such monogenic disorders is important for both therapeutic decisions and genetic counseling. The focus of the present review will be in the discussion of clinical, neuroimaging and genetic factors of monogenic inherited conditions leading to vascular dementia. It will also be exposed recent advances in the neurogenetics of VaD and stroke, as well as their clinical applicability.

Early Cognitive Dysfunctions in Vascular Dementia

Despite this documented high frequency and concomitant medical and sociocultural impact of vascular dementia (VaD), its clinical and pathophysiologic characterizations remain controversial. The most used clinical criteria to diagnose vascular dementia are DSM-IV, ICD-10, ADDTC and NINDS-AIREN. The latter is the most restrictive and DSM-IV the most inclusive.

Early cognitive dysfunctions in VaD are related to its main subtypes. There are three dominant subtypes of VaD: (a) cortical vascular dementia; (b) strategic infarct dementia; and (c) subcortical vascular dementia. Cortical VaD as well as Strategic infarct dementia show some heterogeneity in regard to lesion site and consequent neuropsychologic manifestations. In the case of subcortical VaD, however, its clinical presentation is more homogeneous when compared to the other two subtypes. Subcortical VaD incorporates two entities, both of which involve the concept of small vessel disease, namely lacunar state and Biswanger disease.

In particular, ischemic lesions in the prefrontal cortex, caudate nucleus, pallidum, thalamus, and thalamocortical circuits affect the prefrontal-subcortical network. For this reason, any lesion that affects these circuits may disrupt these circuits and cause similar neuropsychologic manifestations. Hence, regardless of the specific location, often the first neuropsychological manifestation of VaD is executive dysfunction. Even so, there are several subtypes of executive dysfunction, and there is a relatively stable association between the anatomical site of the lesion and the expected subtype of executive function affect.

Therefore, lesions involving the dorsomesial circuit will cause apathy and mental slowing because they may prevent emotional drive to reach the cortex; dorsolateral lesions will cause lack of appropriate planning and task conduction; and vascular lesions to the prefrontal cortex, though rare, may cause a dominant disinhibitictory picture.

From a neuropsychological perspective, there at least four steps to be taken in order to establish a neuropsychological diagnosis: (1) staging the severity of the disorder (*e.g.*, mild

cognitive impairment or dementia); (2) evaluation of the specific location of the cerebrovascular disease; (3) evaluation of affected neuropsychological domains; and (4) examination of secondary factors that may worsen the patient's cognitive function, such as commonly associated vascular depression. The main goal of this chapter is to review the cognitive dysfunctions present in the various subtypes of vascular dementia and top describe their consequences.

Postural Blood Pressure Dysregulation and Vascular Dementia: Evidences for a Vicious Circle

One of the consequences of disturbances on the neurocardiogenic tonus is orthostatic dysregulation Blood Pressure (BP). Postural BP dysregulation is defined by a persistent (≥ 3 minutes) change on BP which is greater than 20 mmHg for its systolic component or ≥ 10 mmHg for its diastolic compound. The most common well-known subtype of orthostatic BP dysregulation is postural hypotension (P.Hypo), but Postural BP hypertension (P.Hype) is also increasingly being recognized to be crossectionally associated with adverse outcomes.

Several lines of evidence point toward a pathophysiologic vicious circle relationship between postural BP dysregulation and Vascular dementia (VaD) . In fact, several studies have shown an association between VaD, in one side, and dysregulation on postural BP, at other side. Sudden and relatively prolonged (more than 3 minutes) changes on brain perfusion caused by P.Hypo may contribute to cause small, asymptomatic, lacunar strokes and may even contribute to leukoaraiosis/White Matter Hyperintensities (WMH) on MRI. It is less clear if disruptions on cerebral blood flow caused by P.Hype can also contribute to brain's small-vessel disease. Microhemorrhages, specially if amyloid angiopathy co-occurs, may be precipitated by sudden, overactive increases on orthostatic BP and, consequently, on cerebral perfusion pressure.

Because the neurovascular BP regulatory control is located at the medulla oblongata, many VaD patients with bulbar ischemic lesions develop dysregulation on orthostatic BP control. In fact, patients with Vascular Dementia (VaD) seem to have important impairment on the regulation of cerebral blood flow. Patients with VaD often have uni- or bilateral carotid stenosis greater than 50-70%. Among patients with bilateral carotid stenosis greater than 70%, routine seated BP levels are inversely associated with ischemic strokes.

Since heart failure can contribute to P.Hypo, interventions that increase both the cardiac output and the neurocardiovascular tonus, such as aerobic exercise, may ameliorate P.Hypo. Moreover, dysregulation of neurocardiovascular tonus, like that existent in overactive P.Hype, may also respond favorably to interventions that aim to improve both the cardiac function and the neurocardiovascular tonus.

Vascular Depression and Dementia: What Do They Have in Common?

Depression twice as common in vascular dementia (~30%) than in Alzheimer´s disease (~15%). Even with the crescent use of the term 'vascular depression' on the scientific literature and the proposal of specific diagnostic criteria, this issue remains controversial. High morbidity, poor response to treatment and a high risk of conversion to dementia are distinctive characteristics of vascular depression.

There is a significant overlap between vascular depression and vascular dementia. Both conditions frequently co-exist and share many aspects, beginning by the common pathophysiology. Clinical presentation also shows some similarities. A broad spectrum of cognitive impairments and disability may occur in vascular depression, making differential diagnosis with vascular dementia often difficult. It have been described that executive dysfunction, attention deficit and slow processing of information occurs more frequently in vascular depression than in other depressive subtypes. Apathy instead than sadness is more common in vascular depression, but apathy is also the main neuropsychiatric symptom of vascular dementia. Poor insight also adds another difficulty in the differential diagnosis between these two diseases.

The clinical picture of vascular depression, including cognitive impairment and lentification, lack of insight, and disability resemble a frontal lobe syndrome, which is also a more frequent manifestation in vascular dementia. Furthermore hypertension, diabetes, hyperlipidemia, smoking, and coronary artery disease are common risk factors for both diseases.

An important issue is: which comes first, vascular depression or vascular dementia? The sharing of a same pathophysiologic and risk factors profile, associated with a similar clinical presentation, make difficult the differential diagnosis. When depressive symptoms arise in the course of vascular dementia, there is no diagnostic dilemma. Most often, however, a unified Vascular Cognitive-affective syndrome would the most appropriated diagnosis. Notwithstanding, adequately treating depression makes possible to evaluate whether cognitive impairment improves together with mood or not. Frequently, there is remission of mood symptoms with pharmacological treatment, but persistence of executive dysfunction. In these cases, if functional impairment is not evident, the most reasonable diagnosis is vascular Mild Cognitive Impairment (vMCI) with depression. Otherwise, if there is impairment in the activities of daily living, the diagnosis of vascular dementia should be considered.

Vascular dementia is also often preceded by both vMCI and vascular depression. Appropriately identifying these conditions and treating associated vascular risk factors are important steps into avoiding vascular dementia.

Vascular Factors on Alzheimer´s Disease

Vascular risk factors (VaRF) have increasingly being recognized as important contributors for late-onset Alzheimer´s disease (AD). VaRF may increase the risk of AD both by increasing both β-amyloid and hyperphosphorized tau burden in the brain through several mechanisms. An increased oxidative stress mileau seems to be propitiate β-amyloid

oligomerization and tau-protein instability. Chronic brain oligemia also seem to trigger neurotoxic amyloidogenic pathways and suppress neurotrophic non-amyloidogenic pathways. These processes might be related to Mesulam´s neuroplastic failure theory of AD. Increased β-amyloid expression on the brain might be a desperate attempt to increase neuroplasticity, but if the net-effect is an imbalance towards the amyloidogenic pathway, thus a positive feed-back mechanism would be pathologically sustained. Several VaRF have already being associated with increased risk for AD, including diabetes mellitus, hypertension, dyslipidemia, (central) obesity, and the metabolic syndrome. Interestingly, these associations seem to be stronger when risk factors are evaluated at midlife, or at least 10 to 20 years prior to the development of AD. Obesity itself, which is often associated with increased caloric intake, may increase oxidative stress and cerebral biological aging, contributing to accelerate AD pathology. Insulin resistance and hyperinsulinism promote endothelial proliferation, microvascular disease, and chronic neuronal oligemia. Sustained hyperglycemia increases the formation of Advanced Glycated Endproducts (AGEs), which, in turn, also promotes β-amyloid misfolding and tau hyperphosporilation. Hypertension produces microvascular sheer stress, lipohyalinosis, and arteriolosclerosis, contributing to WML and chronic brain oligemic states. All of these risk factors may contribute to amyloid angiopathy and microbleeding, further decreasing cognitive reserve. High cholesterol levels may also induce β-amyloid oligomerization. Additionally, VaRF also may decrease the threshold for clinically manifested AD/mixed type dementia by promoting cerebral small-vessel disease and white matter lesions (WML), thus decreasing cognitive reserve. Clinically diagnosed AD is pathologically confirmed by a Braak stage of four or more, out of six stages. There is no consensus to what degree of cerebral microangiopathy would be necessary in order to a vascular component to dementia be diagnosed alone or in association with AD. In this sense, by increasing WMLs, cerebral small-vessel disease may decrease the minimal amount of AD-related neurodegeneration necessary to cause clinically manifested dementia. For these reasons, AD-vascular dementia has being considered by many authors as the most common dementia subtype.

Secondary Prevention of Stroke in Vascular Dementia: Clinical and Ethical Issues

Many studies have proved that strict control of cerebrovascular risk factors (CVRF) is effective in reducing the risk of recurrent stroke (stroke secondary prevention). Recurrent stroke is the cause of multiinfarct dementia, one of the most common forms of Vascular Dementia (VaD). Some controlled trials showed a beneficial effect of lowering blood pressure on the risk of dementia. It would seem logical to suggest that if statins and strict glucose control reduce recurrent stroke there ought to be also a benefit on cognition and dementia in the same way that this was seen with blood pressure lowering. By extrapolation, strict control of hypertension and other CVRF is thought to decelerate progression from vascular Mild Cognitive Impairment (v-MCI) into VaD, and from milder stages of VaD into its more advanced phases. However, due to ethical reasons in conducting such a study, no clinical trial has proved above considerations. Moreover, the specific issue of secondary prevention of stroke in patients with dementia of any type (either pre-existing or new-onset

dementia) is not addressed in any guidelines. Notwithstanding, separated guidelines from American Stroke Association and the European Stroke Organization proposes the management of the following CVRF as effective in generally reducing the recurrence of stroke, by approximate degree of best evidence: blood pressure control (Class I, level A), strict glucose control in diabetes mellitus and other insulin resistance syndromes (I-A), statin therapy for non-cardioembolic stroke (I-A), endarterectomy for carotid stenosis ≥ 80% (I-A), anticoagulation for atrial fibrillation (I-A) and/or cardiomyopathy with left ventricular dysfunction (II-C), aspirin or other antiplatelet agents (IA), discontinuing cigarette smoking (I-C), weight loss in obesity (II-C), and keeping regular physical activity (II-B). Even though hypertension treatment has degree of evidence IA in preventing stroke, some important points of doubt remains in treating older subjects above 80 years, especially those with uni- or bilateral carotid stenosis higher than 70%. Even though stroke secondary prevention at milder stages of VaD is of unquestionable value, physicians often come across with patients with more advanced VaD in whom the issue of strict controlling CVRF, adding antiplatelet agents, and indicating thrombolysis for new strokes is controversial. In fact, secondary prevention of stroke in advanced VaD cases is polemical and involves important ethical issues such the concepts of autonomy, non-maleficence, beneficence, and social justice, besides issues related to quality of life *versus* life expectancy.

Vascular Dementia and Failure to Thrive Syndrome

Vascular Dementia (VaD) often leads to the Geriatric Failure to Thrive (GFTT) syndrome more often than do Alzheimer´s disease. There are several reasons why the prevalence of GFTT syndrome in moderate-to-advanced VaD patients should be higher than among same stage Alzheimer´s disease subjects. Yet, there is a paucity of studies evaluating the relationship between VaD and the GFTT syndrome.

The term ‘failure to thrive’ was imported from pediatrics into geriatrics in the 1970s and refers to a multifactorial syndrome leading to progressive dependence and potentially caused by many chronic-degenerative diseases. GFTT syndrome is considered the final stage of the geriatric frailty syndrome. In a word, whereas frailty refers to those older people who still walk, GFTT syndrome refers to more advanced stage syndrome leading often to a bedridden state. Besides, while frail older people may still be independent for many functional domains, in the GFTT syndrome multiple-domain impairments are the rule. GFTT syndrome affects only about 5-10% of community-dwelling older adults, buts its prevalence is as high as 30-40% among nursing home residents.

The GFTT has four main components, all considered independent predictors of adverse outcomes, namely: severely impaired physical functioning, malnutrition, depression, and cognitive impairment. The contribution of each of these conditions must be assessed in GFTT patients. The GFTT is commonly seen in dementia patients, particularly in moderate to severe cases. However, VaD patients often have severe physical impairment since the beginning of the disease. In fact, neuromotor symptoms caused by ischemic lesions to the fronto-striatal networks, such as small-stepped gait and falls, urge-type incontinence, and pseudobulbar symptoms like dysphagia and dysphonia often precedes cognitive complains in VaD. All

these components of the vascular dementing syndrome, especially dysphagia and gait impairment, may contribute to the earlier-than-usual onset of GFTT syndrome in the vascular type of dementia. In reality, since VaD often initiate by non-cognitive symptoms, it cannot be appropriately considered a dementing disease, but a fronto-striatal vascular syndrome leading to multiple neurological manifestations.

Depression is a common manifestation of both VaD and GFTT syndrome. Depression is more common in VaD than in Alzheimer´s disease because proper affective functions depend on the integrity of the frontostriatal network, usually damaged in VaD. Malnutrition in VaD patients can develop from different causes like dysphagia, anorexia, and depression. However, it seems that the subclinical inflammatory status present in GFTT subjects, allied to a lack of synchronic organic command from the brain cause metabolic changes that prevent individuals from gain weight when high caloric diets are given by a feeding tube. Besides tube feeding increases the risk of pneumonia in these patients, who also have cough mechanisms impaired. Therefore, the decision about tube feeding is usually difficult and controversial, and must be discussed with both the family and the patient, if possible.

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Chapter VIII

Are Vascular, Alzheimer and Mixed Dementias Distinctive Entities?

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Abstract

The coexistence of Alzheimer disease (AD) and cerebrovascular lesions in postmortem studies has been so frequently reported that some authors have begun to consider this pathology as the most common type of dementia, especially in very old persons. An association between the coexistence of those lesions and severity of cognitive impairment has also been reported. Nevertheless, by searching "mixed dementia" in Pubmed (April 17, 2010) only 55 reviews were found (against 1063 for "vascular dementia" (VD)).

Traditionally, patients who showed a combination of Alzheimer disease and vascular dementia (AD+VD patients) were included as VD in epidemiologic studies. Subsequently the attention was focused on AD, which was considered the main cause of dementia. Nowadays, the traditional view has been revitalized with new findings and the re-examination of the vascular hypothesis of AD. However, the term "mixed dementia" is still avoided and the validity of VD is still under discussion.

The concept of mixed dementia only could only have serious foundations if distinctive cognitive patterns in quality, not quantity, were found. Besides, there is an epistemological problem for the taxonomy of AD+VD, and VD. If it is assumed that: a) distinctive and synergistic neuropsychological patterns exist for AD and VD, and b) these patterns can simultaneously or successively be present in a certain patient or group of patients, then the dementia risk (severity and/or progression) should increase. However, as rapid progression is considered a VD characteristic for antemortem studies while VD pathology represents an essential component of AD+VD for postmortem studies, then, the concept of AD+VD for antemortem studies should include the possibility of rapid progression. If AD+VD dementia is defined by the presence of cerebrovascular disease and only gradual progression, some kind of antagonism between AD+VD seems to be unjustifiably assumed. In addition, abrupt onset and stepwise progression has not been

consistently found in patients with vascular lesions. So, and in order to avoid misdiagnosis, the concept of VD for antemortem studies should include the possibility of a slow progression.

If AD+VD condition seems to be the most common type of dementia in postmortem studies, then that combined condition should be the first option to categorize dementia in antemortem studies. But in order to do that, and considering that dementia is not just an anatomical question, distinctive cognitive outputs should be empirically and consistently demonstrated for AD and VD. If not, may be we should only speak about dementia or cognitive impairment.

The coexistence of Alzheimer disease (AD) and cerebrovascular lesions in postmortem studies has been so frequently reported that some authors have begun to consider this pathology as the most common type of dementia, specially in very old persons. An association between the coexistence of those lesions and severity of cognitive impairment has also been reported. Nevertheless, by searching the term "mixed dementia" in Pubmed database (April 17, 2010) only 55 reviews were found (against 1063 for the term "vascular dementia" (VD)). Notably, by July 6, the number of reviews for the term "vascular dementia" had increased to 1072 while the reviews for the term "mixed dementia" remained the same.

Traditionally, patients who showed a combination of Alzheimer disease and vascular dementia (AD+VD patients) were included as VD in epidemiologic studies [1]. For much of the early 20th century, AD was considered just a rare pre-senile condition [2]. Subsequently, the primary focus of attention was changed to AD, which was considered the main cause of dementia or even synonymous of cognitive decline with age.

The period characterized by the hegemonic "presumption of AD" (being considered AD unless proven other etiology) is nowadays changing to the "presumption of mixed dementia" (being considered mixed dementia unless proven a pure etiology). The reason for this change lies on the fact that older adults are often affected by more than one disease [3] and there are prevalent (more than two thirds of patients) comorbidities in aged brains [4]. Remarkably, mixed dementia went from being the least frequent to the most frequent of the neuropathological findings throughout the years. However, the term "mixed dementia" is still avoided.

In the present manuscript the term mixed dementia (as a combination of AD+VD, or as a combination of AD+VD+ any other pathology) will not be avoided.

Schneider, Arvanitakis, Bang, and Bennett [5] postulate that the majority of the community-dwelling older persons have some kind of brain pathology and those with dementia most often have multiple brain pathologies. Besides, as cardiovascular diseases constitute the most common health problem in very old people [6] and the brain is a highly vascular organ, vascular risk factors that impede adequate cerebral flow can substantially impair all aspects of cognitive function with aging [2].

The positive correlation between age and mixed dementia is so high that the difference between early-onset and late-onset dementia tend to be proposed as synonymous of the difference between AD and mixed dementia with a new cut-off point based on age[1]. On the other hand, the traditional view about dementia (in which AD+VD patients are considered as

1 The subdivision of patients into those with late-life dementia or not, may generate a new transitional zone of diagnosis for patients below an age cut-off of, for example, 80 years old.

VD) has re-emerged in view of the revitalization of the vascular hypothesis of AD [7]. Nevertheless, the validity of both VD and mixed dementia are still under discussion.

A factor which represents a risk for cognitive impairment also represents a risk for dementia. Therefore most brain lesions, including the vascular ones, a priori represent a risk for dementia. However, the validity of both vascular lesions and mixed lesions as probable causes of "definite" dementia types has been questioned because no consensus has been reached about their clinical manifestations [2, 4, 8]. Meanwhile, the lack of demonstrations for the clinical manifestations has led to the proliferation of so oversimplified concepts that the controversy has nowadays been translated to the once-definite diagnostic criteria.

Even AD does not seem to be as certain as classically assumed. Consensus criteria for AD diagnosis currently focused on cortical pathology but a subcortical involvement has also been proposed; the amyloid plaques and neurofibrillary tangles (namely the "pure" AD lesions) do not contribute equally to the disease as it would be expected; Lewy body pathology occurs frequently in association with AD; etc.; in addition, the diagnosis of AD was usually made almost independently of the false-positive cases [4]. When the false-positive cases were taken into account, persons with and without dementia did not seem to be as different as previously accepted. In a longitudinal and neuropathological study carried out by Schneider et al [5] persons with and without dementia shared a similar proportion of pure AD lesions at autopsy [2]. Believed or not, the comparison between persons with and without dementia was overlooked "because the consensus clinical criteria were established for making the diagnosis of AD in clinically demented patients" [4].

The definition of dementia syndromes as different clinical entities, the neuro-psychological patterns associated to their hypothetical biological grounds, and the discussion of the sensitivity and specificity are questions that still today remain to be answered.

The use of 2 x 2 contingency tables for validity studies on the basis of conventional and many times mutually dependent categories of analysis has been an invalid (but widespread) approach [9]; redundant does not mean statistically correlated.

In an attempt to find the boundaries between subjects with and without dementia or among subjects with different dementia types, for example, some neuropsychological instruments with their corresponding cut-off points have been proposed. But, from the cognitive point of view, the use of the "known group" criterion to validate neuropsychological tests is incorrect if researchers have classified their patients according to a certain test or collection of tests (as part of the clinical criterion) and then another test has been used to classify these groups. In that case, researchers can be measuring the correlation between those tests rather than the differentiation between those groups, thus producing an ambiguous and overestimated sensitivity and specificity. Anatomically speaking, if a brain injury has been confirmed in neuroimaging studies (as part of the clinical criterion) and then the same brain is observed in neuropathological studies, those observations will be correlated. Similarly, if a cerebrovascular disease is defined, for example, by the presence of evident strokes in antemortem dementia studies then, by definition (not by demonstration), stroke lesions will be discovered in postmortem dementia studies.

Although antemortem and postmortem studies should be complementary, for some reason validity studies and empirical demonstrations were focused on the postmortem lesions as the main factor to understand dementia etiology and pathophysiology. Meanwhile, antemortem dementia causes and expressions, specially the cognitive ones, were either

considered secondary or only viable of a conceptual definition established by consensus. I.e., the subdivision among different dementia types has been established by convention and mainly based in the postmortem discovery of different lesion types (isolated or combined). Even today AD+VD dementia is almost exclusively defined by the discovery of two kinds of neuropathological lesions (the so-called AD and vascular lesions), independently of their actual neuropsychological patterns.

Notably, postmortem findings did not seem to need any gold standard to be validated [9] even when those findings were not always consistent with clinical dementia diagnoses [10]. Some studies have shown that neither abrupt onset nor stepwise progression has consistently been found in patients with vascular lesions [8, 11, 12, 13]. As regards the association between Hachinski ischemic score (HIS) [14] and neuropathological findings, Reed et al [13] stated: "we report on clinical and neuropsychological findings in 18 prospectively studied cases that had substantial pathology-defined cerebrovascular disease (CVD) at autopsy. ... Clinical features were quite variable; only 40% of cases with high CVD levels had elevated Hachinski Ischemia Scale scores and neither abrupt onset nor stepwise progression was found in most high CVD cases, even when AD changes were essentially absent."

Clinical rating scales usually include a combination of physical and psychological manifestations as well as etiological factors but, for research purposes and better definitions of dementia types, such perspective may be ineffective. If dementia progression, initial symptoms, and physical and psychological impairments are considered just as dependent variables, etiological factors would come out more clearly. Alternatively, and at the moment of empirically demonstrating relationships, it may be incorrect to consider one particular combination of lesions "and" one particular combination of symptoms as belonging to only one particular kind of dementia because the concepts established by consensus are just interpretations. The tendency of easily assigning a proper name to interesting combinations of clinical features can be seen as an abuse of the authority criterion if just incipient or case studies are shown as validation. As a consequence, this practice should be avoided as much as possible. Otherwise as many dementia names as lesion types, biochemical indices, neuropsychological patterns, etc. will be described, in particular with the overwhelming advance of the technological assessment[2]. Most important of all is to remember that each hypothetical dementia pattern, either physical or psychological, is not a constant.

There is a need of evidence which empirically demonstrate the presence of dementia and the influence of etiological factors in relative terms.

Some authors have begun to consider the use of probabilistic approaches in their dementia studies. Beach et al [7], for example, stated: "The role of intracranial atherosclerosis in Alzheimer's disease (AD) has been a subject of debate since the first decade of the last century. The initial "vascular hypothesis" of AD was rejected after a series of mid-twentieth century gross anatomical postmortem studies that showed an inconstant relationship between intracranial atherosclerosis and senile dementia. These early studies did not utilize statistical methods, however, and the investigators did not appear to consider the possibility that intracranial atherosclerosis might have a probabilistic, rather than an absolute, effect on AD risk."

2 While in mathematics a case may at most be useful as a counterexample to reject a rule, in human sciences a case may be enough to formulate a rule. Besides, worldwide agreement is not synonymous of demonstration.

Regarding this absolute- versus- relative question, a contradiction between dementia theory and practice can also be observed: while in the conceptual definitions by consensus mainly categorical dementia conditions are described, in the psychological empirical findings only severity and progression are actually observed. Are we talking about stages of the same process or about mutually exclusive categories?

"High- level cognitive functions (involved in the control and direction of lower-level functions) are hierarchically integrated in the brain cortex, especially in the association areas, always deal with memory and learning, and are responsible for the most complex and typical-for-the-species actions. They involve the ability to focus consciousness either with or without effort (to attend), receive relevant stimuli (to perceive), record in an organized way (to memorize), retrieve pre-existing files (either data, situations, sequences, signs, symbols, gnosias, praxias, etc.), understand, connect all this material in coherent thoughts (verbal or non verbal), decide and, finally, respond or execute an action, all pursuant to the motivations and the social and historical contexts of the individual. High- level cognitive functions are supported by (and can not exist without) the integrity of lower-level functions." [15].

Although in the conceptual structure expressed above "the whole is greater than the sum of the parts", the parts obviously contribute to the whole structure function (or dysfunction). When one component or group of components begins to exert a force which is enough to affect the function of the rest of the parts in such a way that a crisis (or catastrophe) occurs, then the whole structure drastically change [16].

Theoretically speaking, this could be the rationale lying behind the notion of dementia as the "collapse of the mind". Plausible or not, the most important goal of this perspective is the dynamics which underlies to the studied structures and not the structures in themselves, as isolated and static categories.

Empirically speaking, it would be unrealistic to think that we can "observe" the exact boundary between health and dementia. So, and for practical purposes, dementia has to be defined as a variable (the cognitive impairment) only observable between those hypothetical end points.

Cognitively speaking, if a certain lesion type is associated to a certain dementia type (specifically, to a certain psychological pattern), then the study of that lesion will be useful for better understanding differential diagnosis. However, if such dementia type has not been empirically and consistently associated to that kind of lesion, the study of that lesion will be irrelevant for differential diagnosis.

Cognitive impairment is affected by the site, side, size, and type of brain lesion as well as by many other factors such as environmental, physiological and biogenetic ones. The functional specialization and heterogeneity of cortical areas also contribute to enlarge the error of cognitive assessments so that it would be impossible to find a "unique" pattern of cognitive impairment (mild or severe) even in patients with, for example, just one type or one site of lesion[3]. The lack of uniqueness does not imply that a variable pattern could not be scientifically identified and predicted according to certain factors of explanation. That some terms (like "mild cognitive impairment" or "vascular cognitive impairment") do not meet the

3 The type and the site of lesion involve two different systems of classification. So, if both of those categories are used together to find a single result, overlap will be the most probable consequence. For example, if indeed VD is associated to a frontal dysfunction, then the distinction between VD and frontal dementia will be almost impossible to be achieved. If such overlap is not observed, then something has to be revised, either in the theory or in the assessment.

expectancies of finding some ideal gathering of symptoms does not mean that they should be classified as "unpredictable". Scientific phenomena are always variable.

The way in which cognitive impairment is expressed according to its biological and environmental grounds has been carefully analyzed by the neuropsychological science [17]. In view of that background a certain pattern of cognitive impairment can be predicted according to, for example, the site of lesion. So, if a brain lesion, namely, atrophy or ischemia, is located in the frontal lobe (or in other brain area strongly connected to the frontal lobe) then some manifestation of the frontal lobe syndrome can be expected to be observed. On the other hand, and considering that the etiological factors for most dementia types are unclear, the key factors for rehabilitation are still the initial symptoms and the cognitive and behavioral strengths analytically described in a neuropsychological diagnosis. The neuropsychological science also teaches that the performance observed in just a few tests is not enough to make a diagnosis. A similar pattern of affected and preserved functions should be corroborated in different series of tests before giving an interpretation. In other words, the analysis of these phenomena is incompatible with a simplistic approach.

In spite of that, dementia studies have compartmentalized dementia and pre dementia syndromes as absolute categories of interpretation even when those syndromes have not been qualitatively identified yet [4]. The lack of different neuropsychological descriptions for dementia processes could be explained not only by this singular methodology but also by the lack of valid neuropsychological tests.

Gustaw-Rothenberg et al [18] affirm that the diagnosis of these symptomatically and clinically almost indistinct processes, as assessed by tests such as the Mini Mental Status Examination [19] or even the Alzheimer's disease assessment scale (ADAS) [20], can be improved by the study of the biochemical biomarkers. "The search for these reliable biomarkers is, in part, a response to the recognition that screening tests for cognitive impairment… may not be sufficiently sensitive…" [18].

A question that emerges from this panorama is why the neuropsychological processes which supposedly triggered the definition of distinguishable dementia types are almost indistinguishable? Do those neuropsychological processes exist at all?

By comparing the cognitive profiles of, for example, two anatomically different samples of patients we can infer if one of the samples mainly represents a distinctive cognitive pattern in magnitude (showing a better or a worse performance than the other sample) or in quality (showing a similar pattern of convergent and divergent functions affected and the same whole level of severity than the other sample). The concept of mixed dementia only could have serious foundations if distinctive cognitive patterns in quality were found and these patterns were present, simultaneously or successively in a certain patient or group of patients. The concept of mixed dementia as a combination of AD+VD only would include two of these patterns but the analysis can be extended to several of these patterns.[5]

Dementia studies were strongly dedicated to find qualitative patterns but, until now, they have not come to fruition. Most of the clinical definitions are characterized by "the presence

[4] This compartmentalization was also extended to some scientific groups, which were preferentially advocated to the understanding of only one of those syndromes almost independently of its eventual counterparts. Many of those groups were more concerned about confirming their methods and theories than about "exploring" the reality by leaving behind consolidated habits or constructs. Fortunately, this trend is nowadays changing.

[5] Within this perspective, that the performance observed in just a few tests (or in just a few case studies) is not enough to make a differential diagnosis should be reiterated.

of dementia" (as a common psychological denominator) followed by the addition of any other not-psychological feature. But the psychological components of those clinical definitions are pretty much the same. A common explanation for this ambiguity is that, as dementia criteria are generally focused on late stages of cognitive impairment (when the whole spectrum of any dementia syndrome has already been developed), the discrimination of different patterns of cognitive impairment is difficult to detect. But the attempt to replace the term "dementia" by the term "cognitive impairment" did not elucidate the ambiguity at all because the main trouble is not the semantics but rather the method.

There is also an epistemological problem for the taxonomy of VD, and AD+VD. If it is assumed that: a) distinctive and synergistic neuropsychological patterns exist for AD and VD, and, as said before, b) these patterns can simultaneously or successively be present in a certain patient or group of patients, then the dementia risk (severity and/or progression) should increase. However, as rapid progression is considered a VD characteristic for antemortem studies while VD pathology represents an essential component of AD+VD condition for postmortem studies, then, the concept of AD+VD for antemortem studies should include the possibility of rapid progression. If AD+VD dementia is defined by the presence of cerebrovascular disease and (only) gradual progressive dementia, some kind of antagonism between AD+VD seems to be unjustifiably assumed. As a consequence, there is potential for misdiagnosis in patients with AD+VD and rapid progressive dementia, in patients without cerebrovascular disease and rapid progressive dementia, or even in patients with pure (not mixed) vascular etiology and a gradual progression to dementia[6]. Thus, the concept of VD for antemortem studies should include the possibility of a slow progression.

Actually, the need of recognizing not only abrupt clinical stroke but also subtle subclinical stroke as the commonest type of cerebrovascular disease has recently been proposed in a forum composed by seven leader groups on stroke [21].

Continuing with this framework of theoretical revisions, Fotuhi, Hachinski, and Whitehouse [2] have noticed: "Recent studies suggest that atrophy in the cortex and hippocampus -now considered to be the best determinants of cognitive decline with aging- results from a combination of AD pathology, inflammation, Lewy bodies, and vascular lesions… Only a small percentage of people beyond the age of 80 years have 'pure AD' or 'pure vascular dementia'."

In a study aimed at analyzing the anatomical factors associated to cognitive dysfunction, Swartz, Stuss, Gao, and Black [22] observed that brain atrophy, subcortical vascular disease, and thalamus and cortical- strategic infarcts contributed independently to the pattern of cognitive disabilities in a sample of overlapped AD and VD patients. Taking into account that Lewy bodies are now considered among the best determinants of cognitive decline, should researchers study three kinds of overlapped samples defined by consensus in order to carry out the same analysis? Would not be better to work directly with the empirical associations and afterwards (not before) classifying those subjects according to the present theory?

If AD and VD (and LB, and so on) are significantly correlated, maybe they are part of the same underlying process. On the other hand, if it is true that the different dementia types have been confirmed by the presence of certain lesions in postmortem studies, ideally the same

[6] Under a correct empirical definition all the cases should be feasible of classification but if real persons are confounded with ideals, many cases will be left out. That is why conceptual definitions should not be used for empirical demonstrations.

dementia types should also be confirmed by the presence of equivalent lesions in antemortem studies.

In a multifactor-multivariate dementia study of our laboratory [23] we observed that patients with and without ischemic lesions seemed to belong to the same population according to the control of numerous variables, including age and the performance in the Mini Mental State [19] and the Blessed Rating Scale [24]. Notably, when the performance in a new series of neuropsychological tasks was analyzed as a whole, a distinctive cognitive pattern in magnitude was mainly observed showing the patients with ischemic lesions a poorer performance than the patients without ischemic lesions. In fact, the two groups appeared to represent two steps of the same underlying deleterious process and ischemic lesions appeared to magnify the cognitive impairment observed in patients without ischemic lesions. Considering that ischemic lesions were associated to severity, our findings can be considered equivalent to those observed in post mortem studies in which the so-called AD and VD lesions coexist [8, 25]. I.e., we directly demonstrated in vivo, with a probabilistic approach, a synergism between ischemic lesions and dementia. In addition, we observed a significant difference between the two patient groups in a series of neuropsychological tasks, which were the first tests validated according to this purpose.

Beyond the severity, the two patient groups also differed in their progression as indicated by the HIS [14]. Patients with ischemic lesions showed, on average, higher scores on the items of abrupt onset, stepwise progression and fluctuating course. Nevertheless this result was only relative and, in the same way as observed in post mortem studies, not all the patients with ischemic lesions showed this pattern. By considering the two groups of patients as a whole and by recoding the HIS into two categories of analysis (cut-off point <4), the total score in the selected neuropsychological tasks was significantly different between patients with degenerative dementia, and patients with vascular-mixed dementia according to the HIS[7]. In summary, we observed that when dementia was present the more were the ischemic lesions, the more was the impairment in severity and progression[8]. Considering our results and the new conceptual trends about mixed dementia, it could be proposed that patients with evident dementia and higher HIS scores are diagnosed as patients with mixed dementia (not VD). The current notion that most patients with late-onset dementia would have a mixture of cerebrovascular and AD-type lesions, among others lesions, [2, 5, 25, 26] could be applied to this sample of 70-year-old participants because only a minority of them (12%) were apparently suffering from mixed dementia.

Although our study paid more attention to the method than to the specific results, those results were in agreement with current knowledge. Thus, those results represented a positive finding for the conceptual validity of the method.

Along with the current knowledge, a question that remains to be answered is whether the pattern observed in patients with ischemic (or more) lesions should be diagnosed as mixed dementia or just as “worse” dementia.

If AD+VD condition seems to be the most common type of dementia in postmortem studies, then that combined condition should be the first option to categorize dementia in antemortem studies. But in order to do that, and considering that dementia is not just an

[7] When the analysis was carried out with the three original HIS categories of interpretation not all the comparisons produced significant differences.

[8] We could not classify the patients with ischemic lesions as having mixed dementia because the current definitions by consensus specify that only VD shows rapid progression.

anatomical question, distinctive cognitive outputs should be empirically and consistently demonstrated for AD and VD. If not, may be we should only speak about old-age dementia or old-age cognitive impairment.

It is usually accepted that under a background of an aged and/or vulnerable brain, some minor environmental changes can cause dementia. It could as well be possible that under a background of stressful environmental conditions, an aged brain with minor lesions can generate other lesions as a defense (or reaction) against the environment[9]. Such defense and lesion interaction might at once generate a second stage in the process towards dementia. Most likely, an interaction between exogenous and endogenous conditions is responsible for accelerating the process towards dementia.

After hundreds of years of study, and the unsuccessful attempt to find "the cause" of dementia, maybe the best formula which can be formulated to decrease dementia risk is "the better the quality (and the fairer the quantity) of life, the better the preservation of the brain".

Considering the factors associated to a better quality of life [2], there are some questions difficult to answer.

For example, which is the boundary between a challenging occupation and stress, between a proper amount of leisure and lack of stimulation, or between happiness (with the preservation of some human "defects") and neurosis (with an obsessive care for everything)?

Besides, in the attempt to reach a better quality of life, the technological and scientific progress has led us to a conflict with nature. While other species try to adapt their wishes to nature, human beings try to adapt nature to their wishes thus producing new problems and diseases. Science is part of our human essence but listening to nature is part of our living essence. Maybe as a result of this unsolved conflict, the more we want to control the time, the more we are burdened by the time.

Psychology rests on two big pillars: the social and the biological. So, in the same way as biological and psychological sciences have these days been joined in the neurosciences, social and biological sciences should also be joined. Going a step further and considering the environment in its broadest sense, the whole planet can be seen as a living organism. Human long-population and over-population seem to be serious problems in this organism. How could we unravel the human paradoxes and convert science in common well-being?

"Senile dementia" always existed but at the present time has expanded so much that we are anxious trying to find its cure. At the same time, millions of persons located throughout the population pyramid could be saved from suffering preventable or already curable diseases. According to new dementia studies (and only for people who can survive until old age) reducing the number of mid-life diseases may result in a lower incidence of dementia (and thus in more life). Nevertheless, what is the point of having one organ of the body in perfect health when the rest of the organs are suffering? Being healthy at the end of life may be important (if this sentence has any sense at all) but being healthy during "all the life" including "the lives of all " is much more important. One thing would not exclude the other[10].

Similarly, human overpopulation and the consequent devastation of everything in its path can be compared to the behavior of a high-grade malignant tumor which only will see its end when its own environment ends. Nevertheless, and considering that the higher the

[9] A long life can become traumatic if the environment becomes every time more complex and the resources to deal with the environment become every time more reduced.

[10] Actually, if one thing excludes the other we are artificially bringing about a contradiction

socioeconomic background, the lower the number of children a woman will be willing to procreate, human overpopulation could immediately be defeated by homogeneously raising the living standard of everybody in the world (i.e., by distributing the "best contraceptive" which human beings have ever invented). Essentially, this is not a question of money but goodwill, specifically, a question of human behavior (the root of most of our problems).

The paradoxical and uncontrollable technological advance that we are going through is a consequence of human intelligence[11]; as well, overpopulation is a consequence of human's dominion over other species. What good are both potentials if they are not harmonically intertwined? Could our potentials be our own condemn? Will we become our own predators by developing self-destructive and unnatural behaviors?

Intelligence (wisdom) is also the ability to find a balance.

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Chapter IX

Biofluid Protein Biomarkers for the Diagnosis of Vascular Dementia

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Abstract

Vascular dementia (VaD) is a heterogeneous disease including several different vascular mechanisms in the brain as well as various clinical manifestations.

Biomarkers to aid in the early and precise diagnosis of vascular dementia (VaD) are in great need. The differential diagnosis between vascular dementia and Alzheimer's disease (AD) or mixed dementia is not always easy in clinical practice. There can also be overlap in symptoms with other neurodegenerative diseases, especially in the early phases of the disease. Biochemical diagnostic markers, which reflect the pathogenetic processes in the brain, would add to the accuracy of the diagnosis for the differentiation between VaD and healthy aging but also for the differential diagnosis between VaD, AD and other neurodegenerative disorders.

This chapter examines the biofluid markers that have been described in the literature as potential markers for VaD.

Cerebrospinal fluid (CSF) levels of amyloid beta 1-42, tau and phosphorylated tau are used routinely to aid in the diagnosis of AD. These markers reflect plaque and tangle pathology and have also shown promise in the differentiation between AD and VaD.

Furthermore, an elevated CSF/serum albumin ratio has been described in VaD, probably reflecting the increased extent of blood-brain barrier damage in VaD.

Circulating inflammatory markers such as C-reactive protein and IL-6 are increased in plasma of VaD patients compared to both AD and healthy controls. In addition, blood levels of coagulation markers such as fibrinogen, factor VIII and fibrin D-dimer were increased in the same manner. Also, elevated levels of the amino acid homocysteine in plasma have been described in patients with VaD compared to both AD patients and healthy controls.

The current available clinical studies describe a wide array of promising markers. There are, however, limitations to many of these studies due to the heterogeneity of

definitions of VaD, small population sizes and differences in analytical methodologies. Ongoing and future validation studies will significantly narrow down the present list of markers with the most robust and reproducible remaining for possible implementation in clinical practice.

Biomarkers: Definition and Criteria

Biological markers or biomarkers can be defined as cellular, biochemical or molecular alterations that are measurable in biological media such as human tissues, cells or fluids. In clinical practice, biomarkers may be used for understanding the prediction, cause, diagnosis, progression or outcome of treatment of a disease [1].

The Working Group on Molecular and Biochemical Markers of Alzheimer's Disease proposed the following criteria for the ideal AD biomarker; these criteria can also be applied to biomarkers for VaD. "The ideal biomarker for AD should detect a fundamental feature of neuropathology and be validated in neuropathologically-confirmed cases; it should have a sensitivity > 80% for detecting AD and a specificity of > 80% for distinguishing other dementias; it should be reliable, reproducible, non-invasive, simple to perform, and inexpensive." [2].

Vascular dementia denotes the development of dementia as a result of vascular lesions in the brain. In principle, the vascular lesions may be ischemic, hemorrhagic or a mixture of both. Dementia may result from a single strategic lesion, multiple lesions or ischemic changes with a more diffuse location in the brain. The diagnosis of vascular dementia is based on the clinical criteria for dementia, in the presence of neuroradiological evidence for vascular lesions, relevant in extent and location, and with a time- and cause relationship between development of vascular lesions and clinical symptoms. Several versions of diagnostic criteria are applied in clinical practice (ICD-10, DSM-IV, NINCDS-AIREN).

As vascular lesions are also seen on cranial CT or MRI in healthy elderly subjects and in patients with other neurodegenerative disorders, the differentiation of VaD from normal aging and AD is challenging. In a substantial fraction of patients with dementia, the cause of dementia is mixed, with both AD and vascular changes playing a significant role.

The development of VaD biomarkers has obvious implications for early diagnosis and may change our clinical definition of the onset of the VaD disease process. Perhaps more importantly, these same markers may also serve as targets for new therapeutic drug development, provide a useful measure of drug efficacy in future longitudinal trials or serve to define target populations for clinical trials.

Biomarkers in CSF

The cerebrospinal fluid (CSF) fills the ventricles and surrounds the external surfaces of the brain, bathing and protecting it [3]. CSF is mainly produced by the choroid plexus and is directly connected to the extracellular fluid. The extracellular fluid surrounds the neurons and glia, and CSF is therefore believed to reflect brain metabolism [4]. In the adult human the total volume of CSF is approximately 140 mL and is produced at a rate of 500 mL per 24 hours [5]. The protein concentration of CSF is roughly 100 times lower than serum, 300-500

mg/L. It is considered that 80% of this protein content derives from blood (through the choroid tissues) and 20% originates directly from the CNS [6].

Changes in CSF composition depend on the blood proteome, CSF circulation alterations, as well as physiological or pathological CNS mechanisms.

Analysis of proteins in the CSF drawn by lumbar puncture (LP) is becoming increasingly important as a diagnostic tool [7]. Furthermore, CSF is a relevant biological material for discovering new biomarkers for diagnosis, prognosis and treatment response in neurological diseases in clinical practice as well as in clinical trials, because of its proximity to the brain.

Lumbar puncture, the standard method for obtaining CSF for biochemical analysis, is a straightforward procedure, associated with minimal discomfort and low (1-2%) incidence of post-LP headache in individuals above the age of 60 [8].

Several preanalytical variables can influence the protein and peptide composition of CSF.

- CSF composition of proteins and other molecules varies with its collection site ventricular or lumbar [9] and also from pre- or post-mortem collection.
- It is recommended to obtain and store CSF in polypropylene tubes, as some CSF proteins such as Amyloid β, tend to adsorb to other plastic surfaces and to glass [10].
- Blood contamination can result in misleading profiling of blood proteins and therefore skewed interpretation of data. Therefore, samples should not contain more than 500 erythrocytes pr μl.
- CSF should be centrifuged to eliminate cells and other insoluble materials and aliquoted within 4 hours after sampling, and the samples should be stored at –80^{o}C [11]. Degradation of some proteins at -20^{o}C has been described [12].
- Addition of protease inhibitors is not necessary due to the short time before freezing of the samples [13]. Also, protease inhibitors can cause artefacts when analysing samples by mass spectrometry [13].
- Repeated freeze/thaw cycles can harm the CSF proteins so aliquoting in several portions is advisable.

Potential Biomarkers in CSF

Amyloid B_{1-42}, Tau and Phosphorylated Tau

CSF levels of Amyloid β_{1-42}, Tau and Phosphorylated Tau are used routinely for the diagnosis of AD and for differential diagnosis from other neurodegenerative diseases. These markers have been recommended as part of the new research criteria for the diagnosis of AD [7].

Amyloid β_{1-42} ($A\beta_{1-42}$) is a 42 amino acid proteolytic peptide from the amyloid precursor protein and the major component of plaques in the brains of patients with Alzheimer's disease. Substantially decreased CSF levels of $A\beta_{1-42}$ have been described in AD as well as Creutzfeldt-Jakob disease (CJD) and amyotrophic lateral sclerosis. In VaD CSF levels of $A\beta_{1-42}$ are mildly to moderately decreased [14].

Amyloid β_{1-40} is the most abundant Amyloid β peptide in the brain. The cerebrospinal fluid (CSF) level of Amyloid β_{1-40} might therefore be considered to most closely reflect the

total Amyloid β load in the brain. CSF aamyloid β_{1-40} levels have been found to be significantly lower in VaD compared to AD [15] and the $A\beta_{1-42}$/ $A\beta_{1-40}$ ratio was the best measure to separate VaD from AD.

Tau is a microtubule-associated protein, primarily located in the neuronal axons. Increased CSF tau protein levels are believed to reflect the intensity of axonal degeneration. In acute conditions such as stroke, there is a marked transient increase in CSF tau that shows a correlation with infarct size [16]. Increased levels of CSF tau have been reported in AD, CJD, and other CNS disorders with neuronal degeneration or damage. Probably due to the axonal degeneration in all these diseases making CSF tau levels a marker for general neurodegeneration. In VaD there have been discrepant results, as some studies found high CSF tau levels but others did not [17;18]. One way to interpret these findings is that a high level of CSF tau in patients with clinical and brain imaging findings indicative of VaD suggest that these patients may have mixed (AD/VaD) dementia.

There are at least 30 phosphorylation sites on the tau protein. The concentration of phosphorylated Tau protein in CSF probably reflects the phosphorylation state of Tau in the brain. There is no increase of phosphorylated Tau in neither stroke or Creutzfeldt-Jakob disease [19]. Elevated levels of CSF phospho-tau are seen in AD but normal levels are seen in other neurodegenerative diseases such as Parkinson's disease, frontotemporal dementia, and dementia with Lewy bodies as well as in VaD [14].

Neuron-Specific Enolase

Neuron specific enolase (NSE) is located in the cytosol of neurons and increased levels of NSE probably reflect ongoing neuronal cytoplasmic damage and neurodegeneration [20].

Levels of NSE in CSF are significantly increased after ischemic stroke and levels correlate with infarct size. Furthermore, NSE levels were also increased in patients with multi infarct dementia compared to controls [21].

One group has described lower CSF levels of NSE in VaD than AD and controls [22], however an other group showed that CSF NSE levels were significantly higher in both AD and VaD compared to healthy controls [23]. Perhaps this discrepancy arises from different diagnostic criteria for VaD, mixed VaD/AD pathology in some patients as well as different analytical methods. More studies are needed to elucidate the value of this marker for diagnostic purposes.

Neurofilament Protein

Neurofilament proteins are elements of the neuronal cytoskeleton and they are involved in maintaining neuronal morphology and neuronal transport. Neurofilaments are composed of three subunits based on the molecular weight, termed high (NF-H), medium (NF-M), and light (NF-L) [24].

The light subunit of the NF protein (NF-L) is mainly localized in the large myelinated axons and the CSF level of NF-L correlates with the degree of white matter changes in the

brain. Levels of CSF NF-L are highly elevated in VaD and also in FTD but only moderately increased in AD [25].

One study also found significantly elevated levels of NF-H in VaD patients compared to controls [26].

The NF-H chain is the most extensively phosphorylated protein in the human brain. Both NF-M and NF-H become highly phosphorylated post-translationally after being transported from the neuronal cell soma to the axon [24]. About 80% of axonal NF are highly phosphorylated and the relatively small (~20%) dynamic pool of non phosphorylated NF is involved in ante- and retrograde axonal transport [27]. Investigations of the potential of these phosphorylated forms of NF to differentiate between different neurodegenerative diseases are ongoing.

Tumor Necrosis Factor-α (TNF-α)

TNF- α is a pro-inflammatory cytokine, which has been shown to exert physiological, neuroprotective and neurodegenerative effects within the nervous system [28]. In the brain, macrophages as well as astrocytes and microglia have the capacity to synthesize TNF-α. TNF-α induces apoptosis in the oligodendrocytes producing myelin and mediates myelin damage in vitro [29]. Several studies have shown elevated CSF levels of TNF-α in VaD patients compared to patients with AD and healthy controls [30;31] supporting the TNF-α toxicity directed towards myelin producing oligodendrocytes.

Vascular Endothelial Growth Factor (VEGF)

VEGF is a glycoprotein, which acts as a highly specific mitogen for vascular endothelial cells capable of inducing angiogenesis. In addition, VEGF is a potent inducer of vascular permeability. The expression of VEGF is upregulated in glia by hypoxia, leading to a compensatory angiogenesis [32]. Increased CSF levels of VEGF have been described in patients with AD and VaD [33]. This supports the idea that vascular factors may play a role in both these diseases.

Transforming Growth Factor-β (TGF- β)

TGF- β is a pleiotropic cytokine, whose cellular site of synthesis and targets are widely distributed throughout the body, including the CNS. Within the CNS, TGF- β is produced by both glial and neuronal cells. TGF- β acts as an anti-inflammatory cytokine, inhibiting the production of the pro-inflammatory cytokines such as TNF-α and IL-6 discussed elsewhere in this chapter [34].

Increased CSF levels of TGF- β have been described in patients with VaD and AD when compared to healthy controls. This finding corroborates the notion that TGF- β is elevated as a response to overproduction of inflammatory cytokines [33].

Alpha- and Gamma-Synuclein

α-synuclein is a cytosolic protein present in the pre-synaptic terminals of almost all types of neurons in the central nervous system. α-synuclein is involved in the regulation of vesicular release, synaptic function and as a molecular chaperone. Over expression of α-synuclein has been described in Parkinson's disease and dementia with Lewy bodies.

γ-synuclein is primarily found in the peripheral nervous system and retina and its function is largely unknown. Over expression studies have shown a possible involvement of γ-synuclein in neuropathophysiological changes and the death of susceptible neurons. Furthermore, γ-synuclein is a marker of tumor progression in breast and colorectal cancer [35]. One study has described elevated levels of both α- and γ-synuclein in the CSF of patients with VaD compared to healthy controls and patients with AD [36] although more studies are needed to validate these findings.

Sulfatide

Sulfatide is the major glycospingolipid in the myelin sheath, which is formed by oligodendrocytes, but it only constitutes a minor component in other brain cells. Degeneration and increased turnover of myelin increase the shedding of membrane fragments, whereby the concentrations of sulfatide are elevated in CSF [37]. Significantly increased CSF levels of sulfatide have been found in patients with VaD compared to both patients with AD, healthy controls and patients with normal pressure hydrocephalus [38;39] suggesting continuous progression of the VaD pathology even if symptoms progress in a stepwise manner.

Biomarkers in Plasma

The search for plasma biomarkers for neurodegenerative diseases has not been as easy as it has been for disorders of other tissues – notably the heart, liver and skeletal muscle. One of the reasons is the presence of the blood brain barrier. The blood brain barrier impedes the transfer of markers from the brain into the bloodstream while the relatively large size of the systemic circulation acts to dilute any marker released [40].

As with CSF, several preanalytical variables can influence the protein and peptide composition of plasma and serum:

- There are differences between protein content and composition when using different anticoagulants such as EDTA, citrate and heparin [41].
- Samples should be aliquoted and stored at - 80^{o}C as soon as possible. Serum proteins start to degrade after 30 minutes but plasma proteins are stable for up to 4 hours [41].
- Great care to avoid hemolyzed samples should be taken, as hemoglobin contamination may cause artifacts in several analyses [42].
- Repeated freeze/thaw cycles can disturb the blood proteome so aliquoting in several portions is advisable [42].

Potential Biomarkers in Plasma

C-Reactive Protein

C-reactive protein (CRP) is primarily synthesized in the liver as part of a coordinated response known as the acute-phase response. In addition, CRP is also synthesized in atherosclerotic tissues [43].

CRP is a sensitive marker of systemic low-grade inflammation. It has been identified as an independent predictor of clinical endpoints, such as myocardial infarction and stroke [44]. Serum CRP has been linked to an increased risk of VaD and AD. These relations were independent of cardiovascular risk factors and disease [45]. Furthermore, high levels of serum CRP have been found to correlate with memory decline and cerebral small vessel disease in community-dwelling and stroke-free elderly individuals [46].

Interleukin-6

Interleukin-6 (IL-6) is a pro-inflammatory cytokine. In the CNS IL-6 is primarily synthesized by astrocytes and exerts multiple functions, which are both neuroprotective and neurodegenerative such as playing an important role in neurogenesis and synaptic plasticity [28].

Elevated levels of plasma IL-6 have been described in patients with VaD compared to patients with AD and healthy controls [47].

Fibrinogen and Fibrinogen Degradation Product

Fibrinogen is a precursor of fibrin and essential component of the coagulation system.

Fibrinogen degradation product (FDP) is a breakdown product formed when plasmin acts on fibrin. High FDP levels correlate with myocardial infarction and stroke [48;49]. Significantly elevated levels of FDP have been described in plasma of patients with VaD compared to both patients with AD and healthy controls [47].

S100B

S100B is a calcium binding protein, and is primarily expressed by astroglia in the brain. This protein may play a dual role in the regulation of cell function, being beneficial to cells at low doses but detrimental at high doses [50]. Structural brain damage causes leakage of S100B into the CSF and blood. A significant correlation has been reported between the plasma and CSF concentrations of S100B protein and the volume of a cerebral infarct [51]. Decreased serum levels of S100B have been described in AD [52], but results for VaD are still scarce. One study described, that high levels of S100B were correlated to poorer cognitive function in neurologically healthy older adults [53], indicating the need for further studies of this protein.

Heat Shock Protein 70

Heat shock proteins are molecular chaperones involved in the folding and degradation of damaged proteins. They interact with multiple components of signalling pathways that regulate cell cycle and inflammation. The expression of heat shock protein 70 (Hsp70) is highly induced in glial cells and neurons following a wide range of noxious stimuli, including ischemia, epilepsy, and hyperthermia as well as other processes associated with cellular resistance to a variety of insults [54].

Plasma levels of Hsp70 were significantly higher in VaD patients compared to patients with AD, MCI and healthy controls [55] suggesting that plasma Hsp70 levels may be related to vascular factors or inflammation.

Tissue Inhibitor of Metalloproteinases-1

Tissue inhibitors of metalloproteinases (TIMPs) are a family of four proteins, TIMP-1 to TIMP-4, that are capable to form inhibitory complexes which inhibit matrix metalloproteinase activity, specifically degradation of the extracellular matrix [56]. Plasma levels of TIMP-1 have been found to be significantly decreased in patients with VaD compared to patients with AD, MCI and other neurodegenerative disorders [57]. This decrease could indicate a failure of regulatory systems resulting in inflammatory brain lesions.

CSF/Serum Albumin Ratio

The integrity and function of the blood-brain barrier (BBB) is evaluated by determining the CSF/serum albumin ratio. An increased CSF/serum albumin ratio indicates reduced BBB function or damage and the ratio increases together with the severity of white matter changes in the brain [58]. Several studies have shown significantly elevated albumin ratios in patients with VaD compared to AD and healthy controls [58-60].

Homocysteine

Homocysteine is a nonessential thiol amino acid biosynthesized from methionine. Plasma homocysteine levels are determined by both genetic and nutritional factors. Homocysteine elevation is associated with microglia activation and proliferation together with immune activation [61]. A relationship between elevated plasma homocysteine and decreased cognitive performance has been reported in the normal aging population as well as in patients with AD and VaD [62]. Several studies have indicated that plasma homocysteine concentrations showed the highest increase in patients with VaD compared to patients with AD, depression or healthy controls. However, after exclusion of renal and vascular disease in all groups it was found that plasma homocysteine concentrations in elderly patients was mainly attributed to the presence of vascular disease and not related to the specific psychogeriatric diagnosis [63].

Conclusion

Vascular dementia (VaD) includes several different vascular mechanisms and changes in the brain, and has different causes and clinical manifestations. Critical to its conceptualization and diagnosis are definitions of the cognitive syndrome, vascular etiologies, and changes in the brain. Variation in these has resulted in different definitions of VaD, estimates of prevalence, and types and distribution of brain lesions. This heterogeneity in definitions may contribute to the negative results in biomarker studies. Furthermore, mixed forms of AD and VaD are very frequent, and there is a risk that markers for AD pathology are misinterpreted as markers for VaD.

Great care in patient selection for both biomarker studies and clinical trials in VaD is imperative for a better understanding of the role of biomarkers in future studies.

As described in this chapter, there are abundant hypothesis generating studies that have resulted in the identification of potential biomarkers that could aid in the diagnosis of VaD. However, differences in the operationalization of diagnostic criteria, different assays and different cut off values for the potential biomarkers make study-to-study comparisons difficult.

Taken together, the potential markers described here show that both inflammatory and degenerative processes are present in VaD, which is also confirmed by neuropathological studies.

In order to improve the performance of the candidate markers, a panel of markers will most likely provide better sensitivity and specificity than any one marker alone.

Validation studies in large well-characterized patient cohorts are necessary to narrow down the present list of candidate markers and to investigate their diagnostic performance not only between patients with VaD and healthy controls but also between patients with VaD, AD and other neurodegenerative diseases.

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Chapter X

Apathy Associated with Subclinical Brain Ischemic Lesions and Vascular Risk Factors

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Introduction

Behavioral and psychological symptoms such as apathy negatively affect cognitive performance, mood, functional status, and prognosis of patients with dementia. Apathy appears to be common in many diseases of the brain, and is associated with distress in caregivers. Apathetic behavior of minor degree seems less problematic than other agitated behaviors such as irritability and restlessness. Nonetheless, apathy has a significant impact on quality of life and activity of daily living in patients with cognitive impairment. Although apathetic behavior in healthy elderly subjects has not been paid much attention until recently, brain magnetic resonance imaging (MRI) studies revealed that silent or subclinical brain ischemic lesions are the basis for apathetic behavior in elderly subjects without dementia. Furthermore, vascular risk factors such as hypertension or vascular diseases per se are known to produce apathy in old age. In this short review, we will discuss about the relationship between apathy and vascular factors in healthy elderly subjects.

Apathy, a Neuropsychiatric Syndrome

Conventionally, apathy is defined as a lack of interest or emotion. The syndrome of apathy is defined as primary absence of motivation, i.e., lack of motivation not attributable to disturbance of intellect, emotion, or level of consciousness [1]. Apathy is a symptom of

dimension of behavior. Abulia or minor degree of akinetic mutism reflects severe apathy. The reliability, internal consistency, and validity of the Starksein's apathy scale were shown for the first time in Parkinson's disease [2]. Also, apathy is frequently evaluated with the apathy items of Neuropsychiatric Inventory [3, 4]. Van Reekum et al. [5] reviewed that apathy appeared very common (60.3%) in Alzheimer's disease (AD) outpatients, less common (32.8%) in AD from the community, and 33.8% in vascular dementia. With the use of 4-item apathy subscale of Hamilton rating scale for depression and the Executive Interview, Marin et al. found that apathy and executive cognitive function was independent of each other in subjects with late-life depression [6]. The frequency of apathy varied across diagnostic groups: many AD, frontotemporal dementia, and progressive supranuclear palsy patients had apathy without depression, while many Parkinson's disease and Huntington's disease patients had depression, and apathy did not correlate with depression in the combined sample of these dementia patients [**7**]. Fones argued that the common overlapping clinical features of apathy with depressive disorder should not justify it being subsumed under the rubric of depression [8]. Apathy, a disorder of motivation rather than mood, should be considered to be a specific neuropsychiatric syndrome that was distinct from depression. In addition to dementia illness with degenerative causes, the working group of Vascular Cognitive Impairment Harmonization Standards recommended that measure of apathy would be important to include, detecting and quantifying apathetic personality changes in subjects with cognitive impairment of vascular origin, which are rather common early manifestations of subcortical vascular disease [9].

Apathy is one of the most common neuropsychiatric symptoms present in 3.2% of general population, 14.7% of subjects with mild cognitive impairment, and 35.9% of subjects with dementia in the Cache County study [10]. Steinberg et al. reported that 69% developed at least one mental or behavioral symptom(s), such as delusion (28%), apathy (21%) and aberrant motor behavior, during 18-months interval in a population-based sample of 355 residents with dementia of Cache County [11]. The results of the Nakayama study replicate findings of the Cache County study: 88.3% of demented community-dwelling subjects had one or more behavioral and psychological symptoms of dementia, and the most common symptoms was apathy/indifference (56.7%) [12]. Participants with Alzheimer's disease were more likely to have delusion, and participants with vascular dementia were more likely to have depression [13, 14]. With regard to vascular dementia patients, apathy was an independent factor associated with functional independence beyond general cognitive abilities [15].

Levy and Dubois defined apathy as a quantitative reduction of voluntary, goal-directed behaviors [16]. They classified apathy as three subtypes as follows: (1) Apathy due to the disruption of 'emotional-affective' processing refers to the inability to establish the necessary linkage between emotional-affective signals and the ongoing or forthcoming behavior. The responsible lesions may be the orbital-medial prefrontal cortex or to the related subregions (limbic territory) within the basal ganglia (e.g. ventral striatum, ventral pallidum). (2) Apathy due to the disruption of 'cognitive' processing refers to difficulties in elaborating the plan of actions necessary for the ongoing or forthcoming behavior. It may be related to lesions of the dorsolateral prefrontal cortex and the related subregions (associative territory) within the basal ganglia (e.g. dorsal caudate nucleus). (3) The disruption of 'auto-activation' processing refers to the inability to self-activate thoughts or self initiate actions contrasting with a relatively spared ability to generate externally driven behavior. It is responsible for the most

severe form of apathy and in most cases the lesions affect bilaterally the associative and limbic territories of the internal portion of the globus pallidus. It characterizes the syndrome of 'auto-activation deficit' (also known as 'psychic akinesia' or 'athymhormia'). This syndrome implies that direct lesions of the basal ganglia output result in a loss of amplification of the relevant signal, consequently leading to a diminished extraction of this signal within the frontal cortex.

Apathy Following Stroke

Starkstein et al. systemically examined the presence and severity of apathy in patients with stroke [17]. Apathy was measured with an abridged version of Marin's apathy scale (the Starkstein's apathy scale). Based on the structured psychiatric examination, 18 of the 80 patients (23%) showed apathy, 9 of whom were also depressed, and additional 18 patients (23%) showed depression in the absence of apathy. Apathy was significantly associated with lesions in the posterior limb of the internal capsule. Mayo et al. examined 408 stroke survivors assessed by their caregivers at 1, 3, 6, and 12 months after stroke: 20% scored in the clinically relevant apathy range, and 8% was concordant for both depression and apathy [18]. High apathy had a significant negative effect on physical function, participation, health perception, and physical health over the first 12 months after stroke, and even minor apathy had a strong negative association with recovery from stroke. The Shimane group used the Starkstein's apathy scale in Japanese translation, and found a reduced regional cerebral blood flow (cortical flow measured with the 133Xe inhalation method) in the right dorsolateral frontal and left frontotemporal regions in serial 40 patients with subcortical infarction [19]. Recently, they observed apathy in 37 of 102 (36%) consecutive patients with brain infarction: the apathy group showed lower cerebral blood flow, determined with quantitative single-photon emission CT, in the bilateral basal ganglia [20]. They also reported in 29 patients with subcortical ischemic stroke, apathy was associated with impaired neural processing of novel events determined with event-related evoked potential (novelty P3) within the frontal-subcortical system [21]. In 100 consecutive patients with anterior cerebral artery infarction, hypobulia/apathy (n=43) was related to involvement of frontal pole, corpus callosum/cingulated gyrus, and superior frontal gyrus, and occurred more frequently with bilateral lesions [22]. Apathy is also caused by strategic infarcts. Acute capsular genu infarction led to an acute confusional state with fluctuating alertness, inattention, memory loss, apathy, abulia, and psychomotor retardation due to presumed disconnection of thalamo-frontal pathway [23]. The acute bilateral infarction of anterior thalamus caused a severe perseverative behavior apparent in thinking, spontaneous speech, and all memory and executive tasks: some dysexecutive features were present in all patients, usually with apathy [24]. In a case of loss of psychic self-activation or apathy after paramedian bithalamic infarction, single-photon emission CT suggested that the neurobehavioral syndrome was most likely caused by disruption of the striatal-ventral pallidal-thalamic-frontomesial limbic loop [25].

Apathy Associated with Subclinical Brain Ischemic Lesions

Recently, we examined effects of vascular risk factors and silent ischemic brain lesions on an apathetic mental condition in community-dwelling elderly subjects [26]. Briefly, brain MRI and other medical examinations were performed on 222 non-demented community-dwelling elderly subjects (96 males and 126 females, average age 70.1 years). The apathy group was defined as the most apathetic quintile determined by an analogue visual version of Starkstein's apathy scale (Figure 1).

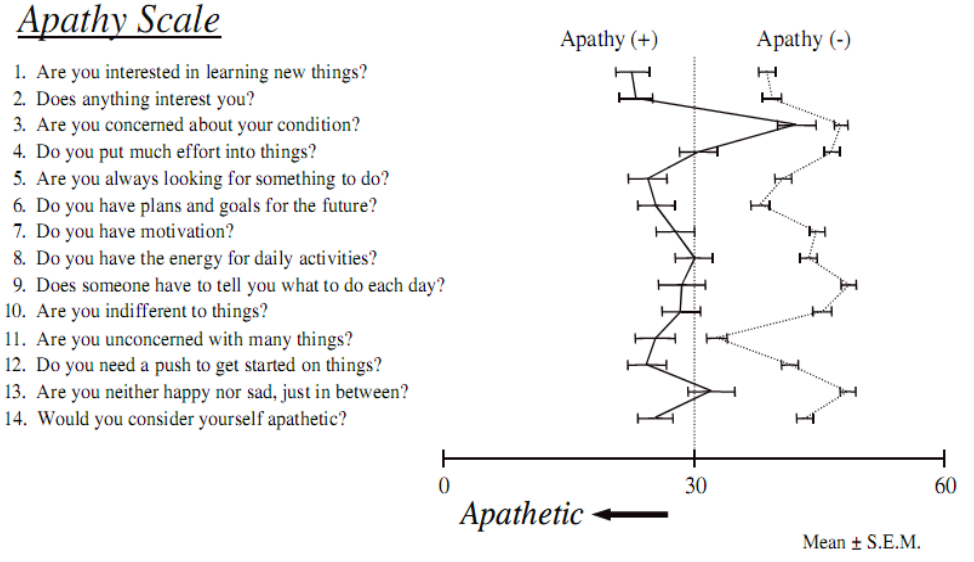

Figure 1. Each item of the Starkstein's apathy scale was quantified on a visual analogue scale where one end of a 60-mm-long line is "absolutely correct" and the other end is "completely wrong". Participants marked where it is appropriate for each question, and then the distance was measured and counted as a score. We used the original arrangement where the directions of apathetic state were changed between the questions No. 8 and No. 9, but this figure shows left side as apathetic. The scores range from 0 to 720; lower scores indicate more severe apathy. Among 14 questions of the apathy scale, item-total correlations of the questions No. 3 and No. 11 were weak (r=0.45 and 0.46, respectively); other questions showed better correlation (r=0.55 - 0.72) with total score. Therefore, we excluded scores of these two questions from the analysis. The subject was judged as having apathy if the total score was below the fifth quintile. Because the apathy group was operationally defined by the self-rating apathy scale, and the clinical diagnosis of apathy was not confirmed by using a psychiatric structured interview. we asked 5 experienced psychiatrists how an assumed "average" apathetic patient with moderate symptoms would score this apathy scale. The upper limit (i.e., mean+2S.D.) of the 5 scores was 320, and the mean score of the apathy group was 321±46 (S.D.) in our study. Therefore, we could have detected mild apathetic behavior in healthy elderly subjects, using the apathy scale modified as analogue visual scale. (Reproduce from Yao H, et al., Hypertens Res 2009;32:586-590).

The combination of T1-weighted images (T1WI), T2-weighted images (T2WI), and fluid-attenuated inversion recovery (FLAIR) images is required to accurately detect both silent brain infarction and mild white matter lesions (WMLs) [27]. The WMLs were defined as isointense with normal brain parenchyma on T1WI, and high signal intensity areas on

T2WI. We used the validated rating scale of deep white matter lesions (DWMLs) by Fazekas et al: Grade 0, absent; Grade 1, punctate foci; Grade 2, beginning confluence of foci; Grade 3, large confluent areas [28] (Figure 2). Silent infarction, DWMLs, and periventricular hyperintensities (PVHs) were detected in 12.2%, 39.2%, and 22.5%, respectively. Linear regression analysis (Pearson) revealed that the scores of apathy scale correlated slightly but significantly with logarithmically transformed scores of the Modified Stroop Test (r=0.135, p=0.045), but not with the Mini-Mental State Examination (MMSE). The apathy group tended to have more high blood pressure (141.6/82.6 mmHg vs. 136.1/79.6 mmHg), less prevalent hyperlipidemia (18% vs. 35%), and lower serum albumin. Multivariate analysis (the forward stepwise method of logistic analysis) revealed an independent correlation between the apathy and the grade of DWMLs (OR 1.826, 95%CI 1.129-2.953 per grade) or diastolic blood pressure (OR 1.055, 95%CI 1.014-1.098 per mmHg) after adjusting for possible confounders. The mean of apathy scale in the diastolic blood pressure≧90 mmHg group was significantly lower (more apathetic) than that in the diastolic blood pressure＜80 group (p=0.011, ANCOVA). The present study showed that hypertension and DWMLs are independently associated with apathy in healthy elderly subjects.

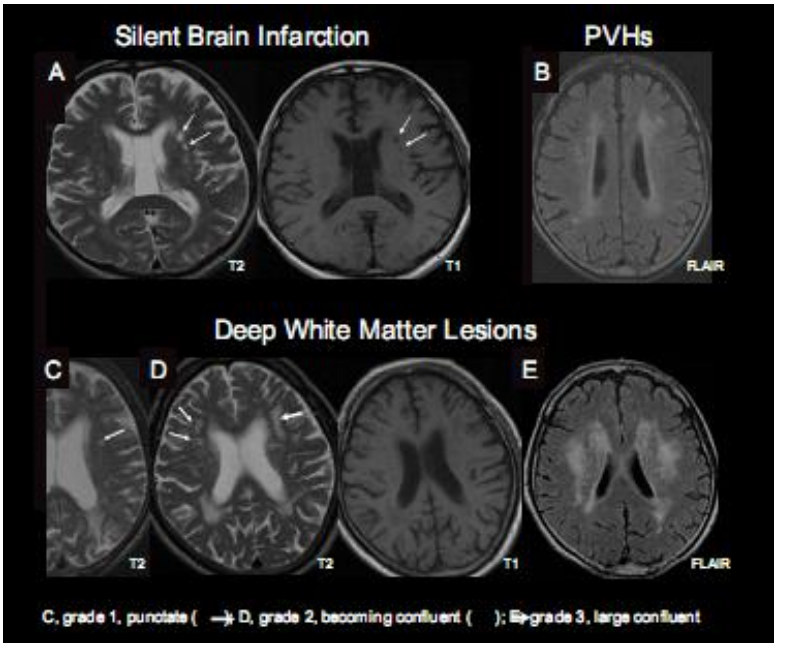

Figure 2. MRI of silent brain infarction (A), periventricular hyperintensities (PVHs)(B), and deep white matter lesions (C, D, and E). The combination of T1WI, T2WI, and FLAIR images is required to accurately detect both silent brain infarction and mild WMLs. Therefore, T1-weighted (TR/TE = 510/12 msec), T2-weighted (TR/TE = 4,300/110 msec), and FLAIR (TR/TI/TE = 6,750/1,600/22 msec) images were obtained with a slice thickness of 6 mm with a 1 mm interslice gap with a brain MRI (1.0T, Shimadzu, Magnex XP, Kyoto, Japan). Brain infarcts were shown as low signal intensities on T1-weighted images, and their size was 5 mm or larger. The WMLs were defined as isointense with normal brain parenchyma on T1-weighted images, and high signal intensity areas on T2-weighted images. We used the validated rating scale of DWMLs by Fazekas et al: Grade 0, absent; Grade 1, punctate foci; Grade 2, beginning confluence of foci; Grade 3, large confluent areas [28]. For PVHs, we determined the presence and severity (Grade 0, absent; Grade 1, pencil thin; Grade 2, smooth halo lining) using FLAIR images.

Several, not many authors have reported the relationship between apathy and subclinical brain ischemic lesions on MRI (Table 1). Lavretsky et al. emphasized the importance of lacunar infarction in community-dwelling older adults as the pathogenesis of late-life neuropsychiatric disorders such as depressed mood, anhedonia, anergia, and apathy [29]. The same authors reported that subjects with both lacunes and depressed mood had the shortest survival among 498 participants recruited from the community [30]. Other reports, including our own, confirmed the association of white matter lesions and apathetic behavior in community-dwelling elderly subjects [26] or dementia patients [31][32]. In our experience, apathy was slightly but significantly associated with frontal lobe dysfunction but not with global cognitive function assessed with MMSE.

Table 1. Apathy associated with subclinical brain ischemic lesions on MRI

Authors / Year	Subjects	N	Age	Apathy scale	Findings
Lavretsky et al. 2008	Community-dwelling older adults	270	mean age 74.4 y.o.	Psychiatric Evaluation Section of the Minimum Uniform Dataset (MUDS)	**Lacunar infarct volume in white matter was associated with the presence of depressed mood, anhedonia, anergia, and apathy.**
Yao et al. 2009	Community-dwelling elderly subjects	22	mean age 70.1 y.o.	Visual analogue version of Starkstein's apathy scale	**Hypertension and deep white matter lesions were independently associated with apathy.**
Starkstein et al. 2009	Alzheimer's disease	9	mean age 71.9 y.o.	Starkstein's apathy scale	**Patients with apathy showed a significant larger volume of frontal white matter hyperintensities.**
Jonsson et al. 2010	Dementia (Alzheimer's diseas, vascular, mixed, mild cognitive impairment)	76	53 - 79 y.o. mean age 70.1 y.o.	Stepwise comparative status analysis (STEP)	**White matter changes in patients with demntia, irrespective of diagnosis, are related to broadly defined apathy.**
Lavretsky et al. 2010	Community Memory clinic	98	mean age 74.5 y.o.	Psychiatric Evaluation Section of the Minimum Uniform Dataset (MUDS)	**Participants with both lacunes and depressed mood had the shortest survival among all congnitive groups.**

In contrast, apathetic subjects were cognitively impaired on multivariate logistic regression analysis [29], or had lower MMSE scores (not significant) than those without apathy [31]. Our apathetic subjects were not depressed in terms of the similar prevalence of depressed mood and insomnia with non-apathetic subjects.

Subcortical vascular disease and DWMLs seen in community-dwelling subjects are associated with cognitive impairment or dementia, depression, and gait disturbance [33]. Likewise, multiple lacunes cause frontal lobe dysfunction including difficulty in shifting set, impaired executive functions, decreased verbal fluency, and apathy [34]. Small-vessel disease is the predominant cause of silent brain infarction and WMLs [35]. Patients with small-vessel vascular dementia (significant WMLs, multiple lacunes, and bilateral thalamic lesions) showed more apathy, aberrant motor behavior and hallucinations than patients with large-vessel vascular dementia (strategic large-vessel infarct, and bilateral hemispheric stroke) [36].

Disruption of the white matter tracts by WMLs and/or multiple lacunes between frontal cortex and relevant limbic input via basal ganglia may result in apathy. Apart from age, the main risk factors for WMLs are vascular risk factors particularly hypertension. Although age was the major factor concerning both deep white matter lesions and PVHs, hypertension was also but less robustly associated with white matter lesions (OR 1.6) compared with silent brain infarction (OR 3.2) [37]. In the PROGRESS study, the risk of new WMLs was reduced by 43% in the active anti-hypertensive treatment group with a reduced blood pressure by 11.2/4.3 mmHg compared with the placebo group [38]. Furthermore, diastolic blood pressure related with apathy independent of WMLs. Taken together, based on the facts of the treatable nature of hypertension and WMLs, it should be emphasized that apathy associated with hypertension and/or DWMLs in the general population is considered to be potentially preventable.

Vascular Risk Factors and Apathy

Hypertension is one of the major risk factors for vascular dementia or vascular cognitive impairment. Although the HYVET trial of lowering blood pressure in subjects aged 80 years or older was stopped early because of a substantial reduction in total mortality and stroke by the treatment, the meta-analysis of HYVET and three similar trials might support antihypertensive treatment to reduce the risk of incident dementia [39]. Epidemiological and clinical studies have established an association between cardiovascular disease and neuropychiatric symptoms such as depression and apathy. In a population-based sample of incident Alzheimer's disease, an exploratory analysis showed that vascular factors such as hypertension and history of stroke were associated with various neuropychiatric symptoms including apathy [40]. In a prospective, population-based study, van der Mast et al. investigated if vascular disease leads to apathy in old age [41]. The 15-item Geriatric Depression Scale (GDS-15) was administered to all subjects with a score≧19 points on the Mini-Mental State Examination. Three major sub-dimensions of the GDS-15 (i.e., apathy, general depressive affect, and satisfaction with life) were evaluated. Their results showed that vascular disease increased the risk of apathy and not depression in a community-based cohort of 85 years old subjects.

A number of cross-sectional studies reported an association between vascular disease and major depression in elderly subjects. However, longitudinal community-based studies have

not found a relationship between vascular risk factors or vascular disease and depression in the elderly. Alexopoulos et al. proposed that cerebrovascular disease might predispose, precipitate, or perpetuate some geriatric depressive syndromes (i.e., vascular depression hypothesis) [42][43]. Patients with vascular depression have common characteristics such as clinical and/or laboratory evidence of vascular disease or vascular risk factors, late-onset depression, cognitive impairment, psychomotor retardation, limited depressive ideation (e.g., guilt), poor insight, disability, and absence of family history of mood disorders. Older age, late onset depressive illness, lower risk of a family history or suicide, higher risk of anhedonia, and functional disability were evident in patients with vascular depression defined on the basis of MRI findings [44]. Elderly patients with depression have white matter hyperintensities more frequently than nondepressed patients. Interestingly, Thomas et al. carried out in vitro MRI study and neuropathological assessment of white matter lesions in 20 elderly subjects who had a history of major depression. They found that all deep white matter hyperintensities on MRI in the depressed subjects were rated as ischemic on the basis of increased macropahge/microglia and/or astrogliosis, and more likely to be in the dorsolateral prefrontal cortex [45]. The Rotterdam Study showed that subjects with atherosclerosis (i.e., severe coronary and aortic calcifications) were more likely to be depressed [46]. These findings strongly support the vascular depression hypothesis. However, in a prospective Leiden 85-Plus Study, although a higher generalized atherosclerosis rating was associated with an accelerated decline of immediate and delayed recall memory, there was no relation between the atherosclerosis and depressive symptoms [47]. In a similar context, the longitudinal PROspective Study of Pravastatin in the Elserly at Risk (PROSPER) Study showed no association between progression of white matter lesions and development of depressive symptoms in elderly subjects with evidence of vascular disease or at high risk of developing vascular disease [48]. Interestingly, the Cardiovascular Health Study found an "unexpected" but significant association between worsening white matter and use of tricyclic antidepressants, suggesting that worsening WMLs could be mediated in part through the side effects of antidepressants [49].

Conclusions

Subclinical ischemic brain lesions (i.e., diffuse white matter lesions and multiple lacunes) appeared to cause apathetic behavior in healthy elderly subjects as has been seen in patients with dementia. Vascular risk factors such as hypertension may aggravate apathy independent of MRI lesions. Future studies should determine whether treatment of vascular risk factors may lead to primary prevention of apathy associated with vascular risk factors and/or subclinical ischemic brain lesions.

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Chapter XI

Caudate Nucleus Volumes in the Leukoaraiosis and Disability in the Elderly Study (LADIS) – A Pilot Case-Control Study of Longitudinal Change

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Abstract

There has been recent interest in the basal ganglia as structural components of small-scale neural networks that may be strategically affected in cerebrovascular disease. The

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neural connectivity, blood and metabolic requirements of the caudate nucleus may make this structure particularly vulnerable to cerebrovascular disease related neurodegeneration. We have demonstrated cross-sectional caudate nucleus atrophy in a Stroke sample, and were interested to investigate whether longitudinal atrophy occurred in a cohort with cerebrovascular disease, to study the disease trajectory. Accordingly, we sought to compare two subsets of longitudinal epidemiological studies investigating confirmed cerebrovascular disease in the form of leukoaraiosis, and a group of healthy age-matched controls.

We manually measured caudate nucleus volume via MRI at baseline and at three year follow-up, in the Stockholm subset of the Leukoaraioisis and Disability in the elderly Study (LADIS). We found that normalized bilateral caudate volume was significantly smaller on follow-up in the LADIS group; and there was an approximately twice higher annual rate of atrophy in LADIS than in healthy age and gender matched controls from Stockholm (Swedish National Study on Aging and Care – Kungsholmen subset) without white matter disease. Leukoaraiosis may be associated with increased caudate nucleus atrophy, a finding which has implications for cognitive and behavioural functions served by this structure. In the context of previous findings of reduced caudate volume associated with white matter hyperintensities in the Sydney Stroke Cohort, these findings support a potential aetiological role for the caudate in cerebrovascular disease.

Introduction

Leukoaraiosis, an age-related rarefaction of white matter related to cerebrovascular disease appears as regions of white matter hyperintensity (WMH) on Fluid Attenuation Inversion Recovery (FLAIR) magnetic resonance imaging (MRI). Disruptions of white matter tracts by leukoaraiosis and cerebrovascular disease may cause neuroplastic change in the caudate (Hannestad et al., 2006, Looi et al., 2009a) affecting affecting frontostriatal circuits and cognition (Looi and Sachdev, 2000).

Frontostriatal circuits comprise a prefrontal region which sends efferent pathways through the neostriatum (caudate nucleus, putamen) or nucleus accumbens, via the globus pallidus, onto the thalamus, and thence to specific prefrontal cortex (Alexander et al., 1986). Such circuits include motor loops originating in the frontal eye fields and supplementary motor cortex, as well as "cognitive loops" arising from dorsolateral prefrontal cortex, anterior cingulate cortex and orbitofrontal cortex (Alexander et al., 1986). Frontostriatal dysfunction may result in characteristic cognitive and behavioral syndromes (Cummings, 1993) observed in vascular dementia (Looi and Sachdev, 2000). Damage to frontostriatal circuits may constitute a subcortical basis for cerebrovascular disease-related cognitive impairment and if, of greater severity, may result in vascular dementia (Looi et al., 2009b).

We have demonstrated that structural components of the frontostriatal circuits, such as the caudate, manifest neuroplastic atrophy due to cerebrovascular disease, and displaying a gradient with increasing disease severity (Looi et al., 2009b). Accordingly, we hypothesized that such changes should be observable in a longitudinal study of patients with established cerebrovascular disease, such as those persons with leukoaraiosis.

We assessed the feasibility of measuring caudate volumetric change in a pilot cohort with leukoaraiosis (LADIS) (Pantoni et al., 2004). We hypothesized:

1. That longitudinal caudate atrophy should manifest in a LADIS subset.

2. That the rate of atrophy would be higher than in normal aging of healthy age-matched controls drawn from the Swedish National study of Aging and Care – Kungsholmen (SNAC-K) (Lagergren et al., 2004).

Methods

Subjects: LADIS Participants

Subjects were drawn from the multicenter Leukoaraiosis and Disability (LADIS) study in which MRI were performed at baseline (2002) and at follow-up (2005) (Pantoni et al., 2004). 639 elderly subjects, who had no or only mild disability in their instrumental activities of daily living (IADLs); stratified for grade of WMH severity, were enrolled. Inclusion criteria were: age between 65 and 84 years; WMH on MRI of any degree, according to the categorization into the 3 severity classes of the Fazekas scale (Fazekas et al., 1987); no or only mild disability; a contactable informant; and informed consent. Major exclusion criteria were: presence of severe systemic, neurologic, psychiatric diseases and leukoencephalopathy of nonvascular origin.

LADIS MRI Acquisition

MRI scans were randomly selected scans of subjects from the local Stockholm-based LADIS cohort with both baseline and follow-up MRI scans completed.

All subjects were studied by MRI following a standard protocol on the same day as the clinical investigation. Thirty scans: 15 baseline and 15 follow-up of the same subjects were analyzed. The 1.5 T MR protocol included the following sequences: T1-weighted 3D MPRAGE (magnetization prepared rapid-acquisition gradient-echo, scan parameters: coronal or sagittal plane, field of view [FOV] 250 mm, matrix 256X256 or 512X512, slice thickness: 1 mm [isotropic voxels], TE: 2 to 7 ms, TR: 9 to 26 ms, FA 10% to 30%), T2-weighted FSE (fast spin echo, scan parameters: axial plane, FOV 250 mm, matrix 256X256 or 512X512, slice thickness: 5 mm, interslice gap 0.5 mm, TE: 100 to 130 ms, TR: 4000 to 8000 ms), and FLAIR (fluid-attenuated inversion recovery, scan parameters: axial plane, FOV 250 mm, matrix 256X256 or 512X512, slice thickness: 5 mm, interslice gap 0.5 mm, TE: 100 to 160 ms, TR: 6000 to 10000 ms, TI: 2000 to 2400).

Controls: SNAC-K

Fifteen healthy controls were drawn from the Swedish National Study on Aging and Care – Kungsholmen subset (SNAC-K) (Lagergren et al., 2004). SNAC-K is a random sample of 60+ year old institutional and community-dwelling individuals in the central Stockholm region of Kungsholmen assessed at baseline March 2001 – June 2004. A random sample had brain MRI scans performed at baseline and six year follow-up (2007-2008). Fifteen SNAC-K

subjects were selected from the cohort as controls, matching for age, gender, absence of white matter lesions on MRI, and availability of baseline and follow-up scans.

SNAC-K Image Acquisition

All controls were studied by MRI following a standard protocol on the same day as the clinical investigation. Thirty scans: 15 baseline and 15 follow-up on the same subjects were analyzed. Imaging guidelines were standardized and subject to quality review. MRI scanning was undertaken on a 1.5T scanner (Philips Intera, Netherlands). 3D MPRAGE (magnetization prepared rapid gradient echo) T1, Axial SE (spin echo) PD/T2, Axial FLAIR (fluid-attenuated inversion recovery) and Axial DTI (diffusion tensor imaging) were acquired. In this study, the 3D MPRAGE T1 images (axial plane, FOV = 240, Matrix = 256X256, Flip angle = 5°, Number of slices = 128, slice thickness = 1.5mm, interslice gap 0mm, TR = 15ms, TE = 7ms) were used for volumetry.

Both studies had institutional ethics approval.

Image Analysis

Image analysis was performed using the software Morphy-Display HERMES (Hermes Medical Solutions AB, Stockholm, Sweden). All images were converted to cubic voxels via interpolation, co-registered to a common Talairach space and adjusted for signal intensity.

Manual Tracing of the Caudate

All brain MRI scans were analyzed blindly to all clinical information by one tracer. A standardized manual tracing protocol with established reliability was used to trace and quantify the volume of the caudate via tracing the axial outline of the caudate serially through successive images, excluding the tail (Looi et al., 2008). The SNAC-K group controls with significant white matter lesions manifest as hypointense regions on MP-RAGE MRI were excluded. LADIS MP-RAGE MRI scans contained hypointense lesions adjacent to the ventricles corresponding to white matter hyperintensities (WMH) on the FLAIR sequences. Such lesions were excluded from the tracings of the caudate. Caudate nucleus volumes were normalized for total intracranial volume (TIV).

Automated Measurement of TIV in LADIS Subjects

We used a locally developed voxel-based morphometric method, VOLSTAT, to automatically quantify intracranial volumes in the baseline LADIS group. In brief, this method used automated segmentation and masking of the brain MRI scans, via statistical (fuzzy *c*-means) cluster analysis (Engman et al., 2006; Watson et al., 2006).

We used statistical cluster analysis methods to "classify" voxels as belonging to certain, predefined, tissue classes. The fuzzy *c*-means (FCM) algorithm is a soft unsupervised

classification procedure that allows sub-classification of individual MR image voxels into two or more groups based on the spectral distance to the cluster centres (*e.g.* a voxel in an image can be classified as 40% grey matter and 60% white matter). We used recently developed methods for automated masking and segmentation of 3D T1 brain MRI (Engman et al., 2006; Watson et al., 2006).

For estimation of T1 and T2 WMH volumes we used automated software, VOLSTAT, running on the HERMES (Hermes Medical Systems AB, Stockholm Sweden) workstation. Combining the information from T1 and T2 (FLAIR) images, the software included a segmentation algorithm based on fuzzy *c*-means cluster analysis (Engman et al., 2006; Watson et al., 2006), which allowed classification of the lesions detected following the co-registration of T1 and FLAIR images according to their signal. Thus, the volumes of white matter (WM), grey matter (GM), and cerebrospinal fluid (CSF) lesions are estimated. Furthermore, using the volumes computed with VOLSTAT one can calculate the brain parenchymal fraction (BPF), thus, the method combines automated brain and WMH volumetry.

All images were converted to coronal orientation with a 256 slice matrix and cubic voxels. The T1 images were registered to a template in the multi modality software in HERMES, using a six-parameter registration with normalized mutual information as a similarity measure. The registered T1 image was masked twice, rendering two different masks; once in order to remove non-brain tissue and once to remove non-brain tissue and cortical-CSF. The masking procedure was based on a region-growing method with a predefined kernel using in-house software (BMAP) also running on HERMES.

Thereafter the T1 mask with remaining cortical-CSF was corrected for inhomogeneities in BMAP, using a combination of quadratic and linear functions to increase the grey matter signal in the peripheral regions (Engman et al., 2006; Watson et al., 2006).

The FLAIR images were registered to the previous registered T1 images and thereafter masked using the binarised T1 mask without cortical-CSF. The mask without cortical CSF was used in order to remove voxels on the border between CSF and GM regions on the FLAIR images that can have high signal and erroneously be classified as white matter lesions. The masked FLAIR images were visually inspected for regions of high signals close to the eye region. If present, these regions were removed by manual masking. As FLAIR lesion volumes varies from non-existent to quite significant between different subjects, the FCM segmentation approach is not ideal as it "assumes" at least a certain volume of each cluster. Therefore, a thresholding approach was implemented to segment the FLAIR lesions and then combining this with FCM segmentation of the T1 images.

Before defining a signal intensity (SI) threshold that separates high SI lesions from normal brain, the FLAIR images SI need to be normalized, as the absolute SI can show quite large variation. A common approach to this is to use whole brain mean signal. We used an approach that takes into account both the mean SI and the total SI distribution. A Gaussian distribution was fitted to the SI distribution (histogram). The Gaussian distribution was used for normalization, and lesions were defined as all voxels with SI over a certain level above the Gauss peak taking into account the distribution shape. We found a reliable way to automatically set this level by defining the peak height (number of voxels) and subsequently the level were the upper end of the Gaussian distribution reached 1% of peak number of voxels. The actual SI threshold was set at 97 % of this "normalized maximum value". These pre-set levels were empirically derived by visual inspection of different level settings on a

small number of test-subjects. The FLAIR lesions were set to 100, rendering a new binary mask.

The binarized lesion mask was now used to further mask the masked T1 image where the CSF was retained. This yielded two set of images. One T1 image where the lesion regions, acquired from the FLAIR, had been removed and one T1 consisting of only lesions.

The two set of images were clustered separately using the fuzzy clustering algorithm to obtain total WM, GM and CSF content (Engman et al., 2006), and, within the lesion volume defined from FLAIR, the T1 lesion content. Total time needed for the automating processing of each subject was approximately 25 minutes.

Manual Measurement of TIV for (SNAC-K) Controls

Custom volumetric software in HERMES was used to acquire total intracranial volume (TIV) using stereologic methods. This is a semi-automatic method for estimating the volume of large brain structures that are too time-consuming to manually trace. The protocol was as follows: any structures inside the inner table of the skull were included: total brain, dura, ventricles and extraventricular CSF, brain stem and cerebellum. The boundary between spinal medulla and brain stem is considered to be at the level of the bottom of the cerebellum.

Volumetrics

Wilcoxon Signed Rank Tests were used to assess the between group differences from baseline to follow-up, analyzing by combined caudate volumes, normalized for intracranial volume. Since the follow-up periods for the LADIS and SNAC-K groups were different, we calculated the annual rate of change by the ratio of caudate volume at follow-up to volume at baseline, divided by the number of years between baseline and follow-up and compared the means by Wilcoxon Signed Rank Tests. Statistical analysis was performed SPSS 15.0 (Chicago, Ill., USA).

Results

Demographic Data (Table 1)

The control group, 15.1 (3.6) years, had more education than the LADIS group, 9.8 (3.8) years.

Caudate Volumes (Table 2)

LADIS Bilateral Caudate Volumes

The Wilcoxon Signed Rank Test revealed a statistically significant reduction in bilateral caudate volume at follow-up, z = -1.988, $P = 0.047$, with a large effect size ($z/\sqrt{N}$) = 0.510. The bilateral caudate volumes (cm^3) were 6% smaller at follow-up (Table).

Table 1. Baseline Group Demographics

		LADIS	SNAC-K
Age		71.1 (4.2)	70.7(4.9)
Gender (M/F)		7/8	7/8
Education years		9.8 (3.6)	15.1 (3.8)
MMSE		26.4 (3.4)	29.5(0.6)
Fazekas'	mild	53%	N/A
	moderate	20%	N/A
	severe	**27%**	**N/A**

Numbers in parentheses indicate standard deviation

N/A: not applicable - For controls manual inspection was conducted to exclude persons with white matter lesions

SNAC-K Bilateral Caudate Volumes

The Wilcoxon Signed Rank Test revealed no statistically significant reduction in bilateral caudate volume at follow-up, z = -1.079, *P* = 0.281. The mean bilateral caudate volumes (cm^3) were less than 2% smaller at follow-up.

Table 2: Caudate Volumes and Annual Volume Change Rate

LADIS	**N**	**Mean**	**Std. Deviation**	**Minimum**	**Maximum**
t1 Bilateral Caudate Volume	15	6.652	1.158694	4.461	8.571
t2 Bilateral Caudate Volume	15	6.277	1.190575	4.937	8.671
SNAC-K CONTROLS	N	Mean	Std. Deviation	Minimum	Maximum
t1 Bilateral Caudate volume	15	6.11880	.845679	4.906	8.354
t2 Bilateral Caudate Volume	15	6.02527	.875674	4.532	8.063
ANNUAL VOLUME CHANGE RATE*	N	Mean	Std. Deviation	Minimum	Maximum
SNAC-K Annual volume change rate	15	.16433	.008139	.152	.176
LADIS Annual volume change rate	15	.31553	.035034	.270	.374

Bilateral caudate volume (cm^3); t1: baseline scan; t2: follow-up scan

*Annual volumetric rate of change calculated by the formula:

[(volume t2/ volume t1)/years between t1 andt2].

Comparison of Longitudinal Rate of Change of Volume between LADIS and SNAC-K

The Wilcoxon Signed Rank Test showed a statistically significant difference in annual bilateral caudate volume rate of change between LADIS and SNAC-K groups, z = -3.408, *P* = 0.001, with a large effect size ($z/\sqrt{N}$) = 0.880. The annual volume change rate in LADIS is approximately twice that in SNAC-K.

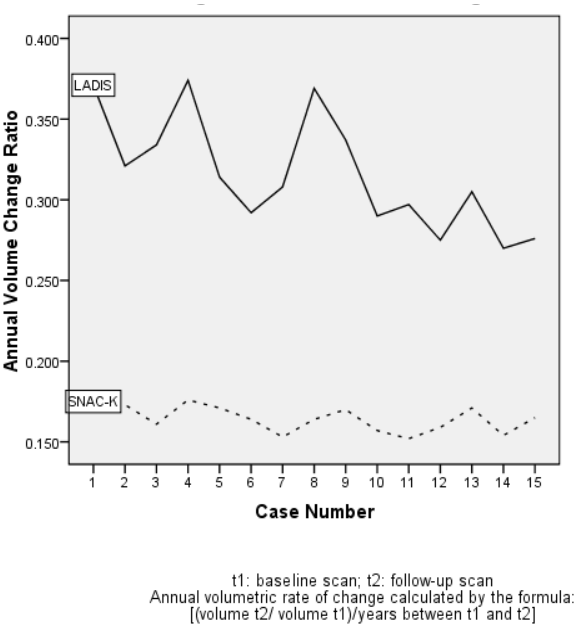

Figure 1. Annual Volume Change Ratio.

Discussion

Longitudinally, bilateral caudate volume decreases significantly in a pilot cohort with leukoaraiosis, supporting our main hypothesis. The annual rate of atrophic change in the LADIS group was almost twice that in SNAC-K group, suggesting leukoaraiosis is associated with caudate atrophy, supporting our secondary hypothesis. Leukoaraiosis and cerebrovascular disease may exert negative neuroplastic effects on the caudate via disconnection and vascular insufficiency. Resultant caudate atrophy, through disruption of frontostriatal circuits, may contribute to cognitive dysfunction found in cerebrovascular disease, manifest as frontal-executive cognitive dysfunction (Looi and Sachdev, 2000). Previously it was shown that there may be a gradient of caudate atrophy in cerebrovascular disease associated with differential cognitive impairment (Looi et al., 2009b), and it will be of interest to further examine if similar findings are apparent longitudinally in larger groups with leukoaraiosis.

We acknowledge these are preliminary results, especially in view of the small sample size and differential follow-up period. A difficulty of assessing longitudinal change within a cohort is selection of an adequate control group. Whilst not ideal, we chose a normal aging

cohort from the same region, precisely age and gender matching, as well as excluding white matter disease – thus random selection was not appropriate. There are demographic differences between the groups, especially in education, as well as differences in scanner protocols, and we acknowledge that this may affect the significance of our findings. Whilst there was variation in measurements of the caudate nucleus volume between time-points, such random measurement errors are likely to minimize longitudinal volume differences within groups, for this reason we compared rates of volumetric change between groups.

In conclusion, this study demonstrated that rate of atrophy of the caudate is greater in leukoaraiosis than in a healthy aging cohort. This, together with our previous cross-sectional study (Looi et al., 2009b), supports a potential role for the caudate as a substrate of neurodegeneration in cerebrovascular disease. Further longitudinal studies of the caudate in larger cohorts with cerebrovascular disease are planned.

Author Contributions

JCLL designed and is the guarantor of the study, performed all caudate measurements and data analysis; and wrote the first draft of the paper. GS assisted in development of protocols, performed inter-rater reliability measurements, assisted in data analysis and coordinated the automatic quantification data with PJ and LS. OL assisted in data analysis. PJ and LS designed the programs Morphy-Display Scaled and Volstat (HERMES). YZ measured TIV in the SNAC-K cohort. LB and EÖ assisted with pre-processing of imaging data. L-OW is the principal investigator for the Stockholm arm of LADIS. All authors contributed to the editing of the paper.

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Disclosures

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Chapter XII

Neuroepidemiology of Vascular Dementia

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1. Introduction

In this chapter, the epidemiology of vascular dementia (VaD) is reviewed; it's prevalence, risk factors, incidence, and prognosis.

1.1. Diagnostic Criteria of VaD

VaD is a dementia of vascular origin including cerebrovascular disease or cerebrovascular insufficiency. However, the agreement on its notion and diagnostic criteria is not achieved as well as the other types of dementias like Alzheimer's Dementia [1]. The primary reason is the difficulty in linking the cognitive dysfunction and cerebrovascular lesions. When we want to know the epidemiological fact of vascular dementia, the effect of diagnostic criteria is a critical problem because different criteria for dementia identify different frequencies and clusters of patients. In addition, differences in defining the vascular cause and etiology may add to the variation.

1.2. Comparison of Different Criteria

Currently, the most widely used criteria for VaD include the *Diagnostic and Statistical Manual of Mental Disorders* (DSM), Alzheimer's Disease Diagnostic and Treatment Centers (ADDTC), *International Statistical Classification of Diseases* (ICD), and National Institute of Neurological Disorders and Stroke–Association Internationale pour la Recherche et

l'Enseignement en Neurosciences (NINDS-AIREN) Criteria. T. Pohjasvaara et al. [2] evaluated the effect of different clinical criteria for VaD in a series of 107 patients with DSM-III poststroke dementia. Different criteria gave different frequency estimates, and overlap in the cases diagnosed was considerable. The origins of these differences included the following: (1) requirement of focal neurological signs and symptoms to be present in DSM-III, ICD-10, and NINDS-AIREN criteria; (2) absence of brain imaging requirements of relevant cerebrovascular disease (CVD) in DSM-III, ICD-10, and DSM-IV criteria; and (3) requirement of patchy or unequal distribution of higher cognitive functions in DSM-III and ICD-10 criteria. Additional factors include (4) qualifying extensive white matter lesions as radiological evidence of relevant CVD in NINDS-AIREN but not in ADDTC criteria and (5) requiring one CT or T1-weighted MRI infarct outside the cerebellum in ADDTC criteria.

2. Prevalence of VaD in Japan

2.1. Higher than the Western Countries?

Most clinicians in Japan used to believe or even now believe that patients with vascular dementia have been frequently encountered in Japan reaching nearly 50% of those with senile dementia, which was equal to or even higher than patients with Alzheimer's disease. And the impression was supported by some population-based studies. [3] [4] This might contrast with a much lower ratio of patients with vascular dementia in the United States. [5] However, this might be the result of overdiagnosis stemming from some problematic diagnosis of VaD or of the frequent use of magnetic resonance imaging to detect cerebrovascular disease in older adults.

2.2. Linking Cerebrovascular Lesions and Cognitive Functions ~ The Osaki-Tajiri Project

2.2.1. Importance of the Diagnostic Algorithm

One of the earliest works that were aware of this issue was the Osaki-Tajiri Project, which is still on-going. [6] [7]

The project targeted all residents aged 65 years and over (n=3,207) in Tajiri Town of Miyagi Prefecture and examined 1,654 participants. The overall prevalence of dementia was found to be 8.5%, of these, 14.9% participants had a past history of stroke. For 113 participants who had a past history of stroke, 18.6% of them were demented. For MRI performed population (n=497), the prevalence of probable VaD by the NINDS-AIREN criteria was 18.8%, whereas that of Ischemic VaD was 31.3% by the ADDTC, being different according to the diagnostic criteria. Rather, the condition of possible Alzheimer disease with cerebrovascular disease was more common.

2.2.2. Cognitive Functions and Cerebrovascular Lesions in VCI-No Dementia

In the project, VCI-no dementia (vascular MCI) was also evaluated, being the prevalence 37.2% among the CDR 0.5 participants.

Compared with the CDR 0, the CDR 0.5 group had more subjects with cerebrovascular lesions of the areas that are involved in the critical cirquits for maintaining cognitive functions. These areas included thalamus, caudaite mucelei, internal capsule, lesions of which may cause critical influence on memory or language. No effects of cerebrovascular disease on MMSE and Geriatric Depression Scale scores were found, but the CDR 0.5/strategic cerebrovascular disease group showed impaired Trail Making Teat-B scores[8]. A VCI-ND population was identified, and executive dysfunction in this population is probably based on an impaired fronto-subcortical circuit [7].

2.3. Population-Based Study Groups in Japan

Ealiest study groups other than the Tajiri Project include the Nakayama study [4], the Hisayama study [3, 9, 10], Radiation Effects Research Foundation Adult Health Study [11] [12].

Recent reports from other study groups include : Wada-Isoe K et al. [13], which is the study of the rural island town of Ama-cho, Konagaya et al. [14], which is the study on presenile dementia, Ikejima C et al. [15], which is the study on early-onset dementia of Ibaraki prefecture (population, 2 966 000).

3. Risk Factors of VaD

3.1. Cardiovascular Risk Factors

3.1.1. Cardiovascular Risk Factors and VaD

VaD shares the risk factors with cardiovascular disease. Cardiovascular risk factors include hypertension, diabetes mellitus, hypercholesterolemia, atrial fibrillation, and smoking. These factors are also found to have correlations to the development of Mild cognitive impairment (MCI), and interestingly, also that of AD. [16-20]

Recent studies showed that AD pathogenesis has a vascular aspect and may be enhanced by vascular risks. [21]

Vascular aspect of AD suggests that CVD including vascular dementia itself may be a risk for AD, and AD vice versa.

3.1.2. VaD and Stroke

History of stroke doubles the risk of dementia. Zhu et al. [22] followed dementia free 1272 subjects (212 poststroke, 1060 stroke free), reporting that baseline history of stroke doubled the risk of dementia (hazard ratio [HR]: 2.0) and adjustment for age, sex, education, and exposure to individual stroke risk factors did not diminish the risk (HR: 2.4).

Dementia itself is also shown to be a risk factor for stroke. According to the study in which a population-based cohort of 1551 subjects with no clinical history or signs of stroke, age 75 years and over at baseline, were followed up for 3 years, an increased incidence of stroke among subjects age 75 years old and over was associated by mild dementia(relative risk[RR] : 2.6) and cognitive impairment (RR: 2.0). [23] The correlation may be explained as

follows. Stroke increases risk of dementia and prior stroke increases risk of a subsequent stroke, mild dementia and cognitive impairment may be a manifestation of clinically unrecognized stroke.

3.2. Demographic Factors

3.2.1. Age

Prevalence of CVD and that of Dementia become higher along with progression of age. From the fact that all the people get weak in cognitive functions to a certain extent, one of the arguments on this matter is whether or not dementia is essentially different from normal aging. Some reports support the notion that dementia should be differentiated from normal aging because dementia is a condition that should be treated. [24, 25]

3.2.2. Education Level

Many reports support the effect of education level that lowers the risk for overall dementia. while the specific effect for VaD is not clear. [18, 26, 27]

There are several possible explanations for an effect of education on cognitive change. (1)Education might be confounded by factors such as illnesses or health habits that are more likely to occur among people with less education (eg. Keeping diet that is not good for health) and that affect cognitive function over time. (2)Alternatively, certain biologic effect of education may be to increase synaptic density in the brain, which will serve as a buffer later in life. (3)Finally, increased levels of education may alter an individual's ability to perform well on tests of cognitive function. [28]

3.2.3. Sex

Current literature is conflicting with regard to sex-specific incidence of both VaD and AD. [27, 29-33]

According to a report from the Rotterdam study, the incidence of vascular dementia is higher for men than for women in all age groups. After 90 years of age the incidence of Alzheimer's disease is higher for women than for men. [32]

3.3. Life Customs

3.3.1. Smoking

Smoking is one of the strongest risk factors for CVD, and is a risk factor for VaD.

According to a study based on the postmortem pathology, higher amount of smoking in the midlife cause higher risk for all the types of dementias of AD, AD with CVD and VaD in the later life, while extreme amount of smoking cancelled the correlation possibly due to the survivor effect. [34]

3.3.2. Alcohol Intake

Despite chronic alcohol abuse causing various injuries on nervous system, several studies have suggested that appropriate amount of alcohol consumption is associated with a decreased risk of dementia or cognitive decline.

In Bordeaux (France), a population-based prospective study found that subjects categorized as moderate drinkers (> 250 and up to 500 ml of wine), the crude odds ratio (OR) was 0.18 for incident dementia ($p < 0.01$) and 0.25 for Alzheimer's disease ($p < 0.03$), as compared to the non-drinkers.[35]

Confirming data were provided from the Rotterdam study. [36] According to the study, light-to-moderate drinking was significantly associated with a lower risk of any dementia (hazard ratio 0.58 [95% CI 0.38-0.90]) and vascular dementia (hazard ratio 0.29 [0.09-0.93]). In this study no evidence that the relation between alcohol and dementia varied by type of alcoholic beverage was found.

Results from several other studies are more or less in concordance with these preceding studies.

A systematic review including meta-analyses of 15 prospective studies[37] evaluated the pooled relative risks (RRs) of AD, VaD, and Any dementia for light to moderate drinkers compared with nondrinkers as 0.72 (95% CI = 0.61-0.86), 0.75 (95% CI = 0.57-0.98), and 0.74 (95% CI = 0.61-0.91), respectively.

3.3.3. Diet

A bunch of studies show that certain patterns of diet may be favorable for lowering the risk of dementia. One of the typical examples is the Mediterranean type diet. The traditional Mediterranean diet is characterized by high consumption of plant foods (vegetables, fruits, legumes, and cereals), high intake of olive oil as the principal source of monounsaturated fat but low intake of saturated fat, moderate intake of fish, low to moderate intake of dairy products, low consumption of meat and poultry, and wine consumed in low to moderate amounts, normally with meals. [38]

Adherence to a Mediterranean type diet has been associated with longer survival, reduced risk of cardiovascular or cancer mortality, and reduced risk of neurodegenerative diseases including Parkinson's disease and Alzheimer's disease. [39, 40]

However, it is difficult to say the association is established. There is a study which showed the association of higher adherence to a Mediterranean diet with slower MMSE cognitive decline but not consistently with other cognitive tests. The study also concludes that higher adherence was not associated with risk of incident dementia. [41]

The effect of diet on the risk of dementia must be prudently discussed, because daily diet is supposed to be confounded by many factors including socioeconomic status, culture, personality, etc.

3.3.4. Physical Activity

Physical activity may help preserve neuronal plasticity, increase synapses and dendritic receptors following injury, and release hormonal factors including brain-derived neurotrophic factor and epinephrine, that may assist in neuronal creation and function. [42]

Some studies found inverse associations between physical activity and dementia [43] [44] [45], but epidemiologic findings are inconsistent. [46, 47]

A report from the Cardiovascular Health Study[48] underlines the importance of apolipoprotein E genotype (APOE) e4 allele.

In the study participants in the highest quartile of physical energy expenditure had a relative risk of dementia of 0.85 compared with those in the lowest quartile, and participants engaging in >=4 activities had a relative risk of dementia of 0.51 compared with those engaging in 0–1 activity. These associations were more marked in APOE e4 allele noncarriers but were absent in carriers.

3.4. Prevalence of Vascular Risk Factors

The Osaki-Tajiri Project found that the prevalence of each vascular risk was evaluated as follows: Hypertension (male 54.4 / female 62.6%), Hypercholesterolemia (31.0/47.0%), Ischemic heart disease (14.3/18.0%)、Diabetes mellitus (12.4/8.1%), Atrial fibrillation (6.7/4.1%). Hypercholesterolemia was significantly higher among females, Hypertension had a tendency to be higher among females, but not significantly. [6]

4. Incidence of Dementia

The Osaki-Tajiri project evaluated the incidence of dementia in Japan. The project followed up the population from 1998 and then evaluated the participants in 2003 and 2005. Overall, 3.9% (8/204) of CDR 0 and 37.0% (20/54) of CDR 0.5 developed dementia during the 5-year period at the evaluation of 2003, whereas 40.2% (113/281) of participants initially evaluated as CDR 0.5 in 1998 developed dementia in 2005 during the 7-year period. Of the participants initially assessed as CDR 0.5 in 1998, 1.8% (1/54) in 2003 and 7.8% (22/28) in 2005 were assessed as CDR 0. The ratio of the number of each type of dementia to that of overall dementia that was found be developed among the participants evaluated in 2005 was 46% for AD according to the criteria of NINCDS-ADRDA, 17% for AD with CVD, 16% for VaD according to the criteria of NINDS-AIREN, 7% for DLB, and 14% for other causes including brain tumor. [7]

5. Prognosis

One of the earliest findings from Finland showed that the proportion of patients with dementia surviving after 6 years was less than half the rate of the general population adjusted for age and sex. The proportion of survivors with AD was reported as 21.1%. For patients with vascular dementia, the proportion was only 11.9% (average age at baseline 78 years for AD and 79.4 years for vascular dementia) [49]. The particularly poor prognosis of VaD was reproduced[50].

According to Ostbye et al., the relative mortality rates for diseases of the vascular system, especially cerebrovascular disease, were highest in vascular dementia. AD groups also had higher relative vascular system mortality rates than the groups with normal cognition. The relative mortality rates for cancer were similar to those with normal cognition. [50]

In contrast to these studies, a study that followed up patients with vascular cognitive impairment without dementia reported that the mortality rate was similar to that of patients with AD[51].

It must be noted that presence of vascular dementia or vascular cognitive impairment strongly suggests the involvement of systemic arteries. In other words, general management including cardiovascular risks may be critical for the prognosis of most of the dementia patients of vascular origin.

Earlier interventions for vascular dementia may be important in spite of the narrow criteria of NINDS-AIREN. [52]

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Chapter XIII

Validation of Two Short Dementia Screening Tests in Indonesia

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Abstract

Background

Dementia is on the increase worldwide, and developing countries are expected to carry the burden of this. Relatively little is known about dementia prevalence in Indonesia. This chapter discusses two short screening tests to assess dementia in rural and urban Indonesian cohorts.

Method

At baseline in 2006/7, 719 elderly were included from rural and urban sites on Java. Large differences appeared in dementia prevalence in those over 60 years of age between urban (3%) and rural sites (7-16%) employing two dementia screening tests also used in Oxfordshire with the same cut-offs. An in depth study was performed on the rural sample from East Java to validate the cut-offs of the tests. For this study, Javanese Indonesian elderly from 4 villages around Borobudur were asked to participate. 113 agreed to

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participate and these were tested in a health center by medical experts and trained research assistants. The screening test cut-offs were validated against consensus based clinical dementia diagnoses by an expert psychiatrist, nurses and GP, which were based on a gold standard diagnostic instrument for dementia diagnoses from Cambridge. In addition, a sub sample of these participants was tested in depth by another psychiatrist using questions from our expert dementia diagnostic system developed at Oxford University.

Results

The adapted memory screening test was shown to have similar cut-offs for dementia (19.5 for controls and 14.5 for cases) as in Oxfordshire and the Mini Mental Status Examination (MMSE) had optimal sensitivity (100%) using a similar cut-off of 24. However, for optimal specificity, the MMSE was shown to require a lower cut-off of 21.5 and MMSE scores were also affected significantly by educational level. It was unclear how many of these cases had vascular dementia (VaD), as stroke, transient ischaemic attack and myocardial infract assessed by self-report were rare (n=1-2) and were only reported by controls, suggesting a recall bias. There was also no difference in diabetes mellitus or (high) blood pressure between cases and controls which could have increased risk. Physical examination suggested no other morbidity driving the dementia (e.g. infectious or lung disease). The cut-offs were also tested by another expert psychiatrist on a sub sample of these participants using her clinical assessment and aided by questions from our expert dementia diagnostic system. Agreement between psychiatrists was high (79%) on diagnoses of these 28 participants, with only 6 disagreed on. Of these, only diagnoses of 2 participants were disagreed on whether these had dementia or were controls. Of 59 elderly patients from the villages who were tested in depth by the second psychiatrist, 17 were thought to have dementia, with most (53%) having Alzheimer's disease (AD) and 6 (35%) suspected of having VaD, with only one mixed (with stroke) case. There was no clear indication of other types of dementia, but two cases with dementia (12%) were thought to be related to systemic disease. The 19 preclinical cases (possible dementia) all had memory complaints, but scored significantly higher on the adapted memory test and MMSE than those with dementia and scored lower than controls (but not significantly so on the memory test), independent of age and gender. Optimal cut-offs for dementia on the memory test were again 19.5 for the total immediate recall (100% sensitivity and 78% specificity) and 24 for the MMSE (88% sensitivity and 96% specificity).

Discussion

This study showed that two short cognitive tests can be used for dementia screening in rural Java. It has been hypothesized that VaD is more prevalent than AD in East Asian countries, but we could not substantiate this. Future studies should investigate in more detail the prevalence of vascular and secondary dementias (due to thyroid or infectious disease, nutritional deficiency etc.). Use of this screening instrument in other ethnic groups in other developing countries also needs to be explored.

Keywords: Alzheimer's disease, dementia, screening, developing countries

Introduction

Dementia has high economic and human costs and its prevalence is predicted to increase substantially over the next decades. Currently it is estimated that 24 million people have dementia worldwide, a number predicted to double every 20 years. Of those afflicted with dementia, 60% is estimated to live in developing countries, with the proportion increasing to 70% by 2040 (Qui, 2007). An older age and low levels of educational attainment are strong risk factors for dementia (Launer, 1999; Karp, 2004). By 2050, 70% of older people will live in developing countries, who are burdened by lack of resources and a combination of non-communicable and communicable disease (Kalache 2003). This leads to increased risks of primary and secondary dementias, increased dependence and lower productivity, as many elderly still contribute to family finances (Kreager, 2006a.b). This combined with a predicted increase in migration from poorer countries (according to recent United Nations population division reports, see UN, 2007) to the North Western more prosperous regions, highlight the importance of cheap, short and cross culturally applicable screening tools for dementia assessment in multi-ethic cohorts

The clinical diagnosis of dementia is based on neuropsychological testing, medical history and examination to rule out systemic, psychiatric, neurological and other causes of cognitive impairment, and to identify the pattern of progression (McKhann, 1984, APA, 1994). However, most clinical screening tools originate from developed countries and do not take into account some of the issues pertaining to many developing countries such as:

i) a general lack of resources (e.g. a lack of trained staff, time and financial constraints)
ii) high rates of illiteracy and cultural/linguistic differences which can affect the validity of neuropsychological tests.

While the brief Mini-Mental Status Examination (MMSE, Folstein, 1975) has often been criticised for its ceiling effects and modification by education, mood and age (Hogervorst, 2002, Iype, 2006), it is still the most widely used short screening test for dementia. The sensitivity of the MMSE is good (its ability to identify cases), but its specificity is low (Hogervorst, 2002, Iype, 2006). However, MMSE specificity can be much improved when used in an algorithm with the Hopkins Verbal Learning Test (HVLT, Brandt, 1991; see Hogervorst, 2002 for a review). Memory is usually one of the first functions to show a decline in Alzheimer's disease (AD), the most common form of dementia in Western countries and is, according to consensus based criteria, a key criteria for the assessment of dementia (APA, 1994). The HVLT, a short memory test, takes about 5 min to administer, is sensitive and well tolerated by patients. In a study (Hogervorst, 2002) of 82 patients with dementia and 114 healthy controls - equivalent in age, years of education and gender ratio - from the Oxford Project To Investigate Memory and Ageing (OPTIMA), the total immediate recall memory score of the HVLT had 87% sensitivity and 98% specificity for dementia, when using a cut-off score of 14.5. Sixty-eight patients had been diagnosed with AD using NINCDS/ADRDA criteria (McKhann, 1984). Using the 14.5 cut-off point, 91% sensitivity and 98% specificity for AD was found. There were no ceiling or floor effects. Regression analyses indicated that HVLT test results did not need to be adjusted for age, gender, education or depression.

These results were robust as a another OPTIMA study (De Jager, 2003) with a different patient group showed 98% sensitivity with 92% specificity, using a cut- off of 18.5 to discriminate between 60 AD cases and 51 controls. The cut-off was higher because in this study the control group did not include participants with mild cognitive impairment (MCI).

People with MCI may be at risk for dementia (Petersen, 1999) but, to simulate clinical reality, this group had been included with controls in our first study (Hogervorst 2002). The HVLT is adept at identifying people with MCI in an early stage. Using a cut-off of 21.5, 78% sensitivity and 80% specificity was detected at baseline between 51 healthy controls and 15 control participants who would develop MCI after a 2-3 year follow-up. A third study (Schrijnemakers, 2007) gave similar data on specificity and sensitivity for AD, MCI and controls, which were maintained at follow-up. To reduce slight learning effects in controls, six parallel versions exist, which have shown good inter-test reliability (Brandt, 1991). Several studies using the test in other cohorts have reported a similar discriminative capacity of the HVLT (see Hogervorst 2002 for a review), including the possibility to distinguish between vascular dementia (VaD) and AD. In our earlier study, 82% sensitivity and 75% specificity of AD versus VaD was found (De Jager, 2003).

On the basis of our prior research in Oxford (de Jager, 2003; Schrijnemakers, 2007; Hogervorst, 2002), the following algorithm was deemed to be most suitable for optimal sensitivity and specificity in screening:

- for dementia: HVLT total immediate recall less than 14.5 and MMSE less than 24.5;
- for controls: HVLT greater than or equal to 19.5, MMSE greater than or equal to 24.5.

Those who fall between cut-offs are questionable and require more in depth assessment. This algorithm was subsequently used for the initial screening in memory clinics in Oxfordshire.

However, it is unclear how cross culturally/ethnically applicable word learning tests are (Morris, 2001). For instance, in an impoverished rural cohort in Arkansas USA, Black Americans scored lower on similar word learning lists than White participants when controlled for age, education, socioeconomic status (SES), sex and mood (Hogervorst, 2004). Systematic distribution differences in education between Blacks and Whites may have confounded results, but were difficult to investigate.

Dementia prevalence is currently unknown in Indonesia, but it has one of the fastest growing aging multi-ethnic populations globally. Currently in Indonesia 8% (17 million) of the population is over 60 years old, which is expected to increase to 13.5% (35 million) by 2025 (Wibowo et al, 2004). Due to the similar demographic pyramid structure and life expectancy, similar dementia prevalence was expected in Indonesia as in India, which is 5% in those over 60 years of age (Biswas et al, 2005).

Methods

Study of Elderly's Memory Impairment and Associated Risk Factors (SEMAR)

Our initial Indonesian cross-sectional study included 719 elderly who were between 52 to 98 years of age from two rural sites (West and Central Java) and an urban site (Jakarta). This study was described in more detail in Yesufu et al (2008). It was set-up as a baseline study in 2006/2007 to be followed-up by a more in depth study to also validate dementia status of participants in 2009. Briefly, prior to the study all village elders and staff at local community health centers or care institutes had been informed of the study and they subsequently forwarded this information to potential participants. Participants had been asked to bring their carers and to arrive in the morning at the local health centers at agreed dates if they were interested in participating. None of the elderly approached refused participation after they had been given information about the study by trained research assistants and all signed the informed consent forms. If a carer was present, they also signed the informed consent form. No incentive was offered. The study was carried out between April and June 2006 in Jakarta and between December 2006 and February 2007 in the rural areas of Borobudur and Sumedang. Ethical approval (University of Indonesia, Jakarta and Loughborough University, UK), governmental and local permits had all been obtained before study-onset, which included a follow-up study two years later.

In West Java, at the Sumedang site, all 207 Sundanese elderly who resided in the village of Citengah (a 1-2 hour drive from Bandung) were invited to come to the community health center and were tested there after they had given informed consent. At the Borobudor site (Central Java, a 2 hour drive from Yogyakarta) all 214 Javanese elderly covered by the Borobudur community heath center were included and were asked to come to the local health center to be tested after they had given informed consent. Those with limited mobility were visited at home (n=2), when they had agreed to be visited there. For the urban area (Central, West and South Jakarta in North-West Java), a sample of 298 elderly with mixed ethnicity was included after giving informed consent (47% Javanese, 17% Sundanese, while other ethnic groups, such as Minangkebau, Chinese, etc., were less prevalent). This ethnic distribution for Jakarta reflected the Indonesian census of 2000. Most of these participants (n=164) were either attending the local community health centers, lived in local care homes for elderly (n=49), or were tested at home (n=1).

Assessment of Demographics and Cognitive Function

Testing was done by trained and supervised research assistants between 8-11am to avoid circadian interference and the effects of heat. Participants were surveyed for demographic and other variables (such as health and lifestyle) using standardized questionnaires. Answers were all substantiated by a carer when present (in about half of the suspected cases and half of controls). To assess memory function, the Hopkins Verbal Learning Test (HVLT, Brandt, 1991) was used. This is a word learning test measuring episodic memory, which consists of 12 words from 3 low frequency categories (for version A: 'human shelter'; 'animals' and

'precious stones'). These words were all repeated 3 times to obtain a total immediate recall measure ('learning ability'). After 20 min, a delayed recall without cues or prompting was done. Some items (of the precious stones category) were changed after a pilot study to adapt to local knowledge, creating a modified Indonesian version of this word list.

The Mini Mental Status Examination (MMSE, Folstein, 1975) consists of a series of questions designed to measure change in cognitive status and to differentiate between normal age-related cognitive decline and the pathological cognitive decline that occurs in dementia. It was slightly adapted for local circumstances (e.g. seasons of the year were scored as wet or dry season, which was similar to the Hindi version developed by Ganguli, 1995). To assess cognitive impairment/possible dementia, the combination of the two cognitive tests was employed, using the total immediate recall of the HVLT and the total score of the MMSE with previously established cut-offs (see above).

Assessment of Ability to Function on a Daily Basis By Carer Report

For carers' confirmation of memory and other cognitive impairment and to identify progression of disease, questions based on the Dementia Questionnaire (Ellis 1998) were included, which is a semi-structured informant (carer) interview for the diagnosis of dementia. It has shown good validity and reliability and relates to the most commonly used consensus based (ICD-10 and DSM-IV, see APA, 1994) dementia criteria. However, according to Yesufu (2009) only the question 'does the person you care for have memory problems and has this been getting worse?' was sufficiently discriminative for cases and controls using factor analyses. None of the questions about other cognitive problems or etiology and course were therefore included in the subsequent analyses. The Barthel Activities of Daily Living (ADL, Barthel and Mahoney, 1965) and Instrumental Activities of Daily Living (IADL, Lawton and Brody, 1969) were used to assess functional capabilities of participants. For this study a slightly modified IADL was implemented using a cut-off of '9' was used (see Appendix)

After pilots and revisions of some contents, the translated forms of cognitive tests and questionnaires encountered no further problems and back-translation from Bahasa Indonesia (in Jakarta), Javanese (in Borobudur) and Sundanese (in Sumedang) to English was also done successfully for all tests.

Consensus based DSM-IV criteria (APA, 1994) for dementia can be summarized as follows:

A. Cognitive impairment including memory and at least one other cognitive function
B. which are a decline from a previous level of function and impact on social and occupational function and activities of daily living, and
C. have gotten progressively (gradually for AD, stepwise for VaD) worse over time,
D. while there are no other medical or psychiatric (E) factors present that are judged to have caused the cognitive impairment.

This was operationalised in our study as:

A. Performance below established cut-offs for cognitive dysfunction (see introduction);

B. Performance below a cut-off of '9' to assess impaired Instrumental Activities of Daily Living (see Appendix)
C. Carer's report of memory impairment (1), which affected IADL (2) and which had gotten (progressively or stepwise) worse over the last year.
D. At this stage physical or mental morbidity affecting cognition could not be reliably investigated, but carers had been asked what they thought the etiology was of the memory problems (old age, stress, depression, sickness etc) and self reported health status (doctors visits, specialists visits, taking medication, perceived health etc) was measured.

Results

Participants from rural areas (all participants covered by the health centres of Borobudur and Sumedang, in Central and West Java, respectively) had substantially higher risk of cognitive impairment/possible dementia using the cut-offs from Oxfordshire (around 43% in both areas scored below cut-offs of both tests using the established algorithm), when compared to the urban areas (with only 11% scoring below both cut-offs in Central and South Jakarta).

	Jakarta	Sumedang	Borobudur
	298	207	214
Below cut-offs	n=34 (11%)	n=88 (43%)	n=93(43%)
IADL <9	n=28 (10%)	n=22 (11%)	n=40 (19%)
Cognition+IADL	n=9 (3-6%)	n=15 (7-9%)	n=34 (16%)

The percentages given below for possible dementia take into account missing data of cognitive tests

	Jakarta	Sumedang	Borobudur
Only 60+	288	203	214
Cognition+IADL	n=9 (3%)	n=15 (7%)	n=34 (16%)
Only 65+	175	143	164
Cognition+IADL	n=8 (4.5%)	n=14 (9.8%)	n=34 (21%)

Rural elderly were thus approximately 4 times more likely to score below cut-off scores. However, participants in Jakarta were on average younger and better educated than those in rural areas. In Central and South Jakarta, the average age of the elderly investigated was 68 years (SD 7.7, range 52 to 90 years). While 13% had obtained no education, 44% of elderly had at least obtained a high school diploma or more. In Sumedang, Citengah, the average age of elderly included was 69 years (SD 7.7 range 52 to 98 years), with 18% not having obtained any education, and only 3% having obtained high school education or more. In Borobudur, the average age of elderly included was 71 years (SD 7.9 range 60-90), and 46% of these elderly had obtained no education, while only 2% had obtained high school education or more. These differences in age and education explained the association of site (urban vs rural) in logistic regression analyses to predict cognitive impairment/possible dementia as

established by cut-offs. Ethnicity, socioeconomic status (occupation and house ownership) and gender were all not significantly associated with cognitive impairment/possible dementia in these analyses, although these variables were associated with performance on the word list, when this was investigated in separate analyses. Distributions of test performance were similar, but average MMSE and HVLT scores of Indonesian controls were slightly lower than those of our Oxfordshire cohort (Hogervorst, 2002; Yesufu, 2008).

Investigating IADL separately, which can be an indicator of dementia, showed regional differences with 1 in 5 of elderly over 60 years of age in Borobudur and 1 in 10 in Sumedang and Jakarta experiencing difficulties in instrumental activities of daily living. Overall, suspected dementia prevalence (taking into account both cognitive impairment and problems in activities of daily living) in those over 60 and 65 years of age was around 8%. This was slightly higher than expected, because of a very high percentage of possible dementia cases in Borobudur, Central Java (16-21%).

However, half of carers stated there were no memory problems in those elderly who scored below the cut-offs on the HVLT and MMSE. It is not clear whether this is an underestimate or whether the low cognitive test scores did actually not reflect deficiencies in every day memory and cognition. Overall, data of n=136 carers indicated that around n=40 (5.6% of the total cohort) of suspected cases had memory problems, of whom n=22 had gotten progressively worse over time and may have had Alzheimer's disease.

Taking these data together indicated that 6-8% of people, who were included in our sample and who were over 60 years of age could be afflicted with dementia. These suspected cases had an average MMSE of 15.5 (SD 9) compared to an MMSE of 23 (SD 6) in controls, as established by the cut-offs. Controls were all reported by carers not to have memory problems. Where activities of daily life and social functioning was affected by cognitive problems according to carers (n=22, 3%), average MMSE scores were 13 (SD 8). Similarly, in an Indian case control study (Iype, 2006), average modified MMSE scores were 23 (SD 5) for controls, and for mild to moderate dementia cases these were around 14 (SD 7). These differences between control cohorts on the MMSE may be related to systematic differences in culture or education between Western and developing countries cohorts.

Discussion

The overall dementia prevalence estimate found in this study is in line with earlier estimates (Biswas, 2005) with around 6% (with a range of 3-8%) of people screened over 60 years of age suspected of having dementia. Taking Indonesian population growth into account (Wibowo, 2004), with a 5% prevalence of dementia, our estimate would render a total predicted 1.8 M dementia cases in 2025 in Indonesia. For the rural areas, our higher estimated prevalence in Borobudur than in Sumedang would be in line with reports from the village elderly and center for health staff when using focus groups and semi structured interviews to assess dementia prevalence.

In Sumedang, only aggression and paranoia (but not memory or any other cognitive impairment) was considered an issue as reported by village elders in perhaps 1 to 3 (of 100 elderly in the village), but this was not considered to be a problem in Borobudur. The most common issues reported in focus groups in Borobudur were those of memory (but not

attention), forgetfulness (e.g. forgetting that they had eaten already) and disorientation (getting lost). These problems were thought to fluctuate and were thought by Health Center staff in Borobudur to be mainly related to deafness, arthritis, and high blood pressure (which were all very common in the elderly). They were not thought to relate to dementia, thyroid disease (which is apparently endemic in the area and monitored in children, although in 100 elderly, only 1 goitre was identified), tuberculosis (only 1-2 known cases in the elderly and this was monitored by the centers and treated for free), or other infectious disease or anaemia (even though most elderly were reported to hardly ever eat meat). There were no tremors/motor problems indicative of Parkinsons's disease (PD). There were 2 stroke cases, but one of these did not have dementia. About 1: 4 of elderly was reported to have wheezing (COPD (?), as all of these were smokers) but none of these cases had signs of 'Pikun' (dementia). Frontotemporal dementia (FTD, e.g. with personality changes, aggression, paranoia, problems in planning) and Lewy Body dementia (LBD, with hallucinations, fluctuations in attention etc) seemed either not to exist or were not recognized. However, according to staff, most elderly could still take care of themselves and memory (forgetting) and some planning problems were considered by them and the villagers to be a normal part of aging. When staff was pressed further, in Borobudur, 3-5 elderly (8-13% of 40 elderly villagers) were perhaps thought to have more than normal issues with memory problems and getting lost. Only 3 elderly had some (but significant) problems with planning and could not support themselves financially, but they still participated in community life (e.g. by sweeping etc). All but maybe 1 or 2 were reported to still be able to handle money and dress/feed themselves. The validity of this rural community consensus on identifying possible dementia cases who need support will be investigated further in a future study. So far, however, only half of the 5 reported cases actually had cognitive performance over the cut-offs of the screening tests, while others with very low scores were not included by the focus group. Whether this reflects poor every day life validity of the tests or a misrepresentation by village elders and center for health staff remains to be determined.

There are several limitations to the current study. Firstly, there was large variation between the sites and systematic demographic differences in the distribution of age, SES, ethnic differences and educational attainments between rural and urban samples may have acted as confounds and are difficult to investigate. In addition, the last APA criteria in our earlier study could not be systematically investigated (whether other medical (D) or psychiatric (E) factors were present that would be judged to have caused the cognitive impairment). It was thus unclear what percentage of those at risk could be counted as secondary preventable dementias or cognitive impairment due to other causes than dementia. According to a more detailed analyses of the course of disease and self reported health factors in this cohort (Yesufu, 2008), Alzheimer's disease (AD) was thought to be less common and vascular or secondary dementias were suspected to be more common. This is important as these may offer treatment possibilities, which are currently not a long term effective solution for AD. However, this was based on self-report data on health, which may be unreliable, particularly in suspected dementia cases due to the very nature of their impairment (see data second study on self report of stroke, etc). Furthermore, community workers (see above staff of Centers for Health) and carers were suspected to under-report (cognitive) problems of both cases and controls, to a somewhat similar magnitude. This may be part of a cultural attitude towards the elderly (one of respect), which needs to be taken into account when using carer report to corroborate patient's self report and cognitive test results. Lastly, in Indonesia, at the

time of testing life expectancy for men was 65 years and for women it was 69 years. People may thus not survive to be old enough to be at risk for dementia, leaving only healthy survivors (of heart disease, stroke etc.) to obtain an old age, who are also at a lower risk for dementia (Clifford, 2009). While our data suggested that this may the case in Jakarta, with fewer than expected cases in the older age strata, rural areas showed more suspected cases based on cognitive cut-offs with increasing age, partly refuting these hypotheses. While access to health care (doctor and health centre visits) was not different between urban and rural areas, medication use and hospitalisation was lower in rural parts, particularly in Borobudur, which also had the oldest population. Confirmation of health and dementia status using a full medical exam was thus required to further investigate this and to validate our initial screening.

Validation Study

In a follow-up study of our cohort in 2009 2 to 3 years after the discussed baseline study which was done in 2006/2007, Dr Fidiansjah Muirsjid and Dr Raden Irawati Ismael trained by dementia expert Dr Martina Nasrun (the 10/66 international dementia screening representative for Indonesia) set out to test whether the algorithm developed in Oxford was valid for the rural cohort in Central Java which had the highest dementia prevalence. The screening was based on the short neuropsychological test-battery consisting of the MMSE and HVLT and the above mentioned questionnaires. This outcome was compared against the judgement of Indonesian clinical dementia experts who used a standardized clinical examination based on the Cambridge Mental Disorders of Elderly Examination (CAMDEX, Roth, 1988) and also the questions from a validated dementia expert system from Oxford (Hogervorst, 2003).

This study was done to establish

i) validity of the HVLT/MMSE algorithm against CAMDEX derived diagnoses
ii) modification of diagnoses by demographic factors (age, education, sex, SES)
iii) a short screening instrument, including a carer's report and questionnaires
iv) to perform a preliminary investigation of prevalence ratios of different types of dementia in Indonesia (e.g. AD vs. VaD and other types of dementias).

Methods

Rural community dwelling elderly of Central Java were included in this survey. All were over 56 years of age and were covered by the local health districts around Borobudur. Some were survivors of our earlier study (Hogervorst, 2008) conducted in 2006. Of these, an estimated 80% could still be contacted for follow-up from Borobudur and Salam districts after the 3 year follow-up in 2009. Follow-up data are discussed in another paper, as this paper concerns the rolling cohort data collected in 2009, which also included novel participants who were over 56 years of age in 2009.

Ethical approval from Loughborough University and the University of Indonesia, governmental permits and informed consent were obtained before data collection. Participants were informed of the research during their visits to the health clinics. They were made aware that non participation would in no way affect their treatment at the health center and that this was not obligatory. However, because of the excellent relationship between health center staff and the elderly, the willingness to participate was high. Participants were collected by car to travel to the research setting at an agreed date with their carer, who also signed the informed consent. This approach had a 96% response rate, as 7 participants (of n=177) could not be included on the day because of frailty or fatigue and a subsequent inability to travel. All others consented to participate. The research was carried out in 5 rooms of the home of one of the administrators in the integrated health unit (Pos Pelayanan Terpadu/Posyandu), which was quiet and which had no distractions.

Assessments

For the validation study, the same survey and test battery (using the same adapted versions of the HVLT, MMSE etc. see above), which were used in the feasibility study were repeated (see above).

Weight, height, waist-hip circumference, non fasting glucose (finger prick) and blood pressure measurements were taken by research nurses. A medical examination and a health resume were conducted by a GP based on the questions from the Cambridge Mental Disorder of the Elderly Examination (CAMDEX, Roth, 1988). This examination consists of a full neurological exam (reflexes, tremors, problems vision/ hearing, gait, Parkinson's disease signs); a psychiatric assessment (interview and observation for depression, psychosis, anxiety, use of psychoactive medication, including those for insomnia, etc.); cognitive testing (orientation, calculation, memory (president Indonesia, family name, children' s name), language etc.) and a carer report, which included a report of cognitive decline, mood, morbidity (cancer, dementia, Parkinson's disease (PD), stroke, myocardial infarct (MI), transient ischaemic attack (TIA), high blood pressure, diabetes), medication use and activities of daily living.

A consultant psychiatrist then made a clinical diagnoses of dementia on the basis of this assessment in consensus with the GP, assistants and health center nurses. The consultant psychiatrist who performed the dementia diagnoses using the CAMDEX questions had assessed 13 cases and controls in Jakarta using the CAMDEX and Kappa agreement on these cases was .81 with another expert psychiatrist and .83 with a GP.

The medical examination was routine and included, for instance, testing of heart rate, lungs, diabetes mellitus (fasting glucose > 7mmol/L or non fasting glucose > 11 mmol/L) and hypertension (systolic> 140 (stage I) or 160 mmHg (stage II) and/or diastolic > 90 (stage I) >100 (stage II) mmHg). Blood samples were taken to assess relevant biochemical variables for differential dementia diagnoses, as suggested by the CAMDEX (such as thyroid hormones, hematocrit, folate, cobalamin, electrolytes, etc) but these are also not included in this chapter as data were not yet assayed by the time of publishing.

Statistical Analyses

All data were checked for missing values and potential outliers. Where necessary, data were (log or square root) transformed to approach a normal distribution. Receiver Operating Characteristic (ROC) curve analyses were performed on the HVLT and MMSE to investigate cut-offs for optimal specificity and sensitivity for clinically established dementia cases versus controls. This was also done by including MCI cases with controls to better mimic the clinic reality. MCI was defined as those elderly participants who had memory or other cognitive problems, but no IADL or social impairment.

Demographic characteristics (age, sex, education) were compared between cases and controls using ANOVA and Chi-square tests. Logistic regression analyses were carried out to investigate whether the diagnoses were associated with cut-off categorisation independent of age, education and gender. All analyses were performed in SPSS 17.0 using a p-value of 0.05.

Results

Descriptive analyses of the cohort are given in the table below. Of the total cohort, of n=113 data on both cognitive tests as well as diagnoses were present. Of these n=113, 33% were considered to have dementia by using the clinical assessment based on the CAMDEX. The two self reported myocardial infarct (MI), the one self reported stroke and the one TIA were all controls. None of participants or carers reported having been diagnosed with dementia, Parkinson's disease, or cancer. 1 case and 1 control reported diabetes mellitus. Of those with high blood pressure, 31% were controls or MCI and 39% were dementia cases (p=.74). 5 controls were considered to show signs of depression, as well as 2 MCI, but only 1 dementia case (p=0.07). In none of these was depression considered to be severe enough to interfere with cognitive function. One dementia case with psychosis had been excluded from analyses. None of participants displayed symptoms of anxiety or other psychiatric morbidity or took medication for this (including medication for insomnia).

	Controls N=31	MCI/Preclin N=45	Probable dementia N=37	
Age	67 (6)	74 (7)	76(10)	p<0.001
No education	10%	35%	54%	p<0.0001
High School or more	80%	20%	0%	
Occupation (present)				
Farmer	21%	27%	51%	p=0.03
Women (%)	29%	30%	40%	(p=0.06)
HVLT	23 (5)	14 (5)	9 (5)	p<0.0001
MMSE	28 (1)	23 (1)	18 (4)	p<0.0001

Average (and median) age of respondents was 73 (SD=8) years. The majority of these Javanese Muslim respondents were women (62%). Almost half (48%) had not obtained any schooling, and most others had only had primary schooling. Most (45%) worked as farmers or labourers and almost all were still actively involved in the community.

Those who were older and had received no education were more likely to be diagnosed with dementia, but there was no significant difference in the female:male distribution. Past

occupation was not different between groups (p=.53), although those who were still working as farmers were twice more likely to be diagnosed with dementia (P=0.03). Education was significantly associated with occupation and to preserve power only education was entered in general linear models along with age and gender. However, the differences in test performance by diagnostic category were shown to be independent of age (p=0.001), education (p=0.08), and gender (ns) in general linear models with diagnoses (controls, MCI and dementia) as a main independent between subject factor [F(2,108)=33,63, p<0.0001] for the HVLT. For the MMSE, there was also a similar significant main effect of diagnoses on performance [F(2, 108)=8.56, p<0.0001] with no effect of age (p=.19) or gender and only a trend for education (p=0.06) to be related to higher test scores.

To assess cut-offs for optimal sensitivity and specifity, Reciever Operating Characteristics analyses were performed. Including only dementia cases versus controls rendered 100% sensitivity and 91% specificity for the HVLT total recall using a cut-off of 19.5. In addition, 100% sensitivity and specificity using a cut-off of 24.5 on the MMSE was found. These cut-offs were similar to those for the cohort in Oxford for both tests (Hogervorst, 2002).

When ROC were performed which included MCI with controls (which is more closely adapated to the clinical reality), results were as follows. A cut off of 19.5 for the HVLT still gave optimal senstivity, but now 14.5 gave a better balance between sensitivity and specificity, which is similar to Oxford data, when we also included MCI with controls against cases with dementia. The MMSE now also required a lower cut-off of 21.5 for optimal sensitivity and specificity. The sensitivity was still high at a cut-off of 24.5, possibly because the MMSE is part of the CAMDEX cognitive tests (CAMCOG).

Test	Sensitivity	Specificity
HVLT		
19.5	100%	43%
14.5	92%	70% (same)
11.5	70%	81%
MMSE		
24.5	100%	54%
21.5	81%	92% (lower)

This suggests that when identifying controls, a cut-off of >24.5 on MMSE should be used, together with a score of > 19.5 on the HVLT. When screening for cases, cut-offs should be a score of < 21.5 on the MMSE and < 14.5 on the HVLT. Thus, MMSE cut-offs for cases were lower in rural Central Java than in Oxford, but adapted HVLT data were similar.

Logistic regression using the optimal cut-offs of 19.5 for the HVLT showed that lower performance (< 19.5) was associated with diagnostic categorisation (OR=0.12, 95% CI=0.02 to 0.08, p<0.0001) independent of age (OR=0.89, 95% CI=0.78 to 1.01, p=0.08), gender (p=0.53) and education (ns, OR=1.38, 95% CI=.59 to 3.26). However, using similar analyses for the MMSE, showed that diagnostic category now was not entered in the equation and only education remained (OR=2.62, 95% CI=1.17 to 5.90), while age and gender also did not significantly contribute to the analyses. This suggests that the MMSE should not be used by itself in screening for dementia particularly in those with a low educational background.

The SF-36 had been assessed to establish quality of life/overall health as an indicator of dementia. While there was a significant difference on the mean SF-36 scores between controls (70) and dementia cases (65) and MCI (73), it was not deemed suitable as a diagnostic (low combined sensitivity and specifity using ROC, p=ns). However, partial correlation analyses (controlled for age, education, gender and occupation) showed that the HVLT was related to the MMSE performance (r=.56, p<0.001) and that both were related to SF-36 (r=.20, and r=.27, respectively, p<0.001), showing worse quality of life with worse cognitive performance.

In Depth Study of a Sub Sample of Rural Borobudur

Dr Raden Irawati Ismail, aided by structured and post mortem validated questions from an expert dementia diagnostic system developed at Oxford University (Hogervorst, 2003), further tested validity of Dr Fidiyansha's diagnoses against the HVLT and MMSE performance in dementia cases and controls to further investigate the cut-offs in a separate study. She also assessed a random number of cases and controls in a more in depth examination to establish ratios of different types of dementia in this sample. For this study, the questions from the decision trees of our post-mortem validated computerized dementia diagnostic system (Hogervorst, 2003) were used. The computer expert system showed to improve inter-rater reliability (between a medical student and an expert neurologist) and improved diagnostic accuracy significantly (in particular specificity) when comparing this to over 200 post mortem confirmed cases and controls. The system can simultaneously diagnose different dementia types using most consensus and research derived criteria within 5 to 10 minutes per patient (Hogervorst, 2003).

This in depth study was carried out in a sub sample of the Borobudur sample of n=59 Javanese elderly who resided in two of the four villages tested. This rendered 17 cases clinically diagnosed with dementia by Dr Ismail. Of these, 2 were considered to be clear Vascular Dementia cases, there was one AD mixed with stroke and focal signs/symptoms and one control who had a stroke with focal and other neurological signs and symptoms, but no signs of dementia. However, carer's history of progression suggested that 6 people had stepwise decline, which may have been suggestive of vascular dementia (35%). Unfortunately, brain scan information was not available (except for the control), so more detailed information needed for the vascular dementia diagnoses was not available. There were no other signs of Binswanger's disease (incontinence, focal signs etc) suggesting that this specific cerebrovascular pathology was also not present. Blood pressure was the same in cases and controls. There was no clear indication of LBD or FTD (no fluctuations in attention, hallucinations, aphasia, or personality change), and it was also judged that there was no psychiatric co-morbidity or substance abuse. All dementia and MCI cases (but one of 19) had memory complaints. Of the 17 dementia cases, 9 were thought to have probable AD (53%) according to NINCDS-ADRDA criteria (memory complaints and at least one other cognitive problem, affecting ADL, no other morbidity explaining disease, gradual and progressive, McKhann, 1984). In addition, 1 patient initially diagnosed as MCI by the psychiatrist was thought to have possible AD. Two cases with dementia were thought to be

related to systemic disease (12%), they had (non Parkinson's disease) tremors, and were clearly ill with sweating and weakness. However, no further diagnostic assessment (blood screen) could be performed. Thyroid disease was suspected as it is endemic in the area (hyperthyroidism ?), but only one of the participants had a goitre. However, using blood samples, we earlier found (Hogervorst, 2009) that half of those afflicted with thyroid disease cases in those over 65 years of age are not detected medically even in developed countries. It was thought that clinicians often rely too much on the clinical signs of thyroid disease, which may not be present in half of the cases.

Controls were significantly younger than cases. However, diagnoses determined test performance on the adapted HVLT [$F(2,54)=6.35$, $p<0.003$], independent of age ($p<0.001$) and gender ($p=.59$). The 19 patients diagnosed as MCI or preclinical dementia all had memory complaints, but all scored significantly higher on the adapted memory test than cases with dementia (on average 5 more words immediately recalled for MCI, compared to the on average 6 more words recalled for the 23 controls). Although a key criteria for amnesic MCI (aMCI), MCI or preclinical dementia cases, as assessed by the psychiatrist scored not significantly different from controls on the memory test ($p=0.33$, on average 1.2 word recalled less by MCI). However, the MMSE showed independent main effects of diagnoses ($F(2, 54)=8.21$, $p=0.001$) and also effects of age ($p=.03$) and a trend for gender ($p=0.06$). Adapted scores (by age and gender) showed that MCI scored significantly lower on this test than controls (on average 2.2. points less) but more than dementia cases (on average 3.1 points more, with controls scoring 5.4 points more than dementia cases). This suggested that aMCI is perhaps less important than other non memory cognitive impairments (e.g. executive dysfunction or other more focal cognitive impairments, such as aphasia, caused by vascular incidents) in the MCI or preclinical group.

Using ROC, optimal cut-offs for dementia (excluding MCI) on the memory test were again 19.5 on the total immediate recall (with 100% sensitivity and 78% specificity, or, with a cut-off of 17.5, this was 76% with 91%, respectively). Optimal cut-offs for the MMSE were 24 (with 88% sensitivity and 96% specificity, or, with a cut-off of 21.5, it was 65% and 96%, respectively).

In the table below, demographics and test scores for different clinical categories are given.

	Normal N=23	MCI/Possible N=19	Probable dementia N=17	
Age	66.3 (5.1)	70 (5.3)	76.6(9)	p<0.001
Women (%)	70%	74%	65%	p=.84
HVLT	22.5 (4.1)	20.0 (5.2)	13.1 (5.8)	p<0.0001
MMSE	26.9 (2.4)	24.0 (3.3)	19.6 (5.4)	p<0.0001
Adjusted for age and gender				
HVLT	21.1 (1)	19.9 (1)	15.2 (1.2)	
95% CI	19.1 to 23.2	17.8 to 22.0	12.7 to 17.6	
MMSE	26.1(.8)	23.8 (.8)	20.7 (.9)	
95% CI	24.5 to 27.7	22.3 to 25.4	18.8 to 22.6	

Including MCI with controls versus dementia still gave a significant area under the curve (.83-.85, $p<0.0001$) and test cut-off scores with their sensitivity and specificity are shown below.

Test	Sensitivity	Specificity
HVLT		
19.5	100%	64%
17.5	77%	72%
15.5	53%	93%
MMSE		
24.5	88%	74%
23.5	88%	79%
21.5	65%	83%

Discussion

These studies showed that similar cut-offs for dementia on two cognitive screening tests could be used for screening relatively affluent, highly educated elderly in Oxfordshire UK and those living in a rural environments on Central Java, Indonesia and that this was independent of age, gender, education and ethnicity. However, this study has several limitations.

Firstly, this algorithm was only validated for elderly Javanese and data should be included on Sundanese, Mingankebau, etc., as Indonesia is a multi ethnic country. However, as cut-offs were the same between elderly in Oxford and those of rural Java, we do not expect much deviation from these findings in other ethnic groups. Similar to Ganguli's version of the MMSE (1995), our modified version also had to be altered to address those who were illiterate and also for the seasons of the year (to 'wet or dry'). However, this did not affect the distribution and ceiling and floor performance was not observed.

Other screening tests developed for multi cultural assessment have shown comparable results. For instance, the Rowland Universal Dementia Assessment scale (RUDAS, Storey, 2004) is another brief dementia screening test, originally developed for a multicultural setting in Australia, which was found to have 89% sensitivity and 98% specificity for dementia. In a study in India (with 58 dementia cases and 58 age and sex matched controls), the RUDAS was found to have similar sensitivity to the MMSE (88% and 90%, using cut-off scores of 23 and 24, respectively), but it also had an educational bias. On the other hand, the RUDAS had better specificity than the MMSE (76% versus 48%, Iype, 2006). In the present study, the MMSE and HVLT seemed to offer a reasonable comparable screening test, but a direct comparison against the RUDAS and also the frequently used (but lengthy) 10/66 algorithm would be useful. For Vascular Dementia and FTD more executive and other function tests, such as the CLOX should be included and future studies will also include the RUDAS executive components to investigate whether this increases resolution and allows better detection of other than AD dementias

A major limitation of the present study was that no blood screens were available and also that no brain scans could be performed to establish more in depth diagnostics. This mimics

the reality of dementia screening in developing countries. In fact, we experienced that even in USA memory clinics, blood screens were not always performed routinely as part of a dementia screening, physicians relying mainly on medical external examinations.

Using our limited data from rural Java suggested a similar profile to many European studies, in that the majority of people with dementia would have had AD followed by VaD, as the second most frequent dementia type. However, as said, this could not be confirmed using brain scans. Whether vascular cognitive impairment is more common than aMCI, AD or FTD/LBD in Indonesia and whether some dementia is secondary and treatable (e.g. related to thyroid and lung disease, infectious disease, nutritional deficiency, toxicity of pesticides etc.) remains to be further investigated and current studies are underway to assess this. It is of interest to include comments from rural health center staff who often view the cognitive impairments as a normal part of aging. They suggested that arthritis and deafness/vision impairments (sensorimotor function) as causing the impairments. These would cause issues in cognitive assessment, which could reflect the high prevalence of dementia in the rural Central Javanese cohort and this needs further confirmation. Visual impairment, for instance, can be an early sign in dementia (Kirby 2010) and we are currently investigating this as a diagnostic marker in both the UK and in Indonesia. When taking into account impaired instrumental activities of daily living, the more realistic prevalence figure for dementia was lower at 11% in this rural area (and 5-6% overall, see above).

Taking into account these discussions with rural health staff and village elders on Pikun (dementia), if an elderly person despite significant cognitive impairment can still maintain their daily life in these relatively uncomplicated settings with few instrumental (with little telephone contact, radios, computers, ATM banking, travel etc.) activities of daily life requirements and sufficient support, the question is whether there is a 'dementia' as such (as in 'impairments that affect daily life', see APA, 1994). According to staff of the center for health, dementia is rare in the rural communities investigated. This contrasts with our reported *higher* prevalence on the basis of cognitive test data which, importantly, was independent of age, occupation and education. So while dementia prevalence is currently low in Jakarta, it may be higher in rural areas but perhaps have less impact in these settings. However, with increasing Westernization, and more young people moving away from rural parts and perhaps even less public investment in health with less center for health staff support, dementia may be an issue for the future with more elderly being isolated in rural areas with little support. This support works both ways. Older people still contribute significantly to family finances (Kreager 2006a.b. Rahardjo, 2007,2008) and dementia means a loss of economic contribution of the person affected, but also of their carers, who need to give 24 hour surveillances in the late stages of the disease. With our estimates of almost 2 million elderly affected by dementia by 2025, this means that at least 4 million of people over 60 years of age, who could substantially contribute to Indonesian economy, can then no longer do so because of dementia.

Even more troubling is that our predicted prevalence estimates could be an underestimate. Data were from a cohort, a generation, who had survived wars, colonialism and famine without much health care and without availability of antibiotics in the vulnerable stages of childhood. This cohort is thus different from the next (perhaps more urbanized) generations who survived childhood disease with antibiotics. In addition, many urban young children from affluent parents in Indonesia now seem obese, eating fast foods and engaging in little physical activity. However, because the more affluent can benefit from good private

health care systems, despite increased risks of these lifestyles on early diabetes mellitus and heart disease also affecting cognition and independence (Clifford, 2009), this generation could be a longer non productive drain on community and health care resources. With increasing health risks at an earlier age, non communicable disorders (NCD) could be predicted to start occurring in very early midlife (the late third decade). Drastic lifestyle interventions are thus needed now for the younger generations. Otherwise, Indonesia could be hit by a double whammy of dementias of a primary nature associated with NCD risk factors (heart disease, diabetes etc) in the rich, and infectious-, nutritional deficient- and systemic disease leading to treatable secondary dementias in the poor.

Prevention of dementia is crucial as no treatment is effective in the longer term and both human and economic costs are high. For the West the current consensus based on longitudinal studies is that midlife is the latest when the most effective lifestyle interventions should be made (Clifford, 2009). If the current trend persists in Indonesia, the more affluent middle classes will experience typical old age disease (diabetes, vascular disease) much earlier, but will also experience this for much longer with improved health care, which is available for those who can afford it. This means that interventions in Indonesia must start earlier, e.g. with more physical activity promotion for middle-class obese children and with better and longer free education for the rural and poor urban children. High education in childhood protects the brain against an earlier onset of dementia and heart disease, possibly because of more reserve capacity (Whalley, 2002; Clifford, 2009) and availability of alternative coping and vocabulary once cognitive impairment sets in. Currently good education in Indonesia is very expensive, however, and more governmental investments need to be made to secure lower costs of poor health related to low education in the long run.

In sum, the studies described in this chapter have led to validation of a short and cheap screening instrument for dementia in rural and urban Java. The data from the validation study combined with those from our first study can give the Indonesian government some indication of the percentage of people afflicted with dementia and can aid in predictive analyses of costs and growth in some areas on Java. This is particularly important given the possible dark scenarios for the development of this disease, that will at least double in prevalence in the next decades, but which may start to occur even earlier for our current young cohort unless drastic lifestyle changes are implemented.

In a group discussion coordinated by Prof Rahardjo and Untung with local policy makers and the medical doctors in Jakarta at the end of this validation study, the consensus was that both validated instruments (the Modified MMSE and HVLT) should be used as screening tools to detect dementia in primary health centers. Subsequently, the implementation and training of the use of these tools was done in Borobudur, Palu and Banda Aceh by Dr Ismail and Dr Fidiansyah in 2010. Recently, it was proposed that from 2011 these tools are to be implemented in the whole of Indonesia, which was supported by the the Ministry of Health and the National Commission for Older Persons of Indonesia which advises directly to the Indonesian President on matters of aging. If this algorithm works in other developing countries and in multi-ethnic cohorts, it can be used by governments to track dementia prevalence, which is an increasing health concern affecting economies worldwide.

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Appendix

Instrumental Activities Of Daily Living (IADL) Based On Lawton And Brody (1969)

No	Activities	Point	Criteria	Comment whether wife/maid does this
Q1	Extending message/using telephone (e.g. there will be meeting tomorrow at Mr. RT's at 10)	0	Unable to extend messages (doesn't have telephone included)	
		1	Message extended partially (capable of answering phone but unable to operate it)	
		2	Able to operate telephone/message extended completely	
Q2	Shopping	0	Unable	
		1	Capable of purchasing up to 3 items, otherwise need help.	
		2	Independent	
Q3	Preparing meal	0	Unable	
		1	Able to cook if the ingredients are ready or can warm cooked food	
		2	Independent	
Q4	Housekeeping	0	Unable	
		1	Able to do light tasks (sweeping, making the bed) only, otherwise needs help.	
		2	Independent (capable of doing all household tasks including mopping and washing clothes)	

Instrumental Activities (Continued)

No	Activities	Point	Criteria	Comment whether wife/maid does this
Q5	Washing clothes	0	Unable	
		1	Able to wash light clothes or ironing, otherwise needs help	
		2	Independent (using washing machine included)	
Q6	Utilization of transportation means	0	Unable to travel with any transportation	
		1	Travels on public transportation/taxi or private car if helped/accompanied by other	
		2	Independent travel	
Q7	Responsibility of taking or preparing own medication	0	Needs help from others to prepare and consume medication.	
		1	Able if medication is previously prepared	
		2	Independent (able to prepare own medication according to prescribed dose and time)	
Q8.	Ability to handle finances	0	Incapable	
		1	Able to arrange daily purchases, but needs help with banking/major purchasing	
		2	Able to manage financial problems (household budget, pays the rent, receipt, bank matters) or to monitor income.	
	Total score			

IADL score: 9 – 16 : Independent/doesn't need any help, 1 – 8 : Needs help, : Unable to do anything

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Chapter XIV

Cognitive Function and Hypertension: Pathophysiology and Mechanisms

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Abstract

According to several longitudinal studies, hypertension appears to predispose individuals to the development of cognitive impairment, dementia and Alzheimer's disease, after a period varying from a few years to several decades. Antihypertensive drug treatment, according to preliminary evidence, may help to reduce the rates of such incidences. Such findings wait to be confirmed by more large therapeutic trials. Understanding the effect of hypertension on cognition is a work in progress. There are several mechanisms of impairment of cognition in hypertensive state. Cerebral hypoperfusion and chronic oxygen deprivation appear to play a pivotal role in cognition deficit and AD pathophysiology due to hypertension. Chronic hypertension alters cerebral endothelium by causing microvascular degeneration. Further, hypertension induces proliferation of smooth muscle cells, basal lamina alterations, luminal narrowing, endothelial hyalinosis, and fibrosis which leads to hypoperfusion, chronic cerebral oxygen insufficiency and deranged glucose homeostasis such as in AD. There are alterations in neurovascular coupling and autoregulatory system which further causes cerebral hypoperfusion. Chronic hypertension also alters peripheral as well as brain renin angiotensin system (RAS) and nitric oxide (NO) pathways which also contribute in cerebral hypoperfusion and hypometabolism. As pathophysiology of hypertension emerges as a contributing risk factor for dementia and AD, it appears that measures directed to control blood pressure will enhance cognitive reserve.

Key words: Hypertension, Dementia, Renin-angiotensin system (RAS), Nitric oxide (NO), Cerebral circulation

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1. Introduction

Dementia of vascular origin i.e. vascular dementia (VD) has gained much attention in the recent times. VD is the loss of cognitive functions of an individual due to ischemic or hemorrhagic cerebrovascular disease (CVD) or from cardiovascular or circulatory disturbances damaging brain regions important for memory, cognition and behavior to such an extent that in advanced stage it begins to encumber with activities of daily life (Román, 2002). Approximately 20% of dementia cases worldwide emanate due to vascular disturbances making VD the second most common form of dementia after Alzheimer's disease (AD) (Dubois and Herbert, 2001). Some European studies showed that the prevalence of VD is 1.6% in the over-65-year-old population as compared with a prevalence of 4.4% of AD in the same studies. North American studies also showed similar estimates averaged together. It was found that prevalence and incidence of VD rises steadily with advancing age (Knopman, 2007). Though prevalence of dementia in developing countries like India and sub-Saharan Africa is less 1-3% (Kalaria et al., 2008), a study found a prevalence of about 39% in population of >65 years in Indian state of Kerala (Shaji, Bose, Verghese. 2005)

Table 1. Risk Factors for Vascular Dementia

- Advanced age
- Isolated systolic hypertension in the elderly
- Chronic and untreated arterial hypertension
- Cigarette smoking
- Hyperhomocysteinemia
- Congestive heart failure
- Recurrent stroke
- Orthostatic hypotension
- Obstructive sleep apnea
- • Coronary artery bypass graft surgery
- Diabetes mellitus
- Hyperlipidemia
- Hyperfibrinogenemia
- Atrial fibrillation
- • Major surgery in the elderly

Among various factors supposedly predisposing to the prevalence of cognitive impairment of vascular origin (Table 1), a history of hypertension has now emerged as an important risk factor for the sporadic, prevalent form of AD in addition to age, diabetes, hypercholesterolemia and genetic factor like apolipoprotein E4 (ApoE4) allele (Hamel et al., 2008). The conclusions of epidemiological studies, that the incidence of cognitive impairment is due to complication of arterial hypertension, vary with the methodology used and depend on the cross-sectional or longitudinal nature of the study, the population included and the method of evaluation of cognitive functions. Cross-sectional studies have found positive (Starr et al 1993; Seux et al 1998) as well as negative (Farmer et al 1987; Scherr et al 1991) correlations between arterial hypertension and cognitive impairment. However, most of the longitudinal studies showed a positive relationship between the presence of hypertension in midlife and the onset of cognitive decline 15–20 years later (Duron and Hanon. 2008).

2. Hypertension and Cognitive Function: An Epidemiological Perspective

Initial support for the view, that hypertension is associated with diminished performance on tests of cognitive function, came from a number of epidemiologic studies. These studies examined the relationship of blood pressure to cognitive loss in late life. Skoog et al. (1996) demonstrated that 70 years old with increased blood pressure show more propensity of developing dementia between the ages of 79 and 85 years as compared with non-demented subjects. However, the relationship between the level of cognitive function and BP values is not simple and has been the subject of numerous conflicts. The conclusions of epidemiological studies vary with different factors like nature of the study i.e. cross-sectional or longitudinal, the population and how cognitive functions are evaluated.

2.1. Cross-Sectional Studies

The data of cross-sectional studies show contrasting results. Some studies show a positive correlation between cognitive decline and BP demonstrating the deleterious effects of hypertension (Gale et al., 1996; Cacciatore et al., 1997; Kilander, 1997; Seux et al., 1998) while other studies, in contrast, show no relationship at all (Farmer et al., 1987; Desmond et al., 1993; Scherr et al., 1991; van Boxtel et al., 1997) or in some cases even a negative effect (Guo et al., 1996).

There are many factors responsible for these conflicting results. Selection bias in the populations of these studies is partly responsible for such divergent results. The results differ with the BP characteristics of the subjects included (normo- or hypertensive), the method of evaluation of BP (several measurements or a single measurement), the type of recruitment (general population or selected sample), the percentage of hypertensive subjects treated, the existence of other cardiovascular risk factors and the method of evaluation of cognitive functions (exploring one cognitive area in particular, or intellectual functioning in general).

2.2. Longitudinal Studies

Longitudinal studies provide more comprehensive and holistic information as they focus how ‘chronic’ hypertension influences cognitive function. Results are more consistent and the majority of them indicate a positive relationship between arterial hypertension and cognitive impairment. In particular, midlife hypertension is a highly predictive sign of subsequent cognitive deterioration; the higher the initial BP, the poorer the subsequent cognitive function. A few such studies observed the effect of blood pressure on subsequent developments of cognitive loss and found an association of hypertension in midlife with neurocognitive loss about 15 to 20 years later (Duron and Hanon, 2008). A prospective Finnish population study of a 21-year follow-up evaluated the impact of midlife elevated blood pressure on the subsequent development of mild cognitive impairment (MCI) in the elderly and observed a tendency toward a significant relation between hypertension and the risk of MCI (Kivipelto et al., 2001).

Hypertension is also a risk factor for AD as it is associated with important AD endophenotypes. A population based longitudinal study with 36 years of follow-up examined the relationship of midlife hypertension with later development of cognitive impairment, vascular dementia and AD in Japanese-American men (Petrovitch et al., 2005). In this study, elevated SBP in midlife was found associated with important endophenotypes such as low brain weight and greater amounts of neuritic plaque in the neocortex and hippocampus. Also, elevated DBP related to greater numbers of neurofibrillary tangles in the hippocampus (Petrovitch et al., 2005). All these studies confirm that hypertension can directly increase the propensity of developing dementia.

The relationship between hypertension and cognition deficit was further accentuated by many studies which proved that the control of blood pressure can modify important endophenotypes and reduce dementia rates in persons with hypertension. Guo and colleagues (1999) showed that antihypertensive treatment caused a significant reduction in the risk of cognitive loss in later years. To determine whether baseline hypertension and antihypertensive treatment predicts cognitive decline in elderly individuals, a fairly large longitudinal population-based study of 1373 elderly individuals was conducted in Nantes (western France). In a 4-year follow-up study, hypertensive patients receiving treatment were compared with a control group and the treatment group had lower cognitive decline at follow-up (Tzourio et al., 1999). Another study with a 5-year follow-up period of 1617 African Americans observed a 38% reduction in cognitive decline in persons with hypertension treated with medication compared with untreated controls (Murray et al., 2002). A number of studies also tried to trace the relationship between antihypertensives and AD. Hanon and colleagues (2006) showed a 42% reduction in the risk of developing AD in persons with hypertension receiving medication, a finding that is also supported by another study, with a large population-based sample of 3308 persons from the Cache County cohort (Khachaturian et al., 2006). Moreover, data from the Honolulu Asia Aging Study showed that the duration of antihypertensive therapy also mattered: For each additional year of treatment, there was a reduction in the risk of dementia incidence (Peila et al., 2006)

2.3. Low Blood Pressure and Cognitive Function

The investigation of the relationship of blood pressure with cognitive decline has focused largely on elevated blood pressure till now; however, emerging evidences suggest that excessively low blood pressure can promote the susceptibility to cognitive decline. Evidence from the NHANES III data suggests that low blood pressure exerts a negative effect on cognitive function, especially in the very old (>80 years). Other studies also show increased incidence of dementia and AD in persons with low SBP or DBP, respectively (Obisesan, 2009). In Kungsholmen project, Qiu *et al* (2003) found that low DBP (<70 mmHg), in 7 years before dementia appears, was associated with increased risk of dementia and AD while baseline evidence of cognitive function was controlled. Patient undergoing treatment for hypertension further supported this finding. Qiu *et al* (2004) showed that a 15 mmHg drop in SBP in subjects whose baseline SBP is less than 160 mmHg increased the propensity of developing dementia and AD, especially in those suffering from vascular disorders such as cerebral vascular accident and diabetes. The 3-year follow-up data from the Gothenburg and Rotterdam studies also support that too much reduction of SBP and DBP increases risk of

dementia among those undergoing treatment for hypertension (Obisesan, 2009). Further, a study by NHANES found that low pulse and blood pressure can promote cognitive loss and risk of AD demonstrating that pulse pressure also affects cognitive function (Lee et al., 2006, Waldstein et al., 2005).

3. Characteristics of the Cerebral Blood Supply

The intracranial cerebral arteries take off from the circle of Willis at the base of the brain and give rise to progressively smaller vessels, termed pial arteries, crawling on the brain surface. Pial arteries branch out into smaller vessels penetrating into the substance of the brain and give rise to arterioles and capillaries. Brain blood vessels are lined with endothelial cells. These endothelial cells constitute blood-brain barrier (BBB). BBB, an interface between brain and blood, consists of endothelial cells stitched tightly together by tight junctions. The endothelial blood-brain barrier limits access of many humoral stimuli to smooth muscle of cerebral blood vessels (Faraci and Heistad, 1998) and is insurmountable to most blood-borne substances (Zlokovic, 2008). Arteries and arterioles have one or more layers of smooth muscle cells (myocytes). In capillaries, myocytes are replaced by pericytes. Cerebral arteries and arterioles are innervated by nerve fibers arising from cranial autonomic and sensory ganglia (Iadecola and Nedergaard, 2007). Smaller arterioles ($\leq$ 100 μm) and capillaries are fully cloaked by the end feet processes of astrocytes (Iadecola and Nedergaard, 2007).

The brain needs an uninterrupted supply of oxygen and energy substrates delivered through blood flow. To ensure that the brain receives an adequate amount of blood at all times, cerebral blood vessels are endowed with adaptive mechanisms. The cerebral blood supply is not homogenous, but depends upon the varying energetic needs of different brain regions. When a brain region is activated, cerebral blood flow (CBF) in that particular region increases, a phenomenon termed functional hyperemia (Iadecola and Nedergaard, 2007). During activation, neurons, astrocytes, and vascular cells release a multitude of vasoactive agents like nitric oxide (NO), carbon monoxide, prostanoids, cytochrome P^{450} metabolites, adenosine, and K^+ ions which act in concert to produce vasodilatation of local arterioles during neural activity. The vasodilatation of local arterioles is accompanied by vasodilatation of upstream pial arteries that supply the activated area (Iadecola and Nedergaard, 2007).

Another prominent feature of cerebral circulation is its ability of autoregulation. Cerebrovascular autoregulation makes CBF independent of changes in arterial pressure within a certain range of 60–150 mmHg mean arterial pressure (Paulson, 1990). In absence of autoregulation, changes in arterial pressure may lead to potentially dangerous increases or decreases in CBF (Mancia et al., 1988). To counteract the effects of blood pressure variations on CBF, cerebral arterioles adjust their resistance according to intravascular pressure. Thus, arterioles constrict when the pressure increases and relax when the pressure decreases. Autoregulation is related to the ability of arterial myocytes to constrict when intravascular pressure rises (myogenic response) (Brayden et al., 2008).

In some vascular beds (e.g., renal, cutaneous, and skeletal muscle), moderately severe levels of hypercapnia and hypoxia have relatively small effects on blood flow, however the same stimuli initiate extreme vasodilatation in the cerebral circulation mediated by nitric oxide (Faraci and Heistad, 1998).

4. Hypertension and Cognitive Function: A Mechanistic Overview

Being a leading cause of stroke and dementia, essential hypertension has devastating effects on the brain,. It alters the structure of cerebral blood vessels and disrupts intricate vasoregulatory mechanisms that promise an adequate blood supply to the brain. These alterations threaten the cerebral blood supply and increase the susceptibility of the brain to ischemic injury as well as Alzheimer's disease. The effects of chronic hypertension on neurocognition and AD appear to be mediated independently or in concert by endotheliahyalinosis, reduced vascular compliance and fibrosis, disturbed neurovascular coupling, altered autoregulatory system and ultimately cerebral hypoperfusion. These changes in vasculature impair the ready flux of important biochemical and synaptic transmission. All these changes affect the blood-brain barrier resulting in increased vascular permeability, protein extravasations in the brain parenchyma, leading to amyloid β protein accumulation. Apart from above factors, the components of renin angiotensin system (RAS) (i.e Angiotensin converting enzyme (ACE) and AT1 receptors) and nitric oxide (NO) pathways play pivotal role in progression of pathogenesis of hypertension and mediate vascular changes in persons with chronic hypertension affecting cognition.

4.1. Cerebral Blood Flow

Studies in humans show that cerebral blood flow and cognitive functions are intricately linked to each other. Reduction in cerebral blood flow significantly affected performance in the Cambridge Cognitive Examination (Tsolaki et al., 2001). Ueda et al (2002) showed that left posterior temporal regional CBF predicted performance on the clock drawing test. Ushijima et al. (2002) found that attention and calculation were affected with decline in CBF in the frontal cortex whereas orientation and recall were associated with attenuation of CBF in posterior brain regions. Moreover, reduced CBF in the right posterodorsal, anterior and superior prefrontal cortex and the inferior parietal cortex was most pronounced in AD patients with rapidly declining cognitive function (Nagahama et al., 2003). Decreased CBF result in chronic deprivation of oxygen in cerebral vasculature and increased susceptibility to hypoxia which is deleterious for cognitive functions. Hypoxia potentiated cyclooxygenase-2 and presenilin-1 gene expression induced by interleukin-1beta and amyloid beta 42 peptide in primary human neural cells (Bazan and Lukiw, 2002). Therefore, it is obvious that cerebral hypoperfusion has an etiologic role in the pathogenesis of cognitive loss.

Both acute and chronic hypertension affects cerebral blood flow. Acute hypertension damages endothelium and impairs dilator responses of cerebral blood vessels, due to endothelium-dependent stimuli such as acetylcholine, in response to systemically administered presser agents (Wei et al., 1985) or experimentally induced head injury (Ellison et al., 1989; Kontos and Wei 1992). Mechanisms that cause impairment of cerebral arterioles to endothelium-dependent stimuli involve production of reactive oxygen species. Production of superoxide anion in brain has been measured in response to acute hypertension (Wei et al., 1985) and fluid percussion injury (Kontos and Wei 1992, 1986; Kontos and Wei, 1992; Thorogood and Armstead, 1996). Local application of superoxide dismutase or deferoxamine

restores vasodilator responses to acetylcholine toward normal, suggesting that inactivation of NO by superoxide anion or hydroxyl radical is responsible for impaired endothelial function (Kontos and Wei 1992; Wei et al., 1985).

Besides, impaired endothelium dependent relaxation in peripheral blood vessels in experimental and human studies, chronic hypertension decreases dilatation of basilar artery (Kitazono et al. 1996; Mayhan, 1990), middle cerebral artery (Vacher et al., 1996), and cerebral arterioles (Mayhan et al., 1987; Mayhan et al., 1988) in response to endothelium dependent agonists like acetylcholine, bradykinin and ADP in spontaneously hypertensive rats (SHRs) and stroke prone SHRs. However, impairment of vascular functions in cerebral vasculature is at level of endothelium and not smooth muscles because cerebral vasodilation in response to endothelium–independent agonists like NO (Yang et al., 1993), nitroglycerin (Yang et al., 1993; Yang et al., 1991a, 1991b), nitroprusside (Baumbach et al., 1994; Kitazono et al., 1993), forskolin (Kitazono et al., 1993), and adenosine (Mayhan et al., 1987, Yang et al., 1993) remains intact.

There are several mechanisms which may account for impaired endothelium-dependent relaxation in cerebral blood vessels during chronic hypertension. Chronic hypertension enhances activity of COX-1 which results in production of a COX-derived endothelium derived contracting factor (EDCF) that counteracts the normal vasodilator effect of endothelium derived relaxing factor (EDRF). This same mechanism leads to altered responses in cerebral arterioles during chronic hypertension because impaired endothelium-dependent responses can be restored to normal using indomethacin (Mayhan et al., 1988) or an inhibitor of PGH2/thromboxane A2 receptors (Mayhan 1992). In contrast to arterioles, impaired endothelium-dependent responses of the basilar artery during chronic hypertension is due to reduced production or activity of EDRF because L-arginine, the substrate for NO synthase, restores responses of the basilar artery to acetylcholine towards normal in older SHRSP (Kitazono et al., 1996).

The functional influence of potassium channels in cerebral vessels is altered during chronic hypertension. Cerebral vasodilatation in response to activators of ATP sensitive potassium channels is impaired in stroke prone SHR (Kitazono et al., 1993, Takaba et al., 1996). A number of studies suggested that a high-salt diet impairs membrane hyperpolarization and relaxation of the middle cerebral artery in response to prostacyclin and hypoxia in *in vitro* (Lombard et al., 1996). Relaxation of the middle cerebral artery to both these stimuli is inhibited by glibenclamide indicating that relaxation is mediated by activation of the ATP-sensitive potassium channel (Fredericks et al., 1994). In contrast, activity of calcium-dependent potassium channels appears to be enhanced during chronic hypertension. Efflux of ^{86}Rb, an index of potassium channel activity, is greater in carotid arteries from SHR than from WKY (Asano et al., 1993). Inhibitors of calcium-dependent potassium channels produce greater contraction of the carotid artery *in vitro* (Asano et al., 1993) and the basilar artery *in vivo* in chronically hypertensive rats suggesting that activity of calcium-activated potassium is enhanced during chronic hypertension (Paterno et al., 1997).

4.2. Neurovascular Coupling

The most distinctive feature of cerebral blood vessels is their close interaction with neurons and glia. Neurons, glia (astrocytes, microglia, oligodendrocytes), and vascular cells

(endothelium, smooth muscle cells or pericytes, adventitial cells) are closely related in development, structure and function. This close spatial and temporal relationship between neural activity and subsequent changes in CBF is termed neurovascular coupling. The magnitude and spatial location of blood flow changes are tightly linked to changes in neural activity through a complex sequence of coordinated events involving neurons, glia, and vascular cells. Many vascular-based functional brain imaging techniques, such as fMRI, rely on this coupling to infer changes in neural activity (Girouard and Iadecola, 2006).

The neurovascular coupling is regulated by a number of mediators like ions, metabolic by-products, vasoactive neurotransmitters and vasoactive factors released in response to neurotransmitters. Among ions, K^+ and H^+ play an important role in regulation of CBF. Elevations in extracellular K^+ up to 8–10 mM, by inward rectifier type K^+ channels on the membrane of arterial smooth muscles cells, cause dilation of arterioles both *in vitro* and *in vivo*. This results in hyperpolarization and subsequent relaxation (Kuschinsky et al., 1972; Nguyen et al., 2000). Further, neuronal activity also induces reductions in extracellular Ca^{2+} which may produce vasodilation (Heuser, 1978). Besides ions, metabolites also affect neurovascular coupling and modulate CBF. Pellerin and colleagues (1998) demonstrated that astrocytes metabolize glucose through glycolysis, leading to lactate production which results in functional hyperemia by increasing H^+ concentration and producing vasodilation (Attwell and Iadecola, 2002), at least in part by opening K^+ channels (Faraci and Sobey 1998). Intracellular signaling induced by activation of neurotransmitter receptors also affect neurovascular coupling by generating vasoactive factors. For example, activation of glutamate receptors produces vasodilation and increases blood flow. In neocortex and hippocampus, exogenous glutamate or *N*-methyl-D-aspartate (NMDA) dilates pial arterioles and/or cerebral microvessels (Faraci and Breese, 1993; Lovick et al., 1999) by elevating Ca^{2+} associated with glutamate receptor activation. The increase in Ca^{2+} activates enzymes like the neuronal NO synthase (nNOS), which produces the vasodilator NO (Yang and Iadecola, 1996).

Hypertension affects brain and its circulation and disturbs neurovascular coupling. Hypertension leads to structural changes in cerebral blood vessels by producing vascular hypertrophy, remodeling and by promoting atherosclerosis in large cerebral arteries and lipohyalinosis in penetrating arterioles thus compromising cerebral perfusion (Dickinson, 2001; Faraci et al., 1990). Hypertension impairs endothelium-dependent relaxation (Faraci and Heistad. 1998) and cerebrovascular autoregulation (Heistad and Kontos 1983) and alters neurovascular coupling. Administration of Ang II to mice increases arterial pressure (20–30 mmHg) and attenuates the increase in CBF in somatosensory cortex produced by whisker stimulation, without reducing resting CBF (Kazama et al., 2003). This disturbance of neurovascular coupling by Ang II is blocked by losartan, indicating the role AT1 receptors (Kazama et al., 2003). This attenuation in functional hyperemia is observed even if the systemic pressor effect of Ang II is avoided by applying the peptide directly to the cerebral cortex (Kazama et al., 2003). The cerebrovascular effect of short-term administration of Ang II is independent of the increase in arterial pressure as elevation of arterial pressure by phenylephrine administration does not reproduce the effects of Ang II on functional hyperemia (Girouard and Iadecola, 2006). There is evidence that hypertension alters functional hyperemia in humans also. The increase in CBF in posterior parietal and thalamic areas produced by cognitive tasks is reduced in patients with chronic untreated hypertension relative to normotensive individuals resulting in a lower cognitive performance (Jennings et

al., 2005). Reduction in resting CBF in hypertensive subjects have also been reported (Fujishima et al., 1995). All these findings confirm that hypertension impairs neurovascular coupling in humans.

Newly emerging evidence suggests that hypertension-induced disturbance of neurovascular coupling can also directly promote Aβ pathology. Disturbed neurovascular coupling, by attenuating the activation of neuronal-related increase in compensatory CBF, directly upregulates Aβ formation. Significant dysregulation of cerebral circulation in mouse models of AD, mutated to overexpress APP and increased Aβ levels, was found indicating derangement of the neurovascular coupling (Iadecola 2004). There is also, attenuation of endothelia dependent vascular response as observed by altered vascular reactivity in isolated vessel of normal mouse exposed to Aβ 1-40 (Niwa et al., 2001; Crawford et al., 1998). Less than optimal CBF also affect Aβ trafficking across the blood-brain barrier and reduce Aβ clearance thus promoting its accumulation in the brain (Zlokovic et al., 2005).

4.3. Hypertrophy, Remodeling and Stiffening of Cerebral Blood Vessels

Hypertension induces hypertrophic and eutrophic remodeling which lead to adaptive changes in systemic as well as cerebral arteries. In hypertrophic remodeling, smooth muscle cells undergo hypertrophy or hyperplasia and grow inward increasing the wall thickness and reducing the lumen of the vessel (Baumbach and Heistad, 1988). In eutrophic remodeling, smooth muscle cells undergo a rearrangement resulting in reduction of the vessel lumen without changes in total vascular mass or wall thickness (Baumbach and Heistad, 1988). Hypertension also leads to vascular stiffening, a process that increases collagen content and rigidity of the vessel wall (Baumbach and Heistad, 1988).

Several factors contribute to hypertrophy in cerebral arteries and arterioles. The sympathetic perivascular innervation exerts a trophic effect on the vascular wall and causes the development of cerebrovascular hypertrophy (Baumbach et al., 1989). Furthermore, mechanical effects of the elevated intravascular pressure on the vascular wall play a role through growth factors, oxidative stress, and NO (Harrison et al., 2006; O'Callaghan and Williams, 2000). Reduced availability of NO, an agent with antiproliferative activity, leads to hypertrophy, as indicated by the vascular growth observed with nitric oxide synthase (NOS) inhibition or in $eNOS^{-/-}$ mice. Oxidative stress also contribute in hypertrophy because mice lacking reactive oxygen species (ROS) scavenger enzyme Cu/Zn superoxide dismutase (SOD1), show hypertrophy even in the absence of hypertension.

Ang II plays a crucial role in the mechanisms of cerebrovascular remodeling (Schiffrin and Touyz, 2004). Treatment of spontaneously hypertensive rats with angiotensin-converting enzyme inhibitors attenuates remodeling independently of effects on blood pressure (Chillon and Baumbach, 1999). ROS induced by Ang II participate in the remodeling of cerebral blood vessels (Schiffrin and Touyz, 2004). Ang II-induced hypertension leads to the activation of matrix metalloproteinase (MMP) and breakdown of matrix proteins that play a critical role in hypertrophy, remodeling, and stiffening (Flamant et al., 2007).

Hypertrophy and remodeling are adaptive responses aimed at reducing stress on the vessel wall and protecting downstream microvessels from the deleterious effect of increased blood pressure (Baumbach and Heistad, 1988; Laurent et al., 2005). Failure of this protective mechanism results in BBB alteration, cerebral edema, and cerebrovascular pathology.

Though, ablation of perivascular sympathetic nerves early in life prevents cerebrovascular hypertrophy in stroke-prone spontaneously hypertensive rats but promotes the development of cerebrovascular lesions (Sadoshima et al., 1983). Remodeling of systemic or cerebral vessels is potentially damaging because it reduces the vessel's lumen and increases vascular resistance, resulting in a greater propensity for vascular insufficiency (Barry, 1985; Mathiassen et al., 2007). Arterial stiffening is also deleterious because it leads to increases in pulse pressure, a good predictor of stroke and cognitive impairment. If hypertension is sustained chronically, the changes in vascular wall composition may lead to reduced distensibility and stiffening. Therefore, duration and magnitude of the blood pressure elevation as well as vessel size are important determinants of the alteration in the vascular wall induced by hypertension (Iadecola and Davisson, 2008)

4.4. Brain Renin-Angiotensin System

Renin-Angiotensin System, conventionally known to regulate systemic blood pressure, fluid homeostasis and hormone secretion, have been shown recently to exert a significant effect on behavioral and cognitive responses (Amouyel et al., 2000; McKinley et al., 2003). The RAS is a complex enzymatic pathway generating several active peptides but the effector peptide of the RAS is angiotensin II which does not cross the blood-brain barrier (von Bohlen und Halbach and Albrecht, 2006). However, peripheral RAS can directly influence cerebral regions such as the circumventricular areas lacking the blood-brain barrier. Apart from peripheral RAS affecting certain brain regions, a complete brain RAS also exists that is distinctly separate from the peripheral system and consists of all necessary precursors and enzymes necessary for the generation and metabolism of the biologically active forms of angiotensin (Wright and Harding 1994; McKinley et al., 2003).

The evidence that RAS is closely intertwined with cognitive function, comes from clinical studies. Amenta et al. (2002) observed in majority of controlled clinical trials that ACE inhibitor treatment (including perindopril, captopril and lisinopril) positively influenced cognitive function independently of its BP-lowering effects and showed better results than diuretics and β-blockers. The Perindopril Protection Against Recurrent Stroke Study (PROGRESS, 2001), conducted among patients with prior stroke or transient ischemic attack, also showed that treatment with perindopril showed a 19% risk reduction in cognitive decline and 34% and 45% reduction in the composite risks of recurrent stroke and cognitive decline respectively. Fogari el al. (2004) found that treatment of elderly hypertensive patients with valsartan and enalapril improved cognitive function besides lowering BP.

Support of interaction of hypertension, RAS and cognition comes from experimental studies also. Brain RAS and its effector peptide i.e. Ang II and its different metabolites like Ang IV and Ang III (Culman et al., 2002; Baranowska et al., 1983; Braszko et al., 1987) exert modulatory effects on learning and memory by influencing the activity of neurotransmitters such as acetylcholine (Barnes et al., 1989). Braszko and his group demonstrated enhancement of learning by Ang II administration, in a number of tasks including passive avoidance and object recognition, (Baranowska et al., 1983; Braszko et al., 1987; Braszko et al., 1988), was mediated by AT1 receptors (Braszko, 2005; Kulakowska et al., 1996). Opposing effects have also been reported; when infused into hippocampus after training, Ang II produced a dose-dependent amnesic effect in the inhibitory avoidance task in rats, which was blocked by the

AT2 antagonist, PD123319, but not by the AT1 antagonist, losartan (Kerr et al .,2005). Another study showed that intrahippocampal administration of Ang II disrupted retrieval of aversive memory in the inhibitory avoidance task, an effect that was mediated via the AT2 receptor (Bonini et al., 2006). Rats with renal hypertension, induced by the method of Goldblatt, showed poor acquisition, retrieval of the learned behavior and disturbance in memory consolidation process and this state was reversed with ACE inhibitor enalapril and AT1 receptor antagonist losartan (Srinivasan et al., 2005). Lifetime treatment of either SHR or WKY with the antihypertensive drug captopril, an ACE inhibitor, significantly attenuated the age-related impairment in learning and memory (Wyss et al., 2003). Recently our group also showed that central AT1 receptors exert an adverse effect on learning and memory in IC STZ induced memory deficit in mice and AT1 receptor blockade by Candesartan at non hypotensive dose improved learning and memory (Tota et al., 2009).

Cholinergic system in the hippocampus plays an important role in memory formation as demonstrated by use of acetylcholine esterase inhibitors in AD to prevent memory decline (Tota et al., 2009). Though cholinergic system is influenced by several factors but RAS also modulates cognitive functions by affecting cholinergic system. Ang II has an inhibitory effect on cholinergic system as it inhibits acetylcholine release from the entorhinal cortex associated with cognitive performance and this effect is reversed by Ang II receptor antagonists (Barnes et al., 1989; Barnes et al., 1990). Ang II also inhibits hippocampal long term potentiation, an effect reversed by AT1 receptor antagonist sarlasin (Denny et al., 1991). RAS inhibition is expected to prevent a cognitive decline in Alzheimer's disease and in the metabolic syndrome, based on animal studies.

Recently, experimental studies proved that RAS inhibition by an ARB prevented the onset of Alzheimer's disease. An ARB, valsartan, attenuated oligomerization of amyloid β peptides into high-molecular-weight oligomeric peptides and attenuated the development of amyloid β-mediated cognitive impairment in Tg2576 mice, an Alzheimer's disease mouse model (Wang et al., 2009). However, there are conflicting reports about the role of angiotensin converting enzyme and metabolism of amyloid β peptides in AD. ACE inhibitors directly reduced Aβ aggregation by degradation, attenuated Aβ fibril formation and prevented neuronal cell death from Aβ-induced neurotoxicity, ultimately reducing susceptibility to AD (Hu et al., 2001). However, in a clinical investigation of the relationship between use of antihypertensive medication and the Alzheimer's disease, the onset of Alzheimer's disease was attenuated by a diuretic agent or calcium channel blocker but an ACE inhibitor failed to prevent Alzheimer's disease, indicating the involvement of ACE in amyloid β deposition (Khachaturian et al., 2006). Relationship between ACE and AD was further supported by study of Kehoe et al (1999) who reported an association between the I/D polymorphism and AD for the first time. In their case–control study, a positive association was found between presence of the I allele and AD (OR 2.43 [95% CI: 1.35, 4.39] for II/ID versus DD genotypes). However, animal study shows contrary result. Amyloid β level in the brain was not changed in ACE-deficient mice, suggesting that further investigations are required to determine whether ACE is actually involved in the decomposition of amyloid β (Kehoe and Wilcock, 2007).

4.5. Nitric Oxide

The discovery of nitric oxide (NO), a lipophilic gaseous molecule constitutively generated from endothelial cells and nerve cells or fibers, opened a new era of understanding the mechanisms underlying the regulation of cardiovascular functions and their disturbances. Nitric oxide (NO) is a free radical produced by a family of nitric oxide synthases (NOS), which includes constitutive neuronal NOS (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS) (Mulsh, 1991).

NO derived from endothelial cells causes vasodilatation, decrease in vascular resistance, low blood pressure, inhibition of platelet aggregation and adhesion, inhibition of leukocyte adhesion and migration and reduction of smooth muscle proliferation, thus leading to prevention of atherosclerosis. These NO functions are mediated by cyclic GMP synthesized by soluble guanylyl cyclase, a heme-containing enzyme, which is directly activated by NO (Moncada et al., 1991). Acting as a messenger molecule, NO (derived from eNOS) mediates the majority of endothelium-dependent responses in the brain like regulating cerebral blood flow (Faraci, 1990), vascular tone, vascular resistance and vascular growth (Baumbach et al., 2004) under resting conditions in various mammals including mice, rats, dogs, pigs, and goats (Toda and Okamura, 2003). Rats injected intracisternally with replication-defective adenovirus containing the bovine eNOS gene developed transient increases in cerebral blood flow (Luders et al., 2000). Acute NOS blockade by intravenous L-NAME injection induced a dramatic decrease in hypothalamic blood flow in rats, whereas chronic oral administration of L-NAME did not alter the blood flow; reversal of chronic NOS blockade by L-arginine infusion evoked hypothalamic hyperemia, suggesting the appearance of a compensatory vasodilator mechanism (Hortoba´gyi et al., 2007).). Cerebral arterioles undergo hypertrophy and remodeling in Wistar-Kyoto (WKY) rats with L-NAME-induced hypertension. Chronic treatment for 3 months with L-NAME in the drinking water increased mean cerebral arteriolar pressure and pulse pressure in WKY rats. The cross-sectional area of the vessel wall was greater in L-NAME-treated WKY than in untreated WKY, and the external diameter was less in L-NAME treated WKY than in untreated WKY (Chillon and Baumbach, 2004)

Apart from NO coming from eNOS, NO liberated from nitrergic nerves under resting conditions also contributes to the control of cerebral blood flow, thus playing important roles in the maintenance of physiological brain functions. Activation of parasympathetic nuclei results in increased NO release from postganglionic nitrergic nerves, leading to cerebral vasodilatation and blood flow increase (Toda and Okamura, 2003). Intraperitoneal injections of 7- Nitroindazole (7-NI), an *in vivo* inhibitor of the neuronal isoform of NOS, lowered baseline cerebral blood flow in unanesthetized rats (Monte´cot et al., 1997; Gotoh et al., 2001), cerebral capillary flow in anesthetized rats (Hudetz et al., 1998) and global cerebral blood flow in cats (Hayashi et al., 2002). Acute administration of 7-NI reduced cerebral blood flow in rats to the same extent in both chronic saline- and L-NAME-treated groups, suggesting that residual NOS activity in brain is sufficient to provide tonic, NO-dependent cerebrovascular dilator tone (Kelly et al., 2000). There was evidence suggesting that NO released from parasympathetic fibers contributes to cerebral vasodilatation during acute hypertension in anesthetized rats (Talman and Nitschke Dragon., 2007). During vibrissal stimulation, regional blood flow equally increased in cortical barrel fields in wild-type and eNOS-knockout mice, and nitro-L-arginine (L-NA) inhibited the response in both groups, suggesting that coupling of blood flow and metabolism seems to be nNOS- dependent but not

eNOS- dependent (Ayata et al., 1996). NMDA (Faraci and Breese, 1993) and kainic acid (Faraci et al., 1994) increased cerebral blood flow via neuronally derived NO. A direct cortical application of NMDA increased regional blood flow and oxygen consumption in rats, and 7-NI attenuated the effects of NMDA on cerebral blood flow and decreased the oxygen consumption during NMDA receptor stimulation (Chi et al., 2003).

Longstanding, untreated, and uncontrolled hypertension acting in concert with the NO system results in cerebral hypoperfusion. In essential hypertension, increased tissue RAS activity is considered to be an important source of reactive oxygen species in oxidative stress. A decrease in NO concentrations is observed under conditions of oxidative stress (Kedziora-Kornatowska et al., 2006). Ang II, the effector peptide of RAS, enhances the production of superoxide anion (O_2^-) by activation of NAD(P)H oxidase located in vessel walls. In a reaction with O_2^-, NO is inactivated and transformed to unstable peroxynitrite ($ONOO^-$), which has strong oxidative properties (Kedziora-Kornatowska et al., 2006). Through the disturbance of basal NO levels, chronic hypertension causes alteration in the endothelium and induces vascular injury **(**Cooke and Dzau, 1997). Further, a decreased bioavailability of NO also induces structural changes in cerebral vasculature. It was found that hypertension induced in mice by chronic NOS inhibition using L-NAME is accompanied by hypertrophy of cerebral arterioles. Even in the absence of increases in arteriolar pulse pressure, cerebral arterioles undergo hypertrophy in homozygous eNOS-deficient mice (Baumbach at el., 2004).

Evidence is also growing to suggest that hypertension-related dysfunction in the NO system acts synergistically with the cholinergic receptors to directly promote AD pathology. It has been shown that the activation of nicotinic acetylcholine receptors with consequent neural vasodilatation contributes to an increase in CBF and NO production in the hippocampus (Lee, 2000; Nakajima et al., 2003). The study by Moro and colleagues (1995) also demonstrated the colocalization of NOS and muscarinic receptors in interneurons in neocortical regions projecting onto cerebral microvessels. Cholinergic denervation of NOS expressing interneurons and cortical microvessels is present especially in the temporal cortex of AD patients (Hamel, 2004; Tong and Hamel, 1999). Dysregulation of this system by chronic hypertension is another important mechanism by which hypertension exerts its deleterious effects on cognitive processes.

4.6. Brain Endothelium-Derived Hyperpolarizing Factor

Recent evidence suggests the presence of other endothelium-dependent dilator mechanism termed as “endothelium-derived hyperpolarizing factor” (EDHF) apart from NO or a cyclooxygenase metabolite (Golding et al., 2002). In cerebral vessels, studies using a combination of inhibitors and eNOS knockout mice demonstrate that a pathway independent from NO does exist in brain. ATP, UTP, substance P, A23187 (Ca^{2+} ionophore) and acetylcholine have been reported to elicit dilations through the release of EDHF in addition to NO (Golding et al., 2002). The EDHF response was shown to exist in human cerebral arteries also (Petersson et al., 1995). The importance of EDHF as a mediator of endothelium-dependent relaxation increases as vessel size decreases (Shimokawa et al., 1996).

The EDHF response in cerebral vessels is different from that in peripheral vessels as evidenced by Dong and coworkers (2000), who reported that while clotrimazole attenuated EDHF-mediated responses in the guinea pig cerebral artery, it had no effect on the mesenteric

artery. Very little is known regarding the identity of EDHF or its mechanism of action in cerebral vessels. In some extracranial arteries, NO (sometimes only at relatively high concentrations) produces hyperpolarization of vascular muscle and thus may function as an EDHF (Cohen and Vanhoutte, 1995). Because relaxation of cerebral blood vessels in response to prostacyclin is antagonized by inhibitors of potassium channels (Faraci and Heistad, 1998), prostacyclin may also act as an EDHF in the cerebral circulation. In many arteries, however, it seems that EDHF is neither NO nor a prostanoid, because inhibitors of NO synthase and COX do not attenuate endothelium-dependent hyperpolarization or relaxation of vascular muscle (Chen et al., 1991; Cohen and Vanhoutte, 1995; Corriu et al., 1996; Nakashima et al., 1993). A substantial body of evidence, obtained primarily from studies of coronary blood vessels, suggests that an EDHF is a product of cytochrome *P*-450 monooxygenase metabolism of arachidonic acid (Bauersachs et al., 1994; Campbell et al., 1996; Cheng and Cheung, 1996). The products of arachidonate that mediate this effect appear to be epoxyeicosatrienoic acids (Campbell et al., 1996; Hrader et al., 1995). Epoxyeicosatrienoic acids are produced in brain (Amruthesh et al., 1992; Ellis et al., 1990; Gebremedhin et al., 1992) and by astrocytes (Alkayed et al., 1996) and some epoxyeicosatrienoic acids produce relaxation of cerebral blood vessels (Amruthesh et al., 1992; Ellis et al., 1990; Gebremedhin et al., 1992). For example, both 5,6-epoxyeicosatrienoic acid and 11,12-epoxyeicosatrienoic acid produce relaxation of the middle cerebral artery *in vitro* (Gebremedhin et al., 1992). Consistent with a possible function as EDHFs, 11,12-epoxyeicosatrienoic acid produces relaxation of the middle cerebral artery, which is inhibited by a high concentration of tetraethylammonium (TEA) ion (Gebremedhin et al., 1992), and 14,15-epoxy- eicosatrienoic acid enhances an outward potassium current in smooth muscle isolated from cerebral microvessels (Alkayed et al., 1996) In contrast, 5,6-epoxyeicosatrienoic acid (but not 11,12-epoxyeicosatrienoic acid) is a potent dilator of cerebral arterioles *in vivo* (Amruthesh et al., 1992; Ellis et al., 1990). Interestingly, effects of 5, 6-epoxyeicosatrienoic acid on cerebral arterioles were inhibited by indomethacin or by superoxide dismutase plus catalase (Ellis et al., 1990), suggesting 5,6-epoxyeicosatrienoic acid does not directly produce relaxation in cerebral arterioles.

Endothelium-dependent relaxation is impaired both in animal models of experimental hypertension and in patients with hypertension. (Vanhoutte, 1996). Fujii *et al.* (1992) showed that EDHF-mediated hyperpolarisation and relaxation were decreased in SHR compared with age-matched normotensive Wistar-Kyoto rats (WKY). Subsequent studies confirmed the impairment of EDHF-mediated responses in mesenteric arteries from genetically hypertensive rats (Onaka et al., 1998; Sunano et al., 1999). Similar observations were also reported in the aorta of two-kidney, one clip renal hypertensive rats (Van de Voorde et al., 1992) and in the renal artery of aged SHR (Bussemaker et al., 2003). These findings indicate that EDHF-mediated responses are impaired in hypertension and the impairment of EDHF pathway may account, at least in part, for the endothelial dysfunction associated with hypertension. On the other hand, it has also been reported that enhanced EDHF effect may compensate for the loss of NO and maintain the vasodilatory response to ACh in mesenteric arteries of Sprague-Dawley rats fed a high salt diet (Sofola et al, 2002)

Besides hypertension, ageing is associated with endothelial dysfunction both in humans and animal models. Reduced NO mediated relaxation and/or increased cyclooxygenase dependent constriction could partially underpin age-related endothelial dysfunction depending on the species and the vascular bed studied (Matz and Andriantsitohaina, 2003). Age related

changes in EDHF-mediated hyperpolarisation and relaxation to ACh were studied in the superior mesenteric arteries from 3-, 6-, 12-, and 24-month-old WKY (Fujii et al., 1993; Fujii et al., 2001). EDHF-mediated hyperpolarisation was significantly smaller in arteries from 12- and 24-month-old rats compared with 3- and 6-month-old rats, with the response tending to be smaller in 24-month-old rats than in 12-month-old rats. EDHF-mediated relaxation also decreased with increasing age. Urakami-Harasawa *et al.* (1997) have reported that EDHF mediated relaxation was reduced with ageing in human gastroepiploic arteries. Thus, the reduced EDHF-mediated responses would also contribute to the age-related endothelial dysfunction in humans.

Despite all this, it still remains to be seen how hypertension influences EDHF in cerebral circulation. Further, in models of cognition deficit and AD role of EDHF in cerebrovasculature remains largely unexplored. In context of EDHF in cerebral circulation we have to find out what is its physiological role? When is it functional? Does it play a role *in vivo* during pathological states? Presently, EDHF is primarily studied following inhibition of both NO and the cyclooxygenase pathway. Only when the mechanism of EDHF-mediated dilations is elucidated, it can be studied in the presence of NO and cyclooxygenase, and only then its relative importance in cerebral vessels during normal physiological and pathological conditions can be determined.

5. NADPH Oxidase: Axis of Evil in Hypertension

Reactive oxygen species (ROS) constitute an integral aspect of the pathogenesis of hypertension and AD (Droge, 2002). Especially, oxidative stress in cerebral blood vessels play a pivotal role in the cerebrovascular dysfunction associated with these conditions (Iadecola, 2004; Iadecola and Davisson, 2008). Although vascular ROS are derived from several sources of, the enzyme NADPH oxidase acts as a major axis and source of vascular oxidative stress (Faraci, 2006). NADPH oxidase is a multiunit enzyme mainly confined in neutrophils but its presence extends in vascular cells, particularly in cerebral blood vessels also (Lambeth, 2007; Miller at al., 2005).

NADPH oxidase-derived superoxide causes cerebrovascular dysfunction associated with the hypertension induced by angiotensin II (Ang II), aging and Aβ (Iadecola, 2004; Park et al., 2007; Iadecola and Davisson, 2008). Ang II, Aβ or aging alter endothelium dependent relaxation and functional hyperemia but mice lacking Nox2 are protected from changes induced by these agents (Iadecola, 2004; Park et al., 2007; Iadecola and Davisson, 2008). Similarly, inhibition of the assembly of NADPH oxidase by a peptide or a pharmacological inhibitor blocks the ROS production and cerebrovascular dysfunction induced by Ang II, aging, and Aβ.(Iadecola, 2004; Park et al., 2007; Iadecola and Davisson, 2008).

The exact details of molecular cascades leading to NADPH activation in hypertension, are still shrouded in ambiguity. In vascular cells, activation of Ang II AT1 receptors leads to activation of protein kinase C, which, in turn, phosphorylates p47phox leading to the assembly of the enzyme and ROS production (Mehta et al., 2007). On the other hand, in AD, Aβ leads to phosphorylation of the guanine nucleotide exchange factor Vav through interaction with the scavenger receptor CD36 and the associated tyrosine kinases Syk and

Lyn in microglia. The downstream target of Vav, the small GTPase Rac1, is GTP-loaded and leads to NADPH oxidase activation (Wilkinson et al., 2006).

6. Hypertension and Cognitive Deficits: What Triggers Cerebrovascular Dysfunction?

Hypertension, AD and aging are often associated with focal (lacunes) or diffuse (laukoaraiosis) white matter abnormalities, which can have a profound impact on the severity of dementia (Kalaria, 2009). Reduced cerebral perfusion affect regions located at the border between different arterial territories like periventricular white matter and the white matter of the basal ganglia and centrum semiovale (Kalaria, 2009; O'Sullivan et al., 2002). Moreover, in AD and hypertension, there is increase in resistance to flow and reduced tissue perfusion in these brain regions due to capillary loss and an increase in microvascular tortuosity (Kalaria, 2009). Therefore, subcortical white matter regions are highly vulnerable to injurious stimuli.

These structural and functional cerebrovascular alterations impact brain regions primarily involved in cognition, such as the neocortex and the hippocampus. Reduced blood flow at rest and during activation impair the delivery of energy substrates and nutrients to active brain cells and is not able to meete energy supply and demand. Furthermore, the clearance of byproducts of brain activity, which is flow dependent, is also likely to be reduced. For example, Aβ is released during synaptic activity (Cirrito et al., 2008) and is cleared via vascular transport mechanisms involving the lipoprotein receptor (Zlokovic, 2008). Therefore, the deficit of cerebrovascular regulation in AD brains may impair the normal clearance of Aβ and facilitate accumulation in brain and blood vessels.

There is alterations in the blood–brain barrier (BBB) in aging, hypertension, and AD (Zlokovic, 2008). These BBB alterations, along with the disturbance in cerebral perfusion, compromise the homeostasis of the cerebral microenvironment and increase the vulnerability of the brain to injury. Lastly, neuronal protein synthesis, a process essential for memory formation and synaptic plasticity, is suppressed by hypoperfusion (Klann and Dever, 2004). Therefore, reduced protein synthesis may also contribute to the cognitive dysfunction by reducing the formation and consolidation of new memories and by altering synaptic plasticity.

7. Antihypertensive Agents and Cognitive Functions

In this conundrum of hypertension and cognitive function, question arises whether all blood pressure-lowering agents are equivalent or whether specific agents may afford brain protection beyond their antihypertensive action. Agents acting on RAS seem to confer more neuroprotection as compared to other antihypertensives. In clinical trials, angiotensin-converting enzyme inhibitors or AT1 receptor antagonists showed greater neuroprotection against stroke or dementia than treatment with other agents despite comparable lowering of blood pressure (Fogari et al., 2004; Dahlof, 2007; Messerli et al., 2007). Among agents acting on components of RAAS, AT1 receptor antagonist, Valsartan, showed better improvements

of episodic memory than angiotensin-converting enzyme inhibitors, Enalapril, in elderly hypertensive patients. Further, the AT1 receptor antagonist valsartan reduced amyloid plaques in a mouse model of AD and improves behavioral performance without altering arterial pressure (Wang et al., 2007).

In the case of stroke prevention, treatment with ARBs as compared with that using ACEis provides better neuroprotection from focal cerebral ischemia in animal studies (Groth et al., 2003). In neuroprotection conferred by ARB, AT2 receptor has an important role. Stimulation of the AT2 receptors in neurons in the brain region adjacent to the infarct area induces vasodilatation by locally synthesized NO and prostacyclin, thus improving cerebral blood flow by collateral circulation (Hanif et al., 2010). Selective blockade of central AT2 receptors abolishes the neuroprotective effect of ARBs (Hanif et al., 2010). This better neuroprotection by AT1 receptor antagonist can be explained on the basis of the hypothesis of Fournier et al. (2004) which states that in stroke prevention, diuretics, calcium antagonists and ARBs, which increase Ang II formation, have an edge over ACEis and β-blockers, which decrease Ang II formation. However, in the HOPE study, ramipril caused a 32% reduction of strokes because it reduced cardiac complications three-fold and prevented plaque destabilization, which otherwise could have caused stroke (Fournier et al., 2004).

However, some studies have not observed a benefit of agents targeting the renin-angiotensin system versus other antihypertensive treatments (Messerli et al., 2007; Staessen et al., 2007). Furthermore, Rho kinase inhibitors and Statins could also have a role in the treatment of the deleterious effects of hypertension on the brain (Chrissobolis and Sobey, 2006; Iadecola and Davisson, 2008). Therefore, more studies are needed on the effects of antagonists of the renin angiotensin system and other antihypertensive agents in the prevention of the cerebral complications in hypertension.

8. Conclusion

Given an increase in the rates of hypertension and memory disorder with advancing age and the relationships of hyper- and hypotension with cognitive loss, available evidence indicates that aggressive control of elevated blood pressure to prevent dementia is necessary. New developments have unveiled new avenues for the treatment of the devastating effects of hypertension on the brain but they also have raised new questions that remain to be addressed. The effects of blood pressure control on stroke risk are well known and emerging data suggest that controlling blood pressure reverses effects of hypertension on cerebral blood vessels. Therefore, treatment of elevated blood pressure should remain the mainstay of preventive approaches to protect the brain and other organs from hypertension. For public health intervention to have a maximal impact, such efforts must be directed at preventing or aggressively controlling hypertension at the earliest possible stage before the establishment of arterial stiffness and the need for higher pressure for optimal cerebral perfusion. In addition, as hypertension is a risk factor for AD and plays a role in β-amyloid neuropathology, controlling hypertension may be valuable for AD prevention as well (Skoog and Gustafson, 2006).

An important question arises about the ‘‘ideal’’ blood pressure that should be achieved in hypertensive patients. The linear relationship between stroke mortality and blood pressure

suggests that the lower the value, the better the outcome, but excessive lowering of diastolic blood pressure may increase the incidence of periventricular white matter lesions (van Dijk et al., 2004). Therefore, Using JNC VII criteria, cognitively beneficial target blood pressure should be in the normal range (<120/80 mm Hg) for persons aged less than 75; for persons aged 75 or older who have new-onset hypertension; and for diabetics irrespective of age. For persons aged 75 or older who have chronic hypertension, blood pressure in the prehypertensive range (120–139 mm Hg) is likely to be cognitively beneficial. Regardless of the duration and history of hypertension, cognitively beneficial target blood pressure for persons aged 80 or older should also remain in the prehypertensive range (Obisesan, 2009). However, the ideal range of blood pressure is still clearly an area that requires further investigation. Anyway, it is logical to expect that measures directed at blood pressure control will enhance cognitive reserve and this must be an important public health goal in present century.

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Index

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

Y

Z