2018

STUDYING VASCULAR MORPHOLOGIES IN THE AGED HUMAN BRAIN USING LARGE AUTOPSY DATASETS

Eseosa T. Ighodaro
University of Kentucky, etigho2@uky.edu
Digital Object Identifier: https://doi.org/10.13023/ETD.2018.049

STUDENT AGREEMENT:

I represent that my thesis or dissertation and abstract are my original work. Proper attribution has been given to all outside sources. I understand that I am solely responsible for obtaining any needed copyright permissions. I have obtained needed written permission statement(s) from the owner(s) of each third-party copyrighted matter to be included in my work, allowing electronic distribution (if such use is not permitted by the fair use doctrine) which will be submitted to UKnowledge as Additional File.

I hereby grant to The University of Kentucky and its agents the irrevocable, non-exclusive, and royalty-free license to archive and make accessible my work in whole or in part in all forms of media, now or hereafter known. I agree that the document mentioned above may be made available immediately for worldwide access unless an embargo applies.

I retain all other ownership rights to the copyright of my work. I also retain the right to use in future works (such as articles or books) all or part of my work. I understand that I am free to register the copyright to my work.

REVIEW, APPROVAL AND ACCEPTANCE

The document mentioned above has been reviewed and accepted by the student's advisor, on behalf of the advisory committee, and by the Director of Graduate Studies (DGS), on behalf of the program; we verify that this is the final, approved version of the student's thesis including all changes required by the advisory committee. The undersigned agree to abide by the statements above.

Eseosa T. Ighodaro, Student

Dr. Peter T. Nelson, Major Professor

Dr. Wayne Cass, Director of Graduate Studies

STUDYING VASCULAR MORPHOLOGIES IN THE AGED HUMAN BRAIN

USING LARGE AUTOPSY DATASETS

Dissertation

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the College of Medicine at the University of Kentucky

By

Eseosa Tinuke Ighodaro

Lexington, Kentucky

Director: Dr. Peter T. Nelson M.D., Ph.D., Professor of Pathology

Lexington, Kentucky

2017

ABSTRACT OF DISSERTATION

STUDYING VASCULAR MORPHOLOGIES IN THE AGED HUMAN BRAIN USING LARGE AUTOPSY DATASETS

Cerebrovascular disease is a major cause of dementia in elderly individuals, especially Black/African Americans. Within my dissertation, we focused on two vascular morphologies that affect small vessels: brain arteriolosclerosis (B-ASC) and multi-lumen vessels (MLVs). B-ASC is characterized by degenerative thickening of the wall of brain arterioles. The risk factors, cognitive sequelae, and co-pathologies of B-ASC are not fully understood. To address this, we used multimodal data from the National Alzheimer's Coordinating Center, Alzheimer's Disease Neuroimaging Initiative, and brain-banked tissue samples from the University of Kentucky Alzheimer's Disease Center (UK-ADC) brain repository. We analyzed two age at death groups separately: < 80 years and ≥ 80 years. Hypertension was a risk factor in the < 80 years at death group. In addition, an *ABCC9* gene variant (rs704180), previously associated with aging-related hippocampal sclerosis, was associated with B-ASC in the ≥ 80 years at death group. With respect to cognition as determined by test scores, severe B-ASC was associated with worse global cognition in both age groups. With brain-banked tissue samples, we described B-ASC's relationship to hippocampal sclerosis of aging (HS-Aging), a pathology characterized by neuronal cell loss in the hippocampal region not due to Alzheimer's disease. We also studied MLVs, which are characterized by multiple small vessel lumens within a single vascular (Virchow-Robin) space. Little information exists on the frequency, risk factors, and co-pathologies of MLVs. Therefore, we used samples and data from the UK-ADC, University of Kentucky pathology department, and University of Pittsburgh pathology department to address this information. We only found MLV to be correlated with age. Lastly, given the high prevalence of cerebrovascular disease and dementia in Black/African Americans, we discussed the challenges and considerations for studying Blacks/African Americans in these contexts.

KEYWORDS: Neuropathology, Arteriolosclerosis, VCID, Stroke, Race, Racism

Eseosa Tinuke Ighodaro

June 13, 2017

STUDYING VASCULAR MORPHOLOGIES IN THE AGED HUMAN BRAIN USING LARGE AUTOPSY DATASETS

By

Eseosa Tinuke Ighodaro

Peter T. Nelson M.D., Ph.D.
Director of Dissertation

Wayne Cass Ph.D.
Director of Graduate Studies

June 13, 2017

This work is dedicated to all patients who suffer from vascular dementia, their families, their physicians, and the scientists who work collaboratively to find a cure.

This work is also dedicated to my father, Godwin E. Ighodaro, who always saw the scientist in me from a very early age and nurtured it.

ACKNOWLEDGMENTS

I would like to thank Dr. Peter T. Nelson, my phenomenal mentor, who took the time to teach me how to become a brilliant and impactful physician-scientist. He instilled in me the four rules of his lab: 1) Ethics, 2) Start projects, 3) Finish projects, 4) Laser-focus on central issues. He nurtured my inquisitive nature and always encouraged me to make “my best” even better. All of my accomplishments and success in graduate school are due to the effective mentorship that I received from Dr. Nelson and I will always be internally grateful. I promise to always live up to these rules in my life-long career as a physician-scientist.

I would also like to thank members of my dissertation committee: Dr. Erin Abner, Dr. Marilyn Duncan, Dr. Anita Fernander, Dr. Frederick Schmitt, and Dr. Linda Van Eldik for their assistance and constructive feedback on my experiments, grants, manuscripts, and graduate school training. I would also like to thanks Dr. Jennifer Hatcher for being my outside examiner.

I would also like to thank Dr. Gregory Jicha for teaching me the logistics of clinical trials and allowing me to shadow him in the UK-ADC clinic.

I would also like to thank Dr. Wayne Cass, Director of Graduate Studies for the Department of Neuroscience, for helping me to complete the PhD program.

I would also like to thank Dr. Susan Smyth and Mrs. Therese Stearns for helping me get through the MD/PhD training program.

I would also like to thank past and present members of the Nelson Lab (Dr. Wangxia Wang, Dr. Sergey Artiushin, Mrs. Wilia Quingwei, Dr. Bernard Rajeev, Dr. Janna Neltner, Dr. Vanessa Smith, Dr. Dana Niedowicz, Mrs. Ela Patel, Mrs. Sonya Anderson, Miss. Angela Wei, Mr. Samuel, Mr. Zsombor Gal, Mr. James Dimayuga) for teaching me useful laboratory skills and making me feel part of the Nelson Lab family.

I would also like to thank members of the Sanders-Brown Center on Aging/ University of Kentucky Alzheimer's Disease Center (SBCoA/UK-ADC) for creating an environment suitable for graduate training.

Lastly, I would like to thank my family and friends for their support during my graduate training.

TABLE OF CONTENTS

List of Tables

List of Figures

Frequently Used Abbreviations

AD = Alzheimer's Disease
FTLD = frontal temporal lobar degeneration
DLB = dementia with Lewy Bodies
PART = primary age-related tauopathy
CARTS = cerebral age-related TDP-43 with sclerosis
VaD = vascular dementia
CADASIL = cerebral autosomal dominant arteriopathy with subcortical infarcts
SVD = small vessel disease
B-ASC = brain arteriolosclerosis
ASC = arteriolosclerosis
MVPs = multi-lumen vascular profiles
NACC = National Alzheimer's Coordinating Center
ADCs = Alzheimer's Disease Centers
HS-Aging = Hippocampal sclerosis of aging
SNP = single nucleotide polymorphism
UK-ADC = University of Kentucky Alzheimer's Disease Center
UPPD = University of Pittsburgh Pathology Department
UKPD = University of Kentucky Pathology Department
VCID = Vascular contributions to cognitive impairment and dementia
MMSE = Mini Mental State Examination
CDR = Clinical Dementia Rating Scale
CDRSoB = CDR Sum of Boxes
ADNI = Alzheimer's Disease Neuroimaging Initiative
ADGC = Alzheimer's Disease Genetic Consortium
CAA = cerebral amyloid angiopathy
ASL = arterial spin labelling
pASL = pulsed arterial spin labeling
NFT = neurofibrillary tangles
α-SMA = alpha smooth muscle actin
H&E = hematoxylin and eosin
TDP-43 = TAR-DNA binding protein
GFAP = glial fibrillary acidic protein

Chapter 1: General Introduction

Dementia is defined as global cognitive impairment in cognitive domains such as memory, learning, language, visuospatial function, executive function, and other aspects of cognition that is severe enough to affect daily functioning [1]. The World Health Organization estimates that 35.6 million people worldwide are living with dementia, with numbers anticipated to triple in 2050 [2]. In Western memory clinic- and population-based series, the dementia frequency averages between 8 – 15.8% with higher prevalence in African Americans compared to White Americans [3]. Millions of new cases of dementia are diagnosed every year imposing a tremendous burden to families and primary caregivers along with financial costs to society [2]. Understanding the pathogenesis of dementia is imperative to treating this devastating clinical syndrome.

Before the advent of clinical-neuropathological correlations from high-quality clinical/autopsy datasets, it was often presumed that Alzheimer's disease (AD) was a major if not the sole pathology contributing to clinical dementia with an increasing prevalence in old age [4]. However, results from clinical-pathological studies have shown that brains of elderly individuals with dementia contain a myriad of other pathologies including cerebrovascular disease, hippocampal sclerosis, frontal temporal lobar degeneration (FTLD), dementia with Lewy bodies (DLB), primary age-related tauopathy (PART), cerebral age-related TDP-43 with sclerosis (CARTS) among others [4-9]. Beside AD, cerebrovascular disease has been recognized to be the 2nd most common cause

of dementia in elderly individuals, responsible for at least 20% of dementia cases [2, 10].

Dementia associated with cerebrovascular disease had been referred to as vascular dementia (VaD) [10, 11]. Vascular contributions to cognitive impairment and dementia (VCID) are the terms currently used to describe the spectrum of severity from clinical prodrome to the full-blown dementia [1, 10-12]. VaD is a clinical syndrome that encompasses a heterogeneous group of brain diseases in which cognitive impairment is attributable to cerebrovascular pathologies [2, 11]. Risk factors for VaD include hypertension, diabetes, and hypercholesterolemia [1, 2, 13-15] that can cause brain damage via hypoperfusion, neuroinflammation, metabolic dysfunction, blood-brain barrier dysfunction, and cerebrovascular hemodynamic dysfunction [1, 16, 17]. As a result, the brain damage can clinically present as VCID with underlying pathologies that can include cerebral amyloid angiopathies, inflammatory vessel diseases, cerebral autosomal dominant arteriopathy with subcortical infarcts (CADASIL), and small vessel disease (SVD) [18]. These VCID related pathologies can be categorized according to the size of blood vessels that they affect within the brain: large vessels (e.g., arteries and veins) and small vessels (arterioles, venules and capillaries). SVDs are the most prevalent vascular pathologies underlying VCID [2, 3, 10, 12, 18].

The brain receives its arterial blood supply from two major sources - the internal carotid arteries and the vertebral arteries which supply the anterior and posterior circulation respectively [19]. The anterior and posterior vascular inputs

are connected within the Circle of Willis at the base of the brain [19]. Above the Circle of Willis, the anterior circulation is responsible for supplying the anterior and medial parts of the brain including the frontal lobes, temporal cortices, parietal cortices, amygdala, and globus pallidus [19]. The posterior circulation is responsible for supplying the posterior regions of the brain including the occipital lobes and thalamus [19]. The Circle of Willis sends vessels called pial arteries that dorsally spread across the surface of the cerebrum within the subarachnoid space [19, 20]. Pial arteries send branches called perforating arteries that penetrate into the brain parenchyma perpendicular to the cerebral surface [19, 20]. As perforating arteries travel deeper into the brain parenchyma, their morphology changes yielding intracerebral arterioles which feed into capillaries and the venous system [20]. [*For a visualization, see Iacedola, 2004, Nature Reviews Neuroscience*].

Normally, arteries consist of three distinct layers which include the intima (innermost layer), the media (middle layer), and the adventitia (outer-most layer) [21]. The intima is composed of a monolayer of endothelial cells on the luminal side and elastic fibers on the peripheral side [21]. In addition, collagen and proteoglycans make up the extracellular matrix within the intima [21]. The media is composed of smooth muscle cells and the adventia with connective tissue and fibroblasts [21]. [*For a visualization, see Lusis, 2000, Nature*]). Eventually, small arteries and arterioles will feed capillaries whose vessel walls only consist of a monolayer of endothelial cells (reference). Capillaries feed into the venous

vessels which also have 3 distinct layers with a smaller media layer and less rigid vascular walls on microscopy (reference).

SVDs mostly affect the small arteries and arterioles of the brain circulation [22]. SVDs include degenerative alterations in the vessel walls which are termed arteriolosclerosis (vessel wall thickening), lipohyalinosis (hyaline substance deposition), and fibronoid necrosis (loss of vessel wall integrity) [2, 3, 22]. SVDs can lead to lacunar infarcts (focal regions of gliosis and cavitation near the basal ganglia), microinfarcts (microscopic focal regions gliosis and cavitation), hemorrhages (gross brain bleeds), and microbleeds (microscopic brain bleeds) which can, individually or more often collectively, damage the brain parenchyma leading to neuronal cell loss and impaired connectivity, ultimately culminating in clinical disease [3, 10, 18, 23]. Therefore, it is important to fully understand the risk factors and cognitive sequelae of SVDs in order to treat and prevent VaD. One major focus of this dissertation was to understand the frequency, risk factors, and cognitive sequelae of brain arteriolosclerosis (B-ASC).

Brain Arteriolosclerosis (B-ASC)

B-ASC is a subtype of cerebral SVD characterized by degenerative wall thickening of arterioles [24-27]. The pathologic changes of B-ASC include hypertrophy and/or death of vascular smooth muscle cells and extracellular deposition of elastin and collagen [22, 28-30]. The true prevalence of B-ASC is unknown but has been observed in 39-80% of autopsied elderly individuals [24, 31, 32]. The pathologic changes of B-ASC include hypertrophy and/or death of

vascular smooth muscle cells and extracellular deposition of elastin and collagen [22, 24, 28, 29]. B-ASC increases in severity with advanced age [33]. B-ASC has been observed in basal ganglia and frontal cortical brain regions [25, 34] with future investigation needed in other brain regions. Hypertension and diabetes are risk factors for arteriolosclerosis (ASC) in the brain and other organs [35-39]. However, the relationship between other conventional vascular risk factors (e.g, obesity, hypercholesterolemia, smoking) and B-ASC is not fully understood. In addition, the relationship between B-ASC and cognition is not well defined [24]. As a result, this dissertation sought to test the associations between B-ASC and conventional vascular risk factors and cognitive status, using information from the National Alzheimer Disease Coordinating Center (NACC) data set. The NACC data set contains research subject information collected from 34 past and present Alzheimer's Disease Centers (ADCs) across the United States, comprising clinical, genetic, and neuropathological (autopsy) data. [40]. These findings are described in **Chapter 2** and published in Ighodaro et al, 2017, JCBFM. This represents one of the most far-reaching studies of B-ASC to date. A second small vessel pathology that was a focus of this dissertation is multi-lumen vascular profiles (MVPs).

Multi-lumen Vascular Profiles (MVPs)

Vascular loops, vascular bundles, vascular convolutes, vascular spirals, vascular multiplications, and vascular glomerular loop formations are terms that have been applied in the literature to describe small blood vessels with multiple

lumens enclosed in a single perivascular space [41-45]. Within this dissertation, the term multi-lumen vascular profile (MVP) is used to describe a single blood vessel consisting of ≥ 3 lumens enclosed in a perivascular space. There are other reports of MVPs being seen in the brains of elderly individuals [42, 44]. It has been suggested that MVPs arise due to hypoxic/ischemic changes in the brain [44, 45]. However, the frequency, vascular risk factors, vascular diseases, and co-pathologies have not been systematically investigated. Therefore, one of the goals of this dissertation was to study this surprisingly common pathologic entity in human brain. To investigate the frequency, risk factors, and co-pathologies of MVPs, brains of individuals who came to autopsy at the University of Kentucky and the University of Pittsburgh were analyzed. The findings are described in **Chapter 3**.

Interestingly, both B-ASC and MVP pathologies are associated with age. Another pathology that is associated with age and shares a common genetic risk factor with B-ASC is a fairly recently described neurodegenerative pathology termed hippocampal sclerosis of aging (HS-Aging) [24, 25] .

Intersection of SVDs and Neurodegeneration

In the elderly population, dementia is commonly due to several pathologies concurrently existing with the brain [5, 7, 47]. Mixed pathologies is a term used in the literature to describe the overlap of different vascular and/or neurodegenerative pathologies within the aging brain [18, 48]. The most commonly discussed and observed mixed pathology involves cerebrovascular

disease and AD [3, 47]. It has been shown that the co-occurrence of cerebrovascular disease lowers the threshold for dementia caused by a single neurodegenerative disease process [10, 49, 50]. However, the relationship between cerebrovascular disease and other neurodegenerative diseases is not fully understood. Some scientists hypothesize that the relationship between cerebrovascular diseases is synergistic while others claim it to be additive [18].

Another goal of this dissertation was to ascertain the upstream relationship between two pathologies often in the aging brain: B-ASC and HS-Aging [25].

Hippocampal Sclerosis of Aging (HS-Aging)

Hippocampal sclerosis of aging (HS-Aging) is a high-morbidity neurodegenerative disease, usually affecting individuals who survive past age 80 [51-57]. HS-Aging is characterized by cell loss, gliosis, and atrophy in the hippocampal formation that is not due to AD-type pathology. A genetic risk factor for HS-Aging is the *ABCC9* SNP rs704180 [59, 60] which has a gene product that is a subunit of ATP-sensitive potassium channels found in vascular smooth muscle cells, including arterioles [24, 61]. As part of this dissertation, the early stages of HS-Aging are described in **chapter 4**.

With respect to cerebrovascular disease, individuals with HS-Aging have worse B-ASC pathology compared to individuals without HS-Aging pathology [24, 25]. Using digital image methods for analysis of arteriolar morphology, HS-Aging cases show larger vessel areas, vessel perimeters, vascular areas, and vessel

wall thicknesses compared to non HS-Aging cases [24, 25]. Because of all these findings collectively, it was hypothesized that the *ABCC9* HS-Aging risk genotype is associated with B-ASC in advanced old age, possibly upstream of the risk for HS-Aging [24, 57]. Findings and the hypothesized relationship between B-ASC and HS-Aging are described in **Chapter 2 and Chapter 4** and have now been published [24, 57].

To study B-ASC, MVPs, and HS-Aging, tissue samples (when available) were investigated along with accompanying clinical and neuropathological information (when available) from NACC, the University of Kentucky Alzheimer's Disease Center (UK-ADC), University of Kentucky Pathology Department (UKPD), and the University of Pittsburgh Pathology Department (UPPD) to test these hypotheses.

Neuropathological Datasets: Challenges to the goal of population-based studies

The advent of large, high-quality neuropathological datasets (e.g., NACC dataset) with longitudinal clinical information have allowed scientists to better understand the pathological conditions associated with dementia [5-7, 47, 62-64]. A major strength of the NACC data set is that it contains research subject information collected from 34 past and present AD Centers (ADCs) across the United States, comprising clinical, genetic, and neuropathological (autopsy) data. [24, 40]. The NACC provides longitudinal and cross-sectional information enabling high-powered clinical-pathological correlation studies [24]. Some weaknesses of the NACC data set are that it is not population-based; is better

characterized as a clinical series of persons enrolled at ADCs, and in addition, carries known biases associated with brain autopsy cohorts [40, 65-67]. As a result, NACC participants are predominantly Caucasian, highly educated, and are drawn predominantly from dementia clinics [65, 68]. In addition, there is little socioeconomic information and small sample sizes of individuals from different ethnic/racial groups. Therefore, the generalizability of results from the NACC data set is limited. However, there is a need for scientists to study dementia-causing pathologies in other racial/ethnic groups in order to decrease health disparities. Cerebrovascular disease has a higher prevalence in African Americans compared to White Americans {Laditka, 2014 #826}. In **chapter 5**, we describe the challenges and considerations for studying African Americans in neuropathological datasets given the current low group representation.

Overall Dissertation Goals

The over-arching goal of this dissertation is to elucidate frequencies, risk factors, co-pathologies, and the cognitive sequelae of two common vascular pathologies (B-ASC and MVPs) using large autopsy datasets and brain repositories. Major dissertation hypothesis are as follows: 1) B-ASC is associated with global cognitive status. 2) B-ASC is associated with conventional vascular risk factors. 3) B-ASC is associated with a single-nucleotide polymorphism (HS-Aging risk factor) in *ABCC9*. 4) MVPs are associated with conventional vascular risk factors. With these hypotheses in mind, it was also important to be aware of the strengths and limitations of using current large autopsy datasets in answering

these research hypotheses. The following chapters describe in detail my work on B-ASC, MVPs, HS-Aging, and strengths/limitations of existing neuropathological data sets.

Chapter 2: Brain Arteriolosclerosis (B-ASC)

Introduction

Cerebrovascular pathologies affecting small arteries and arterioles are common findings in the aged brain [28, 30, 69] and are often seen in patients with dementia [3, 70-72]. Here, this project focused on brain arteriolosclerosis (B-ASC), a vascular pathology characterized by degenerative wall thickening of arterioles [25-27]. The present study sought to better delineate the risk factors and global cognitive status associated with B-ASC pathology among aged individuals.

Chronic hypertension is associated with ASC pathology in the brain, kidney, and other organs [35-37]. Hypertensive animal models have thicker cerebral arteriolar walls, larger vessel cross-sectional areas, and smaller inner arteriolar diameters compared to control animals [73, 74]. Diabetic patients have thicker subcutaneous gluteal arteriolar walls and larger cross sectional areas compared to controls [38, 39]. Diabetic animal models have thicker retinal capillary walls [75] and renal arteriolar glomerular basement membranes [76] compared to controls.

In addition to clinical risk factors, recent studies suggest that a single nucleotide polymorphism (SNP) located in *ABCC9*, ATP-binding cassette sub-family C member 9, may be a genetic risk factor for B-ASC pathology in older adults. Evidence in support of the link between an *ABCC9* SNP and B-ASC pathology includes: 1) The rs704180 SNP located in *ABCC9* is associated with

hippocampal sclerosis of aging (HS-Aging) [59, 60], a neurodegenerative disease affecting individuals > 80 years at death [25, 55]. 2) Individuals with HS-Aging have worse B-ASC pathology compared to individuals without HS-Aging pathology [25]. 3) The gene product of *ABCC9* is a subunit of ATP-sensitive potassium channels found in vascular smooth muscle cells, including arterioles [61]. Thus, by extension, *ABCC9* gene variants may constitute a risk factor for B-ASC pathology in elderly individuals. However, this credible hypothesis has not been tested previously.

As the clinical and genetic risk factors for B-ASC are imperfectly understood, so is the cognitive impairment associated with this pathology. Studies on the global cognitive status of patients with B-ASC have included the analyses of Mini Mental State Examination (MMSE) scores [6], Clinical Dementia Rating Scale (CDR) scores [77], and CDR Sum of Boxes (CDRSoB) scores [47]. The MMSE is an assessment tool used in measuring global cognitive function [78], while the CDR is a clinical interview measure of a person's ability to accomplish activities of daily living and day-to day cognition [64, 79]. The CDRSoB is derived by summing scores from all six CDR domains [80]. Prior analyses of data from 334 elderly individuals did not reveal an association between B-ASC pathology and MMSE scores [6]. However, in an autopsy study with 52 cases, widespread B-ASC pathology in cases with Alzheimer's disease (AD) was associated with worse global CDR scores [77]. Similarly, in an autopsy study using 715 AD cases with CDRSoB information, researchers found that high B-ASC severity was associated with worse CDRSoB scores [47]. Conflicting

results from these studies may be due to a number of experimental factors including small sample size (statistical power), particular cognitive domains affected by small blood vessel pathologies, frequent presence of comorbid pathologies, and other parameters that vary during the human aging spectrum.

In order to gain insight into B-ASC risk factors and global cognitive status while factoring in other dementia associated pathologies, we analyzed a subset of individuals from the NACC data set. Because there is evidence of distinct neurodegenerative outcomes and clinical-pathological correlation differences between the “younger-old” and “oldest-old” persons [4, 81-86], groups were analyzed separately according to ages at death: < 80 years and ≥ 80 years. The goals of the study were to determine if autopsy verified B-ASC is associated with global cognitive status, to assess the association between vascular risk factors and B-ASC pathology, and to determine the relationship between *ABCC9* HS-Aging risk genotype and B-ASC pathology. In order to further test the association between the *ABCC9* HS-Aging risk genotype and B-ASC pathology, the relationship between the *ABCC9* HS-Aging risk genotype and cerebral blood flow (a possible *in vivo* manifestation of B-ASC pathology) were examined. Genetic and neuroimaging data on a sub-sample of individuals from the Alzheimer’s Disease Neuroimaging Initiative (ADNI) data set were used to test this association.

Methods

This study used data from the NACC and ADNI data sets. Patient recruitment and data collection in the NACC data set have been previously described

including details related to Institutional Review Board approvals and patient consent [40, 65, 68, 87]. Research using NACC data was approved by the University of Washington Human Subjects Division. Patient recruitment, neuroimaging acquisition, and data collection on individuals included in the ADNI data set have been previously described with approval by local ethics review boards at each participating site [88, 89].

Study subjects

The NACC data set contains research subject information collected from 34 past and present Alzheimer's Disease Centers (ADCs) across the United States, comprising clinical, genetic, and neuropathological (autopsy) data [40]. More detailed information on data collection is available online (https://www.alz.washington.edu/WEB/dataforms_main.html). Archival data from September 2005 through December 2013 were obtained for this study.

The initial pulled archival data from the NACC data set comprised 29,483 cases. Living cases (n = 26,686) and autopsied cases with missing information which would preclude making an assessment of B-ASC status (n = 407) were excluded from the analysis. For analysis, cases were split into two age at death groups: < 80 years (n = 1,008) and ≥ 80 years (n = 1,382).

The ADNI data set is from a multicenter longitudinal study in the United States and Canada, in which subjects with normal cognition, MCI, and AD were followed with cognitive testing, neuroimaging techniques, and other biomarkers [88]. A convenience sample (n = 15) of rs704180 homozygous (A_A or G_G

genotype) individuals was used for cerebral blood flow (CBF) analysis. No other scans or SNPs were assessed from ADNI.

Cognitive and functional assessment in the NACC data set

MMSE (0-30; 30 = no global impairment) and CDRSoB (0-18; 0 = no global impairment) scores were used as measures of global cognitive status [64, 78, 79]. Scores from the most recent ADC clinical visit before each individual's death were used (median interval between final visit and death: 0.83 years).

Clinical, neuropathologic, and genetic parameters in the NACC data set

Clinical data were obtained from each participant's final ADC clinical visit before death. During clinical research visits, medical histories were obtained from subjects and/or patient records. The following self-reported vascular risk factors and cerebrovascular diseases were used in the analyses: medical histories of hypertension, diabetes, hypercholesterolemia, sex, smoking, stroke, and atrial fibrillation. Responses were coded initially as unknown, absent, recent/active, or remote/inactive, and subsequently, "recent" and "remote" responses were combined into one category (e.g.,, history of a condition) for analytical purposes. Body mass index (BMI) values were derived from height and weight measurements. Pack years were derived from self-reported cigarette packs smoked per day and years of smoking.

Neuropathologic details from all cases included Braak staging [90], CERAD neuritic plaque densities [91], and other parameters as described in detail previously [68]. In the NACC Neuropathology Data Set Coding Guidebook version 9.1 (https://www.alz.washington.edu/WEB/forms_np.html), B-ASC was described as “hyalinosis of the media and adventitia of small parenchymal and/or leptomeningeal vessels.” B-ASC pathology was diagnosed using a semi-quantitative four-tier categorization system with responses scored to indicate “none”, “mild”, “moderate”, or “severe” B-ASC pathology. In the NACC guidebook, neuropathologists were instructed to estimate the overall severity of B-ASC pathology. No specific brain region for B-ASC pathological diagnosis was mentioned; thus, this diagnostic methodology was left to the discretion of each individual neuropathologist and/or research center.

Genetic data were obtained and analyzed as described previously [59, 60]. Briefly, the Alzheimer’s Disease Genetic Consortium (ADGC) accrued genetic data from 29 different ADCs, with multiple iterations of SNP data [92-94], which were analyzed together with neuropathological and clinical data gathered through NACC [66]. The three alleles identified were analyzed according to ADGC SNP nomenclature and were *GRN*.rs5848 (A/G), *TMEM106B*.rs1990622 (A/G; note that other reports have used T/C for this allele: the ‘‘A’’ allele is analogous to ‘‘T’’ allele in other reports, whereas the ‘‘G’’ allele we report is analogous to ‘‘C’’ allele), and *ABCC9.*rs704180 (A/G). [59, 60] *APOE* ε4 genotype information from NACC was also used in the analysis because *APOE*

alleles are known to be associated with cerebral amyloid angiopathy (CAA), which could lead to vascular wall distortions [95-97].

Neuroimaging and genetic parameters in ADNI data set

T_1-weighted brain MRI scans were acquired using a sagittal MP-RAGE sequence following the ADNI MRI protocol [89, 98]. ASL (arterial spin labeling) images were obtained from the ADNI dataset. Data from 15 Caucasian individuals were acquired from six different American research centers using a standardized pulsed arterial spin labeling (pASL) protocol: Field Strength=3.0 tesla; Flip Angle=90.0 degree; Manufacturer=SIEMENS; Matrix X=320.0 pixels; Matrix Y=320.0 pixels; Pixel Spacing X=4.0 mm; Pixel Spacing Y=4.0 mm; Pulse Sequence=EP; Slice Thickness=4.0 mm; TE=12.0 ms; TR=3400.0 ms. Control and label images were subtracted and quantitative CBF (mL/100g/min units) was calculated using in-house Matlab software using the following equation [99, 100] and then correlated with genotyping:

$$\mathrm{CBF} = \frac{\lambda \cdot (\mathrm{SI}_{\mathrm{control}} - \mathrm{SI}_{\mathrm{label}})}{2 \cdot \alpha \cdot M_0 \cdot TI_1 \cdot \exp(-TI_2/T_{1,blood})}\ [\mathrm{ml/100g/min})]$$

Where λ was the brain/blood partition coefficient in mL/g, $SI_{control}$ and SI_{label} were the time-averaged signal intensities in the control and label images, respectively, $T_{1,blood}$ was the longitudinal relaxation time of blood in seconds, α was the labeling efficiency, M_0 was the equilibrium brain tissue magnetization, TI_1 was

post-labeling delay, and TI_2 was the label duration. SNP rs704180 in *ABCC9* and *APOE* ε4 status information came from ADNI.

Statistical analyses:

Exploratory bivariate analyses and regression modeling were used to assess the association between clinical vascular risk factors and B-ASC pathology. Initially, a Chi-square test, a Mann-Whitney U test, or a Kruskal-Wallis test were used to determine possible risk factors for B-ASC severity in the two age at death groups. A Chi-square test was applied for categorical variables, whereas a Mann-Whitney U test or Kruskal-Wallis test was applied for continuous non-normally-distributed variables. Subsequently, clinical variables that yielded a $P < 0.05$ in these analyses were included as independent variables in an ordinal logistic regression to further elucidate the association between clinical variables and B-ASC pathology while controlling for confounding effects. The variables in this logistic regression model included age at death, sex, hypertension, diabetes, smoking pack years, and hypercholesterolemia.

Logistic regression modeling was used to determine the association between the *ABCC9* HS-Aging risk genotype and B-ASC pathology. Age at death, sex, hypertension, diabetes, pack years, and hypercholesterolemia were used as covariates in the models. Mild, moderate and severe B-ASC pathologies were collapsed into one category and treated as a dependent variable in these models.

A linear regression model was used to assess the association between B-ASC pathology with MMSE and CDRSoB scores. B-ASC pathology was treated as the main independent variable. MMSE or CDRSoB scores were treated as dependent variables while adjusting for age at death, sex, Braak neurofibrillary tangles (NFT) stage, presence of any microinfarcts, presence of neocortical Lewy bodies, and presence of HS-Aging pathology. Adjusted mean MMSE and CDRSoB scores derived from the linear regression analyses were reported for each B-ASC severity category and compared using the least squares method.

In order to assess the relationship between the *ABCC9* HS-Aging risk genotype and CBF (a possible manifestation of B-ASC pathology), a Welch's two sample t-test was used to compare CBF measurements between individuals with the *ABCC9* HS-Aging homozygous non-risk and risk genotypes. All statistical analyses were performed using IBM SPSS Statistics 22 Properties and PC-SAS 9.34 (SAS Institute, Inc.; Cary, NC, USA).

Results

The inclusion/exclusion criteria applied to individuals used in our analyses from the NACC data set are shown in **Figure 2.1A.** Individuals used in these analyses were predominantly (>90%) Caucasian (data not shown), and median year at death was 2010 (range: 2005 - 2013). In order to convey examples of blood vessel profiles representing the spectrum of B-ASC pathology, images were obtained from four human brain sections stained with hematoxylin and eosin (H&E) (**Figure 2.2**). The percentage of individuals with B-ASC pathology trended upward with increasing age at death. (**Figure 2.1B**). When stratifying by age at

death, the percentage of individuals with moderate or severe B-ASC pathology was higher in older age at death groups ($P < 0.0001$) (**Figure 2.1C**). These results indicate that B-ASC is a common pathology in the NACC data set, becoming more severe with increasing age at death.

As a result of prior studies showing different outcomes and clinical-pathological correlations in the "oldest old" [81-83], we analyzed the overall cohort in two separate age groups. More specifically, the cutoff of 80 years was chosen because it has been used before to help highlight neurodegenerative disease and/or neuropathologic features that differ – often quite dramatically – among the "oldest-old" [53, 84-86, 101, 102]. Furthermore, this cutoff was close to the overall cohort mean (80.0 years) and median (82.0 years) age at death. Comparing the two age at death groups in the NACC data set, individuals in the ≥ 80 years age at death group were more often female, hypertensive, and less often impaired cognitively when compared to individuals in the < 80 years age at

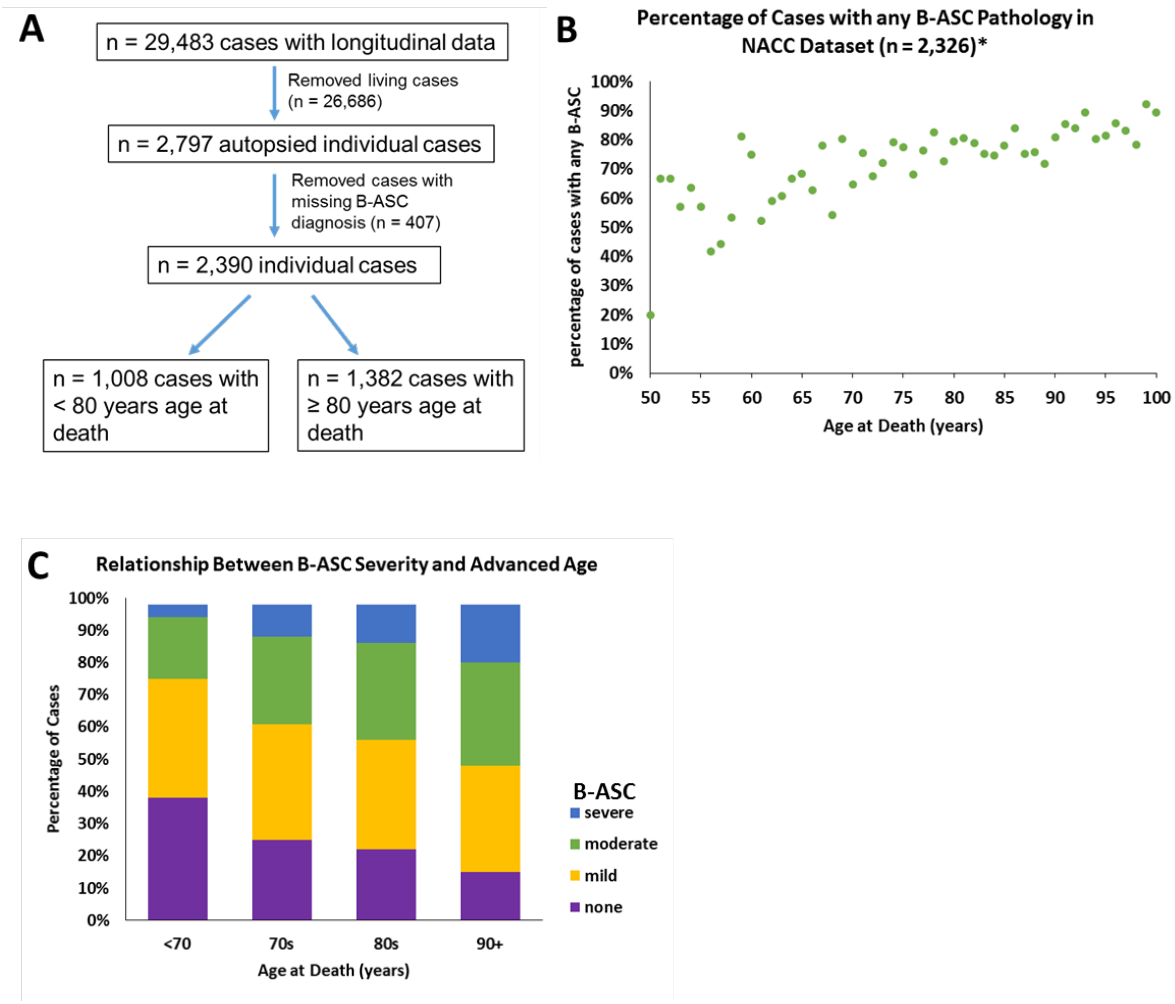

Figure 2.1: Exclusion/inclusion criteria and frequency of brain arteriolosclerosis (B-ASC) pathology in autopsied cases from the National Alzheimer's Coordinating Center (NACC) data set. (**A**) Living cases and autopsied cases with missing information which would preclude making an assessment of B-ASC status were excluded from analysis. (**B**) shows the percentage of cases with any B-ASC pathology (mild, moderate or severe) in the NACC data set. Asterisk (*) indicates that 64 cases with age at death < 50 years or > 100 years were not plotted. **(C)** shows a stacked bar graph representing the relationship between B-ASC severity and age at death, Chi-square p-value < 0.0001.

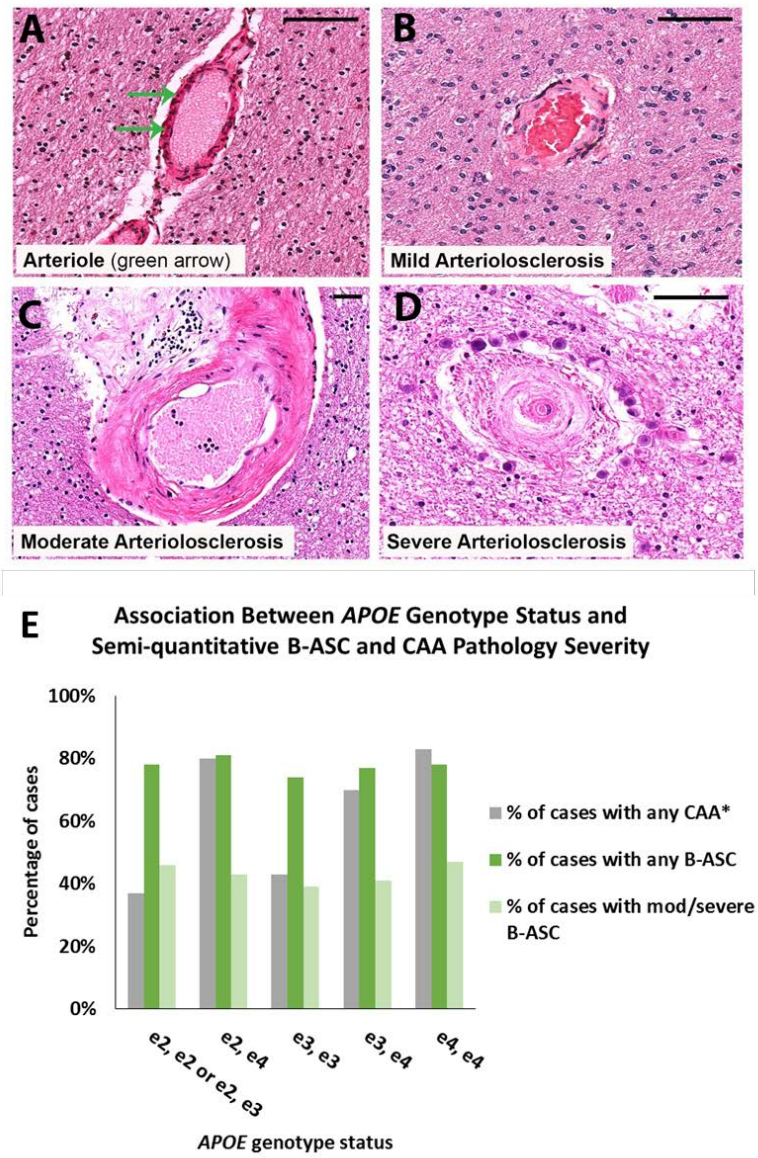

Figure 2.2: Semi-quantitative severity grading of B-ASC pathology and its non-association with *APOE* ε4 allele in the NACC data set. **(A, B, C, D)** show photomicrographs of hematoxylin and eosin stained blood vessels. **(A)** shows what we assume to be a normal arteriole (green arrow). **(B)** shows B-ASC severity grade 1 with relatively mild thickening of vessel wall. **(C)** shows B-ASC severity grade 2 with increased thickening of vessel wall. **(D)** shows B-ASC severity grade 3 with prominent thickening of vessel wall and partly occluded vessel lumen. Scale bars = 100µm. (**E**) shows the association between *APOE* ε4 genotypes and any degree of CAA (mild, moderate, severe) combining both age at death groups: *Chi-square p-value < 0.0001 among 1883 individuals for whom *APOE* genotype data and CAA diagnosis were available. Those with ε2/ε3 and ε2/ε2 genotypes were combined into one category. There were no statistically significant associations that could be determined between B-ASC severity and *APOE* genotype. B-ASC = brain arteriolosclerosis; CAA = cerebral amyloid angiopathy.

death group (**Table 2.1**). In addition, individuals in the ≥ 80 years age at death group were less likely to show “high” levels of AD pathology, but more likely to show B-ASC and HS-Aging pathologies at autopsy, compared to individuals in the < 80 years age at death group. These data, in addition to the prior precedents in the literature, confirmed that the two age groups show differing clinical risk factors, cognitive profiles, and neuropathological autopsy results.

Global cognitive status associated with B-ASC pathology

To analyze the global cognitive status of individuals with B-ASC pathology, linear regression analyses were performed using B-ASC pathology as a predictor, other dementia-inducing pathologies as covariates, and MMSE or CDRSoB scores as outcome variables. After adjusting for age at death, cortical microinfarcts , AD pathology (Braak NFT stage and CERAD neuritic plaque rating), neocortical Lewy bodies, and HS-Aging pathologies, we found that B-ASC was a significant pathological predictor in the MMSE model using cases from the < 80 years age at death group and in the CDRSoB model using cases from both age groups (**Table 2.2 and 2.3**). In the < 80 age at death group, the adjusted MMSE and CDRSoB group means for cases with severe B-ASC were worse compared to that of cases with none, mild, or moderate B-ASC pathology (**Table 2.4**). In the ≥ 80 age at death group, the adjusted MMSE group mean score for cases with severe B-ASC was worse compared to that of cases with none or mild B-ASC pathology. The adjusted CDRSoB group mean score for cases with severe B-ASC pathology was worse compared to that of cases with none, mild,

Table 2.1: Age at Death Group Comparison on Clinical, Cognitive, Neuropathologic, and Genetic Variables.

	< 80 years Age of Death group	≥ 80 years Age of Death group	Missing cases, n	p-value[e]
Sample size	n =1008	n = 1382		
Demographic variables				
Age of death, median (25th, 75th quartile)	70.0 (64.0, 76.0)	88.00 (84.0, 92.0)		<0.0001[c,d]
Gender (%)				<0.0001[c,d]
Male	61.7	48.3		
Female	38.3	51.7		
Clinical Variables				
Hypertension (%)[a]	46.1	64.6	8	<0.0001[c,d]
Diabetes (%)[a]	9.6	13.2	2	0.008[c]
Hypercholesterolemia (%)[a]	47.7	49.8	28	0.315
Pack years median (25th, 75th quartile)[a]	0.0 (0.0, 13.1)	0.0 (0.0, 15.0)	181	0.496
BMI median (25th, 75th quartile)[b]	25.4 (22.8, 28.5)	24.9 (22.2, 27.6)	980	0.004[c]
Cognitive Variables				
MMSE median (25th, 75th quartile)	16.0 (6.0, 24.0)	21.0 (14.0, 27.0)	697	<0.0001[c,d]
CDRSoB median (25th, 75th quartile)	13.0 (7.0, 18.0)	9.0 (2.0, 15.0)		<0.0001[c,d]
Neuropathological Variables				
AD pathology "High*"	47.3	42.5	36	<0.0001[c,d,f]
HS-Aging (%)[b]	7.1	14.2	113	<0.0001[c,d]
B-ASC (%)				<0.0001[c,d,e]
none	31.3	19.9		
mild	37.0	33.9		
moderate	23.8	31		
severe	7.8	15.2		
Genetic Variables				
ABCC9 HS-Aging risk genotype A_A (%)	23.8	25.8	1465	0.508
APOE ε4 allele (%)	48.1	39.9	467	<0.0001[c,d]

In comparing the two age at death groups, P-values for age at death, pack years, and BMI are from Mann-Whitney U analyses. P-values for gender, hypertension, diabetes, hypercholesterolemia, B-ASC, *ABCC9* HS-Aging risk genotype (A_A), and *APOE* ε4 allele are determined from Chi-square tests. Percentages were recorded after excluding missing cases for each variable. AD = Alzheimer's Disease; B-ASC = brain arteriolosclerosis; BMI = body mass index; CDRSUM = Clinical Dementia Rating Sum of Boxes. HS-Aging = Hippocampal sclerosis of aging; MMSE = Mini Mental State Exam.

*NIA/Reagan Institute Criteria, 1997.

[a] Self-reported vascular risk factors.

[b] Derived variables from NACC variables.

[c] P-value < 0.05.

[d] Significant p-value after Bonferroni correction for multiple correction.

[e] Group comparisons exclude cases with missing data.

Table 2.2 shows the p-values from a linear regression analysis used to determine B-ASC global cognitive status using CDRSUM and MMSE scores.

	MMSE Predictors		**CDRSUM Predictors**	
	<80 years	**≥80 years**	**<80 years**	**≥80 years**
Age of death (categorical)	**0.002**	**<0.0001**	**0.010**	**<0.0001**
B-ASC (categorical)	**0.029**	0.098	**0.010**	**<0.0001**
Gender (male vs. female)	0.99	0.13	0.23	0.37
Microinfarcts (vs. no microinfarcts)	0.23	**0.041**	0.057	0.82
Braak stage (categorical)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Neuritic amyloid plaques CERAD (categorical)	0.19	**<0.0001**	0.2	**<0.0001**
HS-Aging (vs. no HS-Aging)	**0.0002**	**<0.0001**	**<0.0001**	**<0.0001**
Neocortical Lewy bodies (vs. no Lewy bodies)	0.13	**0.0017**	0.069	**0.0017**

For the MMSE analysis, 1,649 cases were included in the analysis; excluded observations were missing MMSE or predictor variables. Missing MMSE scores were due to either verbal refusal, physical, cognitive, or behavioral problems. For the CDRSUM analysis, 2,238 cases for were included due to missing predictors. No missing values were observed in CDRSUM scores. * indicates $P < 0.05$. B-ASC = brain arteriolosclerosis; CDRSUM = Clinical Dementia Rating Sum of Boxes; CERAD = Consortium to Establish a Registry for Alzheimer's Disease; HS-Aging = hippocampal sclerosis of aging; MMSE = Mini Mental State Exam.

Table 2.3 shows the beta coefficients from MMSE and CDRSUM linear regression models

	MMSE Beta Coefficients ± S.E		CDRSUM Beta Coefficients ± S.E	
Age at death group	**<80 years**	**≥80 years**	**<80 years**	**≥80 years**
Age of death (1-year increase)	**0.1 ± 0.01**	**0.2 ± 0.05**	**–0.06 ± 0.06**	**–0.1 ± 0.03**
Male (vs female)	0.0002 ± 0.7	–0.7 ± 0.5	–0.5 ± 0.4	0.3 ± 0.3
Microinfarcts (vs. no microinfarcts)	0.9 ± 1.1	**–1.2 ± 0.6**	–1.1 ± 0.6	–0.08 ± 0.4
Braak 0 (vs. Braak 6)	**5.3 ± 1.7**	**6.8 ± 1.7**	–1.7 ± 0.9	**–4.8 ± 1.1**
Braak 1 (vs. Braak 6)	**9.1 ± 1.7**	**8.6 ± 1.2**	**–3.9 ± 0.9**	**–4.9 ± 0.8**
Braak 2 (vs. Braak 6)	**6.2 ± 1.6**	**8.1 ± 1.0**	**–3.5 ± 0.9**	**–5.2 ± 0.6**
Braak 3 (vs. Braak 6)	**8.7 ± 1.8**	**7.5 ± 0.9**	**–4.5 ± 1.0**	**–4.8 ± 0.6**
Braak 4 (vs. Braak 6)	**5.7 ± 1.5**	**6.6 ± 0.8**	**–2.7 ± 0.8**	**–3.9 ± 0.5**
Braak 5 (vs. Braak 6)	1.5 ± 1.2	**2.9 ± 0.7**	**–1.7 ± 0.6**	**–1.5 ± 0.4**
Frequent (vs. no neuritic amyloid plaques)	–2.84 ± 1.5	**–4.8 ± 0.9**	1.5 ± 0.8	**3.7 ± 0.6**
Moderate (vs. no neuritic amyloid plaques)	–0.6 ± 1.39	**–2.3 ± 0.8**	0.2 ± 0.7	**2.0 ± 0.5**
Sparse (vs. no neurites amyloid plaques)	–0.003 ± 1.3	–0.9 ± 0.8	0.3 ± 0.7	0.1 ± 0.5
HS-Aging (vs. no HS-Aging)	**–5.3 ± 1.4**	**–3.0 ± 0.7**	**2.9 ± 0.7**	**2.8 ± 0.4**
Neocortical Lewy bodies (vs. no neocortical Lewy bodies	**–**1.2 ± 0.8	**–1.8 ± 0.6**	0.8 ± 0.4	**1.2 ± 0.4**

Bold values indicates P < 0.05. B-ASC = brain arteriolosclerosis; CERAD = Consortium to Establish a Registry for Alzheimer's Disease; CDRSUM = Clinical Dementia Rating Sum of Boxes; HS-Aging = hippocampal sclerosis of aging; MMSE = Mini Mental State Exam.

Table 2.4 Adjusted Final MMSE and CDRSUM Group Means Associated With Brain Arteriolosclerosis (B-ASC) Pathology

	Age of Death < 80 years		**Age of Death ≥ 80 years**	
B-ASC	**MMSE**	**CDRSoB**	**MMSE**	**CDRSoB**
None	17.0 ± 0.7	10.5 ± 0.4	21.3 ± 0.6	6.8±0.4
Mild	16.8 ± 0.6	10.8 ± 0.3	21.2 ± 0.4	6.8±0.3
Moderate	17.1 ± 0.8	10.3 ± 0.4	20.4 ± 0.5	**7.5 ± 0.3[d]**
Severe	**13.1 ± 1.3[b]**	**12.7 ± 0.7[b]**	**19.5 ± 0.7[c]**	**9.0 ± 0.4[b]**

Means are adjusted for age at death (years), sex, Braak & Braak stage, semi-quantitative ratings of diffuse and neuritic plaques, and dummy indicators for microinfarcts, HS-Aging, and Lewy body pathology. CDRSUM = Clinical Dementia Rating Sum of Boxes. MMSE = Mini Mental State Exam.

[a] $p < 0.01$ versus none, mild, and moderate.

[b] $p < 0.05$ versus none and mild.

[c] $p < 0.05$ versus mild.

or moderate B-ASC pathology (**Table 2.4**). In addition, the adjusted CDRSoB group mean score for cases with moderate B-ASC pathology was worse compared to that of cases with none or mild B-ASC pathology. These results indicate that after adjusting for age and comorbid brain pathologies, individuals with moderate and severe B-ASC pathology had worse global cognition compared to those with none or mild B-ASC pathology.

Clinical vascular risk factors associated with B-ASC pathology

Bivariate analyses and regression modeling were used to assess the relationship between vascular risk factors and B-ASC pathology. Race and ethnicity were not adjusted for in the models because of the low sample size within each group (data not shown). In the < 80 age at death group, hypertension, diabetes, and hypercholesterolemia were associated with B-ASC severity (**Table 2.5**). In the ≥ 80 age at death group, sex and smoking pack years were associated with B-ASC severity. With respect to clinically evident cerebrovascular disease, self-reported stroke history was associated with autopsied confirmed B-ASC pathology in the < 80 years age at death group (P = 0.003) and the ≥ 80 years age at death group (P = 0.033) (data not shown). However, there was no association between atrial fibrillation and B-ASC pathology (data not shown). Five vascular risk factors, along with age at death, were used in an ordinal logistic regression, with B-ASC severity as the ordinal outcome measure, to determine risk factors associated with B-ASC severity. In the < 80 age at death group, age at death, female sex, and hypertension were associated with predicting B-ASC severity. In the ≥ 80

Table 2.5 shows preliminary bivariate analyses on potential risk factors for B-ASC severity in "young" and "old" elderly individuals.

B-ASC Severity	None	Mild	Moderate	Severe	p-value
< 80 years age at death group					
Demographic					
Gender (%)					0.162
Male	32.2	38.1	23.3	6.4	
Female	30.1	35.2	24.6	10.1	
Clinical Variables					
Hypertension (%)	36.5	45.8	53.8	62	<0.0001[a,b]
Diabetes (%)	6.0	10.5	10.4	17.7	0.011[a]
Hypercholesterolemia (%)	40.5	48.6	54.7	50.6	0.010[a]
Pack years, median (25^{th}, 75^{th} quartile)	0.0 (0.0, 12.3)	0.0 (0.0, 22.5)	0.0 (0.0, 15.0)	0.0 (0.0, 25.0)	0.716
BMI, median (25^{th}, 75^{th} quartile)	25.4 (22.8, 28.1)	25.7 (22.8, 29.0)	24.9 (22.4, 24.8)	26.6 (23.7, 30.4)	0.206
Genetic Variables					
ABCC9 HS-Aging risk genotype A_A (%)	24.6	26	20.3	18.8	0.778
APOE ε4 allele (%)	40.7	51.9	50.8	50.0	0.061
≥ 80 years age at death group					
Demographic variables					
Gender (%)					0.024[a]
Male	22.9	33.9	28.2	15	
Female	17.1	33.8	33.7	15.4	
Clinical Variables					
Hypertension (%)	61.8	62.2	68.3	65.7	0.191
Diabetes (%)	13.1	13.2	14.0	11.4	0.847
Hypercholesterolemia (%)	55.0	47.8	47.4	52.4	0.155
Pack years, median (25^{th}, 75^{th} quartile)	0.0 (0.0, 6.3)	0.0 (0.0, 18.8)	0.0 (0.0, 16.5)	0.0 (0.0, 13.8)	0.032[a]
BMI, median (25^{th}, 75^{th} quartile)	25.2 (21.8, 28.2)	24.6 (22.4, 27.3)	25.1 (22.2, 28.1)	24.7 (22.4, 27.6)	0.746
Genetic Variables					
ABCC9 HS-Aging risk genotype A_A (%)	17.2	27.7	26.2	29.4	0.174
APOE ε4 allele (%)	39.0	37.9	43.1	39.0	0.490

P-values for pack years and body mass index (BMI) are from Kruskal-Wallis test. P-values for gender, hypertension, diabetes, hypercholesterolemia, *APOE* ε4 allele, and *ABCC9* HS-Aging risk genotype are determined from Chi-square tests. B-ASC = brain arteriolosclerosis; BMI = body mass index; HS-Aging = hippocampal sclerosis of aging.

[a] P-values < 0.05.

[b] Significant p-values after Bonferroni correction for multiple comparisons.

age at death group, only age at death and female sex remained significant variables in the model, but not hypertension (**Table 2.6**). These findings suggest that age at death and sex are associated with autopsy-proven B-ASC in both younger and older aged individuals. However, hypertension may be a risk factor for B-ASC pathology in young elderly individuals, raising the possibility that other B-ASC risk factors are important in more advanced old age.

Novel B-ASC genetic risk factor: *ABCC9*

Genetic and pathological information from NACC were used to assess the association between the *ABCC9* HS-Aging risk genotype and B-ASC pathology. Of the 2,390 cases included in the analysis, a total of 925 persons had available *ABCC9* SNP information. Individuals with *ABCC9* genotype information were slightly more likely to have AD pathology (45% vs 40%) than those lacking genotype data (data not shown). Among subjects with available *ABCC9* SNP data, bivariate analysis showed that the *ABCC9* HS-Aging risk genotype was associated with the presence of any B-ASC pathology (mild, moderate, and severe combined) in the ≥ 80 age at death group ($P = 0.032$) (**Figure 2.3**). By contrast, *APOE* genotype status was strongly associated with CAA pathology as expected, but not with B-ASC pathology (**Figure 2.2E**).

To account for relevant covariates, a logistic regression analysis was used, treating the presence of B-ASC pathology as a dependent variable, the

	Beta Coefficient ± S.E.	Odds Ratio	C.I.	p-value
< 80 years Age at Death group (n = 922)				
Age at death (1-year increase)	0.04 ± 0.01	1.0	1.0 - 1.1	<0.0001[a]
Gender (male vs. female)	–0.3 ± 0.1	0.8	0.7 - 0.8	0.029[a]
Hypertension (vs. no hypertension)	0.3 ± 0.1	1.4	1.1 - 1.8	0.017[a]
Diabetes (vs. no diabetes)	0.3 ± 0.2	1.4	0.9 - 2.1	0.120
Pack years (1-year increase)	0.0 ± 0.003	1.0	1.0 - 1.006	0.962
Hypercholesterolemia (vs. no hypercholesterolemia)	0.2 ± 0.1	1.3	1.0 - 1.6	0.067
≥ 80 years Age at Death group (n = 1255)				
Age at death (1-year increase)	0.02 ± 0.01	1.0	1.00 - 1.04	0.021[a]
Gender (male vs. female)	–0.2 ± 0.1	0.8	0.7 - 1.0	0.041[a]
Hypertension (vs. no hypertension)	0.2 ± 0.1	1.2	1.0 - 1.5	0.134
Diabetes (vs. no diabetes)	0.001 ± 0.2	1.0	0.7 - 1.4	0.995
Pack years (1-year increase)	0.003 ± 0.002	1.0	1.0 - 1.0	0.102
Hypercholesterolemia (vs. no hypercholesterolemia)	–0.1 ± 0.1	0.9	0.7 - 1.1	0.257

Table 2.6 Brain Arteriolosclerosis and Vascular Risk Factors. An ordinal logistic regression model was applied in both age at death groups using vascular risks factors identified from exploratory analysis. In both age at death groups, the statistical models included cases with available data on all six variables.

[a] P-value < 0.05.

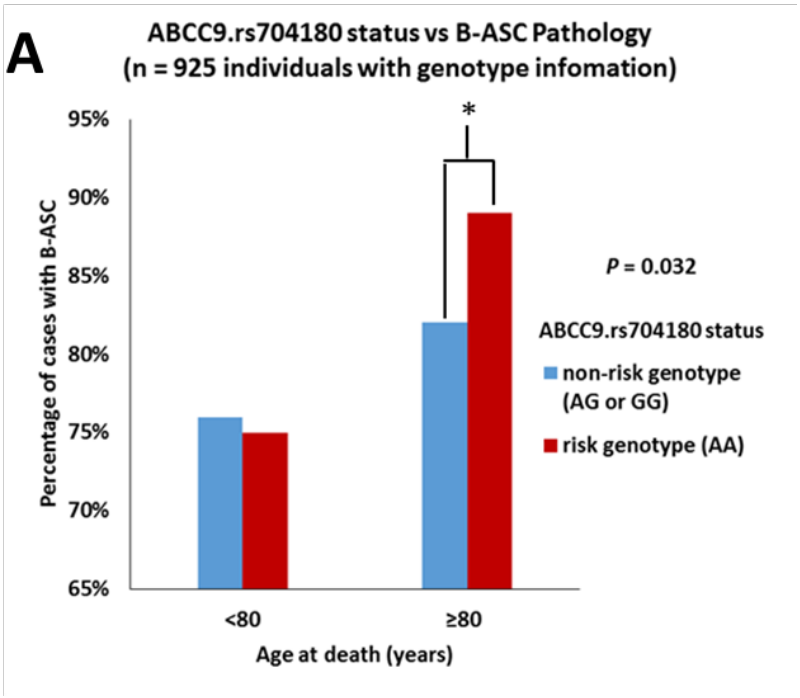

Figure 2.3: Relationship between any degree of brain arteriolosclerosis (B-ASC) and *ABCC9* HS-Aging risk genotype. This figure shows the association between *ABCC9* HS-Aging risks genotype (rs704180 A_A, as determined previously (Nelson *et al.,* 2014, Nelson *et al.,* 2015) and B-ASC pathology, stratifying by age of death. *Chi-square p-value = 0.032.

ABCC9 HS-Aging risk genotype as an independent variable, and age at death, sex, smoking pack years, and history of hypertension, diabetes, and hypercholesterolemia as covariates. Results from this model showed that individuals in the ≥ 80 years at death group with the *ABCC9* HS-Aging risk genotype were 1.9 times more likely to have a diagnosis of any B-ASC pathology (mild, moderate, or severe) compared to individuals without the *ABCC9* HS-Aging risk genotype ($P = 0.04$). There was no association between the *ABCC9* HS-Aging risk genotype and B-ASC pathology in the < 80 age at death group. In a sensitivity analysis adjusting for research center identifications as a fixed effect, the association between the *ABCC9* HS-Aging risk genotype and B-ASC pathology was still observed ($P = 0.04$) in the ≥ 80 years at death group.

ABCC9 and cerebral blood flow

To provide further testing of the association between the *ABCC9* HS-Aging risk genotype and B-ASC pathology, we assessed a neuroimaging modality that we hypothesize detects a clinical manifestation of B-ASC pathology. This was performed by measuring cerebral blood flow (CBF) in convenience sample of individuals (n = 15) from the ADNI data set comparing persons with A_A versus G_G *ABCC9*.rs704180 genotype. Individuals were on average 72 years of age at the time of scan and both groups of rs704180 homozygotes (A_A and G_G) were matched for cognitive status, *APOE* alleles, sex, and age (see **Table 2.7** for complete data on these parameters). Individuals with two *ABCC9* HS-Aging risk alleles (n = 8 with rs704180 A_A) showed lower global CBF

Table 2.7: Data on cases from Alzheimer's Disease Neuroimaging Initiative (ADNI) and Cerebral Blood Flow Data

Case #	*ABCC9* genotype status	Age	Sex	Race	*APOE* ε4 allele status	Diagnosis	CBF (ml/100g/min)
1	2	73	F	Caucasian	0	LMCI	38.11
2	2	71	F	Caucasian	0	Normal Aging	33.65
3	2	82	F	Caucasian	1	Normal Aging	29.78
4	2	79	F	Caucasian	0	Normal Aging	38.72
5	2	76	F	Caucasian	0	Normal Aging	28.88
6	2	81	F	Caucasian	0	LMCI	32.99
7	2	63	F	Caucasian	1	EMCI	35.97
8	2	76	M	Caucasian	1	AD	31.68
9	0	82	F	Caucasian	0	Normal Aging	47.23
10	0	81	F	Caucasian	0	AD	35.33
11	0	64	F	Caucasian	0	Normal Aging	45.19
12	0	66	F	Caucasian	1	LMCI	38.67
13	0	77	F	Caucasian	0	EMCI	46.49
14	0	60	F	Caucasian	0	EMCI	43.61
15	0	73	M	Caucasian	0	Normal Aging	47.44

ABCC9 genotype status: 0 = G_G, 2 = A_A; *APOE:* 0 = no *APOE* ε4 allele present (ε3/ε3, ε2/ε3, ε2/ε2), 1 = one *APOE* ε4 allele present (ε2/ε4, ε3/ε4). None of the individuals were homozygous for the *APOE* ε4 allele. EMCI = early mild cognitive impairment, LMCI = late mild cognitive impairment, CBF = cerebral blood flow

compared to those with two *ABCC9* HS-Aging risk alleles (n = 7 with rs704180 G_G) (**Figure 2.4**). The group level relative difference in CBF was 28%, $P < 0.001$ (**Figure 2.4**).

Discussion

In this study, we describe the global cognitive status, in addition to both clinical and genetic putative risk factors of B-ASC, in autopsied cases from the NACC data set. The presence of severe B-ASC pathology was associated with global cognitive impairment. In addition, a neuroimaging CBF experiment further supports the association between the *ABCC9* HS-Aging risk genotype and B-ASC. Potential risk factors for B-ASC included advanced age at death, hypertension, and sex. In younger individuals (age at death < 80 years), hypertension was associated with B-ASC. However, this association was not observed in older individuals (age at death ≥ 80 years). In the ≥ 80 years age at death group, the *ABCC9* HS-Aging risk genotype was associated with B-ASC pathology. These findings suggest that B-ASC risk factors are age-dependent. For example, hypertension appears to have a strong role in younger elderly individuals, while at least one genetic factor (*ABCC9*) may affect the B-ASC risk in the “oldest-old”.

There are potential limitations in this study. The NACC data set is not a population-based dataset; it is better characterized as a clinical series of persons

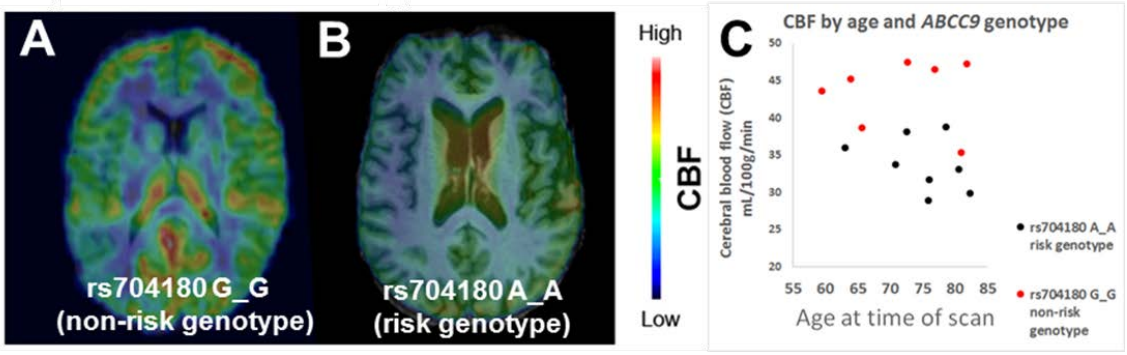

Figure 2.4: Relationship Between *ABCC9* HS-Aging Risk Genotype and Cerebral Blood Flow. Arterial Spin labeling (ASL) neuroimaging indicates that *ABCC9* HS-Aging risk genotype is associated with decreased cerebral blood flow (CBF), compatible with a novel pathogenetic mechanism. (A) shows a representative scan of a 77 year old female with the *ABCC9* HS-Aging non-risk genotype: rs704180 G_G. (B) shows a representative scan from a 76 year old male with rs704180 A_A. (C) Individuals with the rs704180 G_G genotype showed significantly higher global CBF compared to those with the rs704180 A_A genotype, group level relative difference in CBF is 28%, $p < 0.001$. No other scans nor SNPs were analyzed from the ADNI data set. See Table 2.7 for information about *APOE* alleles, sex, ages and cognitive status of subjects.

enrolled at ADCs, and in addition, carries known biases associated with autopsy cohorts [40, 65-67]. As a result, NACC participants are predominantly Caucasian, highly educated, and are drawn predominantly from dementia clinics [65, 68]. Due to the lack of socioeconomic information and low sampling of individuals from different ethnic/racial groups, race and ethnicity were not included in the regression models. The data on clinical disease risk factors are largely self-reported, which can lead to an underestimation of the true disease frequencies [103]. In addition, duration of disease (e.g., hypertension) data was not available, therefore, it was not adjusted for in the regression models. We found that female sex was associated with B-ASC; this finding is potentially confounded by the increased longevity of women (age being a risk factor for B-ASC), and many other covariates that vary with sex. Although this association survived in a regression model that accounted for other factors including age at death, these data should be interpreted with caution and future work is merited in this area. The NACC Neuropathology Data Set Coding Guidebook does not suggest optimal brain sections for B-ASC diagnosis. As a result, B-ASC diagnostic methods are inconsistent across ADCs. Although non-NACC guidelines exist for B-ASC diagnosis [104], different ADCs have reported using the basal ganglia [34], or a “global” [25] criteria in the diagnosis of B-ASC. In a prior, non-ADC autopsy study with 135 vascular dementia brains, B-ASC was seen in the frontal lobe (83.7% of cases), temporal lobe (80.7% of cases), and basal ganglia (89.6% of cases) [105]. Because the diagnosis of B-ASC is presently based on H&E staining, other pathologies that lead to a distortion of vascular walls (e.g.,, CAA)

may mistakenly be diagnosed as B-ASC, leading to a biased estimation of B-ASC frequency. However, we saw a strong correlation between *APOE* status and CAA severity, but no correlation between *APOE* status and B-ASC severity in our sample, indicating that these pathologies are at least partly independent. In the future, improved methodologies and consensus for B-ASC pathologic diagnosis should improve the specificity of B-ASC operationalization. Until then, it can be argued that a multi-center approach, combining data from dozens of neuropathologists, each applying center-specific diagnostic rubrics, is the best way to achieve an outcome that is representative of what any given neuropathologist would define as “brain arteriolosclerosis.”

Despite the challenges inherent to a retrospective cross-sectional study, the NACC and ADNI databases provide relatively high-quality contexts to study clinical, genetic, neuroimaging, and/or pathological correlations. Detailed cognitive assessments, genetic, and neuropathological data have allowed us to study associations of B-ASC with cognitive status and both clinical and genetic variables. These include both “traditional” B-ASC risk factors (hypertension and diabetes), as well as novel genetic aspects (*ABCC9* SNP). Mixed pathologies are frequently seen in the aged brain [5, 47, 106] and detailed neuropathological data from NACC allowed us to adjust for other dementia-inducing pathologies in our analyses. B-ASC is a common cerebrovascular pathology that is often seen in a complex milieu along with other brain diseases [47] and has been associated with motor impairment in advanced old age [32]. In this NACC-ADC data set, the frequency of any B-ASC pathology (mild, moderate, and severe) was 75.3%, and

became more severe with increasing age at death, which is consistent with findings from other autopsy cohorts [31, 33, 107].

We showed that B-ASC pathology was associated with worse MMSE and CDRSoB scores after adjusting for comorbid cognitive impairment-inducing pathologies. Prior studies reporting the cognitive profiles of B-ASC are limited, mostly focusing on patients diagnosed with vascular dementia (a very heterogeneous condition) [3, 71, 72]. Although both age at death groups had MMSE and CDRSoB scores indicative of cognitive impairment, individuals in < 80 age at death group were more globally impaired compared to individuals in the ≥ 80 years age at death group. This finding may be attributed to the differing frequencies of AD and B-ASC pathologies present in the two age at death groups: higher number of individuals with 'high" AD pathology in < 80 years age at death group, and higher number of individuals with moderate or severe B-ASC pathology in ≥ 80 years age at death group. Individuals with AD pathology exhibit greater deficits in memory function and faster rates of information decay compared to individuals with cerebrovascular disease [108]. Our study provided quantitative evidence to support the hypothesis that B-ASC is associated with worse cognitive status independent of other brain changes. Furthermore, it underscores the importance of identifying risk factors, specific neuroimaging abnormalities, and potential treatments of B-ASC, in order to prevent or reverse its development later in life.

Hypertension is a major risk factor for B-ASC [36, 109]. In an autopsy study of 200 cases, Moritz et al observed that B-ASC pathology was more severe

in the hypertensive group compared to the non-hypertensive group [36]. In the < 80 age at death group, we found hypertension to be associated with B-ASC. However, in the ≥ 80 age at death group, there was no association detected between B-ASC and hypertension and these results did not change after adjusting for anti-hypertensive medication use. Similarly, in an autopsy study consisting of 70 cases with B-ASC, 31% of cases were normotensive with 10 of these cases having an age at death ≥ 80 years [109]. These results suggest that hypertension may not be the only risk factor for B-ASC pathology in older elderly individuals.

We hypothesize that the known strong impact(s) of diabetes on brain function may be mediated through a combination of vascular and metabolic etiologies [110]. We did not find support for the direct impact of diabetes on B-ASC, but there was a trend between diabetes and B-ASC in the younger cohort. We note that there was not a distinction between Type 1 or Type II diabetes in the NACC data set, although the majority is presumed to be Type II diabetes. Evidently, beyond the “usual suspects”, there are additional, currently unknown risk factors for B-ASC in advanced old age.

One category of risk factors that may be relevant to B-ASC pathology in the “oldest-old” is genetics, and we here provide support for a specific candidate risk allele. The *ABCC9* SNP rs704180 was previously associated with risk for HS-Aging [59, 60], a hippocampal pathology seen in ~10-25% of autopsied individuals beyond 80 years at death [51, 55, 56, 111]. Recently, we found an association between HS-Aging and B-ASC in three separate cohorts, including

the NACC data set [25]. Using digital image methods for analysis of arteriolar morphology, we found that HS-Aging cases had larger vessel areas, vessel perimeters, vascular areas, and vessel wall thicknesses compared to non HS-Aging cases [25]. Research from a different cohort, using different neuropathological scoring and statistical methods, reported that moderate B-ASC (but not severe or mild) was associated with hippocampal atrophy [112]. Because of all these findings collectively, we hypothesized that the *ABCC9* HS-Aging risk genotype is associated with B-ASC in advanced old age, possibly upstream of the risk for HS-Aging (**Figure 4D**). The present study showed an association between the *ABCC9* HS-Aging risk genotype and B-ASC pathology in cases with an age at death ≥ 80 years. There was no evidence of that association among individuals with age at death < 80 years.

In order to test the association between the *ABCC9* HS-Aging risk genotype and B-ASC, we analyzed CBF in elderly individuals. The rationale for this experiment includes findings that: 1) B-ASC is associated with white matter hyperintensities (WMHs) on MRI scans [113-117], and 2) WMHs have been correlated with CBF decreases [118-120] and cognitive impairment [121-125]. We found the *ABCC9* HS-Aging risk genotype to be associated with decreased CBF in elderly individuals. These findings support the hypothesis that the *ABCC9* HS-Aging risk genotype promotes B-ASC in the oldest-old with decreases in CBF on neuroimaging. *ABCC9* encodes a regulator of ATP-sensitive potassium channels that is expressed in vascular smooth muscle cells [126-129]. The protein is important for vascular tone regulation and reactivity to metabolic factors

and oxidative stress [126, 130-132]. We hypothesize that gene variants in *ABCC9* could result in chronic perturbations of the vascular wall leading to decreases in CBF and B-ASC pathology. Therefore, the brain changes associated with *ABCC9* gene variants may be part of a “brain wide” disease characterized by HS-Aging, TDP-43 pathology, and B-ASC in elderly individuals [57, 133]. Further studies are warranted to test this hypothesis.

Based on the results, it can be concluded that B-ASC is a common vascular pathology with a deleterious impact on global cognition in elderly individuals. Risk factors for B-ASC include hypertension, which has long been considered to be a putative modifiable factor, as well as advanced age. Additional possibly targetable mechanisms involved in the B-ASC pathogenesis are mostly unknown, but the results of this study offer candidate pathways involving *ABCC9* gene products. Furthermore, we provide evidence that ASL neuroimaging is a potential candidate biomarker to indicate *ABCC9*-related variations in CBF that could be useful in a clinical setting. These findings may serve to increase awareness about B-ASC, a common cerebrovascular pathology associated with cognitive impairment.

Chapter 2 Dissertation Work Citation:

Ighodaro ET, Abner EL, Fardo DW, Lin AL, Katsumata Y, Schmitt FA, Kryscio RJ, Jicha GA, Neltner JH, Monsell SE, Kukull WA, Moser DK, Appiah F, Bachstetter AD, Van Eldik LJ, Nelson PT (2017) Risk factors and global cognitive status related to brain arteriolosclerosis in elderly individuals. *J Cereb Blood Flow Metab* **37**, 201-216

Chapter 3: Multi-lumen Vascular Profiles (MVPs)

Introduction

The human cerebral vasculature is responsible for numerous functions such as oxygen loading, nutrient transport, and regulation of blood flow [19, 134, 135]. With aging and cerebrovascular diseases, the structure of blood vessels can exhibit dramatic remodeling [19, 134, 136, 137]. Specific small vessel pathologic features include arteriolosclerosis, arteriovenous malformations, and downstream changes such as lacunar infarcts, leukoaraiosis, micro-infarcts, and hemorrhagic lesions [22, 24, 136, 138, 139]. However, the histomorphologic features related to cerebrovascular malfunction are probably more heterogeneous than is widely appreciated, and here we focus on a subtype of vascular pathology about which there is relatively little information published.

In this dissertation, multi-lumen vascular profiles (MVPs) is a term used to describe a single blood vessel consisting of ≥ 3 lumens enclosed in a perivascular space on a cross-sectional view. The purpose of this study was to investigate the frequency, risk factors, and co-pathologies of brain MVPs incases from the University of Kentucky and the University of Pittsburgh brain banks.

Methods

This study used human brain samples and data from the University of Kentucky Alzheimer's Disease Center (UK-ADC), the University of Kentucky Pathology Department (UKPD), and the University of Pittsburgh Pathology Department (UPPD). Patient recruitment, tissue and data collection in the UK-ADC research

study have been previously described including details related to institutional review board approval and patient consent. [106, 140]. Human tissue samples from UKPD resulted from autopsies that were performed after obtaining informed consent using forms approved by the Institutional Review Board of the University of Kentucky College of Medicine, Lexington, Kentucky. Human tissue samples from UPPD resulted from autopsies that were performed after obtaining informed consent using forms approved by the Institutional Review Board of the University of Pittsburgh, College of Medicine, Pittsburgh, Pennsylvania.

Study subjects

Among the UKPD cases, a set of 39 autopsied tissue samples were collected from the UKPD brain bank. Cases were selected by the investigators (JHN and PTN) to be free of advanced neurodegenerative pathology or any other extensive brain disease that contributed to the patient's death. Hence, exclusion criteria included pathologically confirmed brain tumors and pathologically confirmed neurodegenerative diseases.

Among the UK-ADC cases, all autopsied subjects with detailed quantitative neuropathological data were initially considered for inclusion (n=709). Cases with brain tumors or end-stage neurodegenerative diseases (AD, Parkinson's disease, DLB, prion disease, Picks disease, progressive supranuclear palsy (PSP), multiple sclerosis (MS), HS-Aging, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), and/or frontotemporal lobar degeneration (FTLD) were excluded

from the study. Cases with cerebrovascular disease and controls (free from brain pathology) were included (n=92). The UKPD and UK-ADC cases were combined in order to test the association between age at death and MVP density. Next, the UK-ADC cases were used to determine the association between conventional vascular risk factors, cardiovascular/cerebrovascular diseases, neuropathological conditions, and genetic variables with MVP density.

In two preliminary studies, additional cases from the UK-ADC (n=5) and the UPPD (n=4) were used to observe the relationship between severe cerebral angiopathy and chronic traumatic encephalopathy respectively with MVP density.

Clinical and neuropathologic parameters in the UK-ADC and UKPD data set

Information on clinical and neuropathologic parameters used in this study set was described previously [24] with some changes related to this specific research question. Clinical data were obtained from each participant's final UK-ADC clinical visit before death. During clinical research visits, medical histories were obtained from subjects, caregivers (particularly if the subject was cognitively impaired), and/or patient records. The following self-reported vascular risk factors, cerebrovascular and cardiovascular diseases were used in the analyses: medical histories of hypertension, diabetes, hypercholesterolemia, sex, smoking status, heart attack, congestive heart failure, atrial fibrillation, stroke, transient ischemic attack, angina, arrhythmia, and angioplasty. Responses were coded initially as unknown, absent, recent/active, or remote/inactive, and subsequently, "recent" and "remote" responses were

combined into one category (e.g.,, history of a condition) for analytical purposes. Body mass index (BMI) values were derived from height and weight measurements and dichotomized into the following categories: < 30 BMI and ≥ 30 BMI. The only available information from the UKPD data set was age at death and sex.

Tissue Processing, Immunohistochemistry, and MVP Calculation

To visualize MVPs, brain sections were stained with hematoxylin and eosin (H&E), alpha smooth muscle actin (α-SMA) antibody, and CD34 antibody. α-SMA is a marker for smooth muscle cells and CD34 is a marker for endothelial cells. Brain tissue processing and immunohistochemistry procedures used in the UK-ADC have been described previously in detail [25, 106, 141]. A similar procedure was used in processing UKPD and UPPD tissue samples. Briefly, the brain was sectioned during autopsy, fixed in formalin, and processed in paraffin [106]. Afterwards, sections from archived paraffin-embedded frontal cortex were cut and placed onto glass slides for immunohistochemistry [106]. Frontal neocortex (specifically, Brodmann Area 9) was chosen as a convenience sample that correlates with a brain area where MVPs were described previously [39]. The primary antibodies used during immunohistochemistry were α-SMA (monoclonal mouse anti-human 1A4, Dako, 1:2 dilution) and CD34 (monoclonal mouse anti-human QBEnd 10, Dako, 1:2 dilution). A biotinylated antibody (anti-mouse IgG made in horse, Vector Laboratories) was amplified using avidin-biotin substrate (ABC solution, Vector Laboratories), followed by color development in 3,3'-

diaminobenzidine (DAB; Dako). A detailed protocol is shown in **Table 3.1.** The Aperio ScanScope XT digital slide scanner was used to image the entire stained slide at 40X magnification to create a single high-resolution digital image [106].

The CD34 primary antibody was chosen for calculating MVP density since it appears to label a larger proportion of MVPs compared to the α-SMA primary antibody. Tissue sections were analyzed blind to the demographics, clinical and neuropathological conditions of the corresponding case. First, the entire grey matter was outlined and counted manually using the Aperio ScanScope XT accompanying image analysis software (ImageScope) from Leica Biosystems.

Table 3.1: Immunohistochemistry Staining Protocol for CD34.

Steps	Solution Mixture & Notes	Time needed
1. Deparaffinze slides from xylene to DH2O	*change solutions after 3 times use	Xylene – 3X @ 5min 100% ETOH – 2X @ 5 min 95% ETOH – 2X @ 3 min 80% ETOH – 1X @ 3 min DH2O – 1X @ 5 min
2. Antigen Retrieval in microwave (power 8) citrate buffer pH 9	- 2mL stock pH 9 citrate buffer/ 98mL DH2O *make 100mL per slide holder *balance with blank slides, front left corner	6 min
3. Remove the plastic jar from microwave and cool off on counter	-	10 min
4. Wash in running DH2O	-	5 min
5. Incubate in 3% H2O2	- 10mL stock H2O2 / 90mL methanol	30 min @ RT on shaker
6. Wash in running DH2O	-	5 min
7. Wash in 1X TBS	- 100 mL stock TBS / 900mL DH2O *make fresh daily	2 X @ 5 min
8. Block in 15% horse serum	- 15mL horse serum / 85mL 1X TBS	1 hour @ RT on shaker
9. Incubate with primary CD34 antibody (1:2 dilution); Lot #	- 0.5mL CD34 / 0.5 mL TBS-horse soln *add 2 drops per slide *make 1mL soln per 10 slides *add negative & positive controls if necessary	Overnight @ 4C (cold room)
10. Wash in 1X TBS	-	2 X @ 5 min
11. Incubate with secondary antibody: biotinylated Anti-Mouse IgG made in horse; Lot #	- 5uL 2nd antibody / 15uL horse serum / 1mL 1X TBS *make 1mL soln per 10 slides	1 hours @ RT on shaker
12. Dilute ABC reagent in ABC Standard kit and let stand for at least 30 minutes; Lot #	-1 drop of A/ 1 drop of B / 1mL of 1X TBS *make 1mL soln per 10 slides	Let stand for at least 30 min
13. Wash slides in 1X TBS	-	2 X @ 5 min
14. Incubate slides in ABC soln	-	1 hour @ RT on shaker
15. Wash slides in 1X TBS	-	2 X @ 5 min
16. Add chromogen soln to slides: Dako – DAB; Lot #	- 1 drop of DAB / 1mL kit buffer	-
17. View under microscope & inactive reaction in D2HO	-	-
18. Counterstain with Hematoxylin	- 2 dips in Harris Hematoxylin - rinse in running H2O -1 dip in acid alcohol - rinse in running H2O - 3-5 dips in ammonia water (3 drops/ 100mL DH2O) *filter hematoxylin before use	
19. Rinse in running H2O		5 min
20. Dehydrate, clear, and mount	-	80% ETOH – 1 X @ 20 dips 95% ETOH – 2 X @ 20 dips 100% ETOH – 3 X @ 2 min Xylene – 3 X @ 2 min

Abbreviations: DH2O = distilled water, ETOH = ethanol, H2O2 = hydrogen peroxide, RT = room temperature

Afterwards, the observer counted MVPs present within the region. The formula used to calculate MVP density is:

$$\frac{\boldsymbol{total\ \#\ of\ MLVs}}{\boldsymbol{grey\ matter\ area}\ (\mu\boldsymbol{m^2})} * \mathbf{10^7}$$

Brain Tissue Clearing and Imaging

In a preliminary study, we used a tissue clearing method called SeeDB on one case from the UK-ADC cohort that had the highest MVP density. SeeDB involves a series of increasing concentrations of fructose to match brain tissue to the refractive index of the surrounding medium. The SeeDB method has been described previously [142]. Prior to treatment with SeeDB, tissues were incubated with fluorescein-conjugated lectin, which fluorescently labeled the endothelium of all blood vessels. Next, tissues were imaged using two-photon microscopy to capture 3-D images of the vasculature.

Statistical analyses

Correlations and exploratory bivariate analyses were used to assess the association between demographics, clinical vascular risk factors, cerebrovascular and cardiovascular diseases with MVP density. Initially, a Spearman's rho correlation was used to determine the association between age at death and MVP density using UK-ADC (n=92) and UKPD (n=39) cases. Next, a Mann-Whitney U (Wilcoxon Rank Sum) test was used to determine possible risk factors for MVP pathology using only UK-ADC cases. UKPD cases were excluded from the latter analysis due to lack of data available on clinical risk factors of interest.

Due to multiple comparisons, a significance threshold level of 0.0002 (17 comparisons) was used for this study (Bonferroni correction). All statistical analyses were performed using IBM SPSS Statistics 22 Properties and PC-SAS 9.34 (SAS Institute, Inc.; Cary, NC, USA).

Results

Included individuals from the UK-ADC cohort were predominantly Caucasian (data not shown). Race/ethnicity information was not available for cases within the UKPD brain repository. In order to provide representative blood vessel profiles representing the variation of MVP pathology, images were obtained from four human brain sections stained with either H&E, or antibodies against α-SMA or CD34 (**Figure 3.1**). The quantification method of MVPs in this study is shown in **Figure 3.2**. The overall median age at death for cases used in the study was 84.0 years with an interquartile range of 34.0 years (**Table 3.2**). The overall percentages of females and males within this study was 52.7% and 47.3% respectively (**Table 3.2**). Using all 131 cases, there was a significant linear correlation between age at death and MVP density, shown in **Figure 3.3** (Spearman's rho = 0.60; $p < 0.0001$). In other words, age at death was associated with MVP density.

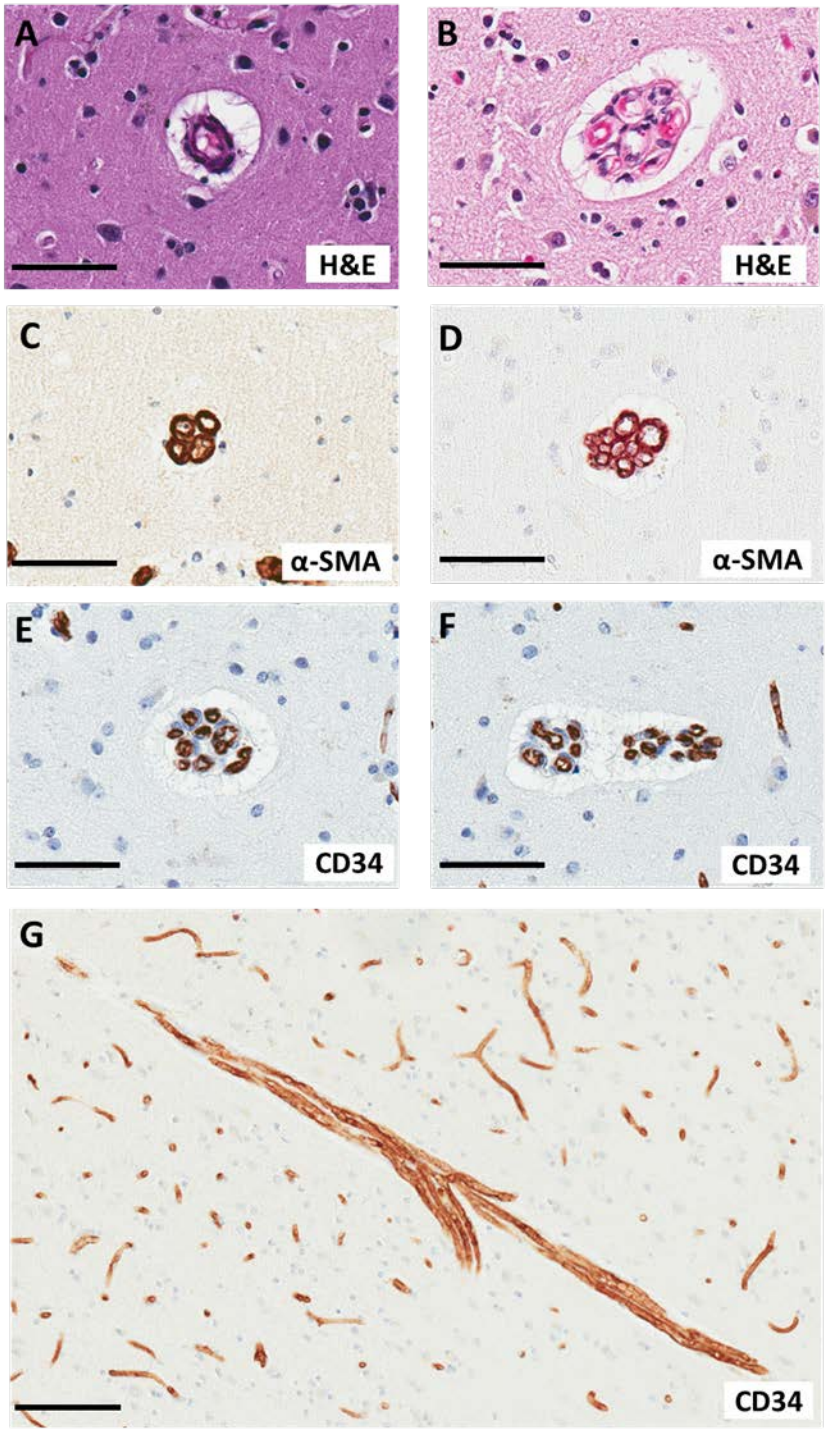

Figure 3.1: Multi-lumen vascular profile (MVP) Pathology. Within this study, MVP is described as a single blood vessel consisting of ≥ 3 lumens enclosed in a perivascular space on a cross-sectional view. (a,b) Photomicrographs of hematoxylin-and-eosin (H&E) stained blood vessels within the grey matter of frontal tissues. (a) Blood vessel shown here can be characterized has a normal arteriole (due to rigid circular structure) from a 42 year-old female. (b) What we describe as a MVP from a 96 year-old female, with at least 8 lumens some of which contain red blood cells. (c,d) Photomicrographs of alpha smooth muscle actin (α-SMA) stained MVPs in cross-section. (c) shows a MVP with at least 4 lumens of similar size from a 91 year-old female. (d) shows a MVP with at least 13 lumens of varying size from a 89 year-old male. (e, f, g) Photomicrographs of CD34 stained MVPs. (e,f) show MVPs with at least 10 lumens in cross-section from a 89 year-old male. (g) shows a MVP cut in a longitudinal direction from a 91 year-old female. Scale bars: a-f = 50µm, g = 100µm

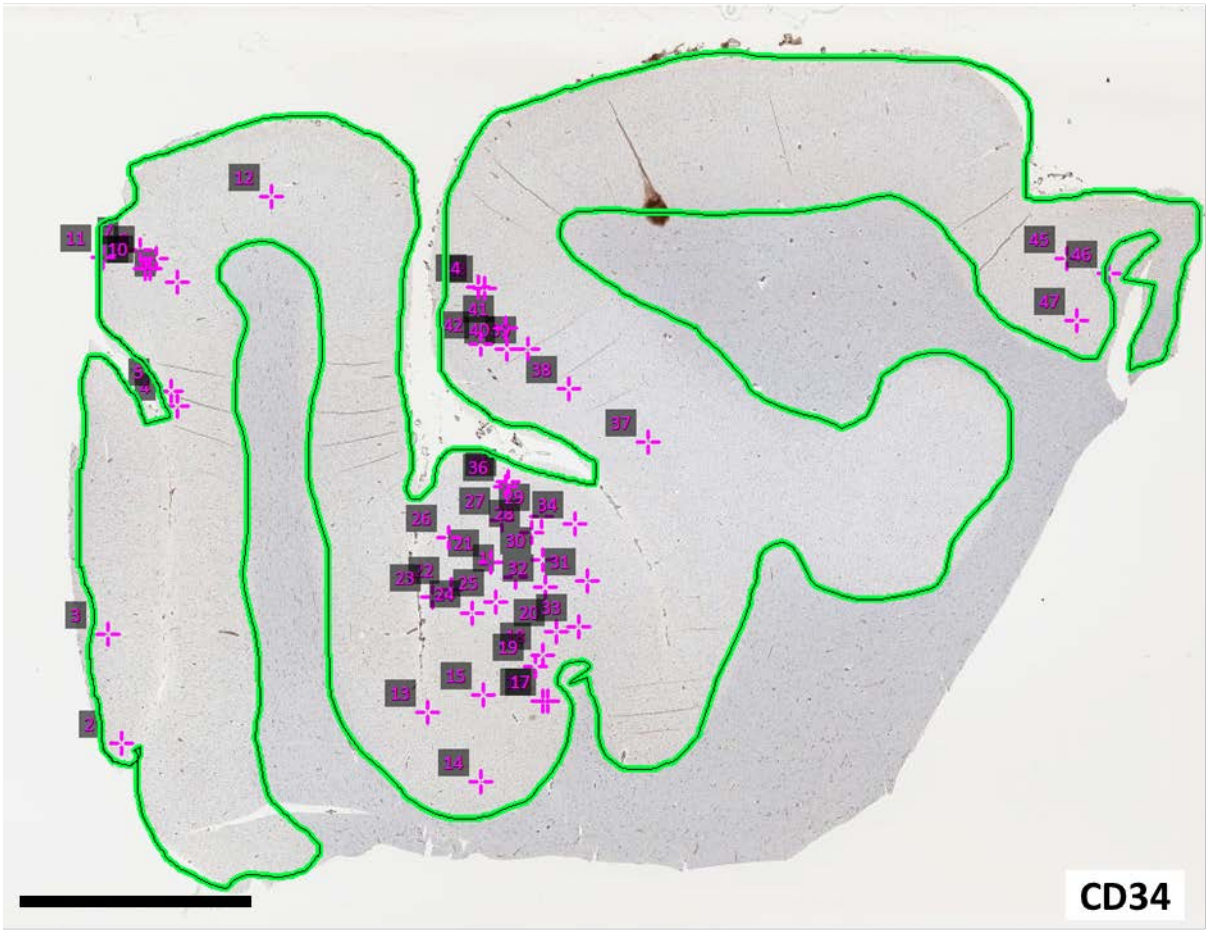

Figure 3.2: Schematic of MVP quantification. The photograph is of a CD34 stained tissue from the frontal cortex (Broadmann area 9) of an 84 year-old female. Using Aperio ScanScope XT digital scanner accompanying image analysis software, the grey matter area was outlined (green-black line). Next, the grey matter area was manually scanned for MVPs which were marked by a counter (pink crosses). The total # of MVPs and the grey matter area for each case were used to calculate an MVP density. Scale bar = 5mm

Table 3.2: Demographics of cases from the University of Kentucky Pathology Department (UKPD) and University of Kentucky Alzheimer's Disease Center (UK-ADC) cohorts

	UKPD cohort	**UK-ADC cohort**	**Overall**
Sample size	39	92	131
Demographic variables			
Age at death, median (IQR)	38.0 (18.0)	87.0 (11.0)	84.0 (34.0)
Sex (%)			
Male	59.0	42.2	47.3
Female	41.0	57.6	52.7

Cases from both cohorts were combined for the MVP study in order to test the association between age at death and MVP density.

Relationship Between Age At Death and MVP Density (n=131)

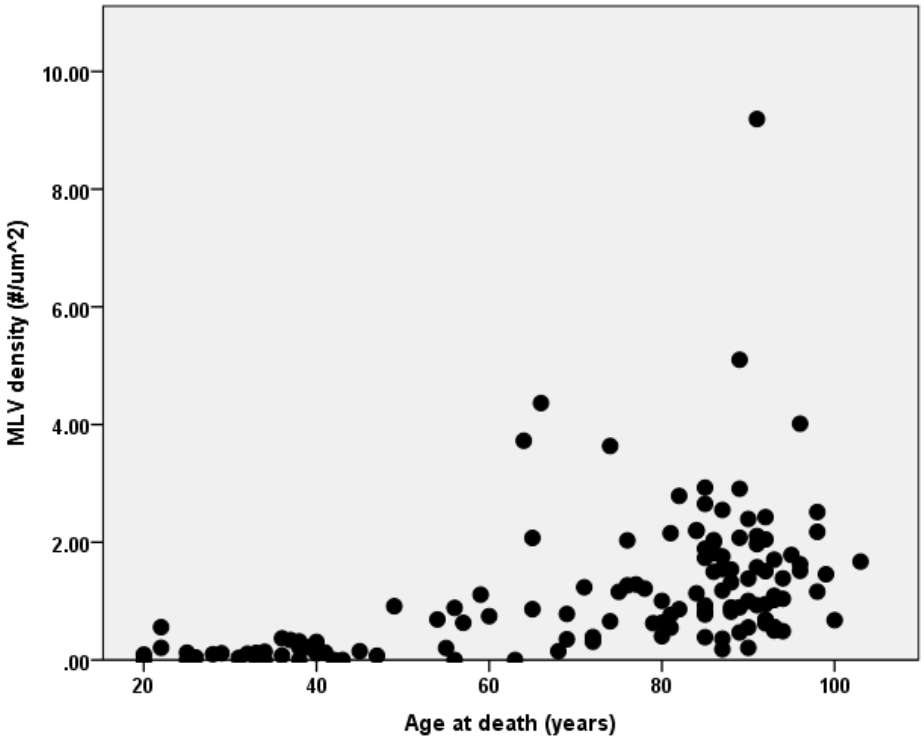

Figure 3.3: Relationship between age at death and MVP density. Cases from the UKPD and the UK-ADC cohort were combined in order to determine the association between age at death and MVP density. A scatter plot was used to show each case's MVP density with corresponding age at death. Using a Spearman's rho test, the correlation between age at death and MVP density was 0.60 with a p-value of < 0.0001.

Using UK-ADC cases (n = 92) with clinical, neuropathological, and genetic information, the majority of cases had hypertension (59.0%), hypercholesterolemia (58.4%), and the APOE ε3/ε3 genotype (64.6%) (**Table 3.3**). In order to determine the clinical vascular risk factors for MVP pathology, a Wilcoxon rank-sum test was performed on 13 clinical variables. None of the clinical variables were significantly associated with MVP density within this cohort (**Table 3.4**). Similar non-significant findings were observed in demographic (sex), neuropathological (name the pathologies), and genetic (*APOE*) variables related to vascular disease (**Table 3.4**).

Further staining was performed on the UK-ADC case with the highest MVPs of the sample group, which was an 89 year-old male. The only condition that he was positive for was hypertension. The gross anatomy of his brain showed small holes in the basal ganglia (**Figure 3.4**). Microinfarcts and a microaneurysm were seen in the patient's putamen (**Figure 3.4**). Using a clearing method, the 3D visualization of this patient's MVPs shows a single large MVP branching into at least 4 smaller MVPs (**Figure 3.5**).

Table 3.3: Clinical, Neuropathological, and Genetic Information of UK-ADC cases used in MVP study

	UK-ADC cohort	Missing cases, n
Sample size	92	
Clinical Variables		
Hypertension (%)	59.0	9
Diabetes (%)	12.5	12
Hypercholesterolemia (%)	58.4	56
Smoking (%)	47.4	14
BMI (%)	22.9	22
Heart atack (%)	26.6	13
Congestive heart failure (%)	24.1	13
Atrial Fib (%)	19.0	13
Stroke (%)	18.8	12
Transient ischemic attack (%)	16.3	12
Angina (%)	19.0	13
Arrhytima (%)	28.4	11
Angioplasty (%)	12.7	13
Neuropathological Variables		
Atherosclerosis (%)		1
zero	5.5	
one	22.0	
two	22.0	
three	26.4	
four	24.2	
B-ASC (%)		6
none	32.6	
mild	46.5	
moderate	18.6	
severe	2.3	
Genetic Variables		
APOE genotype (%)		10
ε2/ε2	1.2	
ε2/ε3	17.1	
ε3/ε3	64.6	
ε3/ε4	17.1	

Clinical variables were self-reported and neuropathological variables were semi-quantitatively graded. There were not individuals with the ApoE ε4/ε4 genotype in our study sample. These variables were used to determine the risk factors for MVP density. The number of cases missing information on a given variable is reported. Clinical, neuropathological, and genetic information on cases from the UKPD were not available.

Table 3.4: MVP Densities for Dichotomous Clinical Variables

Clinical Variables, median density (IQR)	Absent (#/µm)	Present (#/µm)	Significant?
Hypertension	1.24 (1.37)	1.21 (1.28)	NS
Diabetes	1.49 (1.12)	1.03 (1.59)	NS
Hypercholesterolemia	1.73 (1.02)	1.04 (1.08)	NS
Smoking	1.76 (1.34)	1.13 (1.05)	NS
BMI	1.16 (1.06)	1.77 (1.65)	NS
Heart atack	1.15 (1.37)	1.51 (1.11)	NS
Congestive heart failure	1.14 (1.14)	1.51 (1.31)	NS
Atrial Fib	1.19 (1.33)	1.28 (0.91)	NS
Stroke	1.28 (1.24)	0.91 (0.92)	NS
Tranisent ischemic attack	1.16 (1.29)	1.58 (1.39)	NS
Angina	1.18 (1.35)	1.52 (1.19)	NS
Arrhytima	1.35 (1.19)	1.00 (1.26)	NS
Angioplasty	1.13 (1.11)	1.87 (1.13)	NS

In order to associate the clinical and neuropathological variables of interest with MVP density, a Mann-Whitney U (Wilcoxon Rank Sum) test was applied within the analyses. Due to multiple comparisons, an adjusted significance level of 0.00002 (17 comparisons) was used to determine significance. Absent/present references to the absence or present of the disease as self-reported by the research participant. None of the clinical or neuropathological variables of interest yielded a significant result. Abbreviations: IQR = interquartile range; NS = non-significant; MVP = multi-lumen vascular profiles

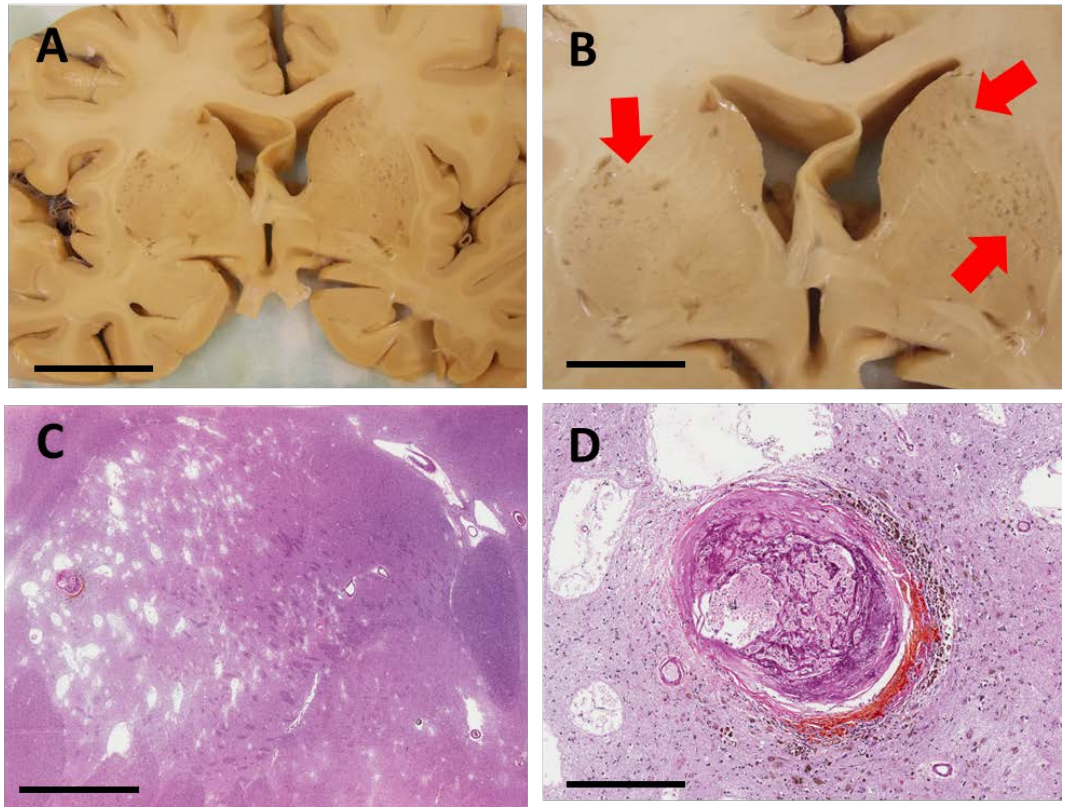

Figure 3.4: UK-ADC Case With Highest MVP Density. (a,b) Photographs showing the gross anatomy of the basal ganglia from a 89 year-old male. (b) Higher magnification of (a) with red arrows indicating holes in the basal ganglia. (c,d) Photomicrographs of hematoxylin-and-eosin stained tissue sections from the putamen of a 89 year-old male. (c) shows regions of infarct tissue. (d) shows what we presume to be a cerebral microaneurysm (Charcot-Bouchard aneurysm). Scale bars: a = 2mm, b =3mm, c = 4mm, d = 500µm

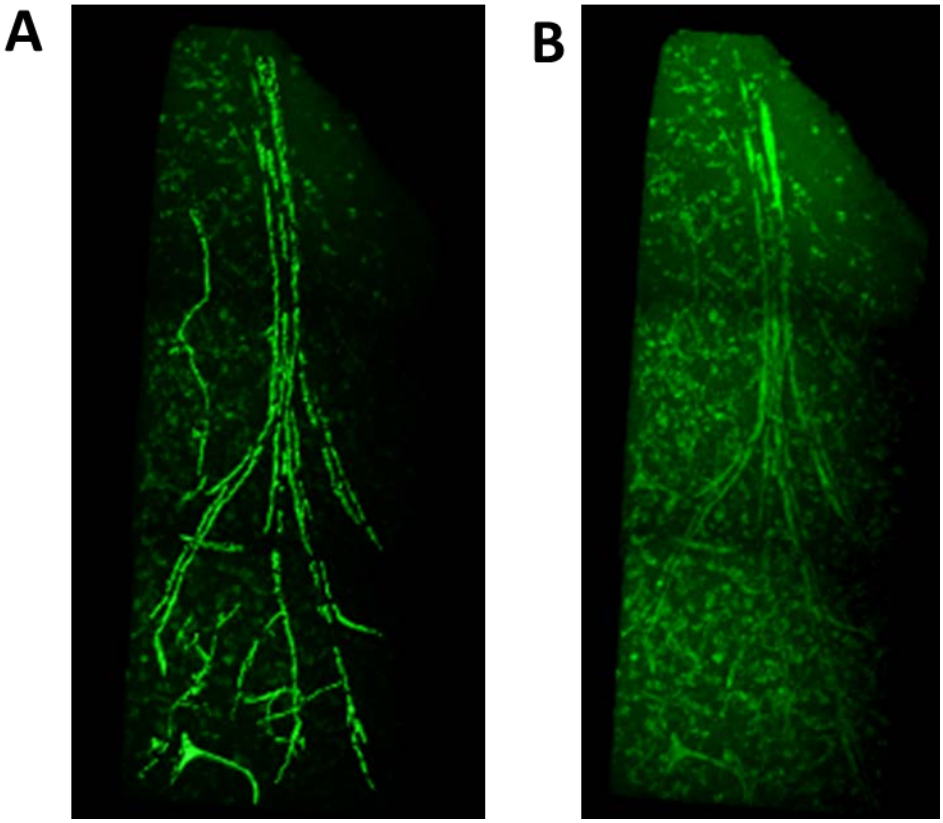

Figure 3.5: 3-D Visualization of MVPs. (a,b) Photographs showing the 3D branching of MVPs within the frontal cortex of a 89 year-old male. Based on the high MVP density of this case (which was analyzed by me), I sectioned this case for tissue clearing. Tissues from this case was subsequently sent to Dr. Andy Shih, Associate Professor at the Medical University of South Carolina, who performed the tissue clearing image capturing.

Relationship Between Age At Death and MVP Density

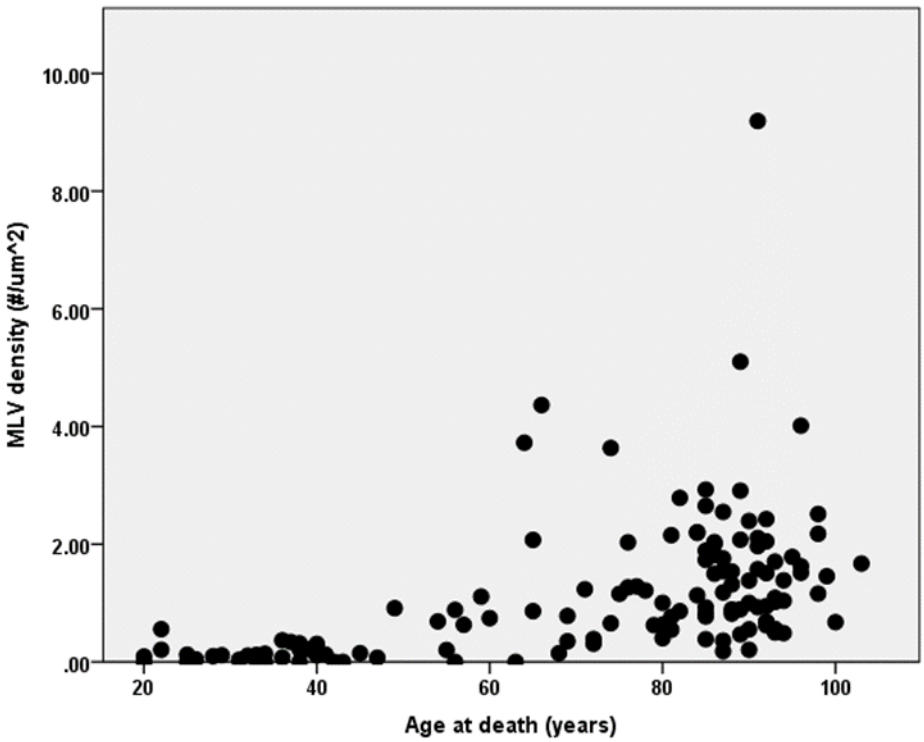

Figure 3.6: Relationship Between Age At Death and MVP Density. Cases from the UKPD and the UK-ADC cohort were combined in order to determine the association between age at death and MVP density. A scatter plot was used to show each case's MVP density with corresponding age at death. Abbreviations: Comb. = combined, U. = university, MVP = multi-lumen vascular profiles

Discussion

In this study, we tested for associations with an understudied small vessel pathology – MVPs -- in cases from the UK-ADC, UKPD, and UPPD brain repositories. We provided quantitative evidence that age was associated with MVP density. When analyzing the association between conventional vascular risk factors (e.g., hypertension, diabetes), cardiovascular diseases (e.g.,, heart attack, arrhythmia) and cerebrovascular disease (e.g.,, stroke, transient ischemic attack), an association between these variables and MVP density was not found. The association between neuropathological (e.g., brain arteriolosclerosis) and genetic (e.g., *APOE*) variables of interests also did not show an association between these variables and MVP density.

There are potential limitations in this study [24]. The UKPD, UPPD, and UK-ADC brain banks do not comprise a population-based samples [140]. The UKPD brain bank is from individuals that came to autopsy from a tertiary-care hospital, which carries known biases associated with hospital affiliated autopsy services. With respect to UKPD cases, some clinical, neuropathological, and genetic information of interest were not available for this study. Therefore, we could not assess MVP risk factors within a younger cohort. The UK-ADC brain repository is a community and clinically based cohort of research subjects associated with an ADC, which carries other known biases [40, 65-67, 140]. As a result, UK-ADC participants are predominantly White Americans, highly educated, at risk for developing clinical AD [65, 140, 143]. Due to the lack of socioeconomic information and low sampling of individuals from different

racial/ethnic groups, race and ethnicity were not included in the analyses. The data on clinical disease risk factors are largely self-reported, which can lead to an underestimation of the true disease frequencies [103]. In addition, duration of disease (e.g., hypertension, diabetes) data were not available.

Despite the challenges inherent to a retrospective cross-sectional study, the combined cohort we used for our study provided brain tissue samples that span a broad aging spectrum (20s – 100s at death). In addition, the UK-ADC provides detailed clinical, neuropathological, and genetic information valuable for studying correlations. The information allowed us to test for associations between conventional vascular risk factors (e.g., hypertension, diabetes), cardiovascular/cerebrovascular diseases (heart attack, stroke), neuropathological diseases, and genes of interests.

For the purpose of this study, a MVP was defined as having ≥ 3 lumens within a single vascular profile. Cervos-Navarros et al characterized MVPs as having up to 10 lumens surrounded by a perivascular space [44]. Hassler et al described MVPs as having ≥ 4 vessels running parallel to each other and surrounded by a perivascular space at a distance 10X the mean diameter of the vessels [41, 42]. Three 3 lumens were chosen as a cutoff because a vessel consisting of 2 lumens on a 2-dimensional glass slide could be due to sectioning blood vessels at vascular branching points. It was previously reported that MVPs showed a tendency to occur in the frontal and parietal lobes [44] and grey matter cortical regions [41]. Our initial survey confirmed these prior reports. Therefore, MVPs were assessed within the grey matter of frontal cortices.

In this autopsy sample, we showed that MVP density is associated with age at death. Prior studies have provided conflicting evidence for the association between age at death and the presence of MVP-type pathologic features. In a study of 231 cases (age of death range: 1 – 90+ years), Hassler et al reported that MVPs were not seen in cases with an age at death of ≤ 39 years [42]. Within the same study, MVPs were recorded in cases with an age of death ≥ 48 years [42]. In a study of 8 cases (age of death range: 26-88 years), Cervos-Navarro et al, showed that all the aged patients (61-88 years) had cerebral MVPs [44]. However, no MVPs were found in the young cases (26 – 48 years) within the same study [44]. In a study of 70 cases (age of death range: 36 – 91 years), Arsene et al observed that MVPs were only present in 2 cases with an age at death of 56 and 80 years respectively [45]. The varying results could be attributed to differences in tissue sampling, staining techniques, MVP characterization/quantification, and cause of death. Instead of dichotomizing the presence of MVPs, we provided a more sophisticated analysis of MVPs by calculating a density score in order to account for grey matter area variability. We also used robust immunohistochemistry methods which allowed for enhanced visualization of blood vessels.

With our immunohistochemistry methods, it was difficult to determine conclusively the types of blood vessel(s) (e.g.,, small arteries, arterioles, capillaries, venules, and/or small veins) that were mostly affected within the aged brain. Moreover, there is not a consensus within the literature as to which vessels are affected [44]. We found MVPs that positively stained for α-SMA and CD34

indicating that MVP profiles usually contain both smooth muscle cells and endothelial cells. Using electron microscopy, Cervos-Navarro et al found that the lumen within each MVP had a continuous endothelial cell layer with tight junctions [44]. In addition, the basement membrane of the endothelial cell layer and smooth muscle cell layer formed a homogenous layer within the MVPs [44]. With these findings, the authors concluded that MVPs are an arteriolar phenomenon [44] which is consistent with our findings.

The biological implications of brain MVPs are not fully understood. It could be part of normal aging, a pathological condition, or a compensatory mechanism, but does not seem to be an artifact of tissue processing. However, the appearance of MVPs could be affected by shrinking of surrounding parenchymal tissue with subsequent vessel distortion [41, 45, 46] or some other agonal or tissue fixation artifact [46]. Other authors have discussed that MVP development could be due to vessel recanalization [44], a result of increased secretion of angiogenic factors in response to chronic ischemia leading to vessel proliferation and/or elongation [44-46], or modified activity of local matrix metalloproteinases [45]. More experiments are needed in order to fully understand the development mechanism of MVPs in the brain.

There is little information published on the risk factors and co-pathologies of MVPs in the brain. In a study of 231 cases (age of death range: 1 – 90+ years), Hassler et al described that a greater proportion of men had MVPs compared to women in that sample [42]. In addition, the author reported that heart weights and arteriosclerosis severity were higher in cases with MVPs [42].

However, statistical analyses were not reported within this article. In our study, we tested the association between conventional vascular risk factors, cardiovascular diseases, and cerebrovascular diseases/pathologies with MVP density. We did not find a statistically significant association between the variables we tested and MVP density. However, a larger sample size or more focused hypothesis-testing may determine that our sample size was underpowered in a statistical sense to identify a true association.

In conclusion, MVPs are an age-related brain pathology whose risk factors may not include conventional vascular risks factors. In our study, we did not find evidence that MVPs are associated with sex, cardiovascular diseases, or cerebrovascular diseases/pathologies. More experiments are needed in order to elucidate the pathogenesis of MVP development within the aged brain.

Chapter 4: Hippocampal Sclerosis of Aging (HS-Aging)

Introduction

Hippocampal sclerosis of aging (HS-Aging) is a high-morbidity neurodegenerative disease, usually affecting individuals who survive past age 80 [51-56]. The diagnosis of HS-Aging rests primarily on neuropathological findings on hematoxylin and eosin (H&E) staining using a consensus-based criteria [51, 58]: cell loss, gliosis, and atrophy in the hippocampal formation that is out of proportion to Alzheimer's disease (AD)-type pathology. HS-Aging is a common pathology among older individuals but its symptoms are often incorrectly attributed to AD in the clinical setting [52, 54, 55, 68]. Hence, a better understanding of the HS-Aging disease spectrum is required.

Although not part of current consensus-based diagnostic criteria, TAR-DNA binding protein 43 (TDP-43) pathology is strongly linked to HS-Aging [51, 55, 144-148]. TDP-43 is a nucleotide-binding protein that is normally enriched in the nucleus of neurons [147, 149]. In HS-Aging, affected neurons display loss of nuclear TDP-43, accumulation of cytoplasmic phosphorylated TDP-43 inclusion bodies, and aberrant TDP-43 in neurites [51]. This aberrant TDP-43 staining is often seen in hippocampal dentate granule cells, CA1 hippocampal sector, subiculum, and amygdala [51, 145]. TDP-43 pathology is a key difference between HS-Aging and other diseases with hippocampal sclerosis (HS) pathology such as epilepsy and vascular insufficiency which lack aberrant TDP-43 staining [51, 150]. When bilateral entorhinal cortex sections are assessed,

approximately 90% of HS-Aging cases show aberrant TDP-43 pathology, whereas only 10% of non-HS-Aging cases show TDP-43 pathology [51]. In individuals with “unilateral” HS-Aging (by H&E stain), aberrant TDP-43 inclusions are observed in both the affected and contralateral sides [51]. This asymmetric HS (one-sided neuronal loss) observed on H&E with bilateral TDP-43 positivity provides insight into potentially early stages of HS-Aging pathology: hippocampal TDP-43 pathology without widespread “sclerosis” [55].

In order to raise awareness of the clinical-pathological features of TDP-43 immunoreactivity with only segmental “sclerotic” changes, findings from two research volunteers in the University of Kentucky Alzheimer’s Disease Center (UK-ADC) longitudinal cohort are described. Segmental HS-Aging is defined as focal cell loss in the CA1 and/or subicular region(s) with astrocytosis seen on some but not all hippocampal sections on H&E and with aberrant TPD-43 immunostaining in the hippocampal formation. Both segmental HS-Aging cases described here were followed from initial cognitively intact status to death. Clinical, cognitive, imaging, and neuropathological findings are presented. A relevant literature review emphasizes that these cases are not being reported as “unusual”.

Methods

Clinical and neuropathological assessments

Details of UK-ADC recruitment, inclusion/exclusion criteria, clinical assessments, cognitive testing, and neuropathological protocols have been described

previously [140, 151]. Briefly, as part of a longitudinal study, the two individuals in this study consented to annual mental status testing, physical examinations, and post-mortem brain donation [106]. All protocols were performed with UK Institutional Review Board approval. Clinical data from annual cognitive assessments were examined using normative values from baseline assessments of 648 healthy aging volunteers. Cognitive assessments reported here included measures of category verbal fluency, immediate memory and learning, and delayed recall scores incorporated from word list learning and paragraph memory tests [140, 152]. Raw scores from each measure were transformed to T-scores [152-154] based on the average baseline performance of the larger sample. The derived T-scores (mean of 50 and standard deviation of 10) incorporated adjustments for age, gender, and education. This method allowed for direct comparisons between each case. Mini Mental State Exam (MMSE) scores [78] are presented as raw scores for comparative purposes. Neuroimaging studies were obtained at the request of the UK-ADC neurologist. Imaging modalities included MRI-T1 weighted imaging with or without contrast, MRI-T2 weighted imaging, and CT imaging without contrast.

Neuropathological assessments were performed at UK-ADC using previously described methodology [51, 106, 110]. At least 28 sections were taken from different brain areas. From both left and right sides, at least four areas were sectioned from the medial temporal lobes: the amygdala, entorhinal cortex, and rostral and caudal hippocampi including the level of the lateral geniculate nucleus. After formalin fixation and paraffin embedding, sections (8 microns)

were stained with H&E. Antibodies to PHF-1 tau (gift from Dr. Peter Davies, Hofstra North Shore-LIJ School of Medicine; 1:500 dilution) and to amyloid-beta (Aβ) (Vector; 1:100 dilution) were used in immunohistochemistry to assess neuritic plaque (NP), Aβ, and neurofibrillary tangle (NFT) pathologies following the NIA-AA consensus recommendations [58]. Alpha-synuclein (Vector; 1:40 dilution) immunohistochemistry was used for assessing Lewy body pathology. Glial fibrillary acidic protein (GFAP; Novacastra) staining was performed to demonstrate reactive astrocytes. The TDP-43 antibody used was anti-phospho TDP-43 (gift from Dr. Manuela Neumann (University of Zurich); 1:500 dilution). The specific tissue staining protocol is similar to the one shown in **Table 3.1.**

Research Subjects

Case “F”

An 82-year-old woman was initially recruited as a cognitively intact research volunteer. Her father, who had been clinically diagnosed with AD, died at age 73. Her medical history included hypertension, coronary artery disease, hyperlipidemia, and hypothyroidism–all of which were treated medically. She had smoked (25 pack/year history) and denied excessive alcohol drinking.

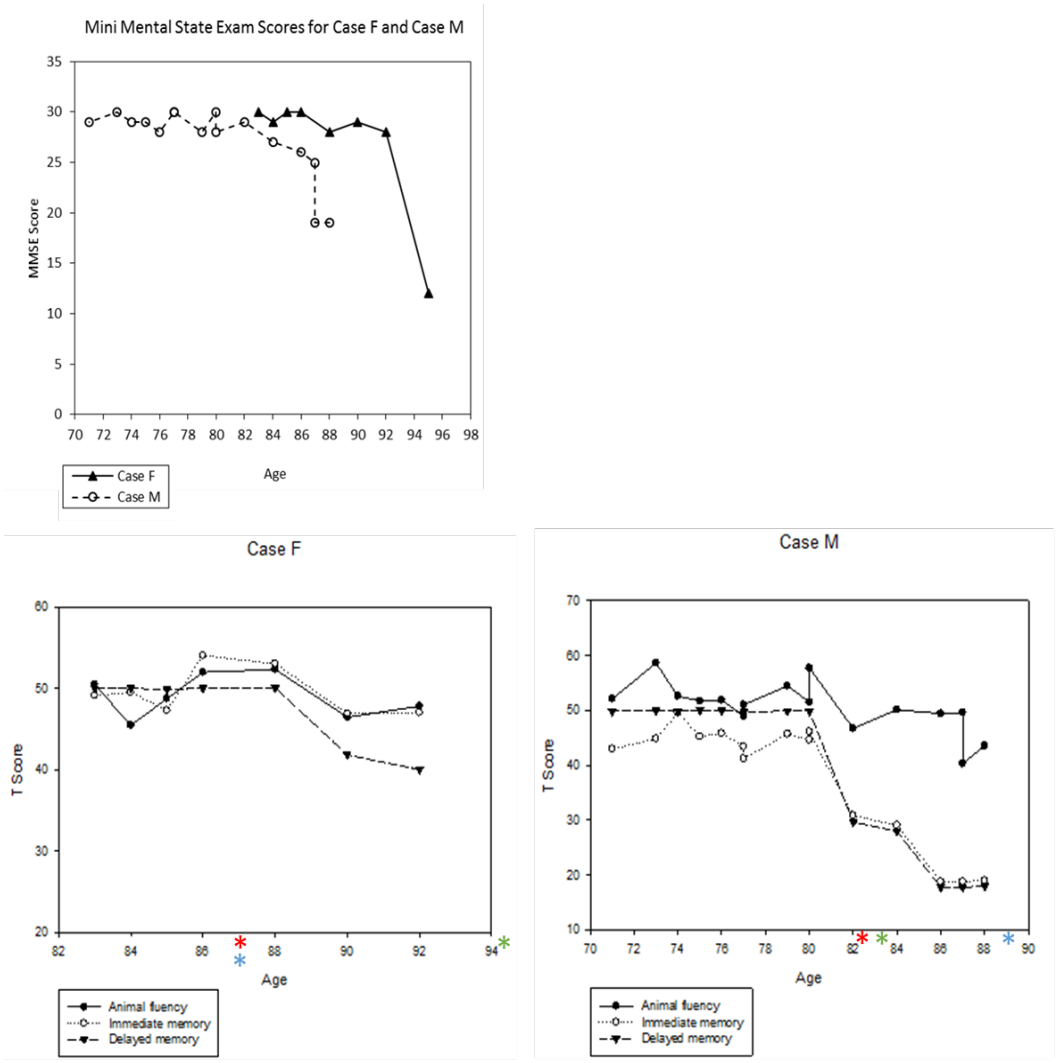

Figure 4.1: MMSE and T-Scores from longitudinal neurocognitive assessments in animal fluency, immediate, and delayed memory tasks. For the T-scores, mean = 50, and STD = 10. Standardization of t-scores is based on results from age-matched non-demented individuals. The age at which MCI diagnosis, neuroimaging scans, and probable Alzheimer's disease diagnosis were made are indicated by red, blue, and green asterisks respectively. Note that memory scores declined more than verbal fluency.

At the age of 88, she received a clinical consensus diagnosis of amnestic mild cognitive impairment (MCI, **Figure 4.1**). Because of her cognitive decline, MRI T1 and T2 weighted images with and without contrast were obtained at age 88 (**Figure 4.2**). Radiological impressions from images included moderate generalized cerebral atrophy with cerebellar atrophy, mild *ex vacuo* ventricular dilatation, and multiple confluent areas of supratentorial white matter hyperintensities. The latter finding was considered consistent with chronic microvascular infarcts.

Near her 90th birthday, she was having difficulties with finances and became paranoid, stating that people were stealing from her. Donepezil was initiated at that time. By age 91, she was no longer driving although she was still performing simple chores in the home. Her cognition continued to decline, and she began to struggle increasingly with higher-order executive tasks. Her cognitive test results showed a steady decline in delayed memory (1 standard deviation below the mean) with preserved immediate memory and animal fluency tasks (**Figure 4.1**). At age 94, she was diagnosed with probable AD and was wheelchair dependent. By this time, she required assistance with all activities of daily living (ADLs) including bathing. She died at age 95.

Neuropathological findings are shown in **Figures 4.3 and 4.4**. For comparison, the hippocampus of a non-demented individual is shown in **Figure 4.3A-C**. An unrelated HS-Aging case is shown in **Figure 4.3D** demonstrating diffuse neuronal loss, neuropil rarefaction (**Figure 4.3E**), and astrocytosis

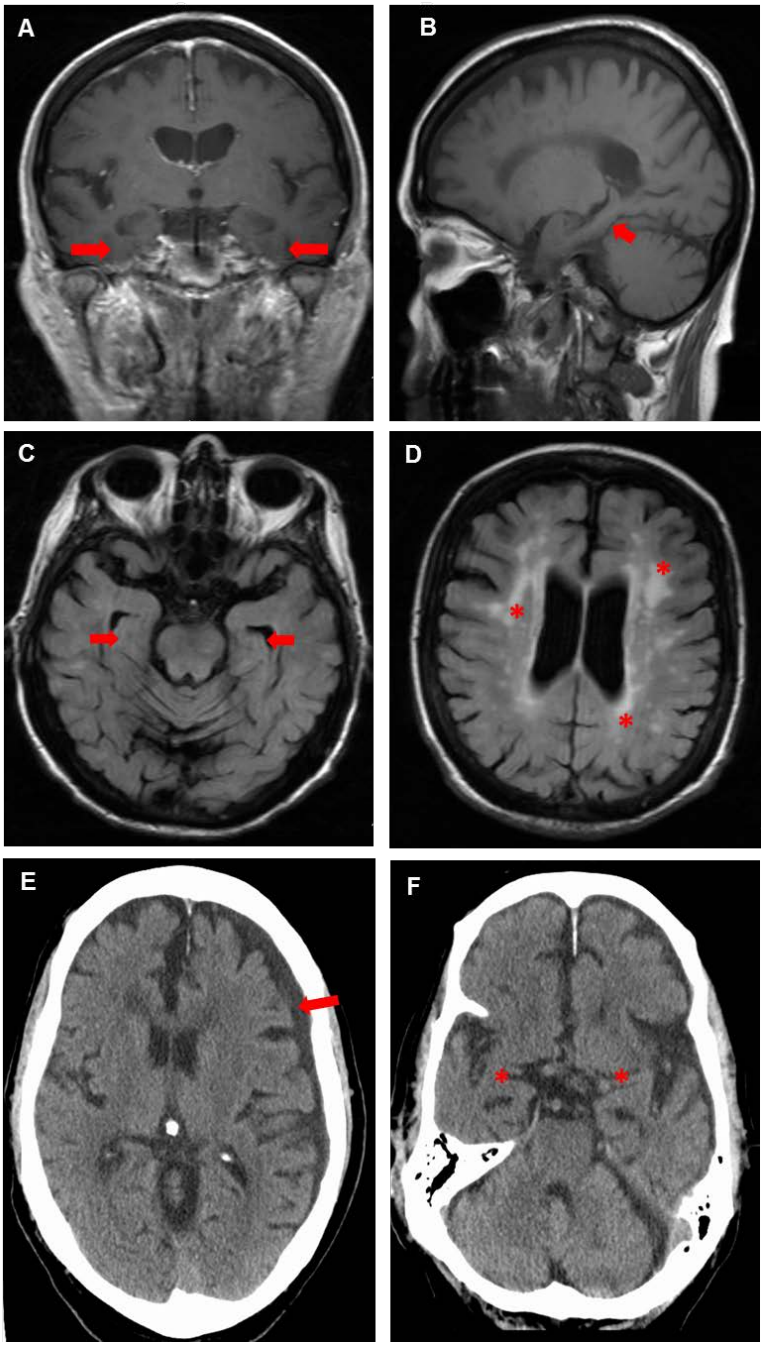

Figure 4.2: Neuroimaging for Case F (A-D) and Case M (E,F). A-D show MRI images for Case F at age 88. **(A)** Coronal T1 weighted image with contrast. Red arrows pointing to the hippocampi. **(B)** Sagittal T1 weighted non-contrast image. Red arrow pointing to hippocampus. **(C)** Transverse MRI T1 weighted image with contrast. Red arrows pointing to the hippocampus. **(D)** Transverse MRI T1 weighted non-contrast image. Red asterisks indicate areas of white matter hyperintensities in the periventricular region. **(D, E)** CT images for Case M at age 98. (**E**) Transverse CT non-contrast image. Red arrows points to chronic subdural collection in the brain indicating a history of previous subdural hemorrhage. **(F)** Transverse CT non-contrast image. Red asterisks indicate hippocampal regions.

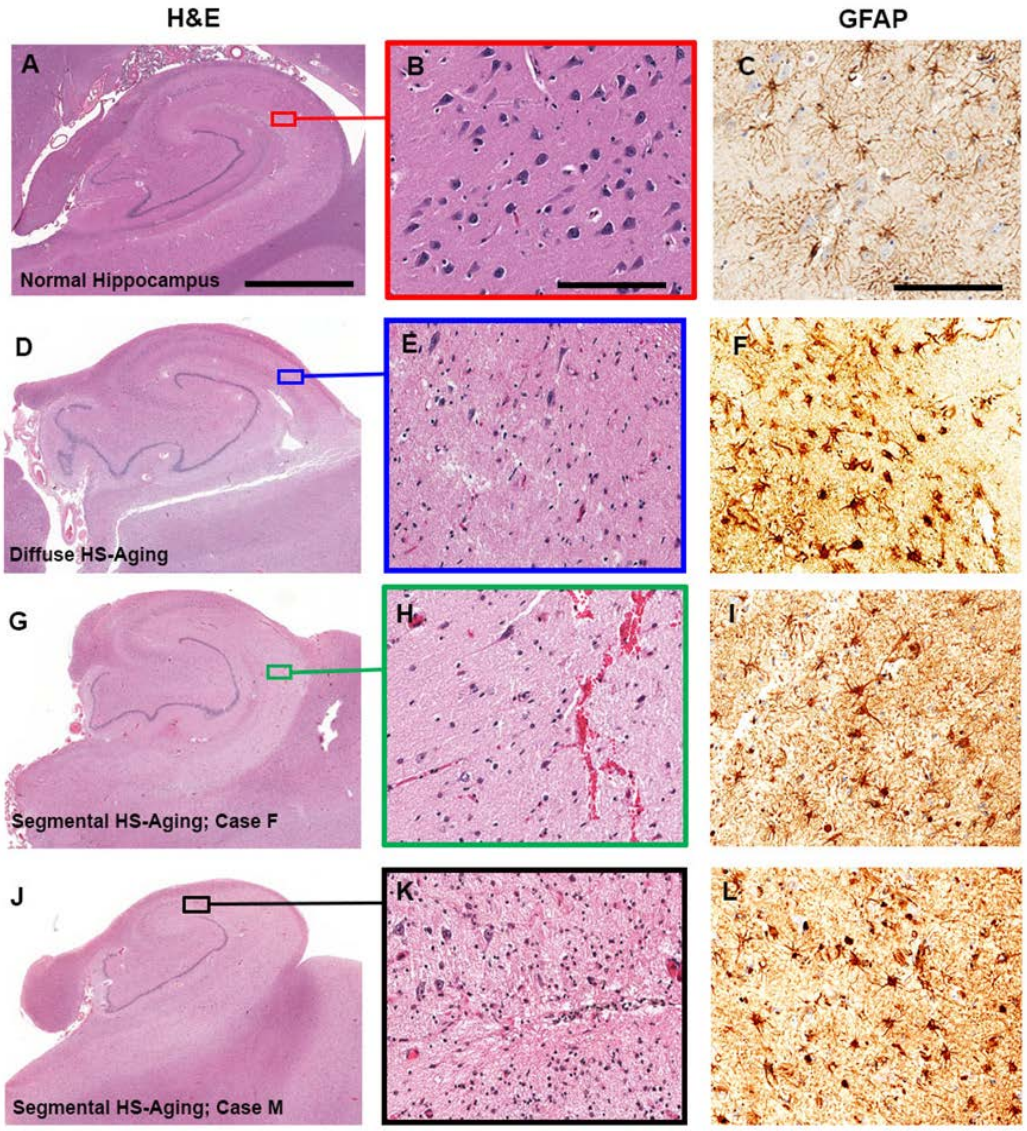

Figure 4.3: Photomicrographs of hematoxylin and eosin (A, B, D, E, G, H, J, K) and GFAP (C, F, I, L) stained hippocampal sections. Images illustrate either a normal hippocampus **(A, B, C)**, diffuse HS-Aging pathology **(D, E, F)**, or segmental HS-Aging pathologies (**G, H, I J, K, L**). (**A**) Low-power image demonstrates normal hippocampal structure. Inset from **B** shows high power image with abundant neuronal cells. Inset from **(C)** shows high power image with little to no reactive astrocytes. **(D)** Low-power image demonstrates diffuse HS-Aging pathology: the hippocampus is shrunken with neuropil rarefaction extending into the subiculum. Inset from **E** shows high power image with severe neuronal cell loss and spongiosis. (**G, J**) Low power images show segmental HS-Aging pathology with a shrunken hippocampus and selective cell loss in CA1. Inset from **H** and **K** show high power images with less neuronal cell loss and spongiosis compared to that seen in the inset from **E**. Insets from **(I, L)** show high power images with reactive astrocytes. Scale bars: 3.5mm **(A, D, G, J)**, 200 microns **(B, E, H, K)**, 150 microns **(C, F, I, L)**.

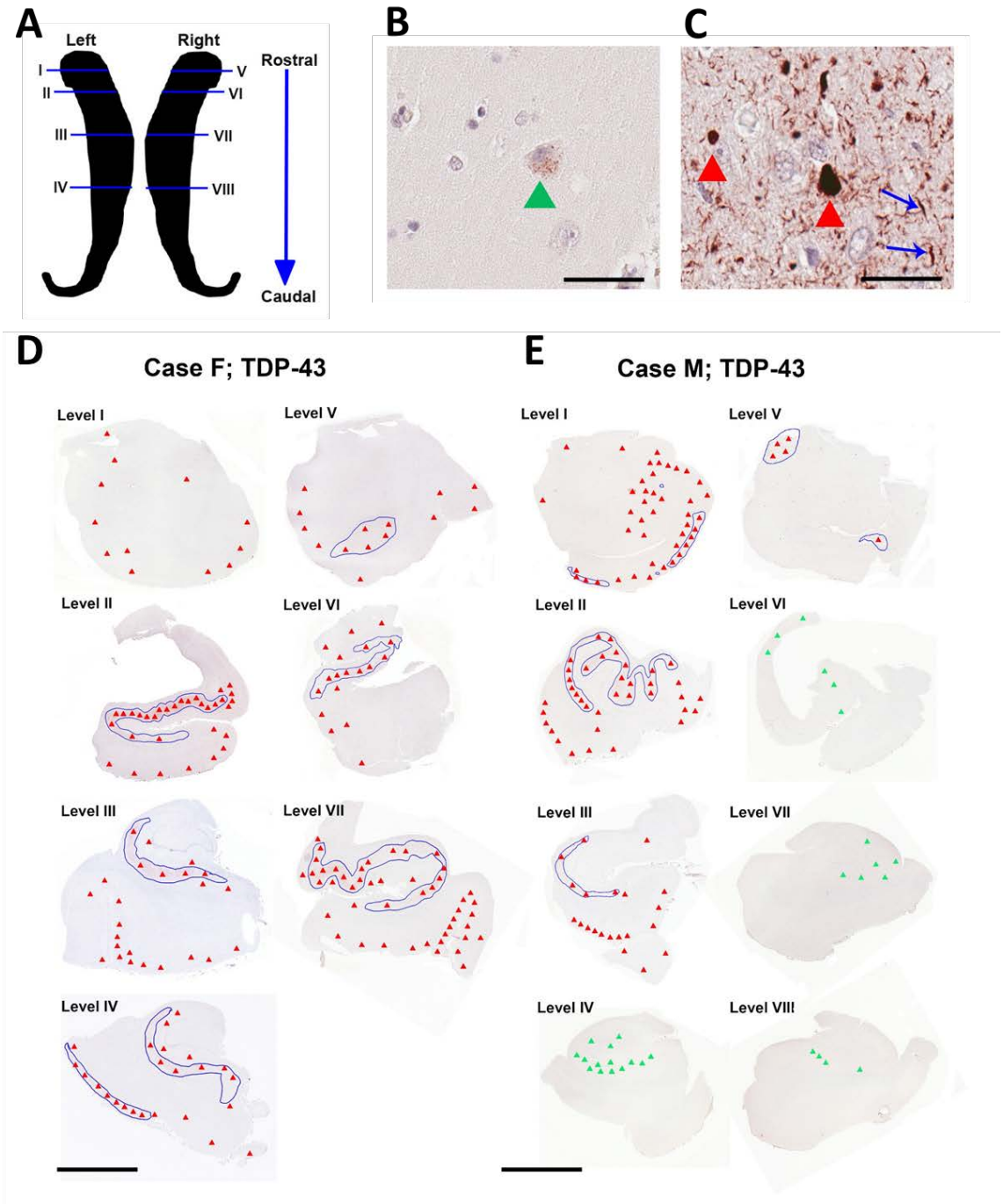

Figure 4.4: Photomicrographs to show TDP-43 immunoreactive pathology. (A) Schematic of the bilateral hippocampal formation levels for Case F and Case M TDP-43 stained sections. The blue horizontal lines indicate approximate sectioning locations of the stained TDP-43 photomicrographs. I, V: amygdala. II, VI: entorhinal cortex. III, VII: rostral hippocampus. IV, VIII: caudal hippocampus. (B, C) Photomicrograph images of TDP-43 pathology. (B) High power photomicrograph of a neuron with TDP-43 positive multiple discrete granular cytosolic accumulations (possible “pre-inclusions”; green triangle), a potential marker of early HS-Aging pathology. (C) High power photomicrograph of TDP-43 positive intracellular inclusion bodies (red triangles) and neurites (blue arrows). (D, E) Photomicrographs of TDP-43 stained serial sections from the hippocampal formation from Case F and Case M respectively. Location of intracellular inclusion bodies or granular cytosolic accumulations are represented by red or green triangles respectively. Location of neurites are enclosed by the blue outline. Level VIII for Case M was not available. Scale bars: (B, C) = 50 microns, (D, E) 5mm.

(**Figure 4.3F**). In contrast, Case F showed less generalized hippocampal shrinkage (**Figure 4.3G**) with segmental neuronal cell loss in the CA1 region (**Figure 4.3H**) and reactive astrocytosis (**Figure 4.3I**). Note that in Case F, HS-Aging pathology was predominantly localized in the CA1 region whereas the comparison case in **Figure 4.3D** shows more widespread pathology extending into the subiculum. Case F showed abundant TDP-43 immunoreactive intracellular inclusions and neurites throughout the amygdala, entorhinal cortex, and hippocampus with bilateral TDP-43 immunoreactivity (**Figure 4.4D**). TDP-43 inclusions were more widespread than neurites (blue outlined areas) in the brain sections (**Figure 4.4D**).

Assessments using the NIA-AA consensus protocol for AD workup [58] revealed low levels of AD neuropathologic changes (**Figure 4.5**)): Braak I, CERAD “sparse”, and Thal stage 3. Also seen at autopsy, her brain showed evidence of multifocal cerebrovascular disease with acute infarcts in the right parietal and temporal lobes, insula, basal ganglia, and entorhinal cortices (the latter in focal areas). Remote microinfarcts were present in the left putamen, along with severe non-occlusive atherosclerosis in Circle of Willis, moderate cortical arteriolosclerosis, widening of Virchow Robin spaces, perivascular rarefaction in scattered areas, and other small blood vessel changes (not shown).

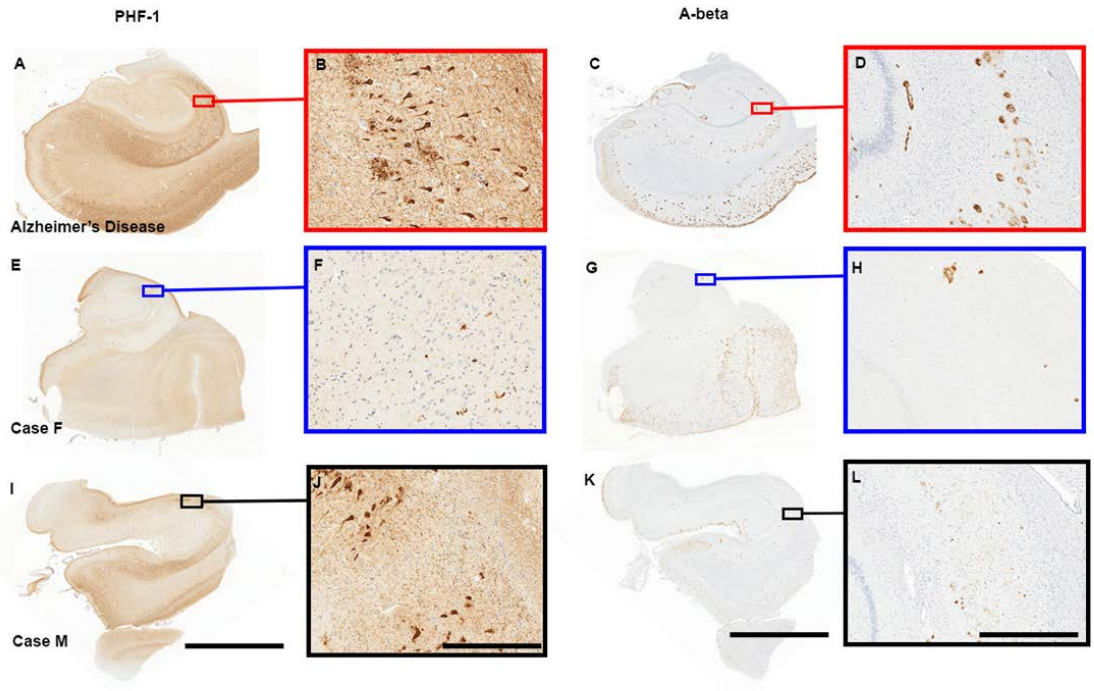

Figure 4.5: Photomicrographs of PHF-1 (A, B, E, F, I, J) and Ab (C, D, G, H, K, L) immunostained human hippocampi from an Alzheimer's disease case, Case F, and Case M. An Alzheimer's disease case (Braak VI, CERAD "frequent", and Thal 5) is shown for comparison **(A, B, C, D)**. **(E, I)** Low-power images demonstrating neurofibrillary tangle pathology for Case F (Braak I) and Case M (Braak IV). Inset from **F and J** shows high power image with relatively sparse or discontinuous neurofibrillary tangle pathology for Case F and Case M respectively. (**G, K**) Low-power images demonstrating neuritic and amyloid-beta plaques for Case F (CERAD "sparse" and Thal 3) and Case M (CERAD "moderate" and Thal 5). Inset from **H and L** show high power images with few neuritic and amyloid-beta plaques in the hippocampal CA1 region for Case F and Case M respectively. Note that the parahippocampal region did show abundant Ab plaques in Case F. Braak stage = neurofibrillary tangle pathology, CERAD = neuritic plaque pathology, Thal stage = amyloid-beta plaque pathology. Scale bars: **(A, C, E, G, I, K)** = 5mm, **(B, F, J)** = 400 microns, and **(D, H, L)** = 1mm.

Case “M”

A 74-year-old male was initially recruited as a cognitively intact research volunteer. He had reported that his mother had dementia with an unknown age of diagnosis. His medical history was significant for hypertension and benign prostatic hypertrophy. He smoked (50 pack/years) and drank alcohol (3-4 drinks/day). His cognitive performance was relatively unremarkable until age 82, when he was diagnosed with MCI based on a consensus review of his symptoms (**Figure 4.1**).

A workup at age 83 led to a diagnosis of mixed probable AD and vascular disease. He began treatment with donepezil at this time. At age 86, he began to struggle with higher order activities including driving. By age 87, he was no longer driving but he was still intact with respect to most other ADLs; memantine was added to his medications at that time. By age 88, he needed help with most ADLs including bathing.

At his last UK-ADC clinic visit, his MMSE score was 19 with mild behavioral issues of impulsivity. Longitudinal cognitive test results showed a steady decline in immediate and delayed memory tasks (3 standard deviations below the mean by age 86) with preserved animal fluency performance (**Figure 4.1**). After a fall at age 89, CT imaging without contrast was obtained. This image revealed a chronic left subdural hematoma, periventricular white matter hypodensity, and generalized brain volume loss (**Figure 4.2**). The patient passed away later that year at age 89.

Neuropathological hippocampal autopsy findings are shown in **Figures 4.3 and 4.4**. Case M showed focal hippocampal shrinkage (**Figure 4.3J**), with segmental neuronal cell loss in the CA1 region (**Figure 4.3K**) and astrocytic gliosis (**Figure 4.3L**). Aberrant TDP-43 immunoreactivity was seen within the amygdala bilaterally, and the left rostral and caudal entorhinal cortices (**Figure 4.4E**). In addition, there were intracellular TDP-43 positive cytoplasmic "preinclusions" (green triangles) in the hippocampus bilaterally and in the right entorhinal cortex (**Figure 4.4E**). TDP-43 intracellular inclusion bodies were more widespread than neurites (blue outlined areas) in the brain sections (**Figure 4.4E**).

Assessments using the NIA-AA consensus protocol for AD workup [58] revealed an intermediate level of AD neuropathologic changes Braak IV, CERAD "moderate", and Thal phase 5 (**Figure 4.5**). In addition to neurodegenerative disease pathologies, there was evidence of multifocal chronic cerebrovascular disease with moderate-to-severe multifocal atherosclerotic disease in the Circle of Willis, arteriolosclerosis and other mild small vessel changes observable on histopathology. In some areas, there was widening of Virchow-Robin spaces and perivascular rarefaction. Incidental Lewy body pathology was found in the olfactory bulb (not shown).

Discussion and Review of the Literature

There is an evolving appreciation of the large impact of HS-Aging on public health, especially among the oldest-old: approximately 10-25% of persons over

age 85 demonstrate this pathology at autopsy [51-55, 68, 111, 146, 155-159]. Here, examination of two segmental HS-Aging cases in detail. They may represent either early HS-Aging pathology, or a subset of cases that develop TDP-43 pathology without widespread hippocampal changes meeting current criteria for hippocampal sclerosis. These segmental HS-aging patterns are frequently seen in the UK-ADC autopsy series – underscored by the observation that approximately 50% of these HS-Aging cases are unilateral on H&E [51] – although their pathogenesis is not well understood.

There are inherent limitations to a study of this nature. The diagnosis of segmental HS-Aging was made primarily using H&E stain, but the “sclerotic” changes are difficult to delineate with precision. Zinc transporter 3 immunostaining, choline acetyltransferase immunostaining, and Hirano silver staining have been used to delineate between CA3 - CA2, CA2 - CA1, and CA1 – subiculum respectively, in HS cases [160]. These stains can be used in future segmental HS-Aging studies to help determine which hippocampal regions are initially and/or most affected. The segmental HS-Aging pathology in these cases may not fully explain the cognitive declines seen years before death. Case F showed evidence of multifocal cerebrovascular disease and Case M showed intermediate AD pathology with severe multifocal cerebrovascular disease, which may help explain the dementia. These cases indicate how frequent comorbidities, especially AD and cerebrovascular neuropathologic changes, could strongly alter the pattern of tested cognitive domains. Both cases in the present study showed TDP-43 pathology in the amygdala, entorhinal cortex, and

hippocampus. Hippocampal TDP-43 pathology, with or without reported HS, contributes to an additive component of cognitive impairment according to reports from different research centers [6, 149, 161-164]. Therefore, it is reasonable to infer that the TDP-43 pathology seen in these individuals may partly explain their cognitive deterioration. Because of the lack of a clear consensus in the field on HS-Aging categorization and "boundary zones" between other neurodegenerative diseases, we highlight HS-Aging in relationship to "pre-HpScl" (a term that has been used to describe early HS-Aging pathology [165]), FTLD with TDP-43 pathology (FTLD-TDP), AD, and cerebrovascular pathologies.

Early HS-Aging, or "Pre-Hp-Scl"

Although information on HS-Aging is quickly accumulating, nomenclature for this disease is not universal. One group refers to a disease category very similar to HS-Aging as "HpScl" [52, 166, 167] and some may refer to this condition as "hippocampal sclerosis dementia" [168]. In a very recent paper, a panel of experts discussed HS-Aging pathologic classification terminology [169]. However, the average age of persons used in this consensus recommendation paper were younger (most <80 y.o.) than when HS-Aging prevalence appears to be highest [51]. Unfortunately, the term "hippocampal sclerosis" is itself potentially misleading. The pathologic features are not fully conveyed by the term "sclerosis", which signifies "hardening". Furthermore, the pathologic changes of HS-Aging generally extend beyond the hippocampus proper. We reiterate the

important point that the term “hippocampal sclerosis” is widely used to describe multiple *distinct* diseases including hippocampal pathology associated with epilepsy, hypoxia, hypoglycemia, FTLD, and others. Well over 95% of “PubMed” citations linked to “hippocampal sclerosis” are unrelated to HS-Aging, HpScl, or HS dementia. Thus to prevent confusion, whatever the eventual terminology ends up being, we recommend that it should include a component different from simply “hippocampal sclerosis”.

Recently, the term “pre-HpScl” was used to describe hippocampal pathology characterized by none to minimal neuronal loss or extracellular neurofibrillary tangles with abundant TDP-43 pathology [165]. This condition may partially overlap with the “segmental” HS-Aging pathology seen in the two cases described here. Both articles present cases that show focal neuronal loss and gliosis in CA1 with more widespread TDP-43 pathology in the limbic system. These findings suggest that HS-Aging could be a progressive disease where the initial stages are characterized by the presence of TDP-43 pathology with or without some focal areas of cell loss (**Figure 4.6**). More broadly, these findings may also be evidence for a presently unnamed “brain-wide” disease that is characterized by a spectrum of hippocampal TDP-43 pathologies (**Figure 4.6**): TDP-43 pathology, TDP-43 pathology with segmental/focal sclerotic-type changes, and TDP-43 pathology with diffuse HS.

FTLD-TDP

Aberrant TDP-43 is seen in 65% to 90% of HS-Aging cases [51, 145]. Because of the high prevalence of TDP-43 pathology in HS-Aging cases, there is some

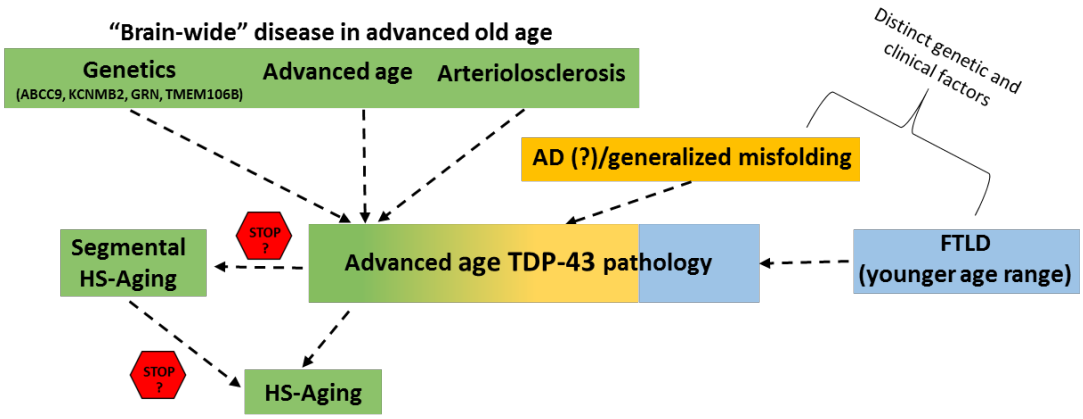

Figure 4.6: Schematic for neurodegenerative disease etiologies of TDP-43 pathology in advanced age. A currently unnamed "brain-wide" disease occurring in advanced age that is associated with certain gene polymorphisms (*ABCC9, KCNMB2, GRN, TMEM106B*) and characterized by a pathologic spectrum that includes arteriolosclerosis, TDP-43 pathology, segmental HS-Aging, and widespread HS-Aging. The disease(s) may comprise a continuous spectrum or 3 separate variants as indicated by the stop signs. A subset of cases may be due to, or exacerbated by, AD pathology leading to misfolding and aggregation of proteins including TDP-43. Furthermore, FTLD may lead to the accumulation of TDP-43 in some cases, even in advanced age. *AD = Alzheimer's disease, FLTD = frontotemporal lobar degeneration, HS-Aging = hippocampal sclerosis of aging.*

support for the hypothesis that HS-Aging is closely related to FTLD-TDP [170]. In one study, the prevalence of hippocampal sclerosis in FTLD-TDP was 42% [171]. Moreover, the slender non-tapering TDP-43 neurites observed in FTLD-TDP hippocampi (“type A” pattern) resemble those seen in HS-Aging [171]. In a different study, >70% of hippocampal sclerosis cases with TDP-43 pathology had neurites and inclusion bodies whose morphology resembled that found in FTLD-TDP [145]. Similarly, in DLB cases with HS-Aging, TDP-43 immunohistochemistry pattern was similar to FTLD-TDP type A pattern [165]. We also note that some human genetic polymorphisms (in *GRN* and *TMEM106B)* are risk factors for both FTLD-TDP and HS-Aging pathologies [59, 60, 162, 166, 172]. The *TMEM106B* polymorphism (rs1990622) was also recently shown to be a risk factor for non-HS TDP-43 pathology [173]. These findings could be argued to support the possibility that HS-Aging and TDP-43 pathologies in older people are either pathogenetically linked, or a frank variant of, FTLD.

Although there are areas of overlap, FTLD and HS-Aging also differ in clinical symptomatology, genetic risk factors (discussed in greater detail below), and pathological characteristics. For example, patients with FTLD pathology show clinical symptoms and die at much younger ages than those with HS-Aging pathology [68, 146]. HS-Aging cases tend to lack either the “bvFTD” or aphasia symptoms (e.g.,, primary progressive aphasia) [51, 68], although the current two cases both (late in their disease course) had features previously linked to frontal cortical dysfunction [174-176]: delusions (Case F) and impulsivity (Case M). HS-Aging patients were previously shown to demonstrate a group-level

neurocognitive profile characterized by higher verbal fluency scores 2-5 years prior to death, as seen in Case F and Case M, dissimilar to non-tauopathic FTLD cases [68].

Pathologically, TDP-43 proteinopathy is non-specific to FTLD-TDP (or HS-Aging) because TDP-43 pathology can be seen in Alexander's disease, low-grade glial neoplasms, AD/Down's syndrome, and brain trauma [150, 177-181]. Thus – perhaps analogous to tau protein and NFTs -- there appears to be a "reactive" aspect to TDP-43 pathology although the pathology also seems deleterious once present. FTLD-TDP cases with HS showed more severe cortical and brainstem atrophy than HS-Aging cases which localized to the hippocampus [146]. HS-Aging cases had lower synaptophysin immunoreactivity, greater astrocytic reactivity and microglial reactivity compared to FTLD-TDP with HS cases [146]. It has been suggested that HS-Aging in aged individuals may be a variant of FTLD-TDP [182]; however, this does not seem to make sense because if this were the case, then there should be a very large cohort of individuals who ultimately express the full-fledged FTLD-TDP picture among the aged, and this is not the case. FTLD is quite rare—there are only approximately 20,000-30,000 cases in America, (around half with FTLD-TDP), versus HS-Aging, which is extremely prevalent, perhaps affecting over a million Americans [183-185]. Furthermore, there is no evidence that in FTLD-TDP cases, the hippocampal pathology shows a clinical-temporal pattern of involvement. These findings support the notion that HS-Aging is a distinct pathology from FTLD-TDP although both diseases show TDP-43 immunoreactivity and HS (**Figure 4.6**).

Alzheimer's Disease

Because TDP-43 pathology is seen in many cases that also have abundant AD pathology, there may be a subset of HS-Aging cases that are best categorized as a variant of AD. TDP-43 positivity can be seen in 14-57% of AD cases [149, 163, 180, 181]. More specifically in one study, TDP-43 pathology was seen in 9% of familial AD cases, 10% in early-onset AD cases, and 29% in late-onset AD cases [180]. Another study showed that increasing TDP-43 immunoreactive pathology correlates with increasing Braak neurofibrillary tangle stages [145]. It has also been shown that some TDP-43 inclusions seen in AD brains co-localize with phospho-tau in the entorhinal cortex and dentate fascia [145]. Because *in vivo* and *in vitro* studies have shown that Aβ and/or tau can promote the misfolding/polymerization of polypeptides (e.g., alpha-synuclein) [186-188], it can be speculated that this misfolding could occur in late stages of AD brain pathology [145]. These pathologies could be coexistent rather than directly linked, since all of these pathologic changes – HS-Aging/TDP-43 pathology, AD, and DLB – are relatively prevalent in community-based samples evaluated to date (in strong contrast to FTLD) [5, 7, 168, 189, 190].

A counterargument against a specific link between HS-Aging and AD pathologies is that HS-Aging has no association with APOE nor with any other AD-related risk allele [52, 56, 110, 191]. In addition, unlike AD where females are more likely to have AD than males, there also does not appear to be a gender-based predisposition for HS-Aging [68]. Since both pathologies are prevalent in

older populations, it would be expected that many individuals would manifest both AD and HS-Aging pathologies at autopsy.

Cerebrovascular Disease

Another brain process that has been associated with HS-Aging pathology is cerebrovascular disease. Dickson and colleagues described cerebrovascular disease pathology in a cohort of 13 individuals with HS-Aging [192]. Arteriosclerosis, atherosclerosis, subcortical arteriosclerotic leukoencephalopathy and cerebral amyloid angiopathy were observed in these cases [53]. A recent study publication from the UK-ADC showed that arteriolosclerosis, characterized by thickened and/or dysmorphic arterioles in the brain, is associated with HS-Aging pathology [25]. In the grey matter, the mean vessel wall thickness was significantly larger in HS-Aging cases compared to that of non HS-Aging cases [25].

There is circumstantial support from genetic studies for a mechanism linking cerebrovascular pathology with HS-Aging. The only two genomics studies that evaluated HS in aged persons as a GWAS endophenotype reported risk alleles at genomic loci in potassium channel regulating genes, unrelated previously to FTLD: *ABCC9* and *KCNMB2* [59, 62]. We recently replicated the observation that *ABCC9* polymorphism is associated with HS-Aging pathology [60]. The ABCC9 polypeptide is physiologically active in arteriolar smooth muscle [126, 130]. Therefore it is credible that ABCC9 dysregulation leads to arteriolosclerosis, which then may contribute to HS-Aging pathogenetically.

ABCC9/SUR2 is an attractive candidate for therapeutic strategies because it is a well-established “druggable target”. Both agonists (nicorandil, diazoxide, iptakalim) and antagonists (sulfonylurea drugs) have been tested in clinical trials for other diseases [193, 194]. Their potential for repurposing for HS-Aging is an active research area in our laboratory.

Coincidentally, both cases in the present study showed cerebrovascular disease including arteriolosclerosis which could partially account for their progressive cognitive decline. In sum, we infer that the association between arteriolosclerosis and HS-Aging provides added support for a “brain-wide” disease that affects both small blood vessels, hippocampal structures, and leads to TDP-43 pathology in the aged brain (**Figure 4.6**).

Conclusions

We presented two cases that show what we consider to be either early stages of HS-Aging pathology or possibly a variant of a brain-wide disease characterized by arteriolosclerosis and TDP-43 pathology affecting the limbic system (**Figure 4.6**). These two cases taken together with other articles describing hippocampal TDP-43 pathology in DLB and AD brains [165, 195] suggest the need for a consensus definition regarding the spectrum of pathologies with TDP-43 and HS seen in the aged human brain. HS-Aging is a prevalent neurodegenerative disease whose specific characteristics -- genetics, neuroimaging, symptomatology, and pathology -- are only beginning to be understood. We conclude that careful clinical-neuropathological correlations may assist in the

overall goal of developing strategies to diagnose and treat individuals who suffer this debilitating illness.

Chapter 4 Dissertation Work Citation:

Ighodaro ET, Jicha GA, Schmitt FA, Neltner JH, Abner EL, Kryscio RJ, Smith CD, Duplessis T, Anderson S, Patel E, Bachstetter A, Van Eldik LJ, Nelson PT (2015) Hippocampal Sclerosis of Aging Can Be Segmental: Two Cases and Review of the Literature. J Neuropathol Exp Neurol 74, 642-652**.**

Chapter 5: Challenges and Considerations Related to Studying Dementia in African Americans

1.1 Introduction

Studying dementia across racial/ethnic populations is a controversial but important area of research. Findings from clinical studies have indicated that Blacks/African Americans are more likely to develop Alzheimer's disease (AD, a major cause of dementia) in comparison to Caucasians (hereafter referred to as Whites) [196-198]. In studies that include autopsy confirmation, the outcomes are less clear: Blacks/African Americans may develop more AD pathology than Whites [199]; however, other studies found either no AD pathological differences between Blacks/African Americans and Whites [200-203] or higher burden of AD pathology in Whites in comparison to Blacks/African Americans [204]. Reasons for differences in the studies' outcomes may be attributable partly to residual confounding related to a failure to take into account historical, cultural, political, sociological, and psychological factors that contribute to health outcomes and health disparities. Perhaps most importantly, clinical research participation in historically marginalized groups (e.g., Blacks/African Americans) has influenced reported research outcomes.

This commentary, is intended to help focus attention on achieving one of the National Institutes of Health (NIH) and the U.S. Department of Health & Human Services (USDHS) objectives to "increase the availability and quality of data collected and reported on racial and ethnic minority populations" [205]. We

outline a selected historical account of the relationship between Blacks/African Americans, medicine, and research, focusing on challenges and relevant factors that affect participation of African Americans in research. We conclude with considerations and topical questions for scientists with a focus on research related to dementia in African Americans.

Note on terminology: hereafter we apply racial categorical terminologies described in the 1997 "Revisions to the Standards for the Classification of Federal Data on Race and Ethnicity" issued by Office of Management and Budget's (OMB):"Black/African American" and "White." For complete definitions, see reference [206].

1.1.2 The Contemporary Consequences of Historical Medical Mistreatment of African Americans

There is a long history of horrific biomedical experimentation on Blacks/African Americans, and the effects linger to this day. A selection of these cases are discussed here for three reasons: 1) to convey that late 19th century/early 20th century scientists designed abusive studies which grossly violate the concept of *primum non nocere* ("first, do no harm"); 2) to provide examples on the dangers of misinterpreting differences between individuals within different racial/ethnic groups; and 3) to demonstrate how the history of racism in the medical sciences and society affect *current* Black/African American participation in clinical research. Nineteenth and early 20th century scientists developed analytical methodologies that lay the foundation for how we conduct 21st century science.

Therefore, the methodological pitfalls are topical and highly relevant to the contemporary setting.

Before the U.S. Civil War, African slaves were regularly used as involuntary test subjects in biomedical experimentations [207, 208], and these practices were supported by law [209]. Often, slave masters offered their slaves to a physician either for biomedical experimentation or because they were too sick, old, or in exchange for medical treatment payment [207]. In addition, some physicians would buy and raise slaves in order to fill their studies with human test subjects [207, 209]. The practice of collecting slaves as research subjects was not an anomaly but a standard practice due to the mainstreaming of racism in much of American society, including science. Some writers use the term 'scientific racism' to describe this perversion of scientific and historical factors to maintain existing social hierarchies based on race [210, 211]. For example, it was believed that Blacks/African Americans could endure more painful stimuli, extreme heat, and were more prone to fevers, syphilis, tuberculosis, and tetanus than White individuals [209, 212]. These 19th and early 20th century scientists used this framework of racism to design flawed experiments that yielded results that further inappropriately justified their use of slaves in biomedical experimentation. For example, lacking a rational scientific reason, Dr. Walter F. Jones repeatedly poured boiling water on naked slaves in four-hour intervals to see if it cured typhoid pneumonia [209, 213, 214]. Dr. John M. B. Haden stripped blood vessels from the limbs of a Black/African American male in order to study vascular morphology [209, 215]. Dr. James M. Sims, "the father of gynecology,"

performed painful experimental gynecological surgery on female slaves without using anesthesia in order to improve his surgical techniques [207, 216, 217]. Dr. T.S. Hopkins gave nitric acid solutions to slaves in order to test its effects on treating asthma [207, 209]. Thomas Jefferson inoculated over 200 slaves with the cowpox vaccine in order to test its efficacy against smallpox [209, 214]. These examples demonstrate how racialized science became part of the justification and practice of human experimentation on Blacks/ African Americans [210].

After the abolition of slavery in the United States, physicians and scientists continued to abuse Blacks/African Americans while conducting unethical biomedical experimentation. One of the most well-known unethical studies was the Tuskegee Syphilis Trials from 1932 – 1972 [209, 218-220]. Funded by the USDHHS, scientists withheld treatment from 400 Black/African American men in order to study the progression of syphilis. One goal was to test the long-standing belief that venereal diseases manifest differently in Black/African American individuals compared to White individuals [209, 218-220]. In an article published in 1937, Dr. Mark Boyd describes conducting an experiment where he infected 470 syphilitic Black/African American individuals with a deadly falciparum strain in order to test new treatments for neurosyphilis [209, 221]. Although some of the individuals died as a direct result from his procedure, he still continued to infect other Black/African American individuals [209, 221]. In 1952, Chester M. Southam of Sloan-Kettering Institute injected at least 396 inmates at the Ohio State Prison (more than 45% of the subjects were Black/African American) with

live human cancer cells [209, 222]. From 1962 – 1966, Dr. Allen Hornblum conducted over 153 experiments using mostly Black/African American men from the Philadelphia's Holmesburg Prison system [209, 223]. Hornblum was paid by pharmaceutical and cosmetic companies to test cosmetics, powders, and shampoos that ultimately caused baldness, scarring, and permanent skin and nail injury in the prisoners [209, 223]. In 1978, without parental consent, physicians from the Medical College of Virginia injected 1,230 children (37% were Black/African American children, 4 times their population representation during that period) with radioactive substances [209]. In summary, the physicians and scientists in these studies performed horrific and sometimes deadly experiments on many Black/African American individuals without regard for their informed consent or well-being. This is by no means an exhaustive list of all the unethical biomedical studies performed on Blacks/African Americans.

The historic and presumed present practice of unethical research on Black/African American individuals constitute a primary reason for the distrust of physicians and scientists within the Black/African American community – a direct factor (among other factors) limiting their desire to participate in biomedical research [218, 224-227]. In a systematic review of barriers and facilitators to minority research participation, George et al., found that 77% (n = 34) of the articles included in their analyses stated that mistrust of the medical system was a barrier for Black/African American participation in clinical human studies [227]. Using a national survey completed by 527 Blacks/African Americans and 382 Whites, Corbie-Smith et al., reported that Blacks/African Americans, compared to

Whites, were more likely to believe that they would be used as a guinea pigs in biomedical experimentation without their consent (79.2% vs. 51.9%, $P < 0.01$) [224].

Due to Blacks'/African Americans' mistrust of the biomedical community and other factors (e.g.,, racism, religious beliefs, access to medical care), they are also less likely to donate their biospecimens (e.g.,, blood) or agree to an autopsy for research [228-233]. Using a national survey completed by 249 Blacks/African Americans and 492 Whites from six U.S. cities, Minniefield et al., found that Blacks/African Americans had a lower total prevalence (63%; $n \approx 156$) of support for organ donation compared to Whites (90%; $n \approx 442$) [233]. More specific to brain donation for clinical research, using a survey completed by 49 Blacks/African Americans and 184 Whites recruited from an AD registry, Jefferson et al., found that only 49% ($n \approx 24$) of Blacks/African Americans compared to 75% ($n \approx 138$) of Whites would agree to brain donation for research ($P > 0.001$) [231]. Therefore, in order to increase Blacks/African American participation, several U.S. Alzheimer's Disease Centers (ADCs) responsible for conducting large-scale longitudinal human studies focused on dementia in elderly individuals, do not require Blacks/African Americans to agree to a brain donation upon enrolling into research studies, while they do require brain donation for White participants [234]. This recruitment strategy can potentially lead to increased autopsy-recruitment bias and limited generalizability of results. Such limitations must be adequately accounted for in analyses and discussed in

research articles when reporting on Blacks/African Americans in clinical research studies.

2.1 Challenges in Studying and Comparing Clinical-Neuropathological Variables Between Black/African Americans and White Americans

In studying the epidemiology of AD and related diseases while comparing Blacks/African Americans to Whites, some pitfalls become apparent: 1) lack of clarity in the operationalization and/or definition of race, 2) using race as a proxy for genetics, 3) failure to account for socio-environmental factors (e.g.,, socioeconomic status, access to healthcare), 4) lack of autopsy validation, and 5) lack of racial/ethnic and scientific diversity within research teams.

Using “race” as a variable in biomedical research is deceptively challenging, due to its vague definition, social implications, confounding factors, and potential for misinterpretation of results [235-237]. The historically evolving definitions of race (skin color, along with other physical and “ancestral” factors) have been discussed by Tishkoff et al.,, 2004; Guthrie RV, 2003; and Williams et al.,, 1997 [238-240]. Within these definitions, it is important to note the lack of clarity and consensus-based implications of the term “race” across various fields of study [235, 239, 241-243]. It has been noted that historic viewpoints of the biological construct of race were not grounded in firm scientific discovery [239, 243, 244] but based on early 19th/20th century racist scientific studies, such as comparing “physiognomy” of Blacks/African Americans and Whites [209, 235, 239, 240, 245]. It has now been well-established that race is, in many senses, a

social construct with categories that change over time due to social policy, cultural beliefs, and political practices [241-243]. Therefore, scholars have suggested that race/ethnicity not be used as a proxy for socio-environmental factors, but deconstructed into specific indicators such as region, language, education, economic level, and access to health care [246-248].

An important consideration is the relationship between race and genetics: variability in genomic phenomena between racial categories and variation of ancestral markers within and between racial groups. There is more genetic variation of individuals within the same racial category than between individuals from different racial categories [249, 250]. Using genetic information on 5,269 Blacks/African Americans, 8,663 Latinos, and 148,780 Whites from 23andMe, Bryc et al., found Blacks/African Americans living in different parts of the United States showed varying frequencies of genetic “African” ancestral markers. For example, a self-described “Black/African-American” living in the South had more “African” ancestral gene markers compared to “Blacks/African-Americans” living in the Northeast, Midwest, the Pacific Northwest, and California [250]. Moreover, self-identified “Blacks/African-Americans” living in the West and Southwest had higher frequencies of “Native American” ancestral gene markers compared to “Blacks/African-Americans” living in other parts of the United States [250]. Thus according to this sample, self-identified “Blacks/African-Americans” across the U.S. have varying proportions of “African” ancestral genetic markers. Evidence from this paper and others support the stance that race is not a dependable proxy for genetics given the sample-to-sample variation of ancestral genetic

backgrounds among individuals within the same category operationalized by self-identification. Further, since many confounders (see below) apply, there is a serious risk of errantly associating a relatively “race-specific” genetic marker with a trait, when it actually is attributable to a regional or social factor. Continuing to use race as a proxy for genetic factors has the potential for detrimental political, social, and medical outcomes as a result of the over-simplification of results based on genetics, including medical stigmatization, racialization, genetic determinism, eugenics, discrimination, and missed/delayed diagnoses [236, 238, 242, 251].

To discuss race-related differences, environmental variables should be collected and accounted for before attributing and discussing genetics as a causal factor. There are many sources of bias and confounders [252]. Associations with race are potentially confounded by socioeconomic status (SES) variables including income level, education, and access to medical care [252-254]. In 2012, the U.S. Census Bureau reported that the median income for Black/African American households was $33,321 compared to $57,009 for White households [255]. In the same 2012 report, the percentage of Black/African American individuals living in poverty was 27.2% compared to 9.7% of White individuals [255]. In 2015, the Centers for Disease Control and Prevention (CDC) conducted a health interview survey that showed that 14.5% of Black/African American individuals were uninsured compared to 8.8% of White individuals [256]. The CDC reported that Black/African American individuals received worse care than White individuals for about 40% of health quality measures [256]. Many

SES variables, socio-environmental exposures, and medical care variables (access, utilization, and discrimination) have been shown to contribute to adverse health outcomes [235, 252, 257, 258]. In our opinion, scientists should not only adjust for these variables, but try to specifically identify the factors that contribute to dementia disparities in order to provide an appropriate intervention.

Examples are appearing in the literature that reveal interactions between socioeconomic status (particularly poverty) and racial-ethnic factors [259-262], resulting in health disparities that can, in turn, influence interpretations of clinical and neuropathological associations. For example, Glymour et al.,, found that childhood and adult social conditions nearly entirely attenuated the association between race and stroke risk in a study population of 3,019 Blacks/African Americans and 17,642 White Americans [261]. Waldstein et al.,, found significant interactions between race and SES composite scores when predicting radiographically-detected white matter lesions in a study population of 85 Blacks/African Americans and 62 White Americans [259]. In a study of 1,019 Blacks/African Americans and 1,438 White Americans, Yaffe et al.,, observed that the incident dementia hazard ratio was greatly reduced and no longer significant when socioeconomic status was added to the statistical model [262]. The results of these studies strongly support the notion that genetic mechanisms are highly unlikely to account entirely for the higher risk of dementia observed in Blacks/African Americans compared to White Americans. Therefore, it is necessary to include SES variables in analyses when studying dementia outcomes among Blacks/African Americans. Failure to do so can result in

misinterpretation of data as to the root causes of dementia outcomes within racial/ethnic groups. Notably, since 1994, NIH guidelines have specifically called for researchers to account for socioeconomic variables such as occupation, education, and income among human subjects [252].

The importance of autopsy-based (neuropathological) data in AD and related dementias has also become increasingly clear. For example, we highlight that both dementia and type II diabetes (T2D) are prevalent in Blacks/African Americans [263]. It is notable that data from different research centers have consistently reported that T2D is a risk factor for AD-type dementia in the clinical (no autopsy) context. By contrast, studies with a single added study design element -- an autopsy -- have shown the opposite result: T2D is not a risk factor for AD pathology [63]. Instead, the autopsies reveal that the clinical diagnosis of AD was not completely accurate, and the T2D appears to exert its impact through a different (potentially additive when comorbid) disorder: cerebrovascular disease characterized by small and medium-sized blood vessel pathology [63]. Thus, a more vulnerable population may be underserved due to a clinical over-diagnosis of AD and an under-appreciation of T2D-related cognitive impairment. This is all the more topical since therapies aimed at diabetes, blood pressure, and cholesterol may exert an impact on cerebrovascular pathology, but not yet AD itself.

According to the 2010 U.S. Census Bureau report, Blacks/African Americans make up 12.6% of the U.S. population [206]. However, Blacks/African Americans make up only ~3.6% of biomedical research faculty, ~4.1% of

physicians, and ~5.9% of social scientist faculty within the United States [264-266]. Improving the representation of Blacks/African Americans across research and clinical disciplines will enable improved outcomes for AD center research teams. Several published studies have provided evidence that diversity of thought and identity among scientists enhances the quality and output of research collaborations, which makes for “better science” [267-269]. For example, Campbell et al., reported that gender-heterogeneous authorship teams received 34% more citations than publications produced by gender-uniform authorship teams [268].

3.1 Addressing the Problem: Considerations for the Field

It is appropriate that all populations within the U.S. be represented in biomedical research studies. In formulating hypotheses and conceptualizing study designs, the ultimate goal should be to improve the health and well-being of the target population [270]. In analyzing data within and across racial/ethnic groups, we encourage scientists to strive for better science by shifting the paradigm away from interpreting clinical/neuropathological results based on the framework of biologic determination to understanding and incorporating both biological and socio-environmental factors known to affect health outcomes. Therefore, Blacks/African Americans should be encouraged and included in biomedical research for the sole purpose of improving their health outcomes, not simply to identify a health disparity. With this paradigm shift in mind, if one is going to embark on this field of investigation, it is necessary to understand and attempt to

account for the anthropological, psychological, sociological, political, biological, and cultural associations/causations attributing to health outcomes in Blacks/African Americans. In **Table 5.1**, we have provided some points of considerations for scientists embarking on this type of investigation. It is important to note that **Table 5.1** contents are not exhaustive but a starting point.

3.1.1 Cultural Competency When Interpreting Research Findings

Alongside adding new variables, we suggest that scientists provide a rationale for their research question and provide adequate discussion of research findings (see Table 1). There should be justification for studying “differences” between racial categories [241, 271], information on categorization of study population (e.g.,, skin color, self-report) [236], analyses and discussions of socio-political factors that can contribute to research findings [218, 236], and explanation of the social, biological, and medical implications of misinterpretation of data within their manuscript [241]. We encourage neuroscientists to solicit expert advice from anthropologists, sociologists, psychologists, African American community leaders, and other individuals who can provide contextual information on contributing factors to health outcomes. Ideally, these individuals can serve as co-authors on clinical-neuropathological manuscripts as suggested by Foster et al., who recommends publishing in cross-discipline journals [241].

Table 5.1. Topical questions and recommendations for a study related to dementia in African Americans

Experimental Design:

i) How to choose and sample the African American population to study?

ii) What is the original hypothesis? If it involves studying "differences," are the strata sufficiently large to allow for adequate power for the detection of effect modification?

iii) What are potential confounders that must be included? How might they affect your hypothesis and experimental design?

iv) How do health disparities in marginalized groups affect participation in the study and potentially the outcomes? Because "refusers" do not actually join the studies, how can potential differences or effects be estimated?

v) What is the justification for studying African Americans? What is the justification for excluding others groups (e.g., multi-racial individuals)

vi) How does composition of the research team affect results?

vii) How does this study contribute to helping underserved populations?

Methods:

i) Establish population stratified sampling methods, possibly similar to those used in cancer registries

ii) Enroll numbers of African Americans to have sufficient power and precision to address the associations under consideration.

iii) How was the comparison group(s) chosen? How was the reference group chosen?

iv) How were the individuals recruited in the study? Was there a difference in recruited strategies for individuals from different racial categories?

v) How was data on racial category assigned and collected? (e.g., self-report)

Data collection:

i) SES variables (income, education, occupation, zip code, etc)

ii) Exposures – social, other environmental

iii) Ancestry (e.g., country of birth, parent's place of birth)

iv) Medical care (insurance status, primary care physician, utilization)

v) Impact of worker collecting the data on the results

Discussion:

i) Implication of results, consequences for misinterpretation

ii) Limitations of study (recruitment bias, autopsy bias, investigator bias)

3.1.2 Recruitment of Research Participants

In terms of context-specific issues, there is a lack of Black/African American representation in clinical-neuropathological datasets. Many articles have published goals and strategies for recruiting Blacks/African Americans and other marginalized groups into clinical studies--one major goal discussed in these articles is to build long-term trust within the Black/African American community [229, 234, 272]. Some recommendations for building trust are as follows: 1) publicly acknowledge the historical mistreatment of Blacks/African Americans in biomedical research [218, 230, 271-273], 2) adequately explain the consent process [218, 230, 274], and protections in place today to prevent mistreatment, 3) engage in ongoing Q&A discussions with the community [218, 224, 234, 272, 273, 275], and 4) create relationships that include the patients, caregivers, healthcare providers, community leaders, researchers, and study coordinators [276].

ADCs shouldbe required to actively enroll Blacks/African Americans, attach specific research questions, and perform power analysis so that it can be ensured that comparisons among individuals from different racial/ethnic groups will be valid. At present, research centers may enroll a percent consistent with the surrounding geographic area as the ADC, but that often just satisfies the “inclusion table” and the group is too small to perform any meaningful comparisons or analyses. Thus, we suggest that the ADCs enroll “enough” subjects to test some intended hypotheses with the expectations of being able to see real associations or interactions/effect modification.

We believe that by implementing some of these strategies, it can help to improve recruitment of Blacks/Africans Americans across research institutions. Moreover, it can help to provide scientists with an appropriate sample size in order to understand dementia progression in Blacks/African Americans. In turn, it has the potential to lead to improved medical and societal solutions decreasing dementia within the Black/African American population.

3.1.3 Data and Brain Tissue Collection

In addition to building trust, we suggest that scientists collect potentially confounding variables to include in data analyses (**see Table 5.1**). Some of these variables include income level, education, zip code, nativity, health insurance status, income level, primary care physician availability, and employment status [236]. The addition of these variables would aid scientists in improving the understanding and analysis of clinical-neuropathological findings in Blacks/African Americans.

An additional point relates to the importance of autopsy-based confirmation of medical diagnoses. Autopsy-based neuropathological diagnoses are central to AD research in general, but may be all the more important in a historically underserved population where clinical and social factors may track differently than among the populations that have traditionally been included in clinical studies. In order to improve brain donation from Blacks/African Americans, we suggest that recruitment strategies incorporate education on the brain donation procedure and protection of human subjects.

3.1.4 Recruiting & Retaining a Diverse Biomedical Research Workforce

It is important to have a culturally diverse group of experts included in the research team [230, 272, 275]. One long-term strategy for ADCs is to increase African American representation among clinicians, scientists, epidemiologists, biostaticians, and study coordinators to aid in experimental design and data analysis of AD and related dementias within the Black/African American population. Some strategies include effective career mentoring [62, 277], addressing unconscious bias and stereotype threat [265], and implementing pipeline and career development programs [278].

4.1 Conclusion

Studying AD and related dementias within the Black/African American population is a complex task due to the historical, cultural, and political factors that play a role in Black/African American participation in clinical studies. This commentary is not an exhaustive list of challenges and considerations, but, instead, aims to help influence movement in the right direction. Moreover, this information can be applied to other underserved populations worldwide. Dementia is a devastating and multi-faceted clinical syndrome. We hope that dementia research centers can improve their recruitment strategies, recognizing the subpopulation-specific challenges and opportunities, and studies can incorporate more of the relevant data. Scientists can create multi-disciplinary teams focused on understanding dementia in Black/African Americans and other marginalized groups, cognizant of the fact that research has the potential to do harm as well as good.

Chapter 6: Conclusion

The overall objectives of my dissertation was were focused on studying two SVD-type pathologies in the aged human brain using large autopsy data sets/brain repositories and understanding the strengths and weakness of these data sets/brain repositories. First, I focused on elucidating the frequency, clinical risk factors, cognitive sequelae, and co-pathologies of B-ASC and MVPs. With respect to B-ASC, we found that hypertension was a risk factor in the < 80 years at death group [24]. In addition, an *ABCC9* gene variant (rs704180), previously associated with aging-related hippocampal sclerosis, was associated with B-ASC in the ≥ 80 years at death group [24]. In terms of cognition as determined by well-established neurocognitive test scores, severe B-ASC was associated with worse global cognition in both age groups [24]. With respect to MVPs, we found that age was associated with MVP density (Ighodaro et al, In Preparation). There is a high frequency of mixed pathologies (vascular disease and neurodegeneration) in the aged brain, and we helped to describe the association between B-ASC and HS-Aging which could be an indicator of a currently unnamed “brain-wide” disease whose upstream pathogenesis involved alterations in *ABCC9* [57]. Lastly, given the fact that African Americans (a group frequently characterized as having higher frequencies of AD-type dementia) are not optimally represented in neuropathological datasets/brain repositories, we wrote a manuscript on the challenges and considerations for studying dementia in Blacks/African Americans using these datasets and brain-repositories.

This dissertation discusses new frontiers in the field of SVD research. These works provide improved understanding of age-related SVDs that will lay the groundwork for improved clinical trial designs and improved treatment options. We discovered novel risk factors for B-ASC in the aged brain [24]. We were one of the first groups to publish quantitative data on the cognitive status of individuals with B-ASC [24]. We provide evidence and make the case for a currently unnamed “brain-wide” disease characterized by both B-ASC and HS-Aging [57]. To the best of our knowledge, we are the first to provide quantitative evidence for the association between MVPs and age using a large autopsy cohort (Chapter 3). In addition, we are the first to describe and show MVPs in 3D using a clearing tissue method (Chapter 3). Lastly, I wrote an imperative and insightful commentary on how neuroscientists can improve their analyses when studying dementia in African Americans which can also be applied to other marginalized groups (Ighodaro et al, *In Revisions*).

Many future studies can result from our current findings discussed. For example, future analyses can be conducted to determine the specific cognitive domains that are associated with B-ASC pathology. Other areas of the brain can be studied in order to understand pathology of MVPs throughout the brain. Instead of calculating MVP density, the number of lumens per MVP can be recorded in order to see if lumen count correlates with vascular risk factors. Cases with high MVP densities can be stained with vasogeneic antibodies in order to understand MVP pathogenesis.

The work presented in this dissertation has provided me with the knowledge and skillset to further my biomedical research career in the field of neuroscience and cerebrovascular disease. With respect to neuropathology, I have learned the fundamentals of cerebrovascular disease, vascular dementia, human tissue bio-banking, human tissue fixation and processing, digital neuropathology image analysis, and immunohistochemistry. With respect to experimental design and data analysis, I have learned the fundamentals of experimental design, strengths/limitations of human longitudinal clinical/neuropathological datasets and regression modeling, and research ethics. With respect to research communication, I have learned the fundamentals of manuscript writing, grant writing, and oral communications (e.g, poster and slideshow presentations). Dementia related to cerebrovascular disease is highly frequent in the elderly population [2, 10] . I hope to be able to aid in the effort to decrease this statistic with my previous work, current knowledge, and skillset.

References

[1] Fillit H, Nash DT, Rundek T, Zuckerman A (2008) Cardiovascular risk factors and dementia. *Am J Geriatr Pharmacother* **6**, 100-118.

[2] Iadecola C (2013) The pathobiology of vascular dementia. *Neuron* **80**, 844-866.

[3] Jellinger KA (2013) Pathology and pathogenesis of vascular cognitive impairment-a critical update. *Front Aging Neurosci* **5**, 17.

[4] Nelson PT, Head E, Schmitt FA, Davis PR, Neltner JH, Jicha GA, Abner EL, Smith CD, Van Eldik LJ, Kryscio RJ, Scheff SW (2011) Alzheimer's disease is not "brain aging": neuropathological, genetic, and epidemiological human studies. *Acta Neuropathol* **121**, 571-587.

[5] Barker WW, Luis CA, Kashuba A, Luis M, Harwood DG, Loewenstein D, Waters C, Jimison P, Shepherd E, Sevush S, Graff-Radford N, Newland D, Todd M, Miller B, Gold M, Heilman K, Doty L, Goodman I, Robinson B, Pearl G, Dickson D, Duara R (2002) Relative frequencies of Alzheimer disease, Lewy body, vascular and frontotemporal dementia, and hippocampal sclerosis in the State of Florida Brain Bank. *Alzheimer Dis Assoc Disord* **16**, 203-212.

[6] Nelson PT, Abner EL, Schmitt FA, Kryscio RJ, Jicha GA, Smith CD, Davis DG, Poduska JW, Patel E, Mendiondo MS, Markesbery WR (2010) Modeling the association between 43 different clinical and pathological variables and the severity of cognitive impairment in a large autopsy cohort of elderly persons. *Brain Pathol* **20**, 66-79.

[7] Schneider JA, Arvanitakis Z, Bang W, Bennett DA (2007) Mixed brain pathologies account for most dementia cases in community-dwelling older persons. *Neurology* **69**, 2197-2204.

[8] Neltner JH, Abner EL, Jicha GA, Schmitt FA, Patel E, Poon LW, Marla G, Green RC, Davey A, Johnson MA, Jazwinski SM, Kim S, Davis D, Woodard JL, Kryscio RJ, Van Eldik LJ, Nelson PT (2016) Brain pathologies in extreme old age. *Neurobiol Aging* **37**, 1-11.

[9] Nelson PT, Trojanowski JQ, Abner EL, Al-Janabi OM, Jicha GA, Schmitt FA, Smith CD, Fardo DW, Wang WX, Kryscio RJ, Neltner JH, Kukull WA, Cykowski MD, Van Eldik LJ, Ighodaro ET (2016) "New Old Pathologies": AD, PART, and Cerebral Age-Related TDP-43 With Sclerosis (CARTS). *J Neuropathol Exp Neurol* **75**, 482-498.

[10] Kalaria RN (2016) Neuropathological diagnosis of vascular cognitive impairment and vascular dementia with implications for Alzheimer's disease. *Acta Neuropathol* **131**, 659-685.

[11] Gorelick PB, Scuteri A, Black SE, Decarli C, Greenberg SM, Iadecola C, Launer LJ, Laurent S, Lopez OL, Nyenhuis D, Petersen RC, Schneider JA, Tzourio C, Arnett DK, Bennett DA, Chui HC, Higashida RT, Lindquist R, Nilsson PM, Roman GC, Sellke FW, Seshadri S (2011) Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the american heart association/american stroke association. *Stroke* **42**, 2672-2713.

[12] Corriveau RA, Bosetti F, Emr M, Gladman JT, Koenig JI, Moy CS, Pahigiannis K, Waddy SP, Koroshetz W (2016) The Science of Vascular Contributions to Cognitive Impairment and Dementia (VCID): A Framework for Advancing Research Priorities in the Cerebrovascular Biology of Cognitive Decline. *Cell Mol Neurobiol* **36**, 281-288.

[13] Gorelick PB (2004) Risk factors for vascular dementia and Alzheimer disease. *Stroke* **35**, 2620-2622.

[14] Knopman DS, Mosley TH, Catellier DJ, Coker LH (2009) Fourteen-year longitudinal study of vascular risk factors, APOE genotype, and cognition: the ARIC MRI Study. *Alzheimers Dement* **5**, 207-214.
[15] Ganguli M, Fu B, Snitz BE, Hughes TF, Chang CC (2013) Mild cognitive impairment: incidence and vascular risk factors in a population-based cohort. *Neurology* **80**, 2112-2120.
[16] Rosenberg GA, Wallin A, Wardlaw JM, Markus HS, Montaner J, Wolfson L, Iadecola C, Zlokovic BV, Joutel A, Dichgans M, Duering M, Schmidt R, Korczyn AD, Grinberg LT, Chui HC, Hachinski V (2016) Consensus statement for diagnosis of subcortical small vessel disease. *J Cereb Blood Flow Metab* **36**, 6-25.
[17] Raz L, Knoefel J, Bhaskar K (2016) The neuropathology and cerebrovascular mechanisms of dementia. *J Cereb Blood Flow Metab* **36**, 172-186.
[18] Ferrer I (2010) Cognitive impairment of vascular origin: neuropathology of cognitive impairment of vascular origin. *Journal of the neurological sciences* **299**, 139-149.
[19] Farkas E, Luiten PG (2001) Cerebral microvascular pathology in aging and Alzheimer's disease. *Prog Neurobiol* **64**, 575-611.
[20] Iadecola C (2004) Neurovascular regulation in the normal brain and in Alzheimer's disease. *Nat Rev Neurosci* **5**, 347-360.
[21] Lusis AJ (2000) Atherosclerosis. *Nature* **407**, 233-241.
[22] Grinberg LT, Thal DR (2010) Vascular pathology in the aged human brain. *Acta Neuropathol* **119**, 277-290.
[23] Kapasi A, Schneider JA (2016) Vascular contributions to cognitive impairment, clinical Alzheimer's disease, and dementia in older persons. *Biochim Biophys Acta* **1862**, 878-886.
[24] Ighodaro ET, Abner EL, Fardo DW, Lin AL, Katsumata Y, Schmitt FA, Kryscio RJ, Jicha GA, Neltner JH, Monsell SE, Kukull WA, Moser DK, Appiah F, Bachstetter AD, Van Eldik LJ, Nelson PT (2017) Risk factors and global cognitive status related to brain arteriolosclerosis in elderly individuals. *J Cereb Blood Flow Metab* **37**, 201-216.
[25] Neltner JH, Abner EL, Baker S, Schmitt FA, Kryscio RJ, Jicha GA, Smith CD, Hammack E, Kukull WA, Brenowitz WD, Van Eldik LJ, Nelson PT (2014) Arteriolosclerosis that affects multiple brain regions is linked to hippocampal sclerosis of ageing. *Brain* **137**, 255-267.
[26] Vinters HV, Ellis WG, Zarow C, Zaias BW, Jagust WJ, Mack WJ, Chui HC (2000) Neuropathologic substrates of ischemic vascular dementia. *J Neuropathol Exp Neurol* **59**, 931-945.
[27] Bridges LR, Andoh J, Lawrence AJ, Khoong CH, Poon WW, Esiri MM, Markus HS, Hainsworth AH (2014) Blood-brain barrier dysfunction and cerebral small vessel disease (arteriolosclerosis) in brains of older people. *J Neuropathol Exp Neurol* **73**, 1026-1033.
[28] Lammie GA (2002) Hypertensive cerebral small vessel disease and stroke. *Brain Pathol* **12**, 358-370.
[29] Gavornik P, Galbavy S (2001) Clinical picture of arteriolosclerosis. *Bratisl Lek Listy* **102**, 326-331.
[30] Román GC, Benavente O (2004) in *Seminars in Cerebrovascular Diseases and Stroke* Elsevier, pp. 87-96.
[31] Chou SH, Shulman JM, Keenan BT, Secor EA, Buchman AS, Schneider J, Bennett DA, De Jager PL (2013) Genetic susceptibility for ischemic infarction and arteriolosclerosis based on neuropathologic evaluations. *Cerebrovasc Dis* **36**, 181-188.
[32] Buchman AS, Yu L, Boyle PA, Levine SR, Nag S, Schneider JA, Bennett DA (2013) Microvascular brain pathology and late-life motor impairment. *Neurology* **80**, 712-718.

[33] Furuta A, Ishii N, Nishihara Y, Horie A (1991) Medullary arteries in aging and dementia. *Stroke* **22**, 442-446.
[34] Buchman AS, Leurgans SE, Nag S, Bennett DA, Schneider JA (2011) Cerebrovascular disease pathology and parkinsonian signs in old age. *Stroke* **42**, 3183-3189.
[35] Baker AB (1941) Structure of the small cerebral arteries in hypertension. *Am J Pathol* **17**, 39-46.31.
[36] Moritz AR, Oldt MR (1937) Arteriolar Sclerosis in Hypertensive and Non-Hypertensive Individuals. *Am J Pathol* **13**, 679-728 677.
[37] Johnson G (1868) 1. On certain points in the Anatomy and Pathology of Bright's Disease of the Kidney. 2. On the Influence of the Minute Blood-vessels upon the Circulation. *Med Chir Trans* **51**, 57-78.53.
[38] Rizzoni D, Porteri E, Guelfi D, Muiesan ML, Valentini U, Cimino A, Girelli A, Rodella L, Bianchi R, Sleiman I, Rosei EA (2001) Structural alterations in subcutaneous small arteries of normotensive and hypertensive patients with non-insulin-dependent diabetes mellitus. *Circulation* **103**, 1238-1244.
[39] Schofield I, Malik R, Izzard A, Austin C, Heagerty A (2002) Vascular structural and functional changes in type 2 diabetes mellitus: evidence for the roles of abnormal myogenic responsiveness and dyslipidemia. *Circulation* **106**, 3037-3043.
[40] Beekly DL, Ramos EM, van Belle G, Deitrich W, Clark AD, Jacka ME, Kukull WA (2004) The National Alzheimer's Coordinating Center (NACC) Database: an Alzheimer disease database. *Alzheimer Dis Assoc Disord* **18**, 270-277.
[41] Hassler O (1965) Vascular changes in senile brains. A micro-angiographic study. *Acta Neuropathol* **5**, 40-53.
[42] Hassler O (1967) Arterial deformities in senile brains. The occurrence of the deformities in a large autopsy series and some aspects of their functional significance. *Acta Neuropathol* **8**, 219-229.
[43] Moody DM, Brown WR, Challa VR, Ghazi-Birry HS, Reboussin DM (1997) Cerebral microvascular alterations in aging, leukoaraiosis, and Alzheimer's disease. *Ann N Y Acad Sci* **826**, 103-116.
[44] Cervos-Navarro J, Gertz HJ, Frydl V (1987) Cerebral blood vessel changes in old people. *Mech Ageing Dev* **39**, 223-231.
[45] Arsene D, Ardeleanu C (2006) Vascular convolutes in the brain: a peculiar entity. *Rom J Morphol Embryol* **47**, 37-41.
[46] Altschul R (1944) THE CONVOLUTED VESSELS OF BRAIN AND SPINAL CORD. *Journal of Neuropathology & Experimental Neurology* **3**, 386-396.
[47] Toledo JB, Arnold SE, Raible K, Brettschneider J, Xie SX, Grossman M, Monsell SE, Kukull WA, Trojanowski JQ (2013) Contribution of cerebrovascular disease in autopsy confirmed neurodegenerative disease cases in the National Alzheimer's Coordinating Centre. *Brain* **136**, 2697-2706.
[48] Jellinger KA, Attems J (2010) Prevalence and pathology of vascular dementia in the oldest-old. *J Alzheimers Dis* **21**, 1283-1293.
[49] Esiri MM, Nagy Z, Smith MZ, Barnetson L, Smith AD (1999) Cerebrovascular disease and threshold for dementia in the early stages of Alzheimer's disease. *Lancet* **354**, 919-920.
[50] Nagy Z, Esiri MM, Jobst KA, Morris JH, King EM, McDonald B, Joachim C, Litchfield S, Barnetson L, Smith AD (1997) The effects of additional pathology on the cognitive deficit in Alzheimer disease. *J Neuropathol Exp Neurol* **56**, 165-170.
[51] Nelson PT, Schmitt FA, Lin Y, Abner EL, Jicha GA, Patel E, Thomason PC, Neltner JH, Smith CD, Santacruz KS, Sonnen JA, Poon LW, Gearing M, Green RC, Woodard JL, Van

Eldik LJ, Kryscio RJ (2011) Hippocampal sclerosis in advanced age: clinical and pathological features. *Brain* **134**, 1506-1518.

[52] Pao WC, Dickson DW, Crook JE, Finch NA, Rademakers R, Graff-Radford NR (2011) Hippocampal Sclerosis in the Elderly: Genetic and Pathologic Findings, Some Mimicking AlzheimerDisease Clinically. *Alzheimer Dis Assoc Disord*.

[53] Dickson DW, Davies P, Bevona C, Van Hoeven KH, Factor SM, Grober E, Aronson MK, Crystal HA (1994) Hippocampal sclerosis: a common pathological feature of dementia in very old (> or = 80 years of age) humans. *Acta Neuropathol* **88**, 212-221.

[54] Zarow C, Sitzer TE, Chui HC (2008) Understanding hippocampal sclerosis in the elderly: epidemiology, characterization, and diagnostic issues. *Curr Neurol Neurosci Rep* **8**, 363-370.

[55] Nelson PT, Smith CD, Abner EL, Wilfred BJ, Wang WX, Neltner JH, Baker M, Fardo DW, Kryscio RJ, Scheff SW, Jicha GA, Jellinger KA, Van Eldik LJ, Schmitt FA (2013) Hippocampal sclerosis of aging, a prevalent and high-morbidity brain disease. *Acta Neuropathol* **126**, 161-177.

[56] Leverenz JB, Agustin CM, Tsuang D, Peskind ER, Edland SD, Nochlin D, DiGiacomo L, Bowen JD, McCormick WC, Teri L, Raskind MA, Kukull WA, Larson EB (2002) Clinical and neuropathological characteristics of hippocampal sclerosis: a community-based study. *Arch Neurol* **59**, 1099-1106.

[57] Ighodaro ET, Jicha GA, Schmitt FA, Neltner JH, Abner EL, Kryscio RJ, Smith CD, Duplessis T, Anderson S, Patel E, Bachstetter A, Van Eldik LJ, Nelson PT (2015) Hippocampal Sclerosis of Aging Can Be Segmental: Two Cases and Review of the Literature. *J Neuropathol Exp Neurol* **74**, 642-652.

[58] Montine TJ, Phelps CH, Beach TG, Bigio EH, Cairns NJ, Dickson DW, Duyckaerts C, Frosch MP, Masliah E, Mirra SS, Nelson PT, Schneider JA, Thal DR, Trojanowski JQ, Vinters HV, Hyman BT, National Institute on A, Alzheimer's A (2012) National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach. *Acta Neuropathol* **123**, 1-11.

[59] Nelson PT, Estus S, Abner EL, Parikh I, Malik M, Neltner JH, Ighodaro E, Wang WX, Wilfred BR, Wang LS, Kukull WA, Nandakumar K, Farman ML, Poon WW, Corrada MM, Kawas CH, Cribbs DH, Bennett DA, Schneider JA, Larson EB, Crane PK, Valladares O, Schmitt FA, Kryscio RJ, Jicha GA, Smith CD, Scheff SW, Sonnen JA, Haines JL, Pericak-Vance MA, Mayeux R, Farrer LA, Van Eldik LJ, Horbinski C, Green RC, Gearing M, Poon LW, Kramer PL, Woltjer RL, Montine TJ, Partch AB, Rajic AJ, Richmire K, Monsell SE, Schellenberg GD, Fardo DW (2014) ABCC9 gene polymorphism is associated with hippocampal sclerosis of aging pathology. *Acta Neuropathol* **127**, 825-843.

[60] Nelson PT, Wang WX, Partch AB, Monsell SE, Valladares O, Ellingson SR, Wilfred BR, Naj AC, Wang LS, Kukull WA, Fardo DW (2015) Reassessment of risk genotypes (GRN, TMEM106B, and ABCC9 variants) associated with hippocampal sclerosis of aging pathology. *J Neuropathol Exp Neurol* **74**, 75-84.

[61] Standen NB, Quayle JM, Davies NW, Brayden JE, Huang Y, Nelson MT (1989) Hyperpolarizing vasodilators activate ATP-sensitive K+ channels in arterial smooth muscle. *Science* **245**, 177-180.

[62] Beecham GW, Hamilton K, Naj AC, Martin ER, Huentelman M, Myers AJ, Corneveaux JJ, Hardy J, Vonsattel JP, Younkin SG, Bennett DA, De Jager PL, Larson EB, Crane PK, Kamboh MI, Kofler JK, Mash DC, Duque L, Gilbert JR, Gwirtsman H, Buxbaum JD, Kramer P, Dickson DW, Farrer LA, Frosch MP, Ghetti B, Haines JL, Hyman BT, Kukull WA, Mayeux RP, Pericak-Vance MA, Schneider JA, Trojanowski JQ, Reiman EM, Schellenberg GD,

Montine TJ (2014) Genome-wide association meta-analysis of neuropathologic features of Alzheimer's disease and related dementias. *PLoS Genet* **10**, e1004606.

[63] Abner EL, Nelson PT, Kryscio RJ, Schmitt FA, Fardo DW, Woltjer RL, Cairns NJ, Yu L, Dodge HH, Xiong C, Masaki K, Tyas SL, Bennett DA, Schneider JA, Arvanitakis Z (2016) Diabetes is associated with cerebrovascular but not Alzheimer's disease neuropathology. *Alzheimers Dement* **12**, 882-889.

[64] Monsell SE, Mock C, Roe CM, Ghoshal N, Morris JC, Cairns NJ, Kukull W (2013) Comparison of symptomatic and asymptomatic persons with Alzheimer disease neuropathology. *Neurology* **80**, 2121-2129.

[65] Morris JC, Weintraub S, Chui HC, Cummings J, Decarli C, Ferris S, Foster NL, Galasko D, Graff-Radford N, Peskind ER, Beekly D, Ramos EM, Kukull WA (2006) The Uniform Data Set (UDS): clinical and cognitive variables and descriptive data from Alzheimer Disease Centers. *Alzheimer Dis Assoc Disord* **20**, 210-216.

[66] Beekly DL, Ramos EM, Lee WW, Deitrich WD, Jacka ME, Wu J, Hubbard JL, Koepsell TD, Morris JC, Kukull WA (2007) The National Alzheimer's Coordinating Center (NACC) database: the Uniform Data Set. *Alzheimer Dis Assoc Disord* **21**, 249-258.

[67] Haneuse S, Schildcrout J, Crane P, Sonnen J, Breitner J, Larson E (2009) Adjustment for selection bias in observational studies with application to the analysis of autopsy data. *Neuroepidemiology* **32**, 229-239.

[68] Brenowitz WD, Monsell SE, Schmitt FA, Kukull WA, Nelson PT (2014) Hippocampal sclerosis of aging is a key Alzheimer's disease mimic: clinical-pathologic correlations and comparisons with both alzheimer's disease and non-tauopathic frontotemporal lobar degeneration. *J Alzheimers Dis* **39**, 691-702.

[69] Zheng L, Vinters HV, Mack WJ, Zarow C, Ellis WG, Chui HC (2013) Cerebral atherosclerosis is associated with cystic infarcts and microinfarcts but not Alzheimer pathologic changes. *Stroke* **44**, 2835-2841.

[70] Esiri MM, Wilcock GK, Morris JH (1997) Neuropathological assessment of the lesions of significance in vascular dementia. *J Neurol Neurosurg Psychiatry* **63**, 749-753.

[71] Erkinjuntti T, Benavente O, Eliasziw M, Munoz DG, Sulkava R, Haltia M, Hachinski V (1996) Diffuse vacuolization (spongiosis) and arteriolosclerosis in the frontal white matter occurs in vascular dementia. *Arch Neurol* **53**, 325-332.

[72] Pantoni L, Garcia JH, Brown GG (1996) Vascular pathology in three cases of progressive cognitive deterioration. *J Neurol Sci* **135**, 131-139.

[73] Baumbach GL, Dobrin PB, Hart MN, Heistad DD (1988) Mechanics of cerebral arterioles in hypertensive rats. *Circ Res* **62**, 56-64.

[74] Baumbach GL, Sigmund CD, Faraci FM (2003) Cerebral arteriolar structure in mice overexpressing human renin and angiotensinogen. *Hypertension* **41**, 50-55.

[75] Danis RP, Yang Y (1993) Microvascular retinopathy in the Zucker diabetic fatty rat. *Invest Ophthalmol Vis Sci* **34**, 2367-2371.

[76] Like AA, Lavine RL, Poffenbarger PL, Chick WL (1972) Studies in the diabetic mutant mouse. VI. Evolution of glomerular lesions and associated proteinuria. *Am J Pathol* **66**, 193-224.

[77] Thal DR, Ghebremedhin E, Orantes M, Wiestler OD (2003) Vascular pathology in Alzheimer disease: correlation of cerebral amyloid angiopathy and arteriosclerosis/lipohyalinosis with cognitive decline. *J Neuropathol Exp Neurol* **62**, 1287-1301.

[78] Folstein MF, Folstein SE, McHugh PR (1975) "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* **12**, 189-198.

[79] Morris JC (1993) The Clinical Dementia Rating (CDR): current version and scoring rules. *Neurology* **43**, 2412-2414.
[80] Lynch CA, Walsh C, Blanco A, Moran M, Coen RF, Walsh JB, Lawlor BA (2006) The clinical dementia rating sum of box score in mild dementia. *Dement Geriatr Cogn Disord* **21**, 40-43.
[81] Savva GM, Wharton SB, Ince PG, Forster G, Matthews FE, Brayne C (2009) Age, neuropathology, and dementia. *N Engl J Med* **360**, 2302-2309.
[82] von Gunten A, Ebbing K, Imhof A, Giannakopoulos P, Kovari E (2010) Brain aging in the oldest-old. *Curr Gerontol Geriatr Res*.
[83] Imhof A, Kovari E, von Gunten A, Gold G, Rivara CB, Herrmann FR, Hof PR, Bouras C, Giannakopoulos P (2007) Morphological substrates of cognitive decline in nonagenarians and centenarians: a new paradigm? *J Neurol Sci* **257**, 72-79.
[84] Ubhi K, Peng K, Lessig S, Estrella J, Adame A, Galasko D, Salmon DP, Hansen LA, Kawas CH, Masliah E (2010) Neuropathology of dementia with Lewy bodies in advanced age: a comparison with Alzheimer disease. *Neurosci Lett* **485**, 222-227.
[85] Bancher C, Jellinger KA (1994) Neurofibrillary tangle predominant form of senile dementia of Alzheimer type: a rare subtype in very old subjects. *Acta Neuropathol* **88**, 565-570.
[86] Lorke DE, Wai MS, Liang Y, Yew DT (2010) TUNEL and growth factor expression in the prefrontal cortex of Alzheimer patients over 80 years old. *Int J Immunopathol Pharmacol* **23**, 13-23.
[87] Weintraub S, Salmon D, Mercaldo N, Ferris S, Graff-Radford NR, Chui H, Cummings J, DeCarli C, Foster NL, Galasko D, Peskind E, Dietrich W, Beekly DL, Kukull WA, Morris JC (2009) The Alzheimer's Disease Centers' Uniform Data Set (UDS): the neuropsychologic test battery. *Alzheimer Dis Assoc Disord* **23**, 91-101.
[88] Mueller SG, Weiner MW, Thal LJ, Petersen RC, Jack C, Jagust W, Trojanowski JQ, Toga AW, Beckett L (2005) The Alzheimer's disease neuroimaging initiative. *Neuroimaging Clin N Am* **15**, 869-877, xi-xii.
[89] Mueller SG, Weiner MW, Thal LJ, Petersen RC, Jack CR, Jagust W, Trojanowski JQ, Toga AW, Beckett L (2005) Ways toward an early diagnosis in Alzheimer's disease: the Alzheimer's Disease Neuroimaging Initiative (ADNI). *Alzheimers Dement* **1**, 55-66.
[90] Braak H, Alafuzoff I, Arzberger T, Kretzschmar H, Del Tredici K (2006) Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. *Acta Neuropathol* **112**, 389-404.
[91] Mirra SS, Heyman A, McKeel D, Sumi SM, Crain BJ, Brownlee LM, Vogel FS, Hughes JP, van Belle G, Berg L (1991) The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology* **41**, 479-486.
[92] Naj AC, Jun G, Beecham GW, Wang LS, Vardarajan BN, Buros J, Gallins PJ, Buxbaum JD, Jarvik GP, Crane PK, Larson EB, Bird TD, Boeve BF, Graff-Radford NR, De Jager PL, Evans D, Schneider JA, Carrasquillo MM, Ertekin-Taner N, Younkin SG, Cruchaga C, Kauwe JS, Nowotny P, Kramer P, Hardy J, Huentelman MJ, Myers AJ, Barmada MM, Demirci FY, Baldwin CT, Green RC, Rogaeva E, St George-Hyslop P, Arnold SE, Barber R, Beach T, Bigio EH, Bowen JD, Boxer A, Burke JR, Cairns NJ, Carlson CS, Carney RM, Carroll SL, Chui HC, Clark DG, Corneveaux J, Cotman CW, Cummings JL, DeCarli C, DeKosky ST, Diaz-Arrastia R, Dick M, Dickson DW, Ellis WG, Faber KM, Fallon KB, Farlow MR, Ferris S, Frosch MP, Galasko DR, Ganguli M, Gearing M, Geschwind DH, Ghetti B, Gilbert JR, Gilman S, Giordani B, Glass JD, Growdon JH, Hamilton RL, Harrell LE, Head E, Honig LS,

Hulette CM, Hyman BT, Jicha GA, Jin LW, Johnson N, Karlawish J, Karydas A, Kaye JA, Kim R, Koo EH, Kowall NW, Lah JJ, Levey AI, Lieberman AP, Lopez OL, Mack WJ, Marson DC, Martiniuk F, Mash DC, Masliah E, McCormick WC, McCurry SM, McDavid AN, McKee AC, Mesulam M, Miller BL, Miller CA, Miller JW, Parisi JE, Perl DP, Peskind E, Petersen RC, Poon WW, Quinn JF, Rajbhandary RA, Raskind M, Reisberg B, Ringman JM, Roberson ED, Rosenberg RN, Sano M, Schneider LS, Seeley W, Shelanski ML, Slifer MA, Smith CD, Sonnen JA, Spina S, Stern RA, Tanzi RE, Trojanowski JQ, Troncoso JC, Van Deerlin VM, Vinters HV, Vonsattel JP, Weintraub S, Welsh-Bohmer KA, Williamson J, Woltjer RL, Cantwell LB, Dombroski BA, Beekly D, Lunetta KL, Martin ER, Kamboh MI, Saykin AJ, Reiman EM, Bennett DA, Morris JC, Montine TJ, Goate AM, Blacker D, Tsuang DW, Hakonarson H, Kukull WA, Foroud TM, Haines JL, Mayeux R, Pericak-Vance MA, Farrer LA, Schellenberg GD (2011) Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. *Nat Genet* **43**, 436-441.

[93] Hollingworth P, Harold D, Sims R, Gerrish A, Lambert JC, Carrasquillo MM, Abraham R, Hamshere ML, Pahwa JS, Moskvina V, Dowzell K, Jones N, Stretton A, Thomas C, Richards A, Ivanov D, Widdowson C, Chapman J, Lovestone S, Powell J, Proitsi P, Lupton MK, Brayne C, Rubinsztein DC, Gill M, Lawlor B, Lynch A, Brown KS, Passmore PA, Craig D, McGuinness B, Todd S, Holmes C, Mann D, Smith AD, Beaumont H, Warden D, Wilcock G, Love S, Kehoe PG, Hooper NM, Vardy ER, Hardy J, Mead S, Fox NC, Rossor M, Collinge J, Maier W, Jessen F, Ruther E, Schurmann B, Heun R, Kolsch H, van den Bussche H, Heuser I, Kornhuber J, Wiltfang J, Dichgans M, Frolich L, Hampel H, Gallacher J, Hull M, Rujescu D, Giegling I, Goate AM, Kauwe JS, Cruchaga C, Nowotny P, Morris JC, Mayo K, Sleegers K, Bettens K, Engelborghs S, De Deyn PP, Van Broeckhoven C, Livingston G, Bass NJ, Gurling H, McQuillin A, Gwilliam R, Deloukas P, Al-Chalabi A, Shaw CE, Tsolaki M, Singleton AB, Guerreiro R, Muhleisen TW, Nothen MM, Moebus S, Jockel KH, Klopp N, Wichmann HE, Pankratz VS, Sando SB, Aasly JO, Barcikowska M, Wszolek ZK, Dickson DW, Graff-Radford NR, Petersen RC, van Duijn CM, Breteler MM, Ikram MA, DeStefano AL, Fitzpatrick AL, Lopez O, Launer LJ, Seshadri S, Berr C, Campion D, Epelbaum J, Dartigues JF, Tzourio C, Alperovitch A, Lathrop M, Feulner TM, Friedrich P, Riehle C, Krawczak M, Schreiber S, Mayhaus M, Nicolhaus S, Wagenpfeil S, Steinberg S, Stefansson H, Stefansson K, Snaedal J, Bjornsson S, Jonsson PV, Chouraki V, Genier-Boley B, Hiltunen M, Soininen H, Combarros O, Zelenika D, Delepine M, Bullido MJ, Pasquier F, Mateo I, Frank-Garcia A, Porcellini E, Hanon O, Coto E, Alvarez V, Bosco P, Siciliano G, Mancuso M, Panza F, Solfrizzi V, Nacmias B, Sorbi S, Bossu P, Piccardi P, Arosio B, Annoni G, Seripa D, Pilotto A, Scarpini E, Galimberti D, Brice A, Hannequin D, Licastro F, Jones L, Holmans PA, Jonsson T, Riemenschneider M, Morgan K, Younkin SG, Owen MJ, O'Donovan M, Amouyel P, Williams J (2011) Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. *Nat Genet* **43**, 429-435.

[94] Lambert JC, Ibrahim-Verbaas CA, Harold D, Naj AC, Sims R, Bellenguez C, DeStafano AL, Bis JC, Beecham GW, Grenier-Boley B, Russo G, Thorton-Wells TA, Jones N, Smith AV, Chouraki V, Thomas C, Ikram MA, Zelenika D, Vardarajan BN, Kamatani Y, Lin CF, Gerrish A, Schmidt H, Kunkle B, Dunstan ML, Ruiz A, Bihoreau MT, Choi SH, Reitz C, Pasquier F, Cruchaga C, Craig D, Amin N, Berr C, Lopez OL, De Jager PL, Deramecourt V, Johnston JA, Evans D, Lovestone S, Letenneur L, Moron FJ, Rubinsztein DC, Eiriksdottir G, Sleegers K, Goate AM, Fievet N, Huentelman MW, Gill M, Brown K, Kamboh MI, Keller L, Barberger-Gateau P, McGuiness B, Larson EB, Green R, Myers AJ, Dufouil C, Todd S, Wallon D, Love S, Rogaeva E, Gallacher J, St George-Hyslop P, Clarimon J, Lleo A, Bayer A, Tsuang DW,

Yu L, Tsolaki M, Bossu P, Spalletta G, Proitsi P, Collinge J, Sorbi S, Sanchez-Garcia F, Fox NC, Hardy J, Deniz Naranjo MC, Bosco P, Clarke R, Brayne C, Galimberti D, Mancuso M, Matthews F, Moebus S, Mecocci P, Del Zompo M, Maier W, Hampel H, Pilotto A, Bullido M, Panza F, Caffarra P, Nacmias B, Gilbert JR, Mayhaus M, Lannefelt L, Hakonarson H, Pichler S, Carrasquillo MM, Ingelsson M, Beekly D, Alvarez V, Zou F, Valladares O, Younkin SG, Coto E, Hamilton-Nelson KL, Gu W, Razquin C, Pastor P, Mateo I, Owen MJ, Faber KM, Jonsson PV, Combarros O, O'Donovan MC, Cantwell LB, Soininen H, Blacker D, Mead S, Mosley TH, Jr., Bennett DA, Harris TB, Fratiglioni L, Holmes C, de Bruijn RF, Passmore P, Montine TJ, Bettens K, Rotter JI, Brice A, Morgan K, Foroud TM, Kukull WA, Hannequin D, Powell JF, Nalls MA, Ritchie K, Lunetta KL, Kauwe JS, Boerwinkle E, Riemenschneider M, Boada M, Hiltuenen M, Martin ER, Schmidt R, Rujescu D, Wang LS, Dartigues JF, Mayeux R, Tzourio C, Hofman A, Nothen MM, Graff C, Psaty BM, Jones L, Haines JL, Holmans PA, Lathrop M, Pericak-Vance MA, Launer LJ, Farrer LA, van Duijn CM, Van Broeckhoven C, Moskvina V, Seshadri S, Williams J, Schellenberg GD, Amouyel P (2013) Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nat Genet* **45**, 1452-1458.

[95] Nelson PT, Pious NM, Jicha GA, Wilcock DM, Fardo DW, Estus S, Rebeck GW (2013) APOE-epsilon2 and APOE-epsilon4 correlate with increased amyloid accumulation in cerebral vasculature. *J Neuropathol Exp Neurol* **72**, 708-715.

[96] Rannikmae K, Kalaria RN, Greenberg SM, Chui HC, Schmitt FA, Samarasekera N, Al-Shahi Salman R, Sudlow CL (2014) APOE associations with severe CAA-associated vasculopathic changes: collaborative meta-analysis. *J Neurol Neurosurg Psychiatry* **85**, 300-305.

[97] Huynh TV, Davis AA, Ulrich JD, Holtzman DM (2017) Apolipoprotein E and Alzheimer's disease: the influence of apolipoprotein E on amyloid-beta and other amyloidogenic proteins. *J Lipid Res* **58**, 824-836.

[98] Leow AD, Klunder AD, Jack CR, Jr., Toga AW, Dale AM, Bernstein MA, Britson PJ, Gunter JL, Ward CP, Whitwell JL, Borowski BJ, Fleisher AS, Fox NC, Harvey D, Kornak J, Schuff N, Studholme C, Alexander GE, Weiner MW, Thompson PM (2006) Longitudinal stability of MRI for mapping brain change using tensor-based morphometry. *Neuroimage* **31**, 627-640.

[99] Wang J, Alsop DC, Song HK, Maldjian JA, Tang K, Salvucci AE, Detre JA (2003) Arterial transit time imaging with flow encoding arterial spin tagging (FEAST). *Magn Reson Med* **50**, 599-607.

[100] Ramage AE, Lin AL, Olvera RL, Fox PT, Williamson DE (2015) Resting-state regional cerebral blood flow during adolescence: associations with initiation of substance use and prediction of future use disorders. *Drug Alcohol Depend* **149**, 40-48.

[101] Brayne C, Richardson K, Matthews FE, Fleming J, Hunter S, Xuereb JH, Paykel E, Mukaetova-Ladinska EB, Huppert FA, O'Sullivan A, Dening T (2009) Neuropathological correlates of dementia in over-80-year-old brain donors from the population-based Cambridge city over-75s cohort (CC75C) study. *J Alzheimers Dis* **18**, 645-658.

[102] Ohm TG, Scharnagl H, Marz W, Bohl J (1999) Apolipoprotein E isoforms and the development of low and high Braak stages of Alzheimer's disease-related lesions. *Acta Neuropathol* **98**, 273-280.

[103] Johnston DW, Propper C, Shields MA (2009) Comparing subjective and objective measures of health: Evidence from hypertension for the income/health gradient. *J Health Econ* **28**, 540-552.

[104] Hachinski V, Iadecola C, Petersen RC, Breteler MM, Nyenhuis DL, Black SE, Powers WJ, DeCarli C, Merino JG, Kalaria RN, Vinters HV, Holtzman DM, Rosenberg GA, Wallin A, Dichgans M, Marler JR, Leblanc GG (2006) National Institute of Neurological Disorders and Stroke-Canadian Stroke Network vascular cognitive impairment harmonization standards. *Stroke* **37**, 2220-2241.

[105] Deramecourt V, Slade JY, Oakley AE, Perry RH, Ince PG, Maurage CA, Kalaria RN (2012) Staging and natural history of cerebrovascular pathology in dementia. *Neurology* **78**, 1043-1050.

[106] Nelson PT, Jicha GA, Schmitt FA, Liu H, Davis DG, Mendiondo MS, Abner EL, Markesbery WR (2007) Clinicopathologic correlations in a large Alzheimer disease center autopsy cohort: neuritic plaques and neurofibrillary tangles "do count" when staging disease severity. *J Neuropathol Exp Neurol* **66**, 1136-1146.

[107] Ferrer I, Bella R, Serrano MT, Marti E, Guionnet N (1990) Arteriolosclerotic leucoencephalopathy in the elderly and its relation to white matter lesions in Binswanger's disease, multi-infarct encephalopathy and Alzheimer's disease. *J Neurol Sci* **98**, 37-50.

[108] Desmond DW (2004) The neuropsychology of vascular cognitive impairment: is there a specific cognitive deficit? *J Neurol Sci* **226**, 3-7.

[109] Lammie GA, Brannan F, Slattery J, Warlow C (1997) Nonhypertensive cerebral small-vessel disease. An autopsy study. *Stroke* **28**, 2222-2229.

[110] Nelson PT, Smith CD, Abner EA, Schmitt FA, Scheff SW, Davis GJ, Keller JN, Jicha GA, Davis D, Wang-Xia W, Hartman A, Katz DG, Markesbery WR (2009) Human cerebral neuropathology of Type 2 diabetes mellitus. *Biochim Biophys Acta* **1792**, 454-469.

[111] Zarow C, Weiner MW, Ellis WG, Chui HC (2012) Prevalence, laterality, and comorbidity of hippocampal sclerosis in an autopsy sample. *Brain Behav* **2**, 435-442.

[112] Crystal HA, Schneider JA, Bennett DA, Leurgans S, Levine SR (2014) Associations of cerebrovascular and Alzheimer's disease pathology with brain atrophy. *Curr Alzheimer Res* **11**, 309-316.

[113] Fazekas F, Kleinert R, Offenbacher H, Schmidt R, Kleinert G, Payer F, Radner H, Lechner H (1993) Pathologic correlates of incidental MRI white matter signal hyperintensities. *Neurology* **43**, 1683-1689.

[114] Scheltens P, Barkhof F, Leys D, Wolters EC, Ravid R, Kamphorst W (1995) Histopathologic correlates of white matter changes on MRI in Alzheimer's disease and normal aging. *Neurology* **45**, 883-888.

[115] Shim YS, Yang DW, Roe CM, Coats MA, Benzinger TL, Xiong C, Galvin JE, Cairns NJ, Morris JC (2015) Pathological correlates of white matter hyperintensities on magnetic resonance imaging. *Dement Geriatr Cogn Disord* **39**, 92-104.

[116] Bal S, Goyal M, Smith E, Demchuk AM (2014) Central nervous system imaging in diabetic cerebrovascular diseases and white matter hyperintensities. *Handb Clin Neurol* **126**, 291-315.

[117] Erten-Lyons D, Woltjer R, Kaye J, Mattek N, Dodge HH, Green S, Tran H, Howieson DB, Wild K, Silbert LC (2013) Neuropathologic basis of white matter hyperintensity accumulation with advanced age. *Neurology* **81**, 977-983.

[118] Oishi M, Mochizuki Y (1999) Differences in regional cerebral blood flow in two types of leuko-araiosis. *J Neurol Sci* **164**, 129-133.

[119] Kobari M, Meyer JS, Ichijo M (1990) Leuko-araiosis, cerebral atrophy, and cerebral perfusion in normal aging. *Arch Neurol* **47**, 161-165.

[120] Promjunyakul N, Lahna D, Kaye JA, Dodge HH, Erten-Lyons D, Rooney WD, Silbert LC (2015) Characterizing the white matter hyperintensity penumbra with cerebral blood flow measures. *Neuroimage Clin* **8**, 224-229.
[121] Albert M, Massaro J, DeCarli C, Beiser A, Seshadri S, Wolf PA, Au R (2010) Profiles by sex of brain MRI and cognitive function in the framingham offspring study. *Alzheimer Dis Assoc Disord* **24**, 190-193.
[122] Au R, Massaro JM, Wolf PA, Young ME, Beiser A, Seshadri S, D'Agostino RB, DeCarli C (2006) Association of white matter hyperintensity volume with decreased cognitive functioning: the Framingham Heart Study. *Arch Neurol* **63**, 246-250.
[123] Luchsinger JA, Brickman AM, Reitz C, Cho SJ, Schupf N, Manly JJ, Tang MX, Small SA, Mayeux R, DeCarli C, Brown TR (2009) Subclinical cerebrovascular disease in mild cognitive impairment. *Neurology* **73**, 450-456.
[124] Williams LR, Hutchinson CE, Jackson A, Horan MA, Jones M, McInnes L, Rabbitt PM, Pendleton N (2010) Clinical correlates of cerebral white matter hyperintensities in cognitively normal older adults. *Arch Gerontol Geriatr* **50**, 127-131.
[125] Silbert LC, Dodge HH, Perkins LG, Sherbakov L, Lahna D, Erten-Lyons D, Woltjer R, Shinto L, Kaye JA (2012) Trajectory of white matter hyperintensity burden preceding mild cognitive impairment. *Neurology* **79**, 741-747.
[126] Nichols CG, Singh GK, Grange DK (2013) KATP channels and cardiovascular disease: suddenly a syndrome. *Circ Res* **112**, 1059-1072.
[127] Zhang HL, Bolton TB (1996) Two types of ATP-sensitive potassium channels in rat portal vein smooth muscle cells. *Br J Pharmacol* **118**, 105-114.
[128] Thorneloe KS, Maruyama Y, Malcolm AT, Light PE, Walsh MP, Cole WC (2002) Protein kinase C modulation of recombinant ATP-sensitive K(+) channels composed of Kir6.1 and/or Kir6.2 expressed with SUR2B. *J Physiol* **541**, 65-80.
[129] Yamada M, Isomoto S, Matsumoto S, Kondo C, Shindo T, Horio Y, Kurachi Y (1997) Sulphonylurea receptor 2B and Kir6.1 form a sulphonylurea-sensitive but ATP-insensitive K+ channel. *J Physiol* **499 (Pt 3)**, 715-720.
[130] Flagg TP, Enkvetchakul D, Koster JC, Nichols CG (2010) Muscle KATP channels: recent insights to energy sensing and myoprotection. *Physiol Rev* **90**, 799-829.
[131] Shi NQ, Ye B, Makielski JC (2005) Function and distribution of the SUR isoforms and splice variants. *J Mol Cell Cardiol* **39**, 51-60.
[132] Teramoto N (2006) Physiological roles of ATP-sensitive K+ channels in smooth muscle. *J Physiol* **572**, 617-624.
[133] Nelson PT, Jicha GA, Wang WX, Ighodaro E, Artiushin S, Nichols CG, Fardo DW (2015) ABCC9/SUR2 in the brain: Implications for hippocampal sclerosis of aging and a potential therapeutic target. *Ageing Res Rev*.
[134] Brown WR, Thore CR (2011) Review: cerebral microvascular pathology in ageing and neurodegeneration. *Neuropathol Appl Neurobiol* **37**, 56-74.
[135] Aaslid R (2006) Cerebral autoregulation and vasomotor reactivity. *Front Neurol Neurosci* **21**, 216-228.
[136] Craggs LJ, Yamamoto Y, Deramecourt V, Kalaria RN (2014) Microvascular pathology and morphometrics of sporadic and hereditary small vessel diseases of the brain. *Brain Pathol* **24**, 495-509.
[137] Polvikoski TM, van Straaten EC, Barkhof F, Sulkava R, Aronen HJ, Niinisto L, Oinas M, Scheltens P, Erkinjuntti T, Kalaria RN (2010) Frontal lobe white matter hyperintensities and neurofibrillary pathology in the oldest old. *Neurology* **75**, 2071-2078.

[138] Brown WR, Moody DM, Thore CR, Anstrom JA, Challa VR (2009) Microvascular changes in the white mater in dementia. *J Neurol Sci* **283**, 28-31.
[139] Charidimou A, Pantoni L, Love S (2016) The concept of sporadic cerebral small vessel disease: A road map on key definitions and current concepts. *Int J Stroke* **11**, 6-18.
[140] Schmitt FA, Nelson PT, Abner E, Scheff S, Jicha GA, Smith C, Cooper G, Mendiondo M, Danner DD, Van Eldik LJ, Caban-Holt A, Lovell MA, Kryscio RJ (2012) University of Kentucky Sanders-Brown healthy brain aging volunteers: donor characteristics, procedures and neuropathology. *Curr Alzheimer Res* **9**, 724-733.
[141] Bachstetter AD, Ighodaro ET, Hassoun Y, Aldeiri D, Neltner JH, Patel E, Abner EL, Nelson PT (2017) Rod-shaped microglia morphology is associated with aging in 2 human autopsy series. *Neurobiol Aging* **52**, 98-105.
[142] Ke MT, Fujimoto S, Imai T (2013) SeeDB: a simple and morphology-preserving optical clearing agent for neuronal circuit reconstruction. *Nat Neurosci* **16**, 1154-1161.
[143] Brenowitz WD, Kukull WA, Beresford SA, Monsell SE, Williams EC (2014) Social Relationships and Risk of Incident Mild Cognitive Impairment in US Alzheimer's Disease Centers. *Alzheimer Dis Assoc Disord*.
[144] Amador-Ortiz C, Dickson DW (2008) Neuropathology of hippocampal sclerosis. *Handb Clin Neurol* **89**, 569-572.
[145] Amador-Ortiz C, Lin WL, Ahmed Z, Personett D, Davies P, Duara R, Graff-Radford NR, Hutton ML, Dickson DW (2007) TDP-43 immunoreactivity in hippocampal sclerosis and Alzheimer's disease. *Ann Neurol* **61**, 435-445.
[146] Amador-Ortiz C, Ahmed Z, Zehr C, Dickson DW (2007) Hippocampal sclerosis dementia differs from hippocampal sclerosis in frontal lobe degeneration. *Acta Neuropathol (Berl)* **113**, 245-252.
[147] Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, Bruce J, Schuck T, Grossman M, Clark CM, McCluskey LF, Miller BL, Masliah E, Mackenzie IR, Feldman H, Feiden W, Kretzschmar HA, Trojanowski JQ, Lee VM (2006) Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* **314**, 130-133.
[148] Arai T, Hasegawa M, Akiyama H, Ikeda K, Nonaka T, Mori H, Mann D, Tsuchiya K, Yoshida M, Hashizume Y, Oda T (2006) TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Biochem Biophys Res Commun* **351**, 602-611.
[149] Keage HA, Hunter S, Matthews FE, Ince PG, Hodges J, Hokkanen S, Highley JR, Dening T, Brayne C (2014) TDP-43 in the Population: Prevalence and Associations with Dementia and Age. *J Alzheimers Dis*.
[150] Lee EB, Lee VM, Trojanowski JQ, Neumann M (2008) TDP-43 immunoreactivity in anoxic, ischemic and neoplastic lesions of the central nervous system. *Acta Neuropathol* **115**, 305-311.
[151] Markesbery WR, Schmitt FA, Kryscio RJ, Davis DG, Smith CD, Wekstein DR (2006) Neuropathologic substrate of mild cognitive impairment. *Arch Neurol* **63**, 38-46.
[152] Mathews M, Abner E, Caban-Holt A, Kryscio R, Schmitt F (2013) CERAD practice effects and attrition bias in a dementia prevention trial. *Int Psychogeriatr* **25**, 1115-1123.
[153] Abner EL, Dennis BC, Mathews MJ, Mendiondo MS, Caban-Holt A, Kryscio RJ, Schmitt FA, Crowley JJ (2012) Practice effects in a longitudinal, multi-center Alzheimer's disease prevention clinical trial. *Trials* **13**, 217.

[154] Mathews M, Abner E, Kryscio R, Jicha G, Cooper G, Smith C, Caban-Holt A, Schmitt FA (2014) Diagnostic accuracy and practice effects in the National Alzheimer's Coordinating Center Uniform Data Set neuropsychological battery. *Alzheimers Dement* **10**, 675-683.
[155] Zarow C, Wang L, Chui HC, Weiner MW, Csernansky JG (2011) MRI shows more severe hippocampal atrophy and shape deformation in hippocampal sclerosis than in Alzheimer's disease. *Int J Alzheimers Dis* **2011**, 483972.
[156] Thom M (2009) Hippocampal sclerosis: progress since Sommer. *Brain Pathol* **19**, 565-572.
[157] Attems J, Jellinger KA (2006) Hippocampal sclerosis in Alzheimer disease and other dementias. *Neurology* **66**, 775.
[158] Kuslansky G, Verghese J, Dickson D, Katz M, Busche H, Lipton RB (2004) Hippocampal sclerosis: cognitive consequences and contribution to dementia. *Neurology* **62**, A128-A129.
[159] Zabar Y, Carson KA, Troncoso JC, Kawas CH (1998) Dementia Due to Hippocampal Sclerosis: Clinical Features and Comparison to Alzheimer's Disease. *Neurology* **50 (4)**, A59-A60.
[160] Hatanpaa KJ, Raisanen JM, Herndon E, Burns DK, Foong C, Habib AA, White CL, 3rd (2014) Hippocampal sclerosis in dementia, epilepsy, and ischemic injury: differential vulnerability of hippocampal subfields. *J Neuropathol Exp Neurol* **73**, 136-142.
[161] Robinson JL, Geser F, Corrada MM, Berlau DJ, Arnold SE, Lee VM, Kawas CH, Trojanowski JQ (2011) Neocortical and hippocampal amyloid-beta and tau measures associate with dementia in the oldest-old. *Brain* **134**, 3708-3715.
[162] Murray ME, Cannon A, Graff-Radford NR, Liesinger AM, Rutherford NJ, Ross OA, Duara R, Carrasquillo MM, Rademakers R, Dickson DW (2014) Differential clinicopathologic and genetic features of late-onset amnestic dementias. *Acta Neuropathol*.
[163] Josephs KA, Whitwell JL, Weigand SD, Murray ME, Tosakulwong N, Liesinger AM, Petrucelli L, Senjem ML, Knopman DS, Boeve BF, Ivnik RJ, Smith GE, Jack CR, Jr., Parisi JE, Petersen RC, Dickson DW (2014) TDP-43 is a key player in the clinical features associated with Alzheimer's disease. *Acta Neuropathol*.
[164] Wilson RS, Yu L, Trojanowski JQ, Chen EY, Boyle PA, Bennett DA, Schneider JA (2013) TDP-43 pathology, cognitive decline, and dementia in old age. *JAMA Neurol* **70**, 1418-1424.
[165] Aoki N, Murray ME, Ogaki K, Fujioka S, Rutherford NJ, Rademakers R, Ross OA, Dickson DW (2014) Hippocampal sclerosis in Lewy body disease is a TDP-43 proteinopathy similar to FTLD-TDP Type A. *Acta Neuropathol*.
[166] Dickson DW, Baker M, Rademakers R (2010) Common variant in GRN is a genetic risk factor for hippocampal sclerosis in the elderly. *Neurodegener Dis* **7**, 170-174.
[167] Probst A, Taylor KI, Tolnay M (2007) Hippocampal sclerosis dementia: a reappraisal. *Acta Neuropathol* **114**, 335-345.
[168] Blass DM, Hatanpaa KJ, Brandt J, Rao V, Steinberg M, Troncoso JC, Rabins PV (2004) Dementia in hippocampal sclerosis resembles frontotemporal dementia more than Alzheimer disease. *Neurology* **63**, 492-497.
[169] Rauramaa T, Pikkarainen M, Englund E, Ince PG, Jellinger K, Paetau A, Alafuzoff I (2013) Consensus Recommendations on Pathologic Changes in the Hippocampus: A Postmortem Multicenter Inter-Rater Study. *J Neuropathol Exp Neurol* **72**, 452-461.
[170] Hatanpaa KJ, Blass DM, Pletnikova O, Crain BJ, Bigio EH, Hedreen JC, White CL, 3rd, Troncoso JC (2004) Most cases of dementia with hippocampal sclerosis may represent frontotemporal dementia. *Neurology* **63**, 538-542.

[171] Hatanpaa KJ, Bigio EH, Cairns NJ, Womack KB, Weintraub S, Morris JC, Foong C, Xiao G, Hladik C, Mantanona TY, White CL, 3rd (2008) TAR DNA-binding protein 43 immunohistochemistry reveals extensive neuritic pathology in FTLD-U: a midwest-southwest consortium for FTLD study. *J Neuropathol Exp Neurol* **67**, 271-279.

[172] Nicholson AM, Finch NA, Wojtas A, Baker MC, Perkerson RB, 3rd, Castanedes-Casey M, Rousseau L, Benussi L, Binetti G, Ghidoni R, Hsiung GY, Mackenzie IR, Finger E, Boeve BF, Ertekin-Taner N, Graff-Radford NR, Dickson DW, Rademakers R (2013) TMEM106B p.T185S regulates TMEM106B protein levels: implications for frontotemporal dementia. *J Neurochem* **126**, 781-791.

[173] Yu L, De Jager PL, Yang J, Trojanowski JQ, Bennett DA, Schneider JA (2015) The TMEM106B locus and TDP-43 pathology in older persons without FTLD. *Neurology*.

[174] Neary D, Snowden JS, Gustafson L, Passant U, Stuss D, Black S, Freedman M, Kertesz A, Robert PH, Albert M, Boone K, Miller BL, Cummings J, Benson DF (1998) Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. *Neurology* **51**, 1546-1554.

[175] Snowden JS, Rollinson S, Thompson JC, Harris JM, Stopford CL, Richardson AM, Jones M, Gerhard A, Davidson YS, Robinson A, Gibbons L, Hu Q, DuPlessis D, Neary D, Mann DM, Pickering-Brown SM (2012) Distinct clinical and pathological characteristics of frontotemporal dementia associated with C9ORF72 mutations. *Brain* **135**, 693-708.

[176] Mendez MF, Joshi A, Tassniyom K, Teng E, Shapira JS (2013) Clinicopathologic differences among patients with behavioral variant frontotemporal dementia. *Neurology* **80**, 561-568.

[177] Walker AK, Daniels CM, Goldman JE, Trojanowski JQ, Lee VM, Messing A (2014) Astrocytic TDP-43 pathology in Alexander disease. *J Neurosci* **34**, 6448-6458.

[178] Ling H, Holton JL, Lees AJ, Revesz T (2014) TDP-43 pathology is present in most post-encephalitic parkinsonism brains. *Neuropathol Appl Neurobiol* **40**, 654-657.

[179] McKee AC, Gavett BE, Stern RA, Nowinski CJ, Cantu RC, Kowall NW, Perl DP, Hedley-Whyte ET, Price B, Sullivan C, Morin P, Lee HS, Kubilus CA, Daneshvar DH, Wulff M, Budson AE (2010) TDP-43 proteinopathy and motor neuron disease in chronic traumatic encephalopathy. *J Neuropathol Exp Neurol* **69**, 918-929.

[180] Davidson YS, Raby S, Foulds PG, Robinson A, Thompson JC, Sikkink S, Yusuf I, Amin H, DuPlessis D, Troakes C, Al-Sarraj S, Sloan C, Esiri MM, Prasher VP, Allsop D, Neary D, Pickering-Brown SM, Snowden JS, Mann DM (2011) TDP-43 pathological changes in early onset familial and sporadic Alzheimer's disease, late onset Alzheimer's disease and Down's syndrome: association with age, hippocampal sclerosis and clinical phenotype. *Acta Neuropathol* **122**, 703-713.

[181] Lippa CF, Rosso AL, Stutzbach LD, Neumann M, Lee VM, Trojanowski JQ (2009) Transactive response DNA-binding protein 43 burden in familial Alzheimer disease and Down syndrome. *Arch Neurol* **66**, 1483-1488.

[182] Onyike CU, Pletnikova O, Sloane KL, Sullivan C, Troncoso JC, Rabins PV (2013) Hippocampal sclerosis dementia: An amnesic variant of frontotemporal degeneration. *Dement Neuropsychol* **7**, 83-87.

[183] Schneider JA, Aggarwal NT, Barnes L, Boyle P, Bennett DA (2009) The neuropathology of older persons with and without dementia from community versus clinic cohorts. *J Alzheimers Dis* **18**, 691-701.

[184] Sonnen JA, Larson EB, Crane PK, Haneuse S, Li G, Schellenberg GD, Craft S, Leverenz JB, Montine TJ (2007) Pathological correlates of dementia in a longitudinal, population-based sample of aging. *Ann Neurol* **62**, 406-413.

[185] Tschanz JT, Treiber K, Norton MC, Welsh-Bohmer KA, Toone L, Zandi PP, Szekely CA, Lyketsos C, Breitner JC (2005) A population study of Alzheimer's disease: findings from the Cache County Study on Memory, Health, and Aging. *Care Manag J* **6**, 107-114.
[186] Giasson BI, Forman MS, Higuchi M, Golbe LI, Graves CL, Kotzbauer PT, Trojanowski JQ, Lee VM (2003) Initiation and synergistic fibrillization of tau and alpha-synuclein. *Science* **300**, 636-640.
[187] Lippa CF, Fujiwara H, Mann DM, Giasson B, Baba M, Schmidt ML, Nee LE, O'Connell B, Pollen DA, St George-Hyslop P, Ghetti B, Nochlin D, Bird TD, Cairns NJ, Lee VM, Iwatsubo T, Trojanowski JQ (1998) Lewy bodies contain altered alpha-synuclein in brains of many familial Alzheimer's disease patients with mutations in presenilin and amyloid precursor protein genes. *Am J Pathol* **153**, 1365-1370.
[188] Rosenberg CK, Pericak-Vance MA, Saunders AM, Gilbert JR, Gaskell PC, Hulette CM (2000) Lewy body and Alzheimer pathology in a family with the amyloid-beta precursor protein APP717 gene mutation. *Acta Neuropathol* **100**, 145-152.
[189] White L, Petrovitch H, Hardman J, Nelson J, Davis DG, Ross GW, Masaki K, Launer L, Markesbery WR (2002) Cerebrovascular pathology and dementia in autopsied Honolulu-Asia Aging Study participants. *Ann N Y Acad Sci* **977**, 9-23.
[190] Bennett DA, Schneider JA, Arvanitakis Z, Kelly JF, Aggarwal NT, Shah RC, Wilson RS (2006) Neuropathology of older persons without cognitive impairment from two community-based studies. *Neurology* **66**, 1837-1844.
[191] Troncoso JC, Kawas CH, Chang CK, Folstein MF, Hedreen JC (1996) Lack of association of the apoE4 allele with hippocampal sclerosis dementia. *Neurosci Lett* **204**, 138-140.
[192] Uchikado H, Lin WL, DeLucia MW, Dickson DW (2006) Alzheimer disease with amygdala Lewy bodies: a distinct form of alpha-synucleinopathy. *J Neuropathol Exp Neurol* **65**, 685-697.
[193] Gribble FM, Reimann F (2003) Differential selectivity of insulin secretagogues: mechanisms, clinical implications, and drug interactions. *J Diabetes Complications* **17**, 11-15.
[194] Lawson K (2000) Potassium channel openers as potential therapeutic weapons in ion channel disease. *Kidney Int* **57**, 838-845.
[195] Josephs KA, Murray ME, Whitwell JL, Parisi JE, Petrucelli L, Jack CR, Petersen RC, Dickson DW (2014) Staging TDP-43 pathology in Alzheimer's disease. *Acta Neuropathol* **127**, 441-450.
[196] Tang MX, Cross P, Andrews H, Jacobs DM, Small S, Bell K, Merchant C, Lantigua R, Costa R, Stern Y, Mayeux R (2001) Incidence of AD in African-Americans, Caribbean Hispanics, and Caucasians in northern Manhattan. *Neurology* **56**, 49-56.
[197] Tang MX, Stern Y, Marder K, Bell K, Gurland B, Lantigua R, Andrews H, Feng L, Tycko B, Mayeux R (1998) The APOE-epsilon4 allele and the risk of Alzheimer disease among African Americans, whites, and Hispanics. *Jama* **279**, 751-755.
[198] Green RC, Cupples LA, Go R, Benke KS, Edeki T, Griffith PA, Williams M, Hipps Y, Graff-Radford N, Bachman D, Farrer LA (2002) Risk of dementia among white and African American relatives of patients with Alzheimer disease. *Jama* **287**, 329-336.
[199] Graff-Radford NR, Besser LM, Crook JE, Kukull WA, Dickson DW (2016) Neuropathologic differences by race from the National Alzheimer's Coordinating Center. *Alzheimers Dement* **12**, 669-677.
[200] Riudavets MA, Rubio A, Cox C, Rudow G, Fowler D, Troncoso JC (2006) The prevalence of Alzheimer neuropathologic lesions is similar in blacks and whites. *J Neuropathol Exp Neurol* **65**, 1143-1148.

[201] Sandberg G, Stewart W, Smialek J, Troncoso JC (2001) The prevalence of the neuropathological lesions of Alzheimer's disease is independent of race and gender. *Neurobiol Aging* **22**, 169-175.
[202] Miller FD, Hicks SP, D'Amato CJ, Landis JR (1984) A descriptive study of neuritic plaques and neurofibrillary tangles in an autopsy population. *Am J Epidemiol* **120**, 331-341.
[203] Wilkins CH, Grant EA, Schmitt SE, McKeel DW, Morris JC (2006) The neuropathology of Alzheimer disease in African American and white individuals. *Arch Neurol* **63**, 87-90.
[204] de la Monte SM, Hutchins GM, Moore GW (1989) Racial differences in the etiology of dementia and frequency of Alzheimer lesions in the brain. *J Natl Med Assoc* **81**, 644-652.
[205] (2015), ed. U.S. Dept. of Health and Human Services OotS, Office of the Assistant Secretary for Planning and Evaluation and Office of Minority Health, Washington, DC.
[206] (2010), ed. U.S. Census Bureau PEPP.
[207] Savitt TL (1982) The use of blacks for medical experimentation and demonstration in the Old South. *J South Hist* **48**, 331-348.
[208] Goodson MG (2003) Enslaved Africans and doctors in South Carolina. *J Natl Med Assoc* **95**, 225-233.
[209] Washington HA (2006) *Medical Apartheid: The Dark History of Medical Experimentation on Black Americans from Colonial Times to the Present*, Anchor Books, New York.
[210] Byrd WM, Clayton LA (2001) Race, medicine, and health care in the United States: a historical survey. *J Natl Med Assoc* **93**, 11s-34s.
[211] Garrod JZ (2006) A brave old world: an analysis of scientific racism and BiDil. *Mcgill J Med* **9**, 54-60.
[212] Savitt TL (2005) Black health on the plantation: owners, the enslaved, and physicians. *OAH Magazine of History* **19**, 14-16.
[213] Jones WF (1854) On the Utility of the Applications of Hot Water to the Spine in the Treatment of Typhoid Pneumonia. *Virginia Medical and Surgical Journal 3*, 108-110.
[214] Randall VR (2006) Slavery, Segregation and Racism: Trusting The Health Care System Ain't Always Easy In *Dying While Black* Seven Principle Press, Dayton, OH, p. 120.
[215] Harden JMB (1846) Some Experiments to Determine the Relative Areas of the Trunks and Branches of Arteries. *Southern Medical and Surgical Journal*.
[216] Ojanuga D (1993) The medical ethics of the 'father of gynaecology', Dr J Marion Sims. *J Med Ethics* **19**, 28-31.
[217] Axelsen DE (1985) Women as Victims of Medical Experimentation: J. Marion Sims' Surgery on Slave Women, 1845-1850. *Sage* **2**, 10.
[218] Corbie-Smith G (1999) The continuing legacy of the Tuskegee Syphilis Study: considerations for clinical investigation. *Am J Med Sci* **317**, 5-8.
[219] King PA (1992) Twenty years after. The legacy of the Tuskegee Syphilis Study. The dangers of difference. *Hastings Cent Rep* **22**, 35-38.
[220] Randall VR (2006) Slavery, Segregation and Racism: Trusting The Health Care System Ain't Always Easy In *Dying While Black* Seven Principle Press, Dayton, OH, p. 121.
[221] Boyd MF, Kitchen, S. F. (1937) Observations on Induced Falciparum. *American Journal of Tropical Medicine 17*, 213-235.
[222] Katz A (1996) in *Philadelphia Bulletin*, p. 1.
[223] Hornblum AM (1998) *Acers of Skin: Human Experiments at Holmesburg Prison*, Routledge, New York.
[224] Corbie-Smith G, Thomas SB, St George DM (2002) Distrust, race, and research. *Arch Intern Med* **162**, 2458-2463.

[225] Hughes TB, Varma VR, Pettigrew C, Albert MS (2015) African Americans and Clinical Research: Evidence Concerning Barriers and Facilitators to Participation and Recruitment Recommendations. *Gerontologist*.
[226] Braunstein JB, Sherber NS, Schulman SP, Ding EL, Powe NR (2008) Race, medical researcher distrust, perceived harm, and willingness to participate in cardiovascular prevention trials. *Medicine (Baltimore)* **87**, 1-9.
[227] George S, Duran N, Norris K (2014) A systematic review of barriers and facilitators to minority research participation among African Americans, Latinos, Asian Americans, and Pacific Islanders. *Am J Public Health* **104**, e16-31.
[228] Guadagnoli E, McNamara P, Evanisko MJ, Beasley C, Callender CO, Poretsky A (1999) The influence of race on approaching families for organ donation and their decision to donate. *Am J Public Health* **89**, 244-247.
[229] Lambe S, Cantwell N, Islam F, Horvath K, Jefferson AL (2011) Perceptions, knowledge, incentives, and barriers of brain donation among African American elders enrolled in an Alzheimer's research program. *Gerontologist* **51**, 28-38.
[230] Bonner GJ, Darkwa OK, Gorelick PB (2000) Autopsy recruitment program for African Americans. *Alzheimer Dis Assoc Disord* **14**, 202-208.
[231] Jefferson AL, Lambe S, Cook E, Pimontel M, Palmisano J, Chaisson C (2011) Factors associated with African American and White elders' participation in a brain donation program. *Alzheimer Dis Assoc Disord* **25**, 11-16.
[232] Siminoff LA, Burant CJ, Ibrahim SA (2006) Racial disparities in preferences and perceptions regarding organ donation. *J Gen Intern Med* **21**, 995-1000.
[233] Minniefield WJ, Yang J, Muti P (2001) Differences in attitudes toward organ donation among African Americans and whites in the United States. *J Natl Med Assoc* **93**, 372-379.
[234] Barnes LL, Shah RC, Aggarwal NT, Bennett DA, Schneider JA (2012) The Minority Aging Research Study: ongoing efforts to obtain brain donation in African Americans without dementia. *Curr Alzheimer Res* **9**, 734-745.
[235] Wyatt SB, Williams DR, Calvin R, Henderson FC, Walker ER, Winters K (2003) Racism and cardiovascular disease in African Americans. *Am J Med Sci* **325**, 315-331.
[236] Wang VO, Sue S (2005) In the eye of the storm: race and genomics in research and practice. *Am Psychol* **60**, 37-45.
[237] Lin SS, Kelsey JL (2000) Use of race and ethnicity in epidemiologic research: concepts, methodological issues, and suggestions for research. *Epidemiol Rev* **22**, 187-202.
[238] Tishkoff SA, Kidd KK (2004) Implications of biogeography of human populations for 'race' and medicine. *Nat Genet* **36**, S21-27.
[239] Williams DR (1997) Race and health: basic questions, emerging directions. *Ann Epidemiol* **7**, 322-333.
[240] Guthrie RV (2003) *Even The Rat Was White: A Historical View of Psychology*, Peasron.
[241] Foster MW (2009) Looking for race in all the wrong places: analyzing the lack of productivity in the ongoing debate about race and genetics. *Hum Genet* **126**, 355-362.
[242] Foster MW, Sharp RR (2002) Race, ethnicity, and genomics: social classifications as proxies of biological heterogeneity. *Genome Res* **12**, 844-850.
[243] Brace CL (2002) The concept of race in physical anthropology In *Physical Anthropology Original Reading on Method and Practice*, Peregrine PN, Ember, C. R., Ember, M. R., ed. Prentice Hall, p. 280.
[244] Montagu A (1962) The concept of race. *American Anthropologist* **64**, 919-928.
[245] Gould SJ (1981) *The Mismeasure of Man*, W. W. Norton & Company, New York.

[246] Dilworth-Anderson P, Hendrie HC, Manly JJ, Khachaturian AS, Fazio S (2008) Diagnosis and assessment of Alzheimer's disease in diverse populations. *Alzheimers Dement* **4**, 305-309.
[247] Manly JJ (2005) Advantages and disadvantages of separate norms for African Americans. *Clin Neuropsychol* **19**, 270-275.
[248] Manly JJ (2006) Deconstructing race and ethnicity: implications for measurement of health outcomes. *Med Care* **44**, S10-16.
[249] Tashiro CJ (2005) The meaning of race in healthcare and research--part 2. Current controversies and emerging research. *Pediatr Nurs* **31**, 305-308.
[250] Bryc K, Durand EY, Macpherson JM, Reich D, Mountain JL (2015) The genetic ancestry of African Americans, Latinos, and European Americans across the United States. *Am J Hum Genet* **96**, 37-53.
[251] Lee SS (2005) Racializing drug design: implications of pharmacogenomics for health disparities. *Am J Public Health* **95**, 2133-2138.
[252] Bleich SN, Jarlenski MP, Bell CN, LaVeist TA (2012) Health inequalities: trends, progress, and policy. *Annu Rev Public Health* **33**, 7-40.
[253] Kawachi I, Daniels N, Robinson DE (2005) Health disparities by race and class: why both matter. *Health Aff (Millwood)* **24**, 343-352.
[254] Mayberry RM, Mili, F., Ofili, E. (2002) Race and Ethnic Differences in Access to Medical Care In *Race, Ethnicity, and Health*, LaVeist TA, ed. Jossey-Bass, A Wiley Imprint, San Francisco, CA, p. 701.
[255] DeNavas-Walt C, Proctor, B.D., Smith, J.C. (2013), ed. Bureau USC U.S. Government Printing Office, Washington, D.C.
[256] Ward B.W. SJS, Freeman G., Clarke T.C. (2015), ed. Statistics NCfH.
[257] Williams DR, Collins C (2001) Racial residential segregation: a fundamental cause of racial disparities in health. *Public Health Rep* **116**, 404-416.
[258] Institute of Medicine Committee on the R, Assessment of the NsSRP, Budget to R, Ultimately Eliminate Health D (2006) Health Disparities: Concepts, Measurements, and Understanding: Unfinished Business In *Examining the Health Disparities Research Plan of the National Institutes of Health: Unfinished Business*, Thomson GE, Mitchell F, Williams MB, eds. National Academies Press (US)

National Academy of Sciences., Washington (DC), pp. 21-33.
[259] Waldstein SR, Dore GA, Davatzikos C, Katzel LI, Gullapalli R, Seliger SL, Kouo T, Rosenberger WF, Erus G, Evans MK, Zonderman AB (2016) Differential Associations of Socioeconomic Status With Global Brain Volumes and White Matter Lesions in African American and White Adults: the HANDLS SCAN Study. *Psychosom Med*.
[260] Dore GA, Waldstein SR, Evans MK, Zonderman AB (2015) Associations Between Diabetes and Cognitive Function in Socioeconomically Diverse African American and White Men and Women. *Psychosom Med* **77**, 643-652.
[261] Glymour MM, Avendano M, Haas S, Berkman LF (2008) Lifecourse social conditions and racial disparities in incidence of first stroke. *Ann Epidemiol* **18**, 904-912.
[262] Yaffe K, Falvey C, Harris TB, Newman A, Satterfield S, Koster A, Ayonayon H, Simonsick E (2013) Effect of socioeconomic disparities on incidence of dementia among biracial older adults: prospective study. *Bmj* **347**, f7051.
[263] Prevention CfDCa (2014), ed. Services USDoHaH, Atlanta, GA.
[264] (2015), ed. National Science Foundation NCfSaES, Arlington, VA.

[265] Valantine HA, Collins FS (2015) National Institutes of Health addresses the science of diversity. *Proc Natl Acad Sci U S A* **112**, 12240-12242.
[266] (2014) Association of American Medical Colleges (AAMC), Washington, DC.
[267] Hong L, Page SE (2004) Groups of diverse problem solvers can outperform groups of high-ability problem solvers. *Proc Natl Acad Sci U S A* **101**, 16385-16389.
[268] Campbell LG, Mehtani S, Dozier ME, Rinehart J (2013) Gender-heterogeneous working groups produce higher quality science. *PLoS One* **8**, e79147.
[269] Freeman RB HW (2014) Collaborating with people like me: Ethnic co-authorship within the US (No. w19905).
[270] Kittles R, Royal, C. (2003) The Genetics of African Americans: Implications for Disease Gene Mapping and Identity In *Genetic Nature/culture: Anthropology and Science beyond the Two-Culture Divide* University of California Press, Berkeley, CA, p. 225.
[271] Dennis BP, Neese JB (2000) Recruitment and retention of African American elders into community-based research: lessons learned. *Arch Psychiatr Nurs* **14**, 3-11.
[272] Schnieders T, Danner DD, McGuire C, Reynolds F, Abner E (2013) Incentives and barriers to research participation and brain donation among African Americans. *Am J Alzheimers Dis Other Demen* **28**, 485-490.
[273] Zaramo CE, Morton T, Yoo JW, Bowen GR, Modlin CS (2008) Culturally competent methods to promote organ donation rates among African-Americans using venues of the Bureau of Motor Vehicles. *Transplant Proc* **40**, 1001-1004.
[274] Dunlop AL, Leroy ZC, Logue KM, Glanz K, Dunlop BW (2011) Preconsent education about research processes improved African Americans' willingness to participate in clinical research. *J Clin Epidemiol* **64**, 872-877.
[275] Darnell KR, McGuire C, Danner DD (2011) African American participation in Alzheimer's disease research that includes brain donation. *Am J Alzheimers Dis Other Demen* **26**, 469-476.
[276] Gorelick PB, Harris Y, Burnett B, Bonecutter FJ (1998) The recruitment triangle: reasons why African Americans enroll, refuse to enroll, or voluntarily withdraw from a clinical trial. An interim report from the African-American Antiplatelet Stroke Prevention Study (AAASPS). *J Natl Med Assoc* **90**, 141-145.
[277] Layton RL, Brandt PD, Freeman AM, Harrell JR, Hall JD, Sinche M (2016) Diversity Exiting the Academy: Influential Factors for the Career Choice of Well-Represented and Underrepresented Minority Scientists. *CBE Life Sci Educ* **15**.
[278] Rodriguez JE, Campbell KM, Mouratidis RW (2014) Where are the rest of us? Improving representation of minority faculty in academic medicine. *South Med J* **107**, 739-744.

Vita

Date Prepared: 06-13-2017

Eseosa Tinuke Ighodaro

UNIVERSITY OF KENTUCKY MEDICAL CENTER

Education

MD Candidate
College of Medicine, University of Kentucky
Expected Graduation Date: May 2019

University of Kentucky, Lexington, Kentucky
Bachelor of Science in Biology
Minors in Chemistry and French
GPA: 3.85, May 2011

Research Experience

Neuropathology May 2013 – Present
Center on Aging in College of Medicine, University of Kentucky
Working with Peter Nelson, MD, PhD on the risk factors, cognitive sequelae, and co-pathologies of two small vessel pathologies in the aged brain

Health Outcomes February 2012 – Present
Department of Behavioral Science in College of Medicine, University of Kentucky
Worked with Hannah Knudsen, PhD (mentor) to design a data collection and survey protocol using RedCap and SPSS to collect and analyze information from participants in 2012 UK College of Medicine Multicultural Health Fair

Neurosurgery April 2012 – Present
Department of Neurosurgery in College of Medicine, University of Kentucky
Worked with Justin Fraser, MD to review literature and write a chart review on different types of stents used in the angiographic treatment of cerebral aneurysms

Chronobiology June 2011 - August 2011
Department of Anatomy and Neurobiology (now Neuroscience) in College of Medicine, University of Kentucky
Aided Marilyn J. Duncan, PhD (PI) in quantifying circadian rhythm gene expression in the suprachiasmatic nucleus of the brain in aging and Alzheimer's mice using in-situ hybridization

Yeast Physiology January 2009 - May 2011
Department of Molecular & Cellular Biochemistry in College of Medicine, University of Kentucky
Assisted Robert C. Dickson, PhD (PI) in studying the signaling pathway responsible for the viability of *Saccharomyces cerevisiae* in iron deficient conditions by conducting lifespan experiments

Neuroimmunology June 2010 - August 2010
University of Lille, Lille, France
Assisted Pierre-Eric Sautiere PhD (mentor) and Michel Salzet PhD (PI) in characterizing surface protein receptor involved in neuronal regeneration in medicinal leech using high performance liquid chromatography.

Bacterial Physiology June 2009 - August 2009
Department of Developmental Biology in School of Medicine, Stanford University
Assisted Grant R. Bowman, PhD (mentor) and Luck Shapiro PhD (PI) in conducting a domain analysis through the use of fluorescence and light microscopy on a protein essential for asymmetrical cell division in *Caulobacter Crescentus*

Research Fellowships

F30 NRSA Individual Predoctoral MD/PhD or Other Dual-Doctoral Degree Fellowship, University of Kentucky, December 2016 – December 2018, $28,505 (2016)

R01 Diversity Supplement: Modulation of microRNA pathways by gemfibrozil as a potential therapy for Alzheimer's Disease, University of Kentucky, January 2015 – December 2016, $69,574

NIH pre-doctoral training grant: Cellular and Molecular Aspects of Brain Aging, University of Kentucky, September 2013 – September 2014, $24,000

Professional Student Mentored Research Fellowship, August 2012 – May 2013, University of Kentucky College of Medicine; $3,000

Summer Research Fellowship, June 2011 – July 2011, University of Kentucky College of Medicine, $800

National Institutes of Health: Minority Health and Healthcare Disparities International Research Training Program (MHIRT), Summer 2010, Université de Lille (covered room, board, stipend, travel, health insurance)

Amgen Scholars Fellowship Program, Summer 2009, Stanford University, (covered room, board, stipend, travel, health insurance)

Appalachian & Minority Science, Technology, Engineering, and Mathematics Research Fellowship August 2009-August 2011, University of Kentucky, $4,000

Louis Stokes Alliance for Minority Participation Research Fellowship, University of Kentucky August 2010 – August 2011, $500

Academic Honors

"Levis D. and Margot D. McCullers Fund for Research and Education on Alzheimer's Disease and Related Dementias" Scholar Award, 2016, Sanders-Brown Center on Aging, $1,000

Robert Terry Award: Honorary Mention for the Best Paper on Neurodegenerative Diseases. June 2015, American Association of Neuropathologist 2015 conference (first-author poster presentation)

Hirano Award for Best Paper on Neurodegenerative Diseases, June 2014, American Association of Neuropathologist 2014 conference (co-author presentation)

Summa Cum Laude, August 2011, University of Kentucky

Honors in Biology, August 2011, University of Kentucky

Honors in Honors Program, August 2011, University of Kentucky

Chellgren Scholars Program, August 2008 – May 2011, University of Kentucky

Kentucky Governor's Scholar Program, June 2006, Morehead University

William C. Parker Diversity Full Ride Scholarship, August 2007 – May 2011, University of Kentucky

Martin Luther King Volunteer and Leadership Award, August 2010, University of Kentucky

Memberships

American Physician Scientist Association (APSA)
Student National Medical Association (SNMA)

Journal Reviewer

Alzheimer's & Dementia, January 2016 - Present

Manuscripts in Preparations

1. Ighodaro ET et al, A Neuropathologic Feature of Brain Aging: Multi-lumen vascular profiles, *In Preparations*

Manuscripts in Revisions

1. Ighodaro ET et al, Challenges and Considerations for Studying Dementia in African Americans, Journal of Alzheimer's Disease, *In Revisions*

Peer-Reviewed Publications

1. Outcomes after diagnosis of mild cognitive impairment in a large autopsy series.
   Abner EL, Kryscio RJ, Schmitt FA, Fardo DW, Moga DC, **Ighodaro ET**, Jicha GA, Yu L, Dodge HH, Xiong C, Woltjer RL, Schneider JA, Cairns NJ, Bennett DA, Nelson PT. Ann Neurol. 2017 Apr;81(4):549-559. doi: 10.1002/ana.24903. Epub 2017 Mar 22. PMID: 28224671

2. Rod-shaped microglia morphology is associated with aging in 2 human autopsy series.
   Bachstetter AD, **Ighodaro ET**, Hassoun Y, Aldeiri D, Neltner JH, Patel E, Abner EL, Nelson PT. Neurobiol Aging. 2017 Apr;52:98-105. doi: 10.1016/j.neurobiolaging.2016.12.028. Epub 2017 Jan 5.PMID: 28131016

3. Overlapping but distinct TDP-43 and tau pathologic patterns in aged hippocampi.
   Smith VD, Bachstetter AD, **Ighodaro E**, Roberts K, Abner EL, Fardo DW, Nelson PT. Brain Pathol. 2017 Mar 9. doi: 10.1111/bpa.12505. [Epub ahead of print] PMID: 28281308

4. Risk factors and global cognitive status related to brain arteriolosclerosis in elderly individuals.
   Ighodaro ET, Abner EL, Fardo DW, Lin AL, Katsumata Y, Schmitt FA, Kryscio RJ, Jicha GA, Neltner JH, Monsell SE, Kukull WA, Moser DK, Appiah F, Bachstetter AD, Van Eldik LJ; Alzheimer's Disease Neuroimaging Initiative (ADNI)., Nelson PT. J Cereb Blood Flow Metab. 2017 Jan;37(1):201-216. Epub 2016 Jan 6. PMID: 26738751

5. "New Old Pathologies": AD, PART, and Cerebral Age-Related TDP-43 With Sclerosis (CARTS).
   Nelson PT, Trojanowski JQ, Abner EL, Al-Janabi OM, Jicha GA, Schmitt FA, Smith CD, Fardo DW, Wang WX, Kryscio RJ, Neltner JH, Kukull WA, Cykowski MD, Van Eldik LJ, **Ighodaro ET**.J Neuropathol Exp Neurol. 2016 Jun;75(6):482-98. doi: 10.1093/jnen/nlw033. Epub 2016 May 21. Review. PMID: 27209644
6. ABCC9/SUR2 in the brain: Implications for hippocampal sclerosis of aging and a potential therapeutic target.
   Nelson PT, Jicha GA, Wang WX, **Ighodaro E**, Artiushin S, Nichols CG, Fardo DW. Ageing Res Rev. 2015 Nov;24(Pt B):111-25. doi: 10.1016/j.arr.2015.07.007. Epub 2015 Jul 28. Review. PMID:26226329

7. Novel human ABCC9/SUR2 brain-expressed transcripts and an eQTL relevant to hippocampal sclerosis of aging.
   Nelson PT, Wang WX, Wilfred BR, Wei A, Dimayuga J, Huang Q, **Ighodaro E**, Artiushin S, Fardo DW. J Neurochem. 2015 Sep;134(6):1026-39. doi: 10.1111/jnc.13202. Epub 2015 Jul 15. PMID: 26115089

8. Hippocampal Sclerosis of Aging Can Be Segmental: Two Cases and Review of the Literature.
   Ighodaro ET, Jicha GA, Schmitt FA, Neltner JH, Abner EL, Kryscio RJ, Smith CD, Duplessis T, Anderson S, Patel E, Bachstetter A, Van Eldik LJ, Nelson PT. J Neuropathol Exp Neurol. 2015 Jul;74(7):642-52. doi: 10.1097/NEN.0000000000000204. Review. PMID: 26083567

9. Disease-related microglia heterogeneity in the hippocampus of Alzheimer's disease, dementia with Lewy bodies, and hippocampal sclerosis of aging.
   Bachstetter AD, Van Eldik LJ, Schmitt FA, Neltner JH, **Ighodaro ET**, Webster SJ, Patel E, Abner EL, Kryscio RJ, Nelson PT.
   Acta Neuropathol Commun. 2015 May 23;3:32. doi: 10.1186/s40478-015-0209-z. PMID: 26001591

10. ABCC9 gene polymorphism is associated with hippocampal sclerosis of aging pathology.
    Nelson PT, Estus S, Abner EL, Parikh I, Malik M, Neltner JH, **Ighodaro E**, Wang WX, Wilfred BR, Wang LS, Kukull WA, Nandakumar K, Farman ML, Poon WW, Corrada MM, Kawas CH, Cribbs DH, Bennett DA, Schneider JA, Larson EB, Crane PK, Valladares O, Schmitt FA, Kryscio RJ, Jicha GA, Smith CD, Scheff SW, Sonnen JA, Haines JL, Pericak-Vance MA, Mayeux R, Farrer LA, Van Eldik LJ, Horbinski C, Green RC, Gearing M, Poon LW, Kramer PL, Woltjer RL, Montine TJ, Partch AB, Rajic AJ, Richmire K, Monsell SE; Alzheimer' Disease Genetic Consortium., Schellenberg GD, Fardo DW.
    Acta Neuropathol. 2014;127(6):825-43. doi: 10.1007/s00401-014-1282-2. Epub 2014 Apr 27. PMID: 24770881

11. Oligomerization and higher-order assembly contribute to sub-cellular localization of a bacterial scaffold.
    Bowman GR, Perez AM, Ptacin JL, **Ighodaro E**, Folta-Stogniew E, Comolli LR, Shapiro L. Mol Microbiol. 2013 Nov;90(4):776-95. doi: 10.1111/mmi.12398. Epub 2013 Oct 7. PMID: 24102805

Books and Manuals

1. Stanley, J.T., Busse, K.L., Arnold, D., Baker, A., Boone, S., Bunch, D., Clements, M., Dewan, R., Gering, C.M., Hester, J., Ifeachor, W.N., Ighodaro, E.T., Imel, J.R., Irvin, M.W., Maijub, J.G., Riesing, R.R., Sawant, D.K., Schlierf, T.J., Starling, M.P., Kochhar, R.S., Duhr, E.F., Todd, T.B., Selegue, J.P., Soult, A.S., Woodrum, K.R., Yates, S.W., Bajue, S.A., "Commonwealth

Chemical Demonstrations" ed. Bramwell, F.B., Empire Science Resources, LLC., 2010. 320 pages.

Invited Guest Speaker Presentations

Invited Guest Speaker: 10th Annual Showcase of Undergraduate Scholars, University of Kentucky, April 2016
Invited Guest Speaker: Center for Equality and Social Justice Symposium on Social Justice within Schools, University of Kentucky, March 2016

Oral Conference Presentations

1. Ighodaro, E.T., Nelson, P.T., (2016, June). Brain Arteriolosclerosis Risk Factors and Cognitive Impairment. Oral presentation given at 2016 Neurology Trainee Research Day, University of Kentucky, Lexington, Kentucky.
2. Ighodaro, E.T., Bowman, G.R., Shapiro, L., (2009, August). Domain Analysis of the Polar Organizing Protein: PopZ. Oral presentation Stanford Summer Research Program Symposium, 2009, Palo Alto, California
3. Ighodaro, E.T., Bramwell, F.B., (2009, April). Enhancing Chemistry Understanding with 21st Century Technology. Oral presentation given at the annual meeting of National Conference of Undergraduate Research (NCUR), 2009, La Crosse, Wisconsin.

Selected Poster Presentations

1. Ighodaro E.T., Abner E.L., Monsell S.E., Kukull W.A., Neltner J.H., Smith V., Fardo D., Nelson P.T., (2016, June) Elucidating Subtypes and Risk Factors of Brain Arteriolosclerosis (B-ASC). American Association of Neuropathologists, Baltimore, MD

2. Bachstetter A.D., Van Eldik L.J., Ighodaro, E.T., Abner E.L., Nelson P.T., (2015, October) Microglia heterogeneity in the hippocampus of Alzheimer's disease, dementia with Lewy bodies, and hippocampal sclerosis of aging. Society for Neuroscience, Chicago, IL

3. Ighodaro E., Abner E., Monsell S., Kukull W., Nelson P., (2015 June) Decoding the Risk Factors and Cognitive Consequences of a Common Vascular Pathology: Brain Arteriolosclerosis. American Association of Neuropathologists, Denver, CO

4. Ighodaro E.T., Abner E.L., Nelson P.T., Brain Arteriolosclerosis: Elucidating Clinical Risk Factors and Neurocognitive Consequences in Aged Individuals Using a Longitudinal Human Dataset (2015, March), UK Center for Clinical and Translational Science 10th Annual Spring Conference, Lexington, KY

5. Nelson P., Abner E., Kukull W., Monsell S., Ighodaro E., Fardo D., (2014 June) Association Between Hippocampal Sclerosis of Aging (HS-Aging)

Pathology and Sulfonylurea Drug Exposure in NACC. American Association of Neuropathologists, Denver, CO

6. Brown, A.T., Kline IV, R.H., Lyons, D.N., Ighodaro, E.T., Nelson, P.T., Miller, C.S., Westlund, K.N., (March 2014) Characterization of Cortical pERK Expression After Trigeminal Inflammatory Compression (TIC) of the Infraorbital Nerve, Center for Clinical and Translational Science Annual Conference, 2014, Lexington, Kentucky

7. Ighodaro, E.T., Alimova, K., White, S., Wright, A., Knudsen, H., (2013, March) Assessing the Outcomes of the 2012 College of Medicine Annual Multicultural Health Fair. Center for Clinical and Translational Science Annual Conference, 2013, Lexington, Kentucky

Volunteer Activities

Community Activist: July 2016 - Present
As vice president of UK Black Graduate and Professional Student Association, I co-lead student efforts in raising concerns and solutions toward improving UK campus racial climate by writing open letters, hosting town halls and organizing student-support activities.
http://www.kentucky.com/news/local/education/article63266732.html - News Article on Town Hall
http://www.kentucky.com/news/local/education/article60687351.html - News Article on Open Letter
https://www.youtube.com/watch?v=PskP5-qbRcc – Video Recording of Town Hall Event (1,000+ views)

Panel Moderator September 2016
Moderated a panel discussion during the “Before I Forget” event hosted by the Sanders-Brown Center on Aging in order to educate and recruit African Americans into dementia clinical trials and longitudinal studies

Diversity and Inclusion Committee Member January 2017 – April 2017
Medical/Graduate student representative on the Diversity and Inclusivity Workgroup committee for the purpose of developing the diversity and inclusion portion of the strategic plan for the College of Medicine at the University of Kentucky

Pre-matriculation Program Co-Chair August 2016 - October 2016
Organizer of a proposal and a committee to implement a pre-matriculation program at the College of Medicine, University of Kentucky

Search Committee Member December 2015 – March 2016

Medical/Graduate student representative on the Anatomy and Neurobiology search committee for the Departmental Chair position at the University of Kentucky