PROMOTERS OF CEREBRAL SMALL VESSEL INTEGRITY FOR COGNITIVE DISORDER PREVENTION

by

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ABSTRACT

The unique metabolic demands of the brain point to the critical nature of cerebral small vessel integrity for overall brain and cognitive health. Given the lack of any disease-modifying treatments, new avenues for Alzheimer's disease (AD) prevention and treatment are urgently needed. This dissertation takes a population neuroscience approach to examine potential promoters of cerebral small vessel integrity for cognitive disorder prevention.

Existing methods of evaluating cerebral small vessel integrity focus on neuroimaging markers distal to small vessel disease and fail to evaluate the vessels themselves. To address this limitation, I developed a method using 7T susceptibility-weighted imaging (SWI) magnetic resonance imaging (MRI) for direct small vein measurement in older adults; I examined associations with potential small vessel integrity promoters cross-sectionally and found that the *APOE*4* allele was associated with small vein tortuosity. In my second paper, a randomized controlled trial, I found that increasing physical activity and brain-derived neurotrophic factor late in life may improve cerebral small vein health profiles as measured by 7T SWI.

In an era when multimorbidity is common among older adults, interactions involving vascular and cardiometabolic risk factors (VCMRF) are critical to evaluate in

order to effectively target preventions and treatments—the promise of precision medicine. I evaluated associations of interactions of interest with incident dementia and cognitive impairment in a large population-based cohort with 10 years of follow-up. I found that the risk of all-cause dementia conferred by stroke was even greater among those with congestive heart failure; the beneficial effects of alcohol consumption on overall cognitive performance varied by stroke history; and in exploratory results, the detrimental effect of age on AD dementia risk was lower among those who walked more. Taken together, my findings point to physical activity and VCMRF reduction as potential strategies to promote cerebral small vessel integrity for cognitive disorder prevention and suggest that growth factors such as brain-derived neurotrophic factor should be evaluated further. These strategies for prevention could reduce late-life cognitive disorder prevalence as well as attendant disability and costs, goals of great public health significance.

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PREFACE

To my committee: I am so grateful for the expert guidance that you have given me over the course of my dissertation. You have allowed me to effectively combine the population approaches of epidemiology, advanced statistical analytic techniques, and state of the art neuroimaging and neuroscience, with aging, neurology, and vascular biology.

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think about epidemiologic methods and how population neuroscience can be applied to dementia research. Abbe, I am fortunate to call you a collaborator since nearly the beginning of my PhD program. You have guided my thinking on biological systems that are represented by sets of molecular markers, measurement approaches, and what these markers can mean in the brains of older adults. Zoltan, my external reader, I feel so fortunate to have met you at the New York Academy of Sciences and again when you visited Pittsburgh and to have worked with you over the past couple of years. As a vascular biologist, you have challenged me to think about pathophysiology in the veins and other groups in whom we should study these phenomena. Each of you on my committee are that amazing combination of smart and kind—exactly the kind of scholar I hope to work with for the rest of my career.

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Judy Saxton deserves extra thanks. I worked for Judy at the Alzheimer Disease Research Center. At her retirement party, as I hugged her goodbye, she told me I needed to go back to school for a PhD, so I finally really considered it. Thank you Judy. You are the reason I am here at this finish line.

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List of abbreviations and acronyms

aβ: β amyloid
AD: Alzheimer's disease
ADL: activities of daily living
AHAB-II: Adult Health and Behavior project, phase II
*APOE*4*: the ε4 allele of the apolipoprotein E gene
ARIC: Atherosclerosis Risk in Communities study
ASL: arterial spin labeling
AUC: area under the curve
baPWV: brachial-ankle pulse wave velocity
BBB: blood brain barrier
BDNF: brain-derived neurotrophic factor
BMI: body mass index
BOLD: blood oxygen level dependent
CADASIL: cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy
CART: classification and regression tree
CBF: cerebral blood flow
CBV: cerebral blood volume
CDR: clinical dementia rating
cfPWV: carotid-femoral pulse wave velocity
CHF: congestive heart failure
CHS: Cardiovascular Health Study
CI: confidence interval

cIMT: carotid intima media thickness
CRP: c-reactive protein
CVR: cerebrovascular reactivity
DBP: diastolic blood pressure
EVA: Epidemiology of Vascular Aging study
FA: fractional anisotropy
FDG: fludeoxyglucose
FDR: false discovery rate
FLAIR: fluid-attenuated inversion recovery
GM: gray matter
HDL: high-density lipoprotein
HbA1c: hemoglobin A1C
HE: health education
HR: hazard ratio
IADL: instrumental activities of daily living
ICA: internal carotid artery
ICAM: intercellular adhesion molecule
ICV: intracranial volume
IL-6: interleukin 6
IMT: intima media thickness
IQ: intelligence quotient
IQR: interquartile range
LDL: low-density lipoprotein
LIFE: Lifestyle Interventions and Independence for Elders
LRT: likelihood ratio test
MAP: mean arterial pressure
mCESD: modified centers for epidemiologic studies-depression
MCI: mild cognitive impairment
MD: mean diffusivity
MI: myocardial infarction
minIP: minimum intensity projection
MMSE: Mini-Mental State Examination
MoVIES: Monongahela Valley Independent Elders Study
MPRAGE: Magnetization-Prepared Rapid Gradient-Echo
MRI: magnetic resonance imaging
MYHAT: Monongahela-Youghiogheny Healthy Aging Team Study
NIH: National Institutes of Health
OR: odds ratio
PA: physical activity
PAR: population attributable risk
PET: positron emission tomography
PH: proportional hazards
PI: principal investigator
PIP: Pittsburgh Imaging Project
PP: pulse pressure
PWV: pulse wave velocity

rCBF: regional cerebral blood flow
rCBV: regional cerebral blood volume
ROI: region of interest
RUN DMC: Radboud University Nijmegen Diffusion Tensor and Magnetic Resonance Cohort
SBI: silent brain infarcts
SBP: systolic blood pressure
SD: standard deviation
SEM: structural equation modeling
STRAIN: (carotid systolic diameter-carotid diastolic diameter)/carotid diastolic diameter; an estimate of heart strain that takes into account distensibility and function
SVD: small vessel disease
SWAN: Study of Women's Health Across the Nation
SWI: susceptibility-weighted imaging
TIA: temporary ischemic attack
TOF: time of flight
VCID: vascular contributions to cognitive impairment and dementia
VCMRF: vascular and cardiometabolic risk factors
VEGF: vascular endothelial growth factor
VO_2 max: maximal oxygen consumption; a measure of aerobic fitness
WEIRD: Western, educated, industrialized, rich, and democratic
WM: white matter
WMH: white matter hyperintensities
WMHV: white matter hyperintensity volume
WML: white matter lesion
3MS: Modified Mini-Mental State Examination
7T: 7 Tesla

1.0 INTRODUCTION

1.1 CEREBRAL SMALL VESSEL INTEGRITY IS CRITICAL TO BRAIN HEALTH

Poor cerebral small vessel health is a key determinant of both neuroimaging features of cerebral small vessel disease (SVD)[1-3] and functional cognitive outcomes such as mild cognitive impairment (MCI) and dementia, including dementia due to Alzheimer's disease (AD).[4,5] The vascular influence on these outcomes has been collectively termed *vascular contributions to cognitive impairment and dementia (VCID)*.[6,7] While at approximately three pounds the human brain comprises only 2% of the body by weight, it is a greedy organ, demanding 20% of the body's oxygen[8] and approximately 60% of the body's glucose.[9,10] With no ability for the brain to store energy, consistent and perpetual blood flow is required to keep it healthy. This metabolic need points to the critical nature of cerebral small vessel integrity for overall brain and cognitive health with implications for AD.

1.2 PUBLIC HEALTH SIGNIFICANCE

Early diagnosis and primary-prevention of AD dementia is a public health priority that is especially urgent among the oldest old. Age is the strongest risk factor for AD dementia,

with individuals aged 80-89 having about seven times greater odds of the disease than those aged 70-79.[11] SVD also increases with age.[12,13] At the same time, this group of older adults is one of the fastest growing segments of the population.[14] Thus, the consequences of this brain aging epidemic on the future of health care systems worldwide could be disastrous. Although new data indicate AD dementia prevalence and incidence may have recently declined,[15] prior projections indicate that the demographic shift would push the US prevalence of AD dementia to triple from 4.7 million among those 65+ in 2010 to 13.8 million people by 2050.[16] Currently, AD-associated disability rates are alarmingly on the rise.[17] Devastating interpersonal suffering is wrought by the disease on individuals diagnosed with it and their caregivers, who often report losing their loved one twice, once when the disease robs them of their personality, memories, and identity, and again when it robs them of life.[18,19] When measured by financial burden, care for AD and other dementias is expensive to society, costing the US between $159 billion and $215 billion in 2010.[20] Thus, finding preventions for AD dementia is of importance, and strategies that target very old adults are also urgently needed.

Despite its importance, the focus placed on vascular impacts on AD and related disorders has fluctuated over time. AD was historically referred to as “hardening of the arteries”, but the discovery of β-amyloid led the pendulum to shift away from vascular influence. More recently, the high-profile failures of multiple drugs aimed at β-amyloid and the amyloid cascade have caused many researchers to reconsider the importance of AD-specific VCID. Given the responsiveness of cardiovascular and cardiometabolic disorders such as hypertension and diabetes to treatment, this line of research is quite timely and could have a large impact on cognitive health at the population level.

Official integration of a defined cross-disciplinary research field studying the intersection of neurovascular health with AD and related disorders is still quite new, but nevertheless, of high priority. The term *vascular contributions to cognitive impairment and dementia* and its acronym, *VCID,* were recently coined, [6,7] and the National Institutes of Health (NIH) only began tracking funding for this field in 2014.[21] Importantly, NIH has recently prioritized finding new biomarkers of small vessel VCID in the Mark VCID study, a collaborative effort of multiple research groups which began in early 2017.[22]

1.3 CEREBRAL SMALL VESSEL DISEASE

Cerebral SVD is an important contributor to VCID. Neurovascular integrity is adversely impacted by cerebral SVD, which includes pathology of the small arteries, veins, and capillaries of the brain. [1-3] However, owing to the difficulty of visualizing these small vessels directly in vivo in humans, SVD has typically been characterized by several neuroimaging markers, which are not direct measures of blood vessels. Among the most common traditional neuroimaging markers of SVD are white matter hyperintensities (WMH) of presumed vascular origin, silent brain infarcts (SBI)/lacunes, and cerebral microbleeds. Adopting the STRIVE consortium[1] terminology and definition, WMH of presumed vascular origin occur in the white matter and appear as areas of hyperintense signal on T2 weighted MRI including fluid attenuated inversion recovery (FLAIR) images. The specifier “of presumed vascular origin” differentiates these hyperintensities from other disorders including multiple sclerosis. Silent brain infarcts / lacunes are termed lacunes of presumed vascular origin by STRIVE.[1]. These are seen as hypointense

cavities 3-15 mm in diameter with hyperintense rims on FLAIR imaging. They are healed small strokes associated with blockage of a deep arteriole. Finally, microbleeds are hypointense round areas ≤ 10 mm in diameter on T2* and SWI. Microbleeds can be due to cerebral amyloid angiopathy, deposition of β-amyloid, in the leptomeninges, media and adventitia of cortical arterioles and small-medium arteries, and occasionally capillaries and veins.[23] Microbleeds may also be due to other SVD pathology (see Ungvari, et al., for a recent review[24]).

In addition to these traditional markers, novel markers of SVD are beginning to be examined. These include cerebral blood flow (CBF), cerebrovascular reactivity (CVR), microstructural integrity via diffusion weighted imaging, and small vessel morphological characteristics. CBF can be measured using multiple modalities including [^{15}O] water positron emission tomography (PET), dynamic contrast MRI, and arterial spin labeling (ASL) MRI. [^{15}O] water PET is an attractive option due to the scan taking only 1 minute. It would be ideal in instances in which other PET imaging is already being obtained, such as in glucose metabolism scanning via [^{15}F] FDG PET or amyloid imaging or in circumstances where a research center has a hybrid PET-MRI scanner. On the other hand, ASL is attractive due to use of magnetically tagged water as a non-invasive tracer. This is ideal for use in older adults who may not withstand exposure to radioactive PET tracers or contrast, and has been shown to correlate well with [^{15}O] water PET.[25] CVR allows for quantification of changes in CBF via ASL or blood oxygen level dependent (BOLD) MRI scanning following breath holding, hypercapnic challenge, or other vasoactive stimulus, thus allowing for measurement of vessel vasodilation and constriction capability. Microstructural integrity of white matter can be measured on

diffusion weighted imaging by fractional anisotropy (FA) or mean diffusivity (MD). These markers are thought to represent very early parenchymal changes associated with SVD. Finally, some of the newest methods include imaging modalities to directly measure the cerebral small vasculature, such as time of flight (TOF) and SWI at ultra-high field strengths. TOF with application of a maximum intensity projection allows for MRI imaging of cerebral arteries, which will appear brighter than the surrounding tissue.[26] Conversely, brain small veins can be measured using SWI and application of a minimum intensity projection to improve visualization of vessel continuity.[27] With this method, veins appear darker than surrounding tissue due to the paramagnetic properties of the deoxyhemoglobin in the deoxygenated venous blood.

1.4 PROMOTERS OF CEREBRAL SMALL VESSEL INTEGRITY

From 2002-2012, 244 experimental drugs for AD dementia were assessed in clinical trials. Only one (Namenda/memantine) made it to market it in that time.[28] Thus, finding promoters of cerebral small vessel integrity has become increasingly urgent as they represent intervention targets for late-life cognitive impairment and dementia. While the literature suggests many candidate promoters, this dissertation will focus on some of the most promising: physical activity (PA), growth factors, and vascular and cardiometabolic risk factor (VCMRF) reduction (Figure 1-1 and 1-2). They will be discussed in the next several chapters.

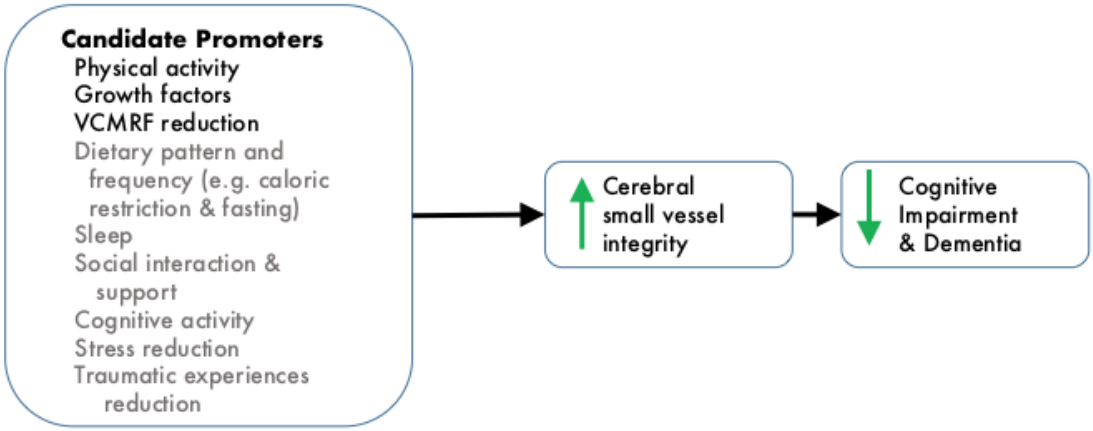

Figure 1-1. Targeting cerebral small vessel integrity offers a promising approach to intervene on cognitive impairment and dementia

Among the many candidate promoters suggest in the literature, the ones in bold will be addressed in this dissertation.

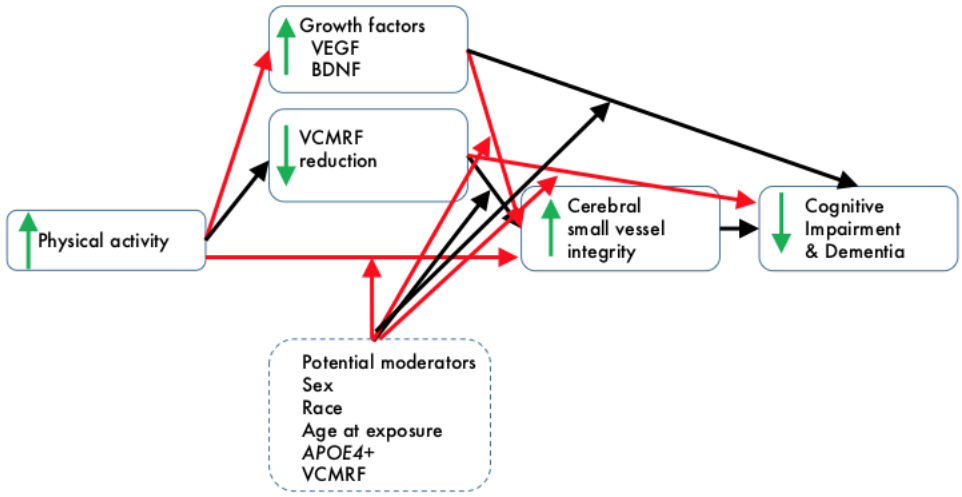

Figure 1-2. Conceptual model

Physical activity, growth factors, and vascular and cardiometabolic risk factor reduction (VCMRF) may impact cerebral small vessel integrity and cognitive impairment and dementia through the direct and indirect paths shown here. These relationships are likely moderated through other non-modifiable factors and other VCMRF. The relationships in red will be evaluated in this dissertation.

2.0 CANDIDATE PROMOTER: PHYSICAL ACTIVITY

PA is a strong candidate promoter of cerebral small vessel integrity. It has beneficial effects on brain changes relevant to both AD and SVD, and the size of PA's potential population level impact on AD dementia is large. The population attributable risk (PAR) of physical inactivity on AD dementia—the proportion of AD dementia in the population that could be eliminated if physical inactivity was eliminated—is estimated to be between 21-32% percent.[29,30] PAR is a function of both relative risk (RR) and prevalence of the risk factor in the population. In order to compare the PAR of several AD risk factors, I calculated a PAR of 26.35% based on a RR of AD dementia of 1.72 for low PA vs. high PA (PA < 3 times per week vs. ≥ 3 times per week)[30] paired with an estimated prevalence of physical inactivity of 49.7% based on the proportion of the Pennsylvania state population 65 years and older not meeting guidelines for 150 minutes weekly of PA.[22] This is compared with the PAR of other risk factors in Figure 2-1. Physical inactivity has one of the largest known PARs of AD-related risk factors including depression, smoking, mid-life hypertension, low education, mid-life obesity, and type 2 diabetes. While many of these risk factors are intertwined, PA nevertheless has a large population effect on AD dementia. Given such a large population-level impact of increasing PA on reducing AD dementia risk, harnessing this intervention and understanding its mechanisms are critically important goals. PA may operate through VCMRF reduction or through a direct effect on small vessel integrity (Figure 2-2).

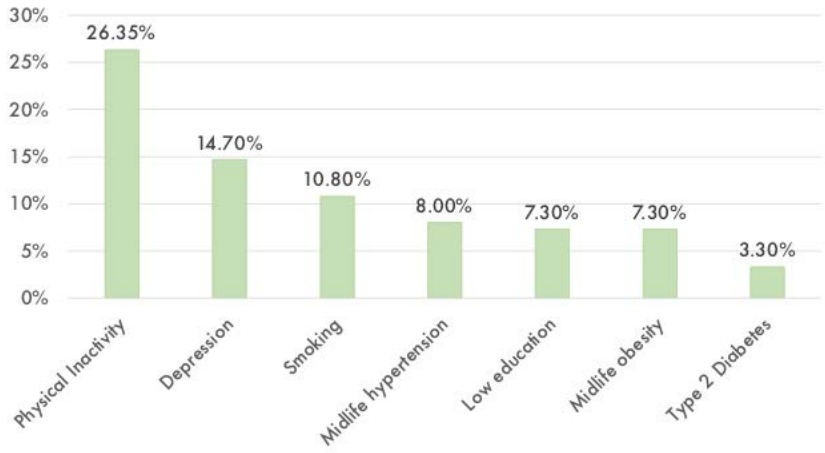

Figure 2-1. Population attributable risks (PAR) for select Alzheimer's disease risk factors

Physical inactivity PAR based on RR of 1.72 and prevalence of those ≥ 65 years of age in Pennsylvania not meeting physical activity recommendations of 150 minutes per week of 49.7%.[22] Other PAR estimates (Based on Barnes, et al.[29] from Beydoun, et al.[30])

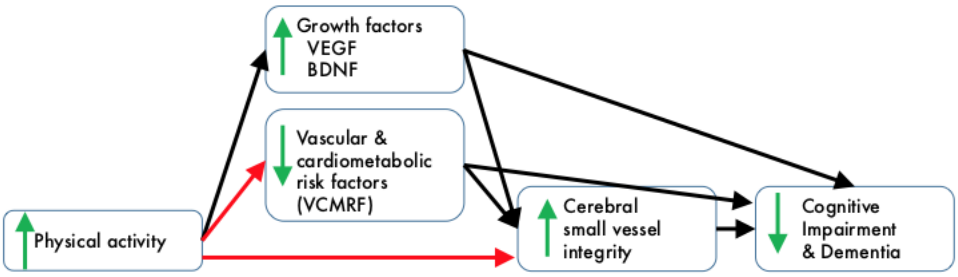

Figure 2-2. Physical activity as a promoter of cerebral small vessel integrity

Physical activity may promote cerebral small vessel integrity through vascular and cardiometabolic risk factor (VCMRF) reduction, by increasing growth factors (discussed in chapter 3), or through other mechanisms shown here as the direct effect of physical activity on cerebral small vessel integrity.

2.1 PA IS BENEFICIAL FOR BRAIN CHANGES RELATED TO AD AND SVD

2.1.1 Hippocampal volume and vascularization

PA is related to lower risk of poor functional cognitive outcomes including MCI[31] and AD dementia,[29,30] but why this is the case is not fully determined. In humans, changes in hippocampal volume in response to PA are seen on MRI.[32-35] The ability of PA to slow or even reverse hippocampal atrophy, a key biomarker of cognitive impairment,[36-42] MCI, and AD, make PA a promising candidate in their prevention. There are many possible functional or morphological changes that could explain the neuroprotective effects of PA including creation of new neurons, other morphologic improvements to existing neurons, better vascularization and nutritional supply of existing neurons, or better functioning or transmission in existing neurons.

2.1.1.1 Creation of new neurons

PA may beneficially impact hippocampal volume through promotion of neurogenesis[43-48] (for a recent review, see Patten et al.[49]). Unique multi-modal studies in animals and humans have been designed to investigate the mechanisms of PA effects on hippocampal atrophy. Several studies from the same group strongly suggest neurogenesis as the mechanism. They found that voluntary* wheel running in mice

* Some murine experiments of the effects of running use forced running. Forced and voluntary running induce different effects. Voluntary runners have been shown to have greater hippocampal brain-derived neurotrophic factor levels and better motor recovery post-stroke,[50] less stress/anxiety, [50,] and possibly better recognition memory and reduced numbers of amyloid plaques in a transgenic model of AD.[52] Forced runners generate more surviving new neurons, but it comes with the cost of greater stress/anxiety.

reduced age-related hippocampal gray matter loss.[53] [54] They have shown that blocking hippocampal neurogenesis via focal irradiation abolishes PA-related hippocampal GM maintenance. [54] When the cellular and vascular correlates of this were evaluated through histologic exam, the factor that explained this best in regression modeling was neurogenesis as opposed to glial proliferation, vascular density or branching, or reduced cell death.[55] Other researchers have shown that in male rats dihydrotestosterone can be synthesized locally in the hippocampus in response to exercise.[56] Dihydrotestosterone binds to androgen receptors, and this mechanism is capable of inducing neurogenesis. These effects were confirmed when neurogenesis was abolished by administration of an androgen receptor antagonist.[56] However, some reports indicate neurogenesis is actually quite rare in older adult humans,[57] thus calling for more investigation of this mechanism.

2.1.1.2 Other morphologic improvements to existing neurons

Research in animals suggests other morphological mechanisms of PA effects on hippocampal atrophy. Combined use of 9.4T MRI and ex-vivo Golgi staining has shown that voluntary wheel running in rats increased hippocampal volume and thin dendritic spine count in dentate gyrus in a rat model of depression.[58] This study did not simultaneously evaluate other mechanisms.

In a highly multi-modal human study incorporating neuroimaging measures of structure, vascular function, and myelination to interrogate pathways of PA effects on the hippocampus in sedentary adults (N=62; mean age: 34), investigators concluded that the hippocampal volume increases induced by PA were more consistent with increases in myelination than with vascular changes.[59]

2.1.1.3 Better vascularization and nutritional supply of existing neurons

PA may induce its beneficial effects through angiogenesis and alterations in small vessel density.[60-64] Examination of neurovascular microstructure in living humans is not yet possible limiting our direct knowledge of PA-induced neurogenesis, spine formation, and the like. Nevertheless, multi-modal studies of humans have attempted to evaluate vascularization impacts of PA on hippocampal plasticity. In a study of 12 weeks of interval treadmill training vs. a stretching control in sedentary older adults, PA induced improvements in aerobic fitness and rCBF and rCBV (when adjusted for age) but not hippocampal head volume.[65] However, across the full sample, percent changes in fitness were positively correlated with percent changes in rCBF, rCBV, and hippocampal head volume; changes in hippocampal head volume were positively correlated with changes in rCBF.[65] Structural equation modeling with these variables indicated that improvements in recognition memory seen in the study could be plausibly mediated either by 1) vascular plasticity indirectly through its benefits on neural plasticity or 2) directly by vascular plasticity.[65] Thus, this study is suggestive of a role for vascular integrity in reducing hippocampal atrophy. It is important to note that this study focused on participants with a far older mean age and had a PA intervention of far longer duration than Thomas, et al., and this may explain their differing conclusions regarding the importance of vascular plasticity.

2.1.1.4 Better functioning or transmission in existing neurons

By combining 9.4T MRI and MR spectroscopy in mice, investigators found that voluntary wheel running in mice was associated with decreased hippocampal glutamate post-intervention, and glutamate was negatively correlated hippocampal volume.[53] Therefore,

the glutamatergic system may be implicated in PA effects on hippocampus, but further studies will need to clarify whether this relationship is causal.

2.1.2 Cerebral small vessel disease

PA may also exert beneficial effects on brain health through impact on cerebral SVD. As part of the review of risk factors for neuroimaging markers of SVD which will be discussed in detail in chapter 4, we reviewed the literature for studies of the relationship between PA and both traditional and novel neuroimaging markers of SVD. We summarize the results below.

2.1.2.1 Traditional neuroimaging markers of SVD

WMH. PA is a promising intervention strategy for SVD. According to a meta-analysis of nine studies in healthy adults > 60 years of age, higher physical fitness and activity levels were associated with lower overall WMH volume.[66]

2.1.2.2 Novel neuroimaging markers of SVD

We found one study evaluating the association of PA with FA/MD.[67] Gow, et al., found that higher levels of physical activity predicted higher FA approximately 3 years later in models adjusted for age, sex, age 11 IQ, and social class. However, when hypertension, cardiovascular disease, and stroke were added to the model, the effect was attenuated,[67] suggesting that VCMRF mediate associations between PA and white matter microstructure. Higher fitness by VO_2 max was associated with higher CVR in periventricular white matter, but lower frontal CVR.[68] The reason for this directionality in

frontal regions is unclear, and much future work remains to be done to fully interrogate this pathway. Older adults who have participated in higher self-reported levels of aerobic PA over the prior ten years have been found to have statistically significantly lower small artery tortuosity and a greater number of cerebral small arteries by time of flight (TOF) compared with less active older adults.[69]

2.1.2.3 Summary

Overall, results regarding physical fitness and activity and CVR remain unclear. Increased levels of physical fitness and activity seem to increase WM microstructural integrity and overall decrease WMH. We found no studies evaluating the PA-SVD relationship in groups <60 years of age. These studies and those evaluating CBF in response to physical fitness and activity should be carried out in the future.

2.2 DOES PA WORK THROUGH VASCULAR/CARDIOMETABOLIC RISK FACTOR REDUCTION?

PA imparts a host of systemic vascular benefits, including reducing blood pressure among individuals with hypertension,[70] improving insulin sensitivity,[71] preventing diabetes,[72] and possibly others.[73,74] Thus improvements in cerebral small vessel integrity could be mediated by vascular risk factor improvements.

2.3 CONCLUSION

PA is a good candidate promoter of cerebral small vessel integrity. There is evidence supporting the mediation of this effect through VCMRF reduction, but there may be other mechanisms of this effect, and if so, these would also be candidate promoters of cerebral SVD. Growth factors are one such mechanism, and they are discussed in detail in the next chapter.

3.0 CANDIDATE PROMOTER: GROWTH FACTORS

3.1 A MECHANISM OF PA EFFECTS ON BRAIN HEALTH

Because of their role in neuro- and angiogenesis, brain-derived neurotrophic factor (BDNF)[75-77] and vascular endothelial growth factor (VEGF)[78-80] have received substantial attention as potential mediators of the neuroprotective effects of PA**.** In animal models, experimental studies show that both BDNF and VEGF increase with increasing PA.[62,64,81-85] Direct administration of BDNF is associated with neurogenesis,[77,86] and direct administration of VEGF is associated with greater brain small vessel density.[87] Importantly, VEGF may be necessary for neurogenesis[88,89] and blocking VEGF has been shown to reduce the effects of PA on neurogenesis.[88] In humans, PA increases BDNF in many studies of older adults,[90-93] while results with PA and VEGF among older adults remain unclear (for a review, see Vital[94]). Higher BDNF levels have been associated with lower risk of AD,[95] and pioneering cross-sectional[96] and experimental studies[97] have also shown a positive relationship of BDNF with hippocampal volume. Studies evaluating a PA/BDNF/cognition pathway[98] have recently emerged in humans, but the relationships of BDNF and VEGF with cerebral small vessel integrity are critically missing from the literature (see Figure 3-1).

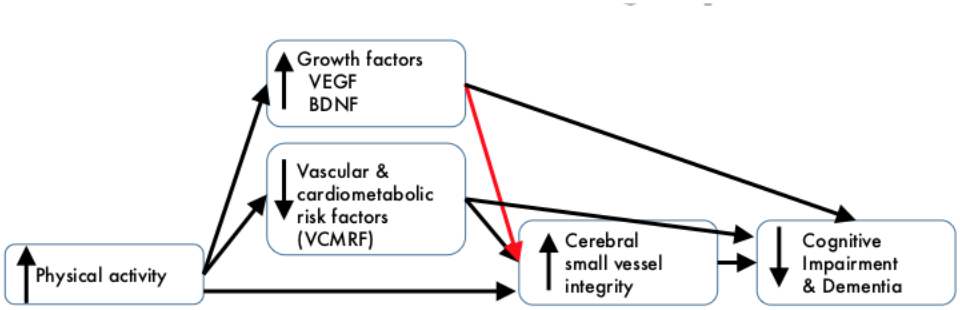

Figure 3-1. Growth factors as candidate promoters of cerebral small vessel integrity

Growth factors such as brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF) are candidate promoters of cerebral small vessel integrity.

3.2 CANDIDATE PROMOTERS IN THEIR OWN RIGHT

Although BDNF and VEGF are the molecular basis for some of the PA's beneficial effects on brain health, they are also important candidate promoters of cerebral small vessel integrity in their own right. Given the large PAR of physical inactivity on AD dementia discussed in chapter 2, explaining even some of that effect could be very beneficial to frail older adults. These individuals are simultaneously the least able to participate in PA and the most at risk for AD dementia. Although we do not have pharmaceutical approaches yet to increase BDNF levels in humans, there is exciting new research in animal models and selected patient populations testing the effectiveness of pharmaceutical inducers of BDNF.[99] If evidence is found supporting a role for these growth factors as promoters of cerebral small vessel integrity, harnessing BDNF and VEGF as pharmaceutical interventions to prevent and treat cognitive impairment and dementia would be a promising intervention, especially among those most at risk.

I discuss the final candidate promoter of cerebral small vessel integrity which this dissertation will consider, VCMRF reduction, in the next chapter.

4.0 CANDIDATE PROMOTER: VASCULAR AND CARDIOMETABOLIC RISK FACTOR REDUCTION

VCMRF reduction may promote cerebral small vessel integrity. Given the modifiability of these factors, reducing this small vessel VCID represents a promising area of prevention for both cerebral SVD and cognitive impairment and dementia. VCMRF have been studied extensively in relation to neuroimaging markers of cerebral SVD.

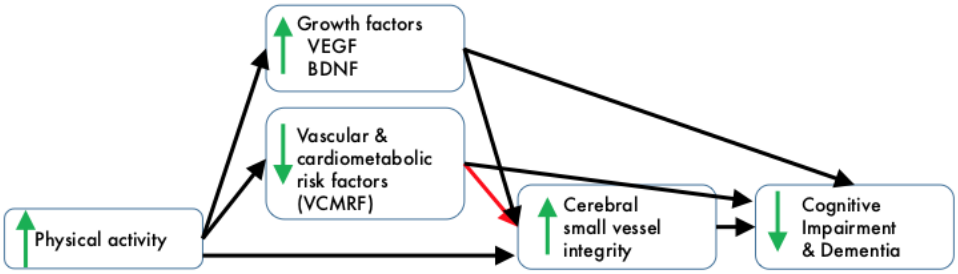

Figure 4-1. Reducing the burden or severity of vascular and cardiometabolic risk factors may promote cerebral small vessel integrity

4.1 VCMRF FOR NEUROIMAGING MARKERS OF SVD

To understand the current state of research and knowledge surrounding relationships of vascular/cardiometabolic risk factors for SVD in otherwise healthy individuals, my co-authors and I carried out a survey of the literature. The resulting review article has since been published.[100] We searched PubMed for review and original articles examining both traditional (WMH, SBI/lacunes) and novel (CBF, CVR, FA/MD, direct vessel measures) neuroimaging markers of SVD and their risk factors. Due to the recent review by Ungvari, et al.,[24] we did not include microbleeds in this review. When available, meta-analyses

were used in lieu of the original articles. Studies that were cited by the articles we included were reviewed and also included if appropriate. Vessel morphology articles were not found using these search terms, and therefore a hand search was carried out. Included populations had to be community-dwelling, neurologically healthy individuals. Exclusion criteria were: a) hospitalized populations or disease state only population without a control group, b) sample size<50, c) narrative reviews, and d) autopsy studies (Figure 4-2). For example, if a study dealt with recent stroke patients, that study was excluded. If a study was carried out only in individuals with diabetes and no controls, that study was excluded. Studies were later stratified by population <60 years of age and ≥60 years of age. Figure 4-3. summarizes the search strategy, which follows PRISMA guidelines (http://www.prisma-statement.org/). Detailed search terms may be found in Appendix A.

Study Inclusion / Exclusion Criteria	
Inclusion	**Exclusion**
Community-dwelling populations	Hospitalized populations
Neurologically healthy populations	Disease state only populations with no control group
	Sample size <50
	Narrative reviews
	Postmortem studies

Figure 4-2. Study inclusion / exclusion for review of vascular and cardiometabolic risk factors for cerebral small vessel disease

Results are summarized below highlighting the number of studies assessing each relationship, the number finding evidence of an association, and the number carried out in populations with a mean age <60. When a study's results differ from the bulk of the evidence, an exploration of the possible reasons is provided. Study designs are described as either cross-sectional or longitudinal. Four longitudinal designs were noted in the

articles included in the review: 1) the predictor precedes the outcome, and each are measured only once; 2) there are repeated measures of the predictor, and the outcome is measure only once at the end of follow up; 3) there are repeated measures of the outcome, with the predictor measured only once at baseline; and 4) there are repeated measures of both the predictor and the outcome. Both designs 3 and 4 allow progression or incident SVD markers to be captured. Given the strength of such designs, these results are emphasized. Figure 4-4 summarizes the results of the review graphically.

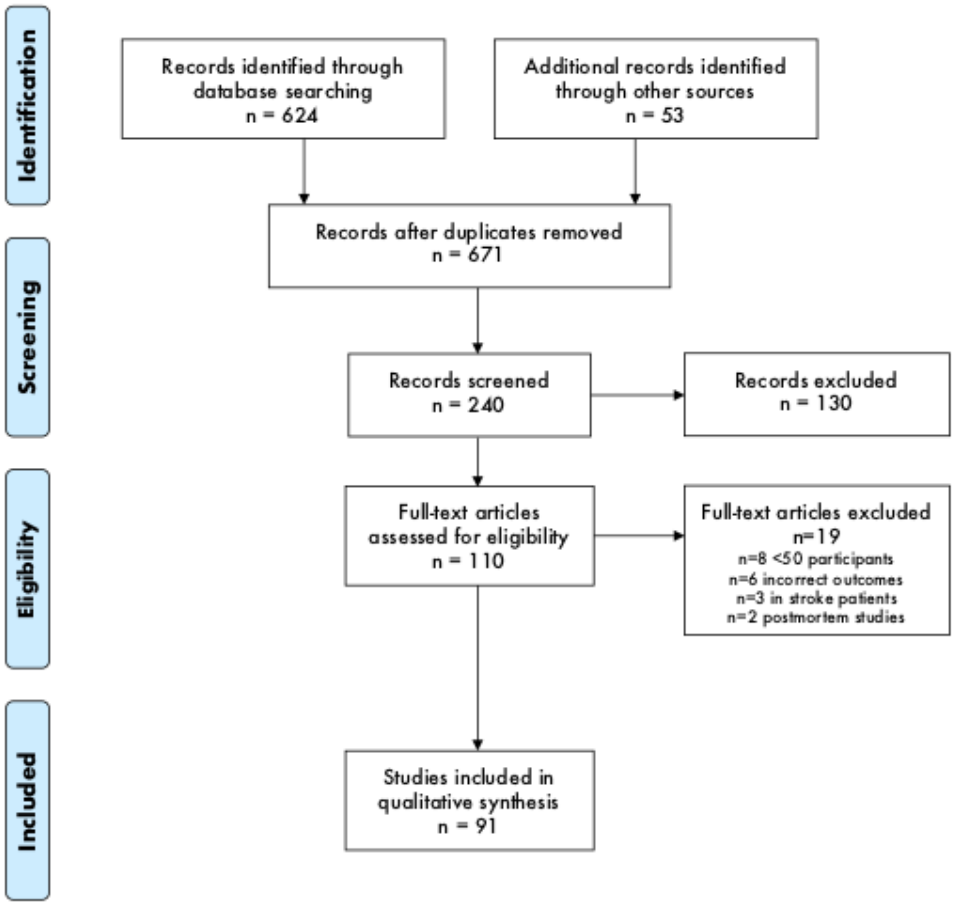

Figure 4-3. PRISMA search strategy for review of vascular and cardiometabolic risk factors for cerebral small vessel disease

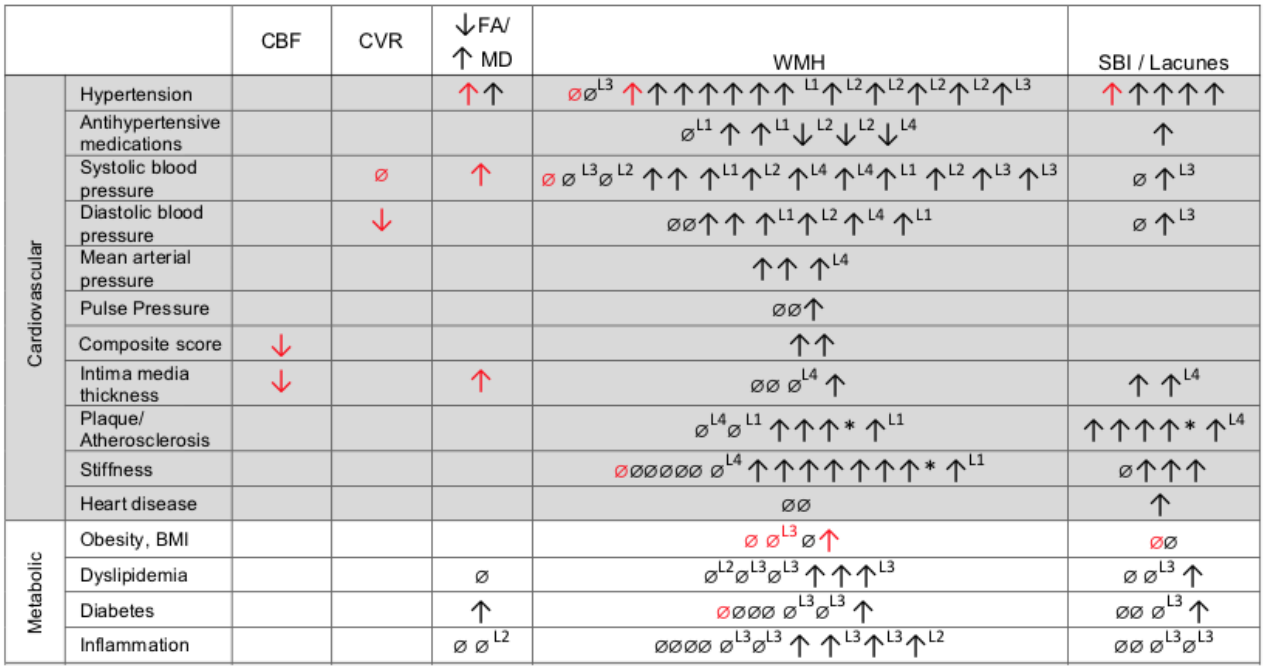

Figure 4-4. Review of associations of vascular and cardiometabolic risk factors with neuroimaging markers of cerebral small vessel disease

L = longitudinal study in one of four designs as follows. L1: predictor precedes outcome, each measured once. L2: repeated measures of predictor, outcome measured only at end of follow-up. L3: predictor measured only once at baseline, repeated measures of the outcome. L4: repeated measures of both predictor and outcome. *: meta-analysis. red: <60 years of age. black: ≥60 years of age. ⌀: no significant association.

4.1.1 Blood pressure related measures

Blood pressure is the most studied SVD risk factor. A variety of blood pressure related measures have been evaluated for associations with SVD. These include hypertension, antihypertensive medication use, systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), and pulse pressure (PP). MAP, a measure of organ perfusion, cannot be measured directly, but an estimate can be calculated based on the following equation: $MAP \cong \frac{SBP+(2xDBP)}{3}$. Normal MAP values range from 70-110 mm/Hg,

and MAP values below 70 mm/Hg can lead to hypoxia. Pulse pressure is calculated by SBP-DBP, and it is a marker of pulsatility.

4.1.1.1 Traditional neuroimaging markers of SVD

WMH. Twelve papers have reported an association of hypertension with WMH.[101-112] Six of these studies were longitudinal,[101,102,104,105,107,110], and one of these examined WMH progression.[104] Only one study reported this association in a population <60 years old.[106] Two studies, one cross–sectional[113] and one longitudinal examining WMH progression in the ARIC study,[114] found no significant association of hypertension and WMH. These results, which differ from the bulk of the literature, may be explained by factors relating to the population under study as well as those relating to study design. First, Nyquist and colleagues' examination of this relationship in 1st degree relatives of individuals with coronary artery disease may explain their null results.[113] This population may have different risk factors for SVD given their high risk of large artery disease. Second, the ARIC study evaluated WMH progression in relationship to baseline hypertension.[114] The follow-up time in this study was 10 years. Studies with long follow-up times can be vulnerable to survival bias. This occurs when those who survive 10 years with hypertension are more resilient than those who survive fewer years and are lost to follow-up. In such a resilient group of participants, the importance of hypertension at baseline would be diminished.

Six studies evaluated the relationship of antihypertensive medication use with WMH. Three longitudinal studies found an association of antihypertensive medication use with reduced WMH risk.[102,107,115] One of these studies reported on WMH progression. Investigators working in the ARIC study reported no significant cross-sectional

association of antihypertensive medication use with WMH after adjustment for multiple comparisons.[112] Counterintuitively, two studies have reported an association of antihypertensive medication use with *greater* WMH. One was cross-sectional,[116] and one was longitudinal.[105] Heckbert, et al. compared WMH among individuals taking different kinds of antihypertensive drugs (calcium channel blockers, loop diuretics, and beta-blockers). It may be that among hypertensive individuals any treatment is better than no treatment. Given their study design, which did not compare antihypertensive drug users to non-users, we cannot draw a clear conclusion.

Ten of twelve studies examining the relationships between SBP and WMH found a positive association.[101,104,105,107,110,112,115,117-119] Eight of these were longitudinal in design,[101,104,105,107,110,115,117,119] and four of these examined WMH progression.[104,115,117,119] One cross-sectional study[120] and one longitudinal study,[121] found no significant association of SBP with WMH. Only one study examined these relationships in a population with a mean age under 60 years old and found no significant association.[120] This study's results are particularly interesting given that the sample is much younger than other studies (mean age 39.8). There are several possibilities for this result. First, it is possible that one measure of SBP is inadequate to capture its effect on WMH. Indeed, certain measures of SBP are more sensitive markers for relationships with WMH. Havlik et al. found that while mean SBP was not significantly related to WMH, moderate and high variability in SBP was associated with 2-fold increased risk of WM lesions.[118] Furthermore, Gottesman, et al. not only found that cumulative mean SBP was a stronger predictor than SBP measured at individual study visits, but that SBP values measured earlier in the course of the study had stronger associations with change in WMH than did

SBP values measured later, thus illustrating the importance of timing and variable definition in evaluation of risk factors.[117] Second, it is possible that people this young have not been exposed to higher SBP levels long enough to see an effect on WMH. In both the Aribisala, et al. and Dickie et al. studies, SBP measures taken earlier in the course of the study were more strongly related to later WMH. [101,121] However, the finding regarding WMH progression did not survive confounder adjustment.[101,121] Importantly, both of these papers are from the Lothian Birth Cohort, making their findings consistent, but also raising the concern that their findings are due to some inherent bias in the study sample. SBP in these two studies was collected at an age generally older than the others (~70 years old). This indicates that other factors such as vascular stiffness, lipids, etc. may be more important for WMH at older ages than measurements of SBP. This is consistent with SBP and hypertension interactions with age seen in other studies.[119,120] Furthermore, a more cumulative exposure to high SBP may be needed to truly assess these relationships.

Six studies found an association of higher DBP with WMH.[105,107,110,112,115,119] Five of the six were longitudinal in design,[105,107,110,115,119] and two of those assessed WMH progression.[115,119] Interestingly, Van Dijk et al. found that both increases and *decreases* in DBP over time were associated with severity of periventricular WM lesions, suggesting that blood pressure variability may be important to this process.[110]

All three of the studies examining the relationship between MAP and WMH found a significant positive relationship.[112,122,123] One of these three studies was longitudinal, examining WMH progression.[122]

Of three studies evaluating the association between PP and WMH, the two longitudinal studies evaluating WMH progression found the relationship between pulse

pressure and WMH progression to be non-significant,[115,122] while the one cross-sectional study found a significant positive relationship.[123] It should be possible that PP could act in much the same way as a cumulative exposure to SBP variable, as prolonged exposure to higher SBP increases arterial stiffness over time and this would be reflected in a wider pulse pressure. We cannot draw a conclusion based on the evidence here though as only one of the three studies that evaluated PP also evaluated SBP; the investigators reported a significant positive association of SBP with WMH progression, but no significant relationship of PP with WMH progression.[115]

There is an important age*blood pressure interaction observed in this literature. Exposure to high blood pressure during midlife has been repeatedly linked to increases in WMH.[104,105] However, with advanced age, high blood pressure can either have no association with WMH or protect against the development of WMH.[119] Given their ischemic origins,[124] proper perfusion is critical to prevent WMH. High blood pressure may be required to maintain proper cerebral perfusion among those with arterial stiffness stemming from long-term hypertension, thus having a protective effect with advanced age. These relationships between hypertension and the development of WMH likely reflect age dependent processes. Hypertension may be an early target for intervention because long-term exposure of the brain to high blood pressure may irreversibly alter the physiologic structure of the arterioles and capillaries leading to a less modifiable state.[125]

SBI/lacunes. All five studies examining the relationship of hypertension with SBI/lacunes found a positive association.[106,126-129] Only one study examined the relationship in a population with a mean age <60 years of age.[106] All of the studies were cross-sectional.

One cross-sectional study of a Japanese population found an association between the odds of SBI and antihypertensive medication use.[130] This may simply be a result of confounding by indication as this study did not adjust for duration of hypertension.

Two articles examined SBP and SBI/lacunes.[119,130] Ochi et al. found a significant relationship between SBP and SBI in a cross-sectional Japanese cohort.[130] van Dijk et al. found no relationship between SBP and incident lacunes over a three year period.[119]

Similar to SBP, two articles examined the relationship of DBP with SBI/lacunes.[119,131] Longstreth et al. found a significant relationship between DBP and SBI in their cross-sectional study.[131] However, van Dijk et al. found no relationship between DBP and incident lacunes over a three year period.[119]

No studies evaluating the association of MAP or PP with SBI/lacunes were found.

4.1.1.2 Novel neuroimaging markers of SVD

Very few studies have evaluated BP related measures and novel neuroimaging markers of SVD. There were especially few studies assessing the range of BP measures with CBF, CVR, and direct vessel measures. One cross-sectional study found no significant relationship between SBP and CVR but a significant association between DBP and CVR due to breath holding or in a ratio of whole brain change and whole brain signal.[132] This study included only people with atherosclerosis, however, and may therefore have limited generalizability.

Results from the Rotterdam Scan Study show that individuals with severe hypertension had decreased FA in 4 of 6 specific association tracts and 1 of 2 commissural tracts as well as increased MD in 4 of 6 specific association tracts, 1 of 2 commissural tracts, and 1 of 2 limbic tracts. These findings were independent of age, sex,

intracranial volume (ICV), and tract-specific volume and WML volume.[133] Results among middle-aged adults (mean age 39.2 (8.4)) were consistent with this, with brain integrity was usually higher in normotensive individuals than in prehypertensive and hypertensive participants.[120] Higher SBP was in this cross-sectional analysis was also linearly associated with decreased FA and increased MD, especially in the anterior corpus callosum, the inferior fronto-occipital fasciculi, and the fibers that project from the thalamus to the superior frontal gyrus.[120] Their results also hint again at important interactions which take place with vascular and cardiometabolic factors. The relationship of hypertensive status with brain microstructural integrity differed as a function of age, such that at younger ages the differences in brain integrity between hypertension, prehypertension and normotensives were greater than at older ages.

4.1.1.3 Summary

The evidence for a positive association of hypertension, SBP, DBP, and MAP with WMH is strong. Overall, antihypertensive medication treatment appears to be protective against WMH, with opposing results likely due to confounding by indication. Few studies have assessed these relationships in populations <60 years old. Multiple studies demonstrate that hypertension is positively associated with SBI/lacunes. Fewer studies have evaluated this outcome in relation to SBP and DBP, and none have evaluated the outcome in relation to MAP or PP. Few longitudinal studies have been carried out to assess relationships of BP-related measures with SBI/lacunes, and few studies have been carried out in populations <60 years old. There is a paucity of literature evaluating novel SVD markers in relation to BP-related measures, with the greatest evidence for a link between hypertension and worse white matter microstructural integrity.

Future studies of BP-related measures of SVD should control for duration of risk factor exposure and incorporate measures of BP-variability and cumulative mean BP. Studies should examine SBP and DBP in conjunction with one another because the response of one to the other may reveal important information about stiffness and compliance of the underlying vascular structure. For example, as DBP increases, SBP is expected to increase but the extent of this increase is determined by the stiffness and compliance of the artery.[134] MAP and PP are measures that combine information from both variables, are easy to incorporate without additional equipment or measurement, and provide more insight into organ perfusion and vascular stiffness. Truly clarifying the nature of the relationships of antihypertensives with SVD outcomes will require: 1) controlling for duration of exposure to hypertension; 2) controlling for confounding by indication; and 3) classifying individuals as not on antihypertensives, treated but poorly controlled, and treated and well controlled.

4.1.2 Dyslipidemia

4.1.2.1 Traditional neuroimaging markers of SVD

WMH. Hypercholesterolemia has been positively associated with WM lesions in cross-sectional PATH analysis.[109] However, it is unclear whether these results are statistically significant and whether these results are adjusted for important covariates. In the Northern Manhattan Study, although total cholesterol was not related to WMH volume (WMHV) approximately 6 years later, conversion from low to high risk total cholesterol as well remaining at a high-risk total cholesterol over the six-year period was associated with greater WMHV on the follow-up MRI.[135] However, when WMH progression over time was

examined, neither hypercholesterolemia in mid-life nor total cholesterol in late life were significantly associated with WMH progression.[104,119] It may be that there is a threshold effect for late-life total cholesterol and/or a critical duration of exposure to high cholesterol that is key for risk of WMH.

Next, we will review the evidence regarding HDL and WMH. A meta-analysis of data from the Dijon and the Epidemiology of Vascular Aging (EVA) studies found that HDL was not significantly associated with WMHV in cross-sectional analysis.[136] In the Northern Manhattan Study, HDL was not related to WMHV approximately 6 years later, however, conversion from low risk to high risk HDL levels over 6 years was associated with a greater WMHV at the 6 year follow up.[135] In the Rotterdam Scan Study, HDL was not associated with WMH progression.[119] However, data from the Lothian Birth Cohort demonstrated that a low ratio of HDL to total cholesterol was significantly associated with WMH progression.[121] These mixed results indicate that more work remains to be done to clarify the role of HDL in WMH. It may be that HDL ratio is more important than absolute value or that there is a critical threshold below which HDL is associated with WMH.

We found no evidence to support a link between LDL and WMH either cross-sectionally[136] or longitudinally with LDL measured 6 years prior to WMHV.[135]

Finally, with regard to triglycerides, Schilling, et al., found that high triglycerides, were significantly associated with greater WMHV in a cross-sectional meta-analysis of data from the Dijon Study and the Epidemiology of Vascular Aging Study (total N=2608).[136] While data from the Northern Manhattan Study showed that baseline triglycerides were not related to WMHV approximately 6 years later, transition from high to low risk triglyceride levels was associated with lower WMHV at 6-year follow-up.[135]

Thus, the evidence overall indicates a positive association between triglycerides and WMHV.

SBI/Lacunes. We found three studies reporting on the association between various lipid profile components and SBI.[119,126,136]

In the Rotterdam Scan Study, total cholesterol was not significantly associated with incident infarcts.[119]

Regarding HDL, in both cross-sectional[126,136] and longitudinal analyses,[119] no significant association of HDL with SBI/lacunes has been reported.

We found one study which assessed the relationship between LDL and SBI/lacunes. This cross-sectional study found no significant association.[136]

Finally, regarding triglycerides, the meta-analysis from the Dijon and EVA studies found that triglycerides were cross-sectionally positively associated with frequency of lacunes.[136] However, in cross-sectional analysis within the Atherosclerosis Risk in Communities Study (ARIC), triglycerides were not significantly associated with silent cerebral infarctions.[126] There are important population differences between the studies that may explain these differing results. For example, the ARIC sample is approximately 5-10 years younger than the two French samples in the meta-analysis. In addition, the ARIC sample is approximately 48% Black, while the French samples are likely all white. 48% of the ARIC sample was hypertensive while 77% of the Dijon sample and 55% of the EVA sample were hypertensive. This is a large difference and given the relationship of hypertension with SBI/lacunes, this alone may explain the different results. Perhaps most importantly, Howard, et al., do not report the proportion of their sample on a lipid lowering drug, while this proportion was >30% in both French cohorts. Differences in

medication prescribing and dietary patterns over time and in the US vs. Europe are likely sources of varying results.

4.1.2.2 Novel neuroimaging markers of SVD

We found no studies evaluating the association of dyslipidemia with CBF, CVR, or direct measures of cerebral arteries or veins. We found one study assessing the relationship of dyslipidemia with WM microstructural integrity. In cross-sectional analyses within the Rotterdam Scan Study, a large cohort of over 4,000, no significant association was found between hypercholesterolemia and WM microstructural integrity in specific association, commissural, and limbic tracts after adjustment for HDL and lipid lowering medication.[133]

4.1.2.3 Summary

Some evidence suggests a role of dyslipidemia in WMH and SBI/lacunes. However, all studies we found assessing the role of dyslipidemia in SVD were carried out in those >60 years of age, and no studies evaluated relationships with novel markers of SVD such as CBF and CVR. This represents a range of modifiable risk factors, and some evidence we reported suggests that intervention on lipids could impact SVD, especially WMH.

4.1.3 Diabetes

4.1.3.1 Traditional neuroimaging markers of SVD

WMH. Cross-sectional and longitudinal assessments have failed to find a significant association of diabetes with WMH.[104,108,112-114,119] It is important to note that when fasting blood glucose was treated as a continuous predictor instead of the dichotomous diabetes,

Knopman, et al., found a positive association with WM grade progression over approximately 10 years in the ARIC cohort.[114] Diabetics with long sleep duration had a greater log-WMHV in cross-sectional analysis in the Northern Manhattan Study.[137] Two additional studies found that insulin resistance was not related to WMH cross-sectionally[106] or WMH progression.[138]

SBI/Lacunes. Longstreth, et al., reported a positive cross-sectional association in the Cardiovascular Health Study.[131] The remaining found no significant association.[119,126,129] These studies were a cross-sectional report from ARIC[126] and both the cross-sectional[129] and longitudinal reports[119] from the Rotterdam Scan Study. The primary difference between the Cardiovascular Health Study and the Rotterdam Scan Study is the racial makeup, with CHS having 16% Black participants at the time of the report and the Rotterdam cohort being 100% white. If the Black participants of CHS were more likely to have diabetes, this may explain differing results. Two additional studies found that insulin resistance was related to single and multiple SBI/lacunes as well as a greater number of SBI/lacunes cross-sectionally[106] and to incident lacunes.[138] Thus, insulin resistance may be a more sensitive risk factor for SBI/lacunes than diabetes itself. Finally, in the ARIC cohort, there was a synergistic effect of fasting glucose with high SBP such that those in the highest tertile of each measure were at far higher risk of incident infarcts than those in the lowest tertile of each measure.[114]

4.1.3.2 Novel neuroimaging markers of SVD

We found no studies evaluating the association of diabetes with CBF, CVR, or direct measures of the vasculature. However, one large study reported that diabetes was

associated with decreased FA in specific white matter tracts including association tracts and forceps major.[133]

4.1.3.3 Summary

Overall, the bulk of the evidence does not support a strong link between diabetes and SVD. However, few studies have been carried out assessing this risk factor in individuals <60 years of age, and few studies have evaluated its association with early markers of SVD. Given the relationship of midlife hypertension with SVD outcomes in later life, assessing younger individuals will be important in future studies to understand whether other vascular risk factors during midlife confer risk of late life SVD. Insulin resistance may be a more sensitive risk factor for SBI/lacunes than diabetes. Diabetes may interact with other risk factors such as sleep and SBP, and these interactions should be evaluated in future studies.

4.1.4 Inflammation

4.1.4.1 Traditional neuroimaging markers of SVD

WMH. The most studied inflammatory factor is C-reactive protein (CRP), and results regarding its relationship with WMH are mixed. Cross-sectional assessment in the Austrian Stroke Prevention Study[139] found no association while the Three City Dijon Cohort showed a positive association of CRP with WMH.[140] Of note, the Three City Dijon cohort is approximately 10 years older on average than the Austrian cohort, so older age or longer duration of exposure to inflammation may explain these differences. When evaluating progression of WMH, there was a positive association with progression of

periventricular WMH in the Rotterdam Scan Study, [141] but not of WMH overall, periventricular WMH, or deep WMH in the Dijon cohort [140] nor with WM lesions in the Austrian Stroke Prevention Study [139]. The Rotterdam Scan Study used a visual rating scale while the Dijon Study used WMH volumes. It is possible that visual rating scales may be prone to more error than volumes, the more sensitive measure, thus resulting in greater misclassification of the outcome. In addition, the Rotterdam Scan Study adjusted results for carotid plaques and IMT, which the Dijon Study did not do. These issues may explain the differing results.

Interleukin 6 (IL-6) has also been evaluated in relation to WMH in both the Dijon Study[140] and the Health ABC study. [142] The overall evidence suggests that one-time, cross-sectional measures of IL-6 may not adequately capture the risk factor of interest for future WMH or WMH progression. Cross-sectional associations were significant in the Dijon Study,[140] but not Health ABC.[142] This could be due to the smaller sample size in the Health ABC study. While no association of IL-6 with WMH progression was found in the Dijon Study,[140] and no association of rate of change of IL-6 over 10 years was found with later WMH in Health ABC, Nadkarni, et al., did find that mean 10 year IL-6 was positively associated with follow-up WMH volume. This suggests that cumulative exposure to IL-6 is more important than exposure at any one time [142]. No other studies found have taken this into account, and it is likely quite important for exposure to all inflammatory factors.

Regarding other inflammatory factors and composite factors, when fibrinogen was evaluated cross-sectionally with WMH, that association was found to be non-significant.[108] When a latent inflammation variable was constructed of the measurement

variables fibrinogen, CRP, and IL-6, no association was found in cross-sectional SEM analysis with a latent WMH variable in the Lothian Birth Cohort.[143] In the Austrian Stroke Prevention Study, intercellular adhesion molecule (ICAM) but not thrombomodulin, tissue factor plasma inhibitor, prothrombin fragments 1 and 2, or D-dimers was positively related to WMH progression, and this survived adjustment for CRP in addition to other typical covariates.[144]

SBI/Lacunes. In the Austrian Stroke Prevention Study, both the cross-sectional baseline association of CRP with SBI/lacunes and the longitudinal association of CRP with incident lacunes were non-significant in models adjusted for age, sex, and vascular risk factors.[139] These results stand in contrast to the other main finding of this study, which demonstrated that higher CRP was associated with greater carotid atherosclerosis. These results may be reflective of the clear inflammatory pathway to atherosclerosis, but a different underlying pathophysiology in SVD. Findings in the Three City Dijon cohort confirm those of Schmidt, et al., with no cross-sectional associations of IL-6 or CRP with SBI/lacunes nor any associations with incident SBI/lacunes. [140]

4.1.4.2 Novel neuroimaging markers of SVD

No studies evaluated the relationship of inflammation with CBF, CVR, or direct vessel measures. One study evaluated the association of interleukin 6 (IL-6) with white matter microstructural integrity in 179 individuals from the Health ABC study.[142] They found no association of IL-6 measured concurrently with FA, rate of change of IL-6 over 10 years, and mean 10-year IL-6 with FA. It is possible that there was no significant association with rate of change of 1L-6 because IL-6 changed so little over the course of the study. However, given that mean 10-year IL-6 was also non-significantly associated with FA in

models well controlled for appropriate confounders, the current state of the evidence indicates no association of IL-6 with FA in older adults.

4.1.4.3 Summary

The state of the evidence is strongest for a positive association of inflammatory factors with WMH. We found no evidence to support an association between inflammatory factors and FA/MD or SBI/lacunes. No studies evaluated inflammatory factors in relation to CBF, CVR, or direct vessel measures, and no studies evaluated these associations in those < 60 years of age. Future studies should focus on those areas and attempt to account for cumulative exposure to inflammatory factors.

4.1.5 Obesity by body mass index (BMI)

4.1.5.1 Traditional neuroimaging markers of SVD

WMH. Among people less than 60 years of age, one study found an association of obesity with WMH, [113] while two found no significant association.[138,145] In people with a family history of early onset coronary artery disease BMI>30 was associated cross-sectionally with extreme WMH scores, however obesity by this definition was not related to WMH *volume* in this study. [113] Conversely, both cross-sectional analysis in a Japanese population, and longitudinal analysis within the ARIC study showed that BMI was not significantly associated with WMH or WMH progression.[138,145] The Framingham Offspring Study demonstrated that among individuals>60 years of age BMI was not associated with WMHV progression.[104]

Thus, these results suggest overall that BMI is not associated with increased WMH. However, more specific measures lead to different conclusions. Though some studies report that waist circumference and waist to hip ratio are not associated with WMH, [104,138], Yamashiro, et al., showed that waist circumference (in unadjusted models) and visceral fat accumulation are associated with WMH among younger Japanese. In fact, there is an independent contribution of visceral fat accumulation beyond BMI and waist circumference when all are included in the same age and hypertension adjusted model.[145]

SBI/Lacunes. No significant associations were found in cross-sectional analyses with Japanese <60 years of age [145] nor with ARIC study participants>60 years of age.[126]

However, as with WMH, other markers of obesity are likely more sensitive for associations with SBI. Yamashiro, et al., showed that large waist circumference and visceral fat accumulation were independently associated with silent lacunar infarcts when adjusted for age, hypertension, and obesity by BMI cutoff.[145] Dearborn, et al., found that waist circumference was positively associated with incident lacunes >7mm, and waist to hip ratio was associated with all incident lacunes. [138]

4.1.5.2 Novel neuroimaging markers of SVD

We found no studies evaluating the association of obesity with CBF, CVR, WM microstructural integrity, or direct small vessel measures.

4.1.5.3 Summary

These results suggest that visceral fat may be most important in relation to SVD and likely represents the most sensitive measure of central fat mass, an important factor in

endocrine and cytokine signaling, inflammation, and subclinical cardiovascular disease.[146-148] Few studies have evaluated these relationships, however. Future studies should examine relationships of earlier markers of SVD in relation to visceral fat mass.

4.1.6 Smoking

4.1.6.1 Traditional neuroimaging markers of SVD

WMH. In both individuals <60 years of age,[113] and those older than 60,[104,112,119,121,149] smoking is associated with WMH. Two of these studies examined WMH progression.[104,119] Results may be dependent on the treatment of the WMH progression variable. When Debette, et al. examined progression continuously, associations with smoking were non-significant.[104] But when they were examined as a dichotomous variable of extensive progression (>1.34 cm^3, the equivalent of one grade on the CHS visual rating scale) vs. non-extensive progression (≤1.34 cm^3), associations were significant.

SBI/Lacunes. Both ARIC and CHS investigators found a cross-sectional relationship of smoking with SBI/lacunes,[126,150] while both a cross-sectional and longitudinal assessment of this relationship in the Rotterdam Scan Study demonstrated no significant association.[119,129] The stronger longitudinal design shows no association, but these study samples have differing sizes and characteristics which may also explain differing results. CHS and ARIC were far larger studies, evaluating 3660 participants and 1737 participants respectively potentially affording more statistical power to detect an effect if one is present. Rotterdam evaluated associations in 1077 (cross-sectional) and 668 (longitudinal). CHS and Rotterdam had a similar mean age around 71, but the sample

in ARIC was nearly 10 years younger. CHS and ARIC had a greater proportion of females and non-white participants, while Rotterdam has only white participants. If associations of smoking and SBI/lacunes are stronger in young-old adults, females, and non-whites, this could explain differing results. Finally, scanning protocols were different in the studies, and this could also explain differing results.

4.1.6.2 Novel neuroimaging markers of SVD

There were no studies evaluating the relationship of smoking with CVR or direct vessel measures. One study evaluating the relationship of smoking and CBF showed no significant cross-sectional association among individuals <60 years of age.[151] Two studies evaluated the relationship of smoking with WM microstructure.[133,149] In cross-sectional analyses in the Radboud University Nijmegen Diffusion Tensor and Magnetic Resonance Cohort (RUN DMC), smoking was associated with higher MD values in both normal appearing white matter (NAWM) and WM lesions.[133,149] RUN DMC data show that associations of smoking with lower FA and relationships of smoking with FA/MD in WM lesions were not significant.[149] This may indicate that macrostructural damage is significant enough that associations with microstructural integrity cannot be detected. The associations within the Rotterdam Scan Study were within specific WM tracts.[133] More years since quitting smoking was associated with better WM microstructural integrity in NAWM, but not within WM lesions.[149]

4.1.6.3 Summary

Smoking is associated with WM microstructural integrity, WMH, and potentially with SBI/lacunes. Results may be affected by differing sample sizes, characteristics, and scanning protocols. Future studies in individuals <60 and diverse cohorts are needed.

4.1.7 Subclinical vascular disease measures

4.1.7.1 Traditional neuroimaging markers of SVD

WMH. We found eighteen analyses evaluating the relationship between large vessel characteristics and WMH. Nine found a positive association [152] [123,153-157] [158,159] and ten found non-significant.[101,112,119,122,123,152,153,155,156,160] One study examined relationships in a population with a mean age under 60 years old [161], two were meta-analyses [158,159], eight were cross-sectional [101,112,123,152,153,157,160,161], one was a case-control [156], three were longitudinal [154,155] [119], with one examining progression of neuroimaging markers [119]. Many different measures of subclinical vascular disease were used across studies, for simplicity we will present the results from three major categories: Atherosclerosis, IMT, and PWV.

Atherosclerosis. Atherosclerosis, as measured by atherosclerotic lesions, calcified deposits, and/or plaques, were examined in 4 studies [119,152,155,159]. Two original investigations [152,155] and a meta-analysis }[159] found significant relationships between Atherosclerosis and WMH cross-sectionally [152] and longitudinally [155,159]. By contrast, van Djik et al [119] did not find a significant association longitudinally; however, in this study participants with both MRIs were younger and healthier overall than those who were ineligible for a second MRI. Thus, participants with both MRIs were less likely to have plaques, potentially explaining the null results.

It is worth mention that the study of de Leeuw et al. had somewhat mixed findings: where aortic atherosclerosis during midlife was associated with periventricular WML about 20 years later, but if the measure of aortic atherosclerosis occurred later in life, closer to the time of the MRI there was no longer an association between aortic atherosclerosis and WMH.[155]

IMT. Four studies examined carotid IMT and WMH, three of which found it to be non-significantly related [112,119,152] and only one finding a significant relationship [156]. The Hajdarević et al. study used a case-control design with age, gender, and risk factor matching. The risk factors on which cases and controls were matched are not explicitly stated, but there was no statistical difference in hypertension, diabetes, hyperlipidemia, atrial fibrillation, coronary artery disease, peripheral artery disease, smoking, alcoholism, or previous TIA between the groups.

PWV. Four of six studies including one meta-analysis found that vascular stiffness, (as measured by carotid-femoral pulse wave velocity (cfPWV), brachial-ankle PWV (baPWV), aortic pulse wave velocity) found a significant relationship with WMH,[123,156-158] two studies found no significant relationships.[122,161] Tsao et al. was one of the few studies to examine progression, while Nakano et al. was the only study in a population with a mean age under 60 years old. Furthermore, Nakano was in a Japanese population setting it apart from the other studies.[161] In the meta-analysis which pooled the analysis of 5 cross-sectional studies with mean ages of 41-67 years, it was found that for every 1 SD increase in arterial stiffness the odds of having WMH increased by 30% (OR 1.30 (95% CI: 1.16-1.46).[158]

Other measures. Wardlaw et al. found no association with a component measure of large artery disease measure, which included: ischemic heart dx, peripheral vascular disease, other circulatory problems and currently measured ankle brachial pressure index, carotid stenosis, and IMT [160]. Other non-significant findings included Carotid lumen diameter [152], ICA pulsatility index,[101] carotid artery stiffness [153,156], an index of STRAIN [153], and brachial artery endothelial function.[123] However, one study found that increased carotid diastolic diameter was associated with higher log WMH volumes.[153]

SBI / Lacunes. All seven analyses examining the relationship between large artery characteristics and SBI found significant relationships.[119,123,128,150,152,153,159] Five of these studies were cross-sectional,[123,128,150,152,153] one was longitudinal looking at progression of markers,[119] and one was a meta-analysis.[159]

Atherosclerosis. Three out of five studies which examined the relationship between atherosclerosis and SBI / lacunes found a positive relationship between atherosclerosis, atherosclerotic lesions, or plaques and SBI / lacunes.[119,128,150,152,159] Uehara fount the relationship to be significant in the basal ganglia but not white matter.

IMT. CIMT was found to be related to progression in a longitudinal study[119], but not to prevalence of SBI / lacunes cross-sectionally [152].

PWV. Additionally, CFPWV was found to be related to SBI / lacune.[123]

Other measures. Carotid diastolic diameter[153] and increasing carotid lumen diameter[152] were both found to be related to SBI / lacunes.

4.1.7.2 Novel neuroimaging markers of SVD

CBF. One cross-sectional study examined the relationship between CBF and subclinical vascular disease measures [151]. Jennings et al. found that increased carotid intima-media

thickness was related to lower total CBF in a population with a mean age under 60. This relationship remained after adjustment for age, race, current smoking status, sex, total brain volume, CMR measure, metabolic syndrome, or the Framingham index.

CVR. We found no studies evaluating the association of large vessel characteristics with CVR.

FA/MD. We found one study investigating the relationship between FA/MD and subclinical vascular disease.[162] In this cross-sectional study, they found that increases in carotid-femoral pulse wave velocity was associated with decreased regional FA, in both the corpus callosum and the corona radiata (8.7 and 8.6 cc, respectively, $P<0.001$), in a population with a mean age less than 60 years old.

4.1.7.3 Summary

In summary, there have been many studies examining subclinical vascular disease and SVD outcomes. The majority of these studies have been in populations older than 60 years old, are cross-sectional, and have examined WMH or SBI. It seems that some large vessel characteristics may be related more strongly to WMH than others (e.g. vascular stiffness and plaques compared to IMT), while almost all studies of various components of subclinical vascular disease and SBI found significant relationships. More prospective studies are needed to examine why some large vessel characteristics appear to have stronger relationships to certain markers of SVD than others, as well as, in younger populations and evaluating earlier markers or brain health. For example, everyone at an older age may have advanced IMT, limiting the variation and ability to detect associations in older populations. However, as IMT reaches the limits of progression, plaque will occur making it a better late stage marker for advanced vascular disease in older populations.

The opposite might be true as well, where IMT may be better at detecting risk of SVD if measured in younger populations, while plaque would be extremely rare at young ages.

4.1.8 Composite Cardiovascular Disease Risk Scores

4.1.8.1 Traditional neuroimaging markers of SVD

WMH. A composite score of cardiovascular disease including diabetes, hypertension, heart disease, and clinical stroke was cross-sectionally associated with higher WMH burden, particularly in Blacks.[163] Wardlaw et al. had similar results when using a composite measure of vascular risk history including hypertension, diabetes, hypercholesterolemia, smoking, and currently measured blood pressure, hemoglobin a1c (HbA1c), and plasma cholesterol. Importantly, although this cross-sectional relationship was statistically significant, the vascular risk factors explained only ~2% of variance in WMH, suggesting that non-vascular factors have more explanatory power.

SBI/lacunes. No studies evaluated composite cardiovascular disease risk scores with SBI/lacunes.

4.1.8.2 Novel neuroimaging markers of SVD

No studies evaluated the relationship of composite cardiovascular disease risk scores with CVR, FA/MD, or direct vessel measures. In a community-based study with a mean age of 42.6, Jennings, et al. found that increased Framingham risk scores were significantly associated with decreased total CBF, even after adjustment for age, race, current smoking status, sex, total brain volume, CMR measure, and metabolic syndrome.[151]

4.1.8.3 Summary

Few studies examined the relationship between composite cardiovascular disease risk scores and markers of SVD. Only one study examined this relationship in a population with a mean age <60.[151] Among these studies, cardiovascular disease risk was consistently related to increased risk of SVD markers, however vascular risk factors may only explain a small amount of the variance in SVD markers,[160] and therefore, other explanatory factors and markers of subclinical disease should be investigated.

4.1.9 Clinical Vascular Disease

4.1.9.1 Traditional neuroimaging markers of SVD

WMH. Two cross-sectional studies found no significant relationships with clinical vascular disease and WMH.[108,112]

SBI/lacunes. In a cross-sectional study in a Japanese population ischemic heart disease was related to an increase in SBI within the basal ganglia but not within the WM.[128]

4.1.9.2 Novel neuroimaging markers of SVD

No studies evaluating the association of clinical vascular disease with novel markers of SVD were found.

4.1.9.3 Summary

The overall findings suggest that clinical vascular disease is not related to markers of SVD. However, there are several reasons that this may be an artifact rather than the true

relationship. First and foremost, this could be an artifact of the inclusion/exclusion criteria of this literature review. The included studies were selected to evaluate relationships in healthy populations, and specifically excluded any group that was hospitalized or recovering from an acute event. Second, it could be that many individuals with overt disease are too ill to participate in research, leaving only the healthiest and most resilient individuals in the reviewed studies. Finally, it could be that other factors confound this relationship, especially given that all the reviewed studies were cross-sectional. Future prospective studies are needed to clarify the nature of the relationship between clinical vascular disease and SVD.

5.0 BARRIERS TO PROGRESS AND GAPS IN KNOWLEDGE

There are several barriers preventing progress in the field, as well as gaps in knowledge that this dissertation will address.

5.1 SVD NEUROIMAGING CAPTURES LATE-STAGE CHANGES

First, traditional neuroimaging markers of SVD capture late-stage changes, and these have become substituted for direct knowledge of diseased vessels. This quote from Pantoni is an apt summary of the problem:

> "Unlike large vessels, small vessels cannot be currently visualised in vivo; therefore, the parenchyma lesions that are thought to be caused by these vessel changes have been adopted as the marker of small vessel disease, and small vessel disease has become a synonym of brain parenchyma lesions."[3]

While WMH demonstrate parenchymal damage (myelin pallor, axonal loss, gliosis, and edema), they also show reduced CBF and abnormalities of the small penetrating vessels seen on post-mortem exam, such as tortuosity, venous collagenosis, and arteriolar thickening.[2] However, researchers have been unable to measure the vessels in vivo in humans.

5.2 RISK FACTOR INTERACTIONS ARE NOT EVALUATED

Second, prior studies of VCMRF and brain health typically do not examine interactions of the VCMRF with one another nor with important other non-modifiable factors such as sex, age, and *APOE*4* positivity. This is likely because these factors tend to be correlated, and the number of interactions of interest is large. But we are living longer with multiple chronic conditions, and such multimorbidity increases with age.[164,165] In this era of multimorbidity among older adults, these interactions are critical to evaluate in order to understand which combinations of risk factors and non-modifiable factors increase risk the most. This will allow us to target specific preventions and treatments to specific sub-groups based on risk profile, moving us toward the promise of precision medicine.

6.0 PROPOSED SOLUTIONS TO ADDRESS GAPS IN KNOWLEDGE

6.1 PROPOSED SOLUTION FOR PROBLEM 1: SVD NEUROIMAGING CAPTURES LATE-STAGE CHANGES

Ultra-high field neuroimaging such as 7T MRI has emerged as a way to image the small deep medullary veins in the brain. The high strength of the magnet increases signal to noise ratio,[166] resulting in exceptionally clear images. SWI uses both phase and amplitude information for endogenous contrast. It exploits the paramagnetic properties of the deoxyhemoglobin in deoxygenated blood to visualize the cerebral small veins. Although seminal SWI papers describe the ability of the modality to visualize these veins,[27,167] they describe neither how to measure them nor their implications for brain health. To address this limitation, I develop a method using SWI for direct small vein measurement in older adults. Relationships of small vein characteristics with potential neurovascular integrity promoters are evaluated cross-sectionally.

Second, whether neurovascular integrity promoters are related over time with improvement in direct measures of small vessel health is unknown. In the second analysis, within the context of a randomized controlled trial, I evaluate whether candidate neurovascular promoters including physical activity and growth factors can improve cerebral small vein health profiles as characterized by this 7T SWI method.

6.2 PROPOSED SOLUTION FOR PROBLEM 2: RISK FACTOR INTERACTIONS ARE NOT EVALUATED

Existing evidence demonstrates that there are some known interactions of interest such as those of VCMRF with age and sex. Female sex is associated with SBI/lacunes[119,150,168] progression of WMH[119] in those >60 years of age. This impact of sex on brain health appears to vary by age, although few studies have been designed to evaluate this. In addition, there are specific interactions of VCMRF which have been suggested by existing literature. These include interactions of diabetes with SBP[169] and sleep.[137] An understanding of interactions is crucial given the propensity of older adults to have multiple chronic health conditions. Evidence regarding these interaction effects can be used for both risk-stratification and to tailor interventions. To address this gap, I carry out a study across 10 years of follow-up in an observational cohort to 1) evaluate several a priori interactions of interest as described above and 2) explore new interactions via a classification and regression tree (CART).

7.0 POPULATION NEUROSCIENCE FRAMEWORK

The three papers presented next in this dissertation are based on a population neuroscience framework, and as such, it is important to understand some background regarding this approach and how the papers of this dissertation fit into that framework. The bodies of literature in both human psychology[170] and neuroscience[171] have been critiqued as being based on those who are Western, educated, industrialized, rich, and democratic (WEIRD). It is also worth pointing out that studies of human neuroscience are generally small and often use convenience samples of healthy college undergraduates as study participants. Of primary interest in this dissertation is the problem this poses when our population of interest turns to those who are older and less healthy.

It is in this context that the field of population neuroscience of aging has evolved. Population neuroscience applies epidemiological methods and translational research to draw conclusions that are more generalizable or more adequately leverage population heterogeneity to study determinants of health and disease.[172,173] Falk, et al. point out that in this approach, sampling strategy and mechanisms of health and disease are emphasized.[172] Other features include: multilevel views of exposures including environment, behavior, medical conditions, and molecular markers; well-characterized study samples; life-course and longitudinal study designs; and both descriptive and interventional epidemiology. Such an approach requires multidisciplinary teams of researchers from epidemiology, biostatistics, psychology, neuroscience / neuroimaging, and other fields to carry out studies fusing brain and behavior.

Ganguli, et al. have recently suggested re-conceptualizing neuroepidemiology and psychiatric epidemiology studies of dementia as population neuroscience.[174] This dissertation focuses on promoting cerebral small vessel integrity in order to prevent cognitive impairment and dementia—exactly the kind of work that can be done with a population neuroscience approach. Papers 1 and 2 present descriptive and intervention work respectively in the context of an MRI sub-study within a randomized controlled trial of physical activity. The focus is on understanding of biologically plausible pathways to cerebral small vessel integrity. These studies employ careful characterization of the study participants, use of molecular markers, and advanced neuroimaging methods. Paper 3 is a population-based study of incident cognitive impairment. Participants were selected from voter rolls to be representative of the population. Once again, careful characterization of the study participants, including detailed neuropsychological testing, is used. What unites these three papers is the population neuroscience framework. Carrying out the work represented in the three papers together has provided me with the skills and insights that prepare me to carry population neuroscience research forward in the next stage of my academic career.

8.0 PAPER 1: CROSS-SECTIONAL RELATIONSHIP OF PA AND GROWTH FACTORS WITH CEREBRAL SMALL VESSEL INTEGRITY

In Vivo Imaging of Venous Side Cerebral Small Vessel Disease in Older Adults: An MRI Method at 7T

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ABBREVIATIONS

SVD = small vessel disease; AD = Alzheimer's disease; WMH = white matter hyperintensities; aβ = β amyloid; *APOE4* = Apolipoprotein E e4 allele; VEGF = vascular endothelial growth factor; BDNF = brain-derived neurotrophic factor; LIFE MRI = Lifestyle

Interventions and Independence for Elders Magnetic Resonance Imaging study; 3MS = Modified Mini-Mental State Examination

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8.1 ABSTRACT

Background and Purpose. Traditional neuroimaging markers of small vessel disease focus on late-stage changes. We aimed to adapt a method of venular assessment at 7Tesla for use in older adults. We hypothesized that poorer venular morphological characteristics would be related to other small vessel disease neuroimaging markers and higher prevalence of small vessel disease-Alzheimer's disease risk factors.

Materials and Methods. Venules were identified in periventricular regions of interest on SWI and defined as tortuous or straight. Tortuosity ratio was defined as total tortuous venular length divided by total straight venular length. WMH burden (visually rated from 0 to 3) and number of microbleeds (0, 1, >1) were determined. Differences in tortuous and straight venular lengths were evaluated. Relationships with demographic variables, *APOE4*, growth factors, pulse pressure, physical activity, and Modified Mini-Mental State examination were assessed via Spearman correlations.

Results. Participants had 42% more tortuous venular tissue than straight (median [95% CI]: 1.42 [1.13, 1.62]). *APOE4* presence was associated with greater tortuosity ratio (rho=0.454, p=0.001), and these results were robust to adjustment for confounders and multiple comparisons. Associations of tortuosity ratio with sex and vascular endothelial growth factor did not survive adjustment. Associations of tortuosity ratio with other variables of interest were not significant.

Conclusion. Morphological measures of venules at 7T could be useful biomarkers of early stages of small vessel disease and Alzheimer's disease. Longitudinal studies should examine the impact of *APOE* and VEGF on risk of venular damage.

8.2 INTRODUCTION

Cerebral small vessel disease (SVD) increases dementia risk[175] and vulnerability to Alzheimer's disease (AD) neuropathology.[176] Neuroimaging methods investigating SVD have traditionally relied on WM hyperintensities (WMH). However, WMH is a marker of late-stage SVD, reflecting advanced parenchymal damage, reduced CBF, and abnormalities of the small penetrating vessels.[2] Thus, there is a need for radiological markers that capture the earlier stages of SVD relating directly to vessel health.

With aging and hypertension, arteries have reduced ability to absorb flow pulsatility, thus transmitting highly pulsatile flow to the venules. Venular walls are well equipped to handle low pulsatile and slow flow, but not highly pulsatile flow. Pulsatility-related damage can induce venular morphological changes such as collagenosis, leading to loss of elasticity and lumen narrowing/occlusion, which in turn promote ischemia. Both collagenosis and tortuosity lead to reduced CBF and increased upstream resistance, exacerbating arterial pathology. Extravasation and inflammatory response, including focal perivascular parenchymal infiltration, can also occur facilitated by the lack of tight junctions on the venous side circulation.[177] Inflammatory cascades further damage the vasculature, reduce CBF, and compromise the BBB. These phenomena can become apparent as morphological changes such as tortuosity, collagenosis, and thicker basal lamina. Such changes have been seen in vivo in AD,[178] and in postmortem studies appear more common with older age and in proximity to regions with WMH.[179-181] Although it has not been tested directly, venular morphological alterations are considered to precede radiologically overt WMH.

Ultra-high field (7Tesla) MRI has emerged as a non-invasive method to visualize venous microcirculation.[27,167] Specifically, SWI exploits the paramagnetic properties of deoxyhemoglobin to visualize venules without a contrast agent. Methods to quantify venules in multiple sclerosis,[182] sickle cell anemia,[183] CADASIL,[180,184] and recently AD[185] have been reported. However, venular characteristics in relation to cerebral SVD in aging are unknown.

Our primary aim was to demonstrate the feasibility of adapting published methods[182,183] to study venular characteristics in older adults. Our secondary aim was to evaluate relationships of venular characteristics with neuroimaging markers of SVD—WMH and microbleeds—and variables relevant to SVD and AD. We hypothesized that poorer venular morphological characteristics would be related to other SVD neuroimaging markers and higher prevalence of SVD-AD risk factors.

8.3 METHODS

8.3.1 Participants

The Lifestyle Interventions and Independence for Elders MRI study (LIFE MRI) is a neuroimaging study within a randomized controlled trial, which demonstrated that physical activity prevents major mobility disability in at-risk community-dwelling older adults vs. health education control (hazard ratio, 0.82, p=0.03).[186] The study protocol was approved by the University of Pittsburgh Institutional Review Board. All participants

provided written informed consent. The present study (N=53) uses images from the baseline visit.

The LIFE study design was previously reported.[187] Supplemental Table 1 shows inclusion/exclusion criteria. Participants were not screened for MRI based on caffeine use due to minimal reported average caffeine-related signal change of veins in white matter (-2±1.2%).[188]. Supplemental Figure 1 shows participant flow.

8.3.2 Sample characteristics

Age, race, and sex, self-reported by participants, were evaluated because of their association with SVD and AD.[11-13,189,190] Apolipoprotein E (*APOE*) was genotyped using TaqMan (TaqMan probe C__904973_10, Applied Biosystems, Life Technologies, CA) and Pyrosequencing.[191] *APOE4* is the strongest genetic risk factor for late-onset AD.[192] Pulse pressure (systolic blood pressure-diastolic blood pressure (average of two seated measurements)) and physical activity were assessed because of their associations with AD[30,193] and SVD.[194,195] Physical activity was measured for seven days using hip-worn accelerometry (GT3X, Actigraph, LLC) as minutes per day of moderate physical activity. Finally, Modified Mini-Mental State Examination (3MS)[196] was included as a measure of global cognition.

8.3.3 Growth factors

The angiogenic factors vascular endothelial growth factor (VEGF)[79] and brain-derived neurotrophic factor (BDNF)[197] were measured via the Luminex system using kits (Bio-

Rad Human Cancer Panel EMD Millipore Human Neurodegenerative Disease Panel). Fasting blood, collected by venipuncture, remained at room temperature for 30-60 minutes to clot, and was then centrifuged at 1600xg for 15 minutes at 4°C. Serum was aliquoted and immediately frozen at ≤-70°C and stored until analysis. Concentrations were determined with two sets of standard curves, with final values calculated according to standardized procedures we have validated.[198]

8.3.4 Potential Confounders

Self-reported antihypertensive medication use, which may affect pulse pressure, was recorded. Blood hemoglobin was measured as it may affect venular conspicuity on SWI. We also recorded SWI voxel size, which varied among the participants.

8.3.5 Outcome Variables

8.3.5.1 Venular characteristics

Axial SWI MRIs were obtained at the University of Pittsburgh MR Research Center using a Siemens 8-Channel head coil on a 7Tesla scanner (TR=2000 ms; TE=15 ms; 64 slices). Voxels were 0.25x0.25x1.50 mm (x, y, z; N=40). Some scans were initially acquired with 0.23x0.23x3.00 mm voxels, inter-leave gap=0.60 mm (N=12), or with 0.50x0.50x1.00 mm voxels (N=5) and were resampled to 1.50 mm slice thickness.

A 4X1cm ROI was placed in each hemisphere, one slice below the uppermost slice on which ventricular CSF was visible. To maintain consistency, the ROI was placed based on native-space landmarks, centered along the anterior-posterior length of the ventricle,

and placed on the ventricle's lateral wall (Supplemental Figure 2). The ROI was chosen because it corresponds to regions known to be vulnerable to SVD,[178,184,185] is consistent with published methods,[182,183] and allows for the greatest consistency in vessel orientation, with a clear course perpendicular to the length of the lateral ventricles. The minimum intensity projection (MinIP) was applied over three slices (4.5 mm) to improve visualization.[27]

Three raters (CES, DRJ, NAM), were trained and overseen by a certified neuroradiologist (JM) and the study PI (CR). First, published protocols were studied and discussed among the raters, neuroradiologist, PI, and coinvestigators (HJA, RLM). Next, the same five MRIs were rated by the raters, each blinded to the tracings of the other two. Last, each venular tracing was discussed among the raters with the neuroradiologist and PI regarding presence/absence and straight/tortuous course. This was done until the raters were proficient in tracing and the results of their consensus were consistent with the judgment of the neuroradiologist/PI. Tracing was done using OSIRIX.[199] Criteria to identify a venule were: a linear structure of intensity darker than the surrounding parenchyma; length $\geq$3 mm; and coursing through the ROI for $\geq$ 3mm (to reduce inter-rater variability of inclusion for vessels along the edge of the ROI). Most venules could be followed to obvious deep veins, and the dark appearance and orientation axial to the ventricles and deep within the WM also helped to identify the origin of the vessels as venous. Venules were traced across their full length, even if they continued outside of the ROI, to avoid artificial truncation. After all venules were traced, presence/absence of a venule was adjudicated by consensus among raters. A venule was included only if $\geq$2 of the three raters had traced it (Figure 1). Next, venular course (straight/tortuous) was rated

during the consensus meeting. A vessel that ran free of inflexion points ≥30° for the majority of its total length (> 60%) was defined “straight”; otherwise the vessel was defined “tortuous”. The length of each venule was computed as the median value of the lengths measured by the raters tracing that venule. Number and length of all consensus-traced venules were summed and total length and average length (total length/venule number) obtained for each participant. Tortuous venules are present in areas with WMH,[179] thus we evaluated tortuous and straight venules separately. Tortuosity ratio was calculated as total tortuous length divided by total straight length. Thus, a tortuosity ratio>1 indicates greater tortuous venular length than straight length. Due to BOLD-related signal blooming, measures of diameter may not be accurate, thus we did not quantify diameter.

8.3.5.2 White matter hyperintensities

WMH was imaged using T2WI (TR=12500 ms; TE=55 ms; voxel size=0.5x0.5x6.0 mm) and MPRAGE (TR=3430 ms; TE=3.54 ms; voxel size=0.7mm^3 isotropic) and rated by consensus of two raters (CR, HJA) using a 0-3 modified Fazekas rating scale.[200] Ratings consisted of the following: 0=none: no punctate hyperintense areas or periventricular rims; 1=mild: few punctate hyperintense areas and/or limited amount of hyperintense rims around the ventricular horns; 2= moderate: multiple punctate hyperintense areas and/or larger rims around the ventricular horns; or 3=severe: confluent subcortical hyperintense areas and/or rims all around the ventricles, including horns and sides. Only 4/53 had no WMH (WMH=0), leaving 92% with at least mild WMH; thus, we combined 0 and 1 to create a “none/mild” category. Because the distinction between periventricular and deep WMH is not consistently meaningful, we did not differentiate between them.[201]

8.3.5.3 Microbleeds

We classified cerebral microbleeds based on Greenberg, et al.[202] Two trained raters (NAM, ELT) characterized microbleeds under the supervision of a neuroradiologist (JM). Microbleeds were defined as: black or substantially hypointense on SWI; round or ovoid (confirmed on adjacent slices); and at least half surrounded by brain parenchyma. To take advantage of the 7T magnet's ability to capture quite small microbleeds, no minimum size criterion was used. Final ratings were based on consensus with disagreements mediated by the neuroradiologist. We counted total number of microbleeds across all 64 slices of the axial SWI and categorized totals as 0, 1, or >1 microbleed.

8.3.6 Statistical Analysis

Descriptive statistics were calculated as counts and percentages, means and standard deviations (SD), or medians and interquartile ranges (IQR). Differences were tested with t-tests, Wilcoxon rank sum tests, or chi-square tests, $\alpha=0.05$. We also determined the median tortuosity ratio and calculated the 95% CI using 10,000 bootstrapped samples.

We explored relationships of tortuosity ratio with other neuroimaging markers of SVD including WMH and microbleeds; non-modifiable factors including demographic variables (age, race, and sex) and *APOE4*; potentially modifiable factors including growth factors (VEGF and BDNF), pulse pressure (adjusted for antihypertensive medication use), and physical activity; 3MS; and hemoglobin, with Spearman correlations, $\alpha=0.10$. Significant correlations with tortuosity ratio were re-evaluated as partial correlations adjusted for hemoglobin and voxel size. A false discovery rate (FDR) of 0.10 was used to correct for multiple comparisons.

Statistical analysis was performed in SAS version 9.4[203] and SPSS version 22.[204]

8.4 RESULTS

MRI study participants were younger and less likely to be non-Hispanic white than the non-MRI study participants (Table 1). Of MRI study participants, 15/47 with *APOE* data had at least one copy of the *APOE4* allele. Thus, representation of *APOE4* was higher than the 14% estimate among controls worldwide,[205] but did not differ significantly from non-MRI participants. No or mild WMH were seen in 58.5% of participants while 20.8% each had moderate and severe WMH. Regarding microbleeds, 39.6% of the sample had 0, 20.8% had 1, and 24.5% had >1.

Total length of tortuous vessels ranged from 26.25-246.36 mm, while total straight length ranged from 16.72-217.65 mm. Overall length of tortuous venules was greater than that of straight venules (Table 2). Total tortuous length was 42% greater than total straight length (median tortuosity ratio [95% bootstrapped CI]: 1.42 [1.13, 1.62]); (Supplemental Figure 3). To examine whether this was due to venular number or average length, we evaluated differences in those measures. Total number of tortuous venules ranged from 4-32, while total straight venules ranged from 2-24. There were more tortuous venules than straight venules. The range of average tortuous length was 6.56-10.93 mm while the range of average straight length was 4.35-11.57 mm, and these average lengths were not significantly different. Thus, the difference in total tortuous and straight venular lengths was driven by greater numbers of tortuous venules.

Correlations between neuroimaging markers of SVD and tortuosity ratio were not significant. WMH correlated at rho= -0.125, p=0.37 and microbleeds correlated at rho= -0.059, p=0.70.

Among non-modifiable variables associated with AD and SVD, sex was associated with tortuosity ratio (Table 3). Males had a higher tortuosity ratio (median (IQR), 2.15 (0.98)) than females (median (IQR), 1.31 (0.71)). Those with at least one copy of the *APOE4* allele had a higher tortuosity ratio (median (IQR), 2.15 (1.78)) than those without (median (IQR), 1.21 (0.75)). Associations with age and race were not significant (p>0.10).

Among modifiable factors potentially influencing venular characteristics, higher VEGF was associated with lower tortuosity ratio. There were no significant associations with BDNF, pulse pressure (adjusted for antihypertensive use), physical activity, or 3MS score (p>0.10). Results were similar when using a ratio of vessel counts instead of the ratio of total lengths.

The relationship of *APOE4* with venular tortuosity, but not the other findings, remained significant after FDR correction of the p-value (p=0.01). Further adjustment for hemoglobin and voxel size did not modify the association with *APOE4*.

8.5 DISCUSSION

We found that application of 7T SWI is feasible to image cerebral venular characteristics in vivo in older adults. This method is a novel way to visualize an understudied component of the cerebral vasculature. Given associations of venular tortuosity with SVD[179] and AD,[206,207] as well as increases in microvascular changes with age,[181] venular tortuosity

may serve as a marker of declining cerebrovascular integrity. This method may afford earlier detection of SVD and has the advantage of characterizing venular morphology without contrast.

We also found that *APOE4* was associated with higher tortuosity ratio, and this association was robust to adjustment for potential confounders and multiple comparisons. This result supports studies implicating *APOE4* in reduced vascular integrity. APOE4 protein can directly damage the vasculature.[208] *APOE* is associated with neuroimaging manifestations of SVD,[209,210] and there are indications that it is associated with microvascular changes. Mice expressing transgenic human *APOE4* have altered basement membrane protein expression.[211] In humans with AD, *APOE4* is associated with BBB disruption.[212] *APOE4* is associated with both increased deposition and reduced clearance of aβ.[208] Clearly, *APOE4* is central to development of AD pathology, and our results suggest it could be implicated in venular damage. It is possible that aβ deposition induces venular damage. An AD mouse model showed that as aβ built up in the arterioles beginning at 5 months of age, venular mural cells were damaged by 7 months of age.[207] However, it is also possible that venular damage induces aβ deposition. In this same experimental model, further venular mural cell damage led to increased arteriolar aβ deposition, and interestingly, induction of venular tortuosity.[207] Temporality of venular damage and aβ deposition remains an open question. We were unable to collect amyloid imaging. Hence, future multimodal neuroimaging studies need to evaluate timing and relationship of aβ burden and venular tortuosity.

Although our result is remarkably consistent with proposed *APOE4*-mediated reduction of vascular integrity,[208] our study cannot clarify the underlying mechanism(s).

This limitation notwithstanding, the fact that *APOE4* is associated with venular tortuosity indicates the potential for risk stratification as an intervention strategy. Thus, other factors should be evaluated to offset *APOE4*-related risk.

We found a non-significant association of tortuosity ratio with WMH and microbleeds, which could be due to lack of sensitivity in our visual ratings or the small sample size. Future larger studies should evaluate associations of tortuosity ratio with WMH volume, a more sensitive measure as compared to visual ratings. Alternatively, this lack of association may indicate that tortuosity ratio is capturing novel, early information regarding vascular integrity. Future work should examine relationships of tortuosity ratio with other SVD neuroimaging markers and related cognitive and mobility impairment and clarify temporal order of venular damage and other SVD neuroimaging manifestations. We predict that venular damage comes before traditional neuroimaging markers of SVD.

Our study has several limitations. The sample was not selected to have particularly low or high SVD burden. Future studies should compare venular tortuosity ratio in those two groups. Larger samples will be needed to confirm associations with sex and VEGF. The venular measures are also two-dimensional, and therefore do not account for venules running out of the plane. However, this bias is non-differential across our sample. Finally, MRI participants were younger and had a higher proportion of non-whites indicating this sample may differ from the general community-dwelling older adult population.

Despite these limitations, our study has notable strengths. We applied ultra-high field neuroimaging with higher SNR than typically used to visualize novel venular characteristics. This allows for smaller sample sizes at ultra-high field than would be required at lower field strength. Because this neuroimaging study was also within a

randomized controlled trial, these participants were extremely well-characterized, allowing us to control for potential confounding factors.

8.6 CONCLUSIONS

SWI at 7T offers a non-invasive method to image markers of cerebral venular integrity and fills an important gap in knowledge. Morphological measures of venules at 7T could be useful biomarkers of early stages of SVD and AD. Risk and protective factors, especially those that are modifiable, for these pathophysiologic changes should be evaluated. Future longitudinal multimodal studies characterizing venular integrity at 7T are warranted.

8.7 ACKNOWLEDGEMENTS

The authors thank Erica Lynn Tamburo for neuroimaging assistance and Joshua Michel for implementing standardized Luminex assays.

8.8 FIGURES AND TABLES

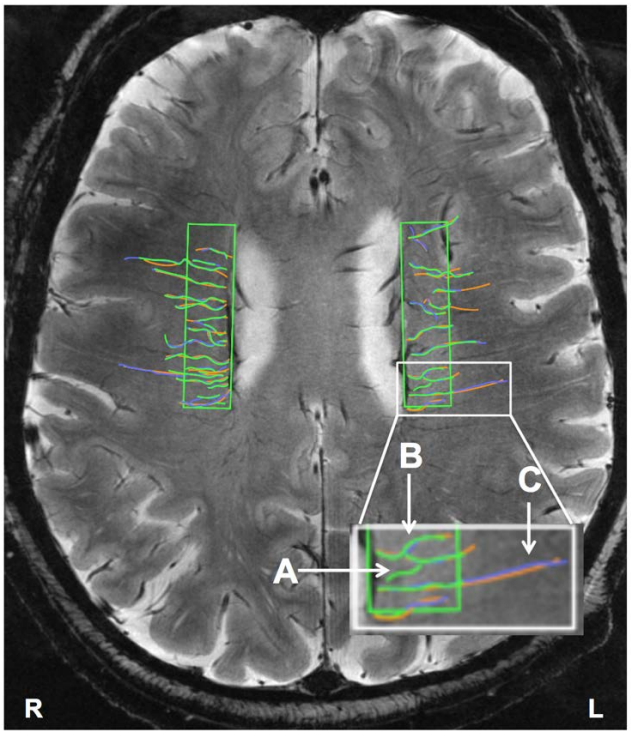

Figure 8-1. A sample consensus venular tracing on SWI MRI at 7 Tesla across ROIs in both hemispheres in the LIFE MRI study

Each rater traces the venules. A different color (green, purple, orange) is assigned to each rater, and the three sets of tracings are then overlaid. Inset in white is shown at larger magnification at the bottom of the figure to illustrate: A) an example of a venule that would not be included in the dataset because it was traced only by one of the three raters (green); B) an example tortuous venule; and C) an example straight venule.

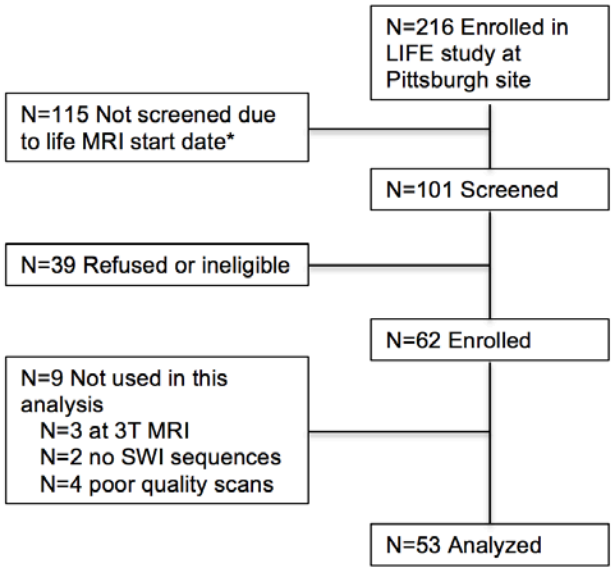

Figure 8-2. Supplemental Figure 8-1. LIFE MRI study flow at baseline

*LIFE study began in March 2010 and LIFE MRI began in December 2010.

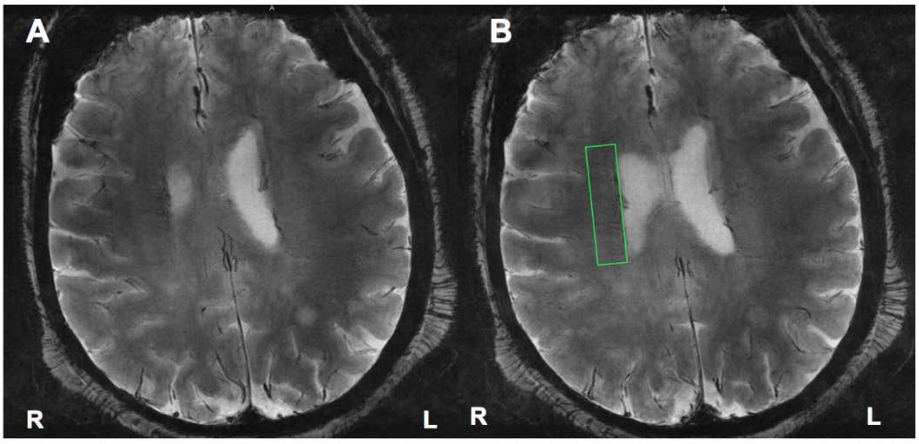

Figure 8-3. Supplemental Figure 8-2. Example ROI placement in the right hemisphere

A: The last slice on which CSF is visible is found. B: Then the 4X1cm ROI (green box) is placed on the slice where the ventricle is visible, one slice below A. The minimum intensity projection is used over three slices (4.5 mm) to improve visualization of continuity of low intensity venous structures.

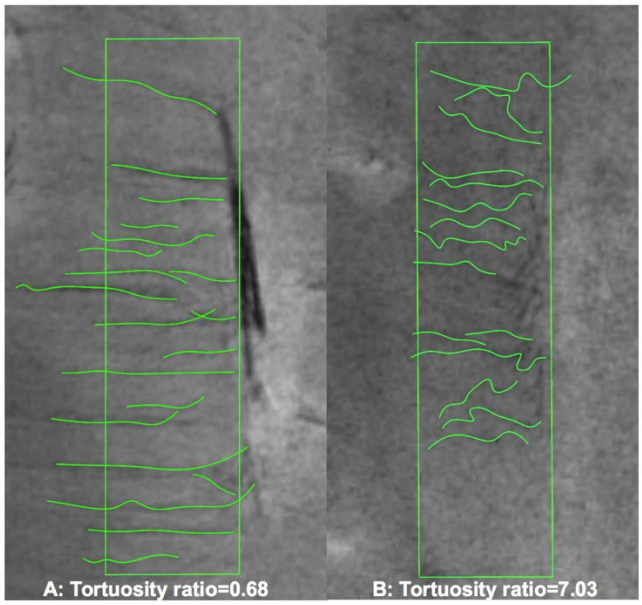

Figure 8-4. Supplemental Figure 8-3. Example right hemisphere ROIs of individuals with low (A) and high (B) tortuosity ratios

Table 8-1. Study sample characteristics in the LIFE study at the Pittsburgh Site

	MRI Study N=53	Non-MRI Study N=163	p-value
Age (years), Median (IQR)	76.0 (5.8)	79.4 (9.0)	**<0.01**
Race, non-Hispanic white, N (%)	30 (56.6)	124(76.1)	**<0.01**
Sex, female, N (%)	42 (79.2)	123 (75.5)	0.57
APOE4 allele presence,[a] N (%)	15 (31.9)	27 (20.0)	0.10
VEGF, pg/mL, Median (IQR)	414.61 (370.17)	--	
BDNF, pg/mL, Median (IQR)	1978.30 (27492.00)	--	
Pulse pressure, median (IQR)	53 (13)	57 (18)	0.06
Physical Activity—Minutes of daily moderate activity, Median (IQR)	24.6 (31.6)	18.3 (22.7)	0.05
3MS, Median (IQR)	93 (7)	92 (9)	0.46
Severe WMH burden,[b] N (%)	11 (20.8)	--	
No microbleeds,[c] N (%)	21 (39.6)	--	
Confounders			
On antihypertensive medication, N(%)	39 (73.6)	119 (73.0)	0.93
Hemoglobin, g/dL,[d] Median (IQR)	12.7 (1.2)	13.2 (2.0)	0.22

Note: [a]Available on N=47 (MRI) and N=135 (non-MRI). [b]WMH: white matter hyperintensities, rated as 0= none/mild, 1= moderate, 2= severe. [c]Available on N=45 due to scan quality or motion; the remaining 24 were split nearly evenly between 1 and >1. [d]Available on N=47 (MRI) and N=139 (non-MRI). APOE4: Apolipoprotein E e4 allele. 3MS: Modified Mini-Mental State Examination. VEGF: Vascular endothelial growth factor. BDNF: Brain-derived neurotrophic factor.

Table 8-2. Venular length measures in LIFE MRI (N=53) for tortuous and straight venules separately

	Straight	Tortuous	P-value*
Total length of venules (mm), mean (SD)[a]	156.87 (53.18)	111.41 (50.11)	**<0.0001**
Number of venules, mean (SD)	18.09 (5.87)	13.11 (5.34)	**<0.0001**
Average length of venules (mm), mean (SD)	8.64 (0.87)	8.30 (1.42)	0.07

Note: *P-values based on paired t-tests comparing tortuous and straight venule characteristics; [a] Venule lengths: For each participant, venules are traced in 4 cm^2 regions of interest (one in each hemisphere), their length measured by three raters, and median length computed for each vessel. Venules are characterized as straight or tortuous. The total straight and tortuous venular length in mm is calculated for each participant. [b] Tortuosity ratio: Total tortuous venular length in mm divided by total straight venular length in mm.

Table 8-3. Spearman correlations of tortuosity ratio with variables of interest to small vessel disease and Alzheimer's disease in LIFE MRI (N=53)

	rho	p
Age	-0.023	0.87
Race	0.202	0.15
Sex	-0.304	**0.03**
APOE4	0.454	**0.001**
Pulse pressure[a]	0.206	0.14
VEGF	-0.236	**0.096**
BDNF	0.227	0.11
Hemoglobin	0.266	**0.07**
Physical Activity—Minutes of daily moderate activity	-0.187	0.20
3MS	0.199	0.15

Note: [a] Partial correlation of pulse pressure and tortuosity ratio adjusted for antihypertensive drug use. *APOE4*: Apolipoprotein E e4 allele. VEGF: Vascular endothelial growth factor. BDNF: Brain-derived neurotrophic factor. 3MS: Modified Mini-Mental State Examination.

Table 8-4. Supplemental Table 8-1. Inclusion / exclusion criteria for the LIFE MRI study

Inclusion	Exclusion
Parent Study Criteria	
1) age 70-89 2) sedentary activity level: <20 minutes per week of regular physical activity in the past month and <125 minutes per week of moderate physical activity 3) high risk of mobility disability: ≤ 9 on the Short Physical Performance Battery 4) ability to walk 400 m in less than 15 minutes without sitting, leaning, or getting assistance	1) 3MS score ≥ 1.5 standard deviations below education and race-specific norms 2) inability to safely participate in the intervention as determined by medical record review, physical exam, and electrocardiogram
LIFE MRI Specific Criteria	
1) willingness to complete an MRI scan at study baseline and after two years	1) meeting any MRI exclusion criteria including metal in the body or claustrophobia

Note: 3MS=Modified Mini-Mental State examination

9.0 PAPER 2: PA INTERVENTION ON CEREBRAL SMALL VESSEL INTEGRITY

Physical Activity and Cerebral Small Vessel Integrity in Older Adults

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9.1 ABSTRACT

Identifying promoters of cerebral small vessel integrity is important to counter vascular contributions to cognitive impairment and dementia. In this preliminary investigation, the effects of a randomized 24-month physical activity (PA) intervention on changes in cerebral small vessel integrity were compared to those of a health education (HE) control. Cerebral small vessel integrity was measured in 24 older adults (n=8, PA; n=16, HE) using ultra-high field MRI before and at the end of the 24-month intervention. Deep medullary veins were defined as straight or tortuous; percent change in straight length, tortuous length, and tortuosity ratio were computed. Microbleed count and white matter hyperintensities were also rated. Accelerometry-based values of PA increased by 17.2% in the PA group but declined by 28.0% in the HE group. The PA group, but not the HE group, had a significant increase in straight veins length from baseline to 24-month follow-up (p=.02 and p=.21, respectively); the between group difference in percent change in straight length was significant (median (IQR) increase: 93.6%(112.9) for PA, 28.4%(90.6) for HE; p=.07). Between group differences in other markers were non-significant. Increasing PA in late-life may promote cerebral small vessel integrity. This should be confirmed in larger studies.

KEYWORDS: cerebral small vessel disease, physical activity, susceptibility-weighted imaging, vascular contributions to cognitive impairment and dementia, ultra-high field MRI

9.2 INTRODUCTION

Loss of cerebral small vessel integrity is increasingly recognized as a key vascular contributor to cognitive impairment and dementia (VCID), including Alzheimer's disease (AD).[4,5,175,213,214] Thus, focus on identifying promoters of cerebral small vessel integrity is increasing,[6] as they could represent new intervention targets for cognitive impairment. Physical activity (PA) has gained interest as a good candidate promoter of cerebrovascular integrity. In addition to well-known effects on the hippocampus,[35,97,215] recent work suggests that PA may have effects on neuroimaging markers of lesions of presumed vascular origin.[66-68] A limitation of current studies is their use of *indirect* markers of *late-stage* cerebral small vessel disease. Research directly examining the influence of PA on cerebral small vessel integrity has been limited to one small, observational study of self-reported high vs. low aerobic exercise level over the past 10 years on cerebral arteries of healthy older adults by MR angiogram.[216] Longitudinal observational studies with prospective, repeated measures of PA and cerebral small vessel integrity as well as even short-term intervention studies of PA effects on cerebral small vessel integrity are critically missing from the literature as are studies with more rigorous measures of PA. Thus, the effects of PA on in vivo direct measures of early stages of cerebral small vessel abnormalities are currently unknown.

Several growth factors have been proposed as potential mechanisms underlying the beneficial effects of PA on brain vasculature, including brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF). VEGF is a well-recognized angiogenic factor;[78-80] emerging evidence from animal models suggests an angiogenic role also for BDNF[217,218] in addition to its neurogenic effects.[75-77] We have recently shown

in cross-sectional analysis that lower peripheral blood levels of VEGF are associated with a higher tortuosity ratio of deep medullary veins in older adults.[219] Abnormalities in deep medullary venous morphologic characteristics including tortuosity have been found on neuropathological exam in areas affected by cerebral small vessel disease[179] and associated with AD in both animal models[207] and recent human studies.[178,206] Whether PA and PA-related changes in BDNF and VEGF are associated with changes in cerebral small vein integrity has not been tested.

In this preliminary investigation, we conducted a secondary analysis of a randomized controlled trial of 24-month PA vs. health education (HE) control to evaluate the effects of PA on several markers of cerebral small vessel abnormalities. We have recently shown a beneficial effect of PA on hippocampal volume in this cohort.[35] In this analysis, we hypothesized that the PA intervention would be associated with less morphological change of deep medullary veins (e.g. less tortuosity) and less accrual of white matter hyperintensities and microbleeds. Our secondary exploratory aim was to assess the association between PA-related changes in BDNF, VEGF, and neuroimaging markers of cerebral small vessel integrity.

9.3 MATERIALS AND METHODS

9.3.1 Participants

Study participants were from the Lifestyle Interventions and Independence for Elders (LIFE) randomized controlled trial in which 1635 participants were randomized either to PA or HE. The study demonstrated a beneficial effect of the PA intervention on the prevention of major mobility disability.[186] Participants in this analysis are from the magnetic resonance imaging (MRI) sub-study carried out at the Pittsburgh field center. Both the parent and sub-study inclusion/exclusion criteria have been previously reported.[186,219] Briefly, participants had to be sedentary, community-dwelling, 70-89 years old, at risk of mobility disability as demonstrated by a Short Physical Performance Battery[220] score of ≤ 9, but able to walk 400 meters, and agreeable to completing an MRI at study baseline and at the 24-month follow-up visit. Participants were excluded if they were cognitively impaired based on the Modified Mini-Mental State Examination,[196] determined unsafe to participate in the study based on medical record review, or if they met any MRI exclusion criteria such as claustrophobia or metal in the body. The University of Pittsburgh Institutional Review Board reviewed and approved the study protocol, and informed consent was carried out prior to completion of any study procedures.

9.3.2 Intervention

A detailed description of the intervention has been previously reported.[186,187] In summary, participants were randomized either to PA intervention or HE control. PA was multi-

component, involving aerobic activity (walking), light resistance training, and flexibility exercises. PA training included two clinic visits per week and three to four days per week of at-home PA. Moderate intensity walking was defined based on a rate of perceived exertion of 13 / “somewhat hard” on the Borg scale.[221] In a sub-group (N=14; n=7 PA group, n=7 HE group), accelerometry was collected as a more objective measure of PA completion. The HE group received healthy aging classes weekly for the first six months and twice monthly thereafter. Classes covered topics like health screenings, preventive services, and the like, and specifically avoided PA-related topics.

9.3.3 Physical activity

PA was characterized by PA intervention as well as minutes of moderate PA. At study baseline, 6 months, 12 months, and 24 months, daily minutes of moderate PA were measured across seven days using the GT3X hip-worn accelerometer by Actigraph (Pensacola, FL). Moderate PA by accelerometry was defined by a 760 count per minute cutoff.[186]

9.3.4 Sample characteristics

Age, race, and sex were self-reported by participants. *Apolipoprotein E* (*APOE)* genotyping was carried out using TaqMan (Applied Biosystems, Life Technologies, Foster City, California) and Pyrosequencing. [191] We present results here for *APOE*4* allele presence.

9.3.5 Growth factors

Blood was collected while participants were fasting and was centrifuged at 1600 x g for 15 minutes at 4°C after clotting at room temperature for 30-60 minutes. Serum was then aliquoted and stored at ≤ -70°C until analysis. We used Luminex with multiplex kits (EMD Millipore Human Neurodegenerative Disease Panel, Danvers, MA; Bio-Rad Human Cancer Panel, Hercules, CA) to test BDNF and VEGF levels. We used two sets of standard curves to determine concentrations and standardized procedures we have validated in a variety of clinical settings to calculate final values.[198,222,223]

9.3.6 Cerebral small vessel integrity

The full method for characterizing cerebral small veins was previously reported.[219] In summary, we traced deep medullary veins in periventricular regions of interest in both cerebral hemispheres on susceptibility-weighted MRI at ultra-high field strength (7T). Veins were characterized as either straight or tortuous, and using a consensus method with three raters (CES, DRJ, NAM) under the direction of the study PI (CR) and neuroradiologist (JM) we determined total straight and tortuous venous length across both hemispheres for each participant at both baseline and 24-month follow-up. To summarize all information in one metric, we also calculated the tortuosity ratio for each participant. It was defined as total tortuous venous length over total straight venous length.

9.3.7 Microbleeds

Our method for analyzing microbleeds has been previously published.[219] Briefly, under the direction of the neuroradiologist (JM), two raters (NAM, ET) counted microbleeds across 64 axial slices on 7T susceptibility-weighted imaging following Greenberg, et al.,[202] with no minimum size criterion. This allowed for the 7T imaging to capture smaller microbleeds than is possible at lower field strength. Raters came to agreement on counts through a consensus process.

9.3.8 White matter hyperintensities

Our method for analyzing WMH has been previously published.[219] In summary, WMH on T2 weighted MRI and MPRAGE at 7T were rated by two raters (CR, HJA) using a consensus process. Ratings ranged from 0-3 (none-severe) based on a modified Fazekas rating scale.[200]

9.3.9 Other variables

Certified raters administered the Modified Mini-Mental State Examination.[196] This is a global measure of cognition. The score ranges from 0-100 with higher scores indicating better performance. The 4-meter walk from the Short Physical Performance Battery was used to calculate gait speed in meters per second. Participants were asked to walk at their usual pace.

9.3.10 Statistical analysis

Differences between the PA and HE group were evaluated to determine whether randomization of baseline variables held within this sub-study. Differences between the group with MRI at both study baseline and 24-month follow-up and the group with MRI at baseline only were also evaluated. For each of these comparisons, non-parametric tests including the Mann Whitney U test and chi-square tests were used.

To account for baseline values in analyses, percent change from baseline to 24 months was computed for all vein outcomes. These variables were calculated for each individual as (follow-up – baseline) / baseline * 100. Thus, a positive percent change indicates an increase from baseline to follow-up, while a negative percent change indicates a decline from baseline to follow-up. We calculated summary statistics for baseline, 24-month follow-up, and percent change as median (interquartile range (IQR)). Non-parametric tests of median comparisons were used to evaluate differences from baseline to 24 months within intervention groups and differences in percent changes between intervention groups for vein outcomes. Variables indicating worsening microbleed count and WMH grade were created. We defined worsening as present if the 24-month follow-up microbleed count or WMH grade was greater than the baseline value for that variable. Fisher's exact tests were used to assess differences in worsening microbleed count and WMH grade by intervention group.

We visually inspected scatterplots of associations of vein outcomes with PA. Given the small sample size, we repeated the plots with the most extreme values withheld to confirm that the direction of association was maintained. Spearman partial correlations were used to assess relationships of percent change in vein outcomes with PA adjusting

one at a time for percent change in BDNF and VEGF. As a sensitivity analysis, we repeated these correlations of percent changes of vein outcomes with total minutes of moderate PA by accelerometry. For this analysis, the PA variable was a cumulative exposure variable to minutes of moderate PA created by summing accelerometry minutes of moderate PA across all study visits. Finally, we carried out exploratory analyses with growth factors. Percent change variables were created and non-parametric tests of median comparisons within and between intervention groups were carried out in the same way as with the vein outcomes. For any percent change vein outcome found to be related to PA, we visually inspected scatterplots of the percent change vein outcome by the percent change in growth factors across the full combined study sample. These were repeated with extreme values withheld. We used Spearman correlations to assess relationships of percent change in relevant vein outcomes with percent change in growth factors across the full sample. These were repeated as partial correlations adjusting for PA. Due to the small sample size, alpha was set at 0.10 to reduce likelihood of false negatives. When studies are preliminary and exploratory in nature, adjustment for multiple comparisons is likely to result in false negatives and abandonment of potentially promising but nascent lines of inquiry.[224] Thus, in order to preserve interesting results in some of the earliest work in this area, we have not adjusted for multiple comparisons. Statistical analysis was performed in SAS version 9.4.[203]

9.4 RESULTS

This study sample consisted of 24 participants who had 7T MRIs at both baseline and 24-month follow-up. Eight were randomized to PA intervention, and 16 were randomized to HE control. Figure 9-1 illustrates the participant flow. Overall, the sample had a median (IQR) age of 76.0 (6.7), was 58.3% non-Hispanic white, and was 83.3% female. Medians and interquartile ranges or numbers and percentages of baseline characteristics are presented in Table 9-1. The PA and HE groups were well-balanced on demographic and general health characteristics. Similarly, participants with MRIs at baseline only were not significantly different from those with MRIs at baseline and 24-month follow-up (Table 9-2). Moderate PA minutes by accelerometry increased by 17.2% in the PA group but declined by 28.0% in the HE group. Regarding the cumulative totals, the PA group had a median of 158.8 minutes of total daily moderate PA across all study visits, while the HE group had 86.7 total PA minutes.

Figure 9-2 shows an example of baseline and 24-month follow-up vein tracings in one participant from each intervention group. The PA group, but not the HE group, had a significant percent increase in straight length from baseline to 24-month follow-up (p=.02 and p=.21, respectively; Table 9-2). Most participants in the PA group had an increase in straight venous length from baseline to the 24-month follow-up (Figure 9-3). There was a significant between group difference in percent change of straight vein length such that the PA group had a greater percent increase than the HE group (p=.07; Table 9-3).

Tortuosity ratio declined from baseline to follow-up by 33.2% within the PA group and 10.8% within the HE group. There were no significant within or between group differences for percent change in tortuous length or tortuosity ratio. PA did not have a

significant effect on worsening microbleed count or WMH grade. Approximately 42.9% of the PA group and 41.7% of the HE group had a worsening microbleed count (Fisher's exact p>.99). None of the PA group had a worsening WMH grade, while 18.8% of the HE group worsened by at least one WMH grade (Fisher's exact p=.53; Figure 9-3).

The PA intervention was correlated with percent increases in straight venous length, and adjusting for percent change in BDNF and VEGF did not attenuate this relationship (Table 9-4). The PA intervention was not significantly associated with percent change in tortuous venous length or tortuosity ratio. These associations remained similar in sensitivity analyses using total moderate PA minutes by accelerometry in lieu of intervention group assignment; the association of PA with increases in straight length remained significant (unadjusted rho=0.499, p=.07) while the association of PA minutes with decreases in tortuosity ratio became significant (unadjusted rho= -0.538, p=.05). *APOE*4* presence was not significantly related to any of the vein outcomes.

Within and between group differences in BDNF and VEGF were not significant (p>0.1 for all). Percent changes in BDNF were positively correlated with percent changes in straight venous length (rho=0.404, p=.07), and adjusting for intervention arm did not attenuate this association. Percent change in VEGF was not associated with percent change in straight venous length.

9.5 DISCUSSION

In this preliminary study, participating in a 24-month randomized PA intervention was associated with greater percent increases in straight length of deep medullary veins than the HE control. This could suggest that one pathway of PA's beneficial impacts on the brain is through its role as a promoter of cerebral small vessel integrity. If these results can be replicated in the context of a larger trial designed to test this hypothesis, we can conclude that cerebral small vessel integrity can be altered through PA. These results begin to lend support to existing evidence that PA is associated with cerebral artery health and lower burden of neuroimaging lesions of presumed vascular origin.[66,216,225,226] Associations of PA with the cerebral vasculature have been evaluated previously in a small, observational study comparing differences between high and low aerobic PA groups among healthy older adults (mean ages: 64, high PA; 68, low PA).[216] PA assessment was based on self-report and generally mapped onto ≥180 minutes per week of moderate PA over the prior 10 years (high PA) or <90 minutes of weekly PA with no specific PA program over the prior 10 years (low PA). The results indicated lower tortuosity and increased numbers of vessels <0.6 mm in diameter in the high PA vs. low PA group. This study captured mid-sized arteries, while our study focuses on small deep medullary veins.

We found no significant difference in worsening microbleed count by intervention group. More participants in the HE group demonstrated worsening WMH grade, although this was not statistically significant. Others have found that PA may be associated with lower burden or severity of WMH of presumed vascular origin, an indirect marker of later stage cerebral small vessel disease. A meta-analysis of nine studies (all with mean age

>60) found a small protective effect of PA and physical fitness on WMH volume.[66] Even among adults 40-65 years old (mean age 59) with risk factors for AD dementia, greater fitness was associated with reduced WMH burden.[225] While none of these studies evaluated impact on WMH progression, which would require multiple MRIs, a secondary analysis of an RCT with pre- and post-intervention MRIs found that resistance training reduced WMH progression among women with baseline WMH (mean age 69).[226] Our differing results may be due to our small sample size and lack of power to detect such small differences. Nevertheless, our results suggest that veins of even older, sedentary adults can be altered, and taken together, these results suggest that PA may have the capacity to promote cerebral small vessel integrity across several decades of mid- to late-life.

We found that straight venous length increased in both the PA and HE arms, although the increase in the HE arm was non-significant. This raises two questions. The first is whether PA can actually increase straight venous tissue as opposed to just reducing the loss of straight veins. In addition to promoting endothelial function, PA can also result in increased production or bioavailability of growth factors which could beneficially impact blood vessels.[227] While we did not find PA-related increased peripheral serum BDNF and VEGF, our results do not rule out increased central production or bioavailability of these factors. The second question is why both intervention groups would demonstrate increasing straight venous length. These results could be due to the exposure of the HE group to social activity. The HE classes were carried out in groups, potentially exposing HE participants to increased social activity, and social activity is

beneficial for brain health.[228,229] If this is the case, social activity may also be beneficial for cerebrovascular health. This result should be evaluated further in additional studies.

Interestingly, PA was associated with straight venous length but not tortuous venous length. This may indicate two separate pathophysiological pathways for the cerebral small veins: 1) decreasing straight venous length, which our results suggest PA may be able to counter, and 2) increasing tortuous venous length, which our results suggest PA does not counter. It is possible that through PA's action to generate rhythmic pulsing of the veins, either through the increased heart rate seen with aerobic activity or through rhythmic stretching of the vessels that may be seen with resistance activity, PA effectively maintains shear stress and flow parameters which help to promote endothelial function and nitric oxide production thus keeping the vessels healthy.[227,230] It is feasible for this to preferentially benefit straight vessels as shear stress is altered in tortuous vessels.[231]

We found that *APOE*4* was not associated with changes in vein outcomes over time. We had shown in our cross-sectional analyses that *APOE*4* was associated with greater tortuosity ratio.[219] Together these results suggest that *APOE*4* is associated with one's starting point with regard to tortuosity, but not how rapidly tortuosity changes over time. This result should be confirmed in a larger study with multiple MRIs over time.

While we found no difference in BDNF between the intervention groups, we found that percent change in BDNF was positively associated with percent change in straight venous length. We interpret these results cautiously given that they are the result of exploratory analyses. If confirmed, these results may indicate that much like BDNF's beneficial impact on hippocampal volume[97] and functional connectivity,[232] it may also be

a promoter of cerebral small vessel integrity. This supports animal evidence of BDNF's role in angiogenesis and vessel health,[217,218] and work in humans finding that a genetic predisposition to lower and less efficient BDNF levels[233] was associated with greater WMH volume in older adults (mean age 70).[234]

Several limitations to this study should be kept in mind while interpreting these results. First, the IQRs we present here for BDNF and VEGF are quite large. Serum markers like these are known to have high variance, and this variance can make differences hard to detect or significant values hard to trust. Future studies incorporating such markers should be designed with a large enough sample to account for this. Second, this study was a secondary analysis of an RCT, and included exploratory analyses. As such we interpret these findings cautiously. For these reasons, the results of this study should be tested in a larger study as part of a pre-specified analytic plan.

There are several strengths of this study. First, our study design incorporates many advantages over existing studies. While most prior studies of PA and cerebral small vessel integrity and disease have relied on observational studies, ours is the first study of the impact of a randomized controlled PA intervention on deep medullary veins. In addition, most existing studies have been cross-sectional analyses or longitudinal analysis with MRI only at study follow-up. However, this study evaluates change over time through use of MRI at baseline and 24-month follow-up. In addition, our assessments of PA are more objective than retrospective self-report. We have collected data on PA both by random assignment to the PA intervention group and by cumulative moderate minutes of PA based on accelerometry. Second, we studied a novel, direct vessel neuroimaging marker of cerebral small vessel integrity as opposed to traditional late-stage markers of

parenchymal damage of presumed vascular origin. This allows us a closer appreciation of the vessels themselves in studying promoters of cerebral small vessel integrity. Third, randomization was maintained in this sub-study. Thus the results of our primary analytic aim are controlled for potential confounding baseline factors.

Our study represents some of the earliest work in this important area of research. Future larger studies will be needed to confirm our results.

9.6 ACKNOWLEDGEMENTS

The authors thank Erica Lynn Tamburo for neuroimaging assistance and Joshua Michel for implementing standardized Luminex assays.

9.7 FIGURES AND TABLES

Table 9-1. Baseline characteristics in the LIFE study

	Physical Activity N=8		Health Education N=16		p
Age (years)	74.3	(5.5)	76.1	(6.9)	0.98
Race, non-Hispanic white	5/8	(62.5)	9/16	(56.3)	>0.99
Sex, female	7/8	(87.5)	13/16	(81.2)	>0.99
*APOE*4* allele presence	3/8	(37.5)	5/15	(33.3)	>0.99
Modified Mini-Mental State Examination	94.5	(10.5)	91.5	(8.0)	0.88
Gait speed, m/s	0.80	(0.27)	0.82	(0.20)	0.81
VEGF, pg/mL [a]	482.9	(603.3)	391.3	(255.8)	0.77
BDNF, pg/mL [a]	18143.1	(23782.0)	19780.3	(24036.0)	0.87
Physical activity minutes[b, c]	41.7	(14.9)	28.4	(37.6)	0.64
Severe WMH burden,[d] No./ Total No. (%)	3/8	(37.5)	3/16	(18.7)	0.71
No microbleeds, No./ Total No. (%)	5/8	(62.5)	7/14	(50.0)	0.58

Notes: Numbers are median (IQR) or No. / Total No. (%). [a]N=15, Health Education. [b]Minutes of daily moderate physical activity by accelerometry.
[c] N=13, Health Education. [d]WMH rated as 0= none, 1=mild, 2= moderate, 3= severe.
Abbreviations: *APOE*4*, Apolipoprotein E e4 allele; BDNF, brain-derived neurotrophic factor; VEGF, vascular endothelial growth factor; WMH, white matter hyperintensities.

Table 9-2. Baseline characteristics in those with and without follow-up venous outcomes

	Venous outcomes at both visits N=24		Baseline venous outcomes only N=29		p
Age (years)	75.2	(6.8)	77.0	(5.9)	0.37
Race, non-Hispanic white	14/24	(58.3)	16/29	55.2	0.82
Sex, female	20/24	(83.3)	22/29	(75.9)	0.74
*APOE*4* allele presence	8/23	(34.8)	7/24	(29.2)	0.68
Modified Mini-Mental State Examination	92.0	(8.5)	94.0	(6.0)	0.96
Gait speed, m/s	0.81	(0.20)	0.80	(0.20)	0.37
VEGF, pg/mL[a]	391.3	(324.6)	484.07	(506.2)	0.33
BDNF, pg/mL[a]	19780.3	(24036.0)	20707.9	(28312.0)	0.89
Physical activity minutes[b, c]	36.0	(25.3)	23.50	(33.5)	0.26
Severe WMH burden, No./ Total No. (%)[d]	6/24	(25.0)	5/29	(17.2)	0.70
No microbleeds, No./ Total No. (%)	12/22	(54.6)	9/23	(39.1)	0.25

Notes: Numbers are median (IQR) or No. / Total No. (%). [a]N=23 for both visits; N=28 for baseline only. [b]Minutes of daily moderate physical activity by accelerometry. [c]N=21 for both visits; N=26 for baseline only. [d]WMH rated as 0= none, 1=mild, 2= moderate, 3= severe.

Abbreviations: *APOE*4*, Apolipoprotein E e4 allele; BDNF, brain-derived neurotrophic factor; VEGF, vascular endothelial growth factor; WMH, white matter hyperintensities.

Table 9-3. Impact of the physical activity intervention on venous outcomes

	Physical Activity (N=8)				Health Education (N=16)				
	Baseline	Follow-up	%Δ	p	Baseline	Follow-up	%Δ	p	between arm p
Straight venous length	99.9 (49.9)	153.2 (82.7)	93.6 (112.9)	**0.02**	105.0 (104.8)	144.9 (131.6)	28.4 (90.6)	0.21	**0.07**
Tortuous venous length	147.2 (93.3)	154.7 (52.0)	11.6 (64.5)	0.31	137.7 (69.5)	155.9 (79.9)	-12.9 (49.9)	0.63	0.41
Tortuosity ratio	1.4 (1.1)	1.1 (0.7)	-33.2 (63.5)	0.15	1.2 (1.1)	1.0 (1.5)	-10.8 (105.9)	0.98	0.38

Note: Tortuosity ratio=total tortuous length / total straight length. Due to small samples sizes, all values presented as median(IQR) and non-parametric tests.

Table 9-4. Unadjusted and partial Spearman correlations assessing relationships of physical activity with venous outcomes

	% Change Straight Venous Length		% Change Tortuous Venous Length		% Change Tortuosity Ratio	
	Rho	p	Rho	p	Rho	p
PA Group[a]	**0.383**	**0.07**	0.179	0.40	-0.192	0.37
Adjusted for %Δ BDNF[b]	**0.426**	**0.06**	0.247	0.30	-0.200	0.40
Adjusted for %Δ VEGF[b]	**0.415**	**0.07**	0.185	0.44	-0.191	0.42

Abbreviations: BDNF, brain-derived neurotrophic factor; PA, physical activity; VEGF, vascular endothelial growth factor.
[a]N=24. [b]N=21.

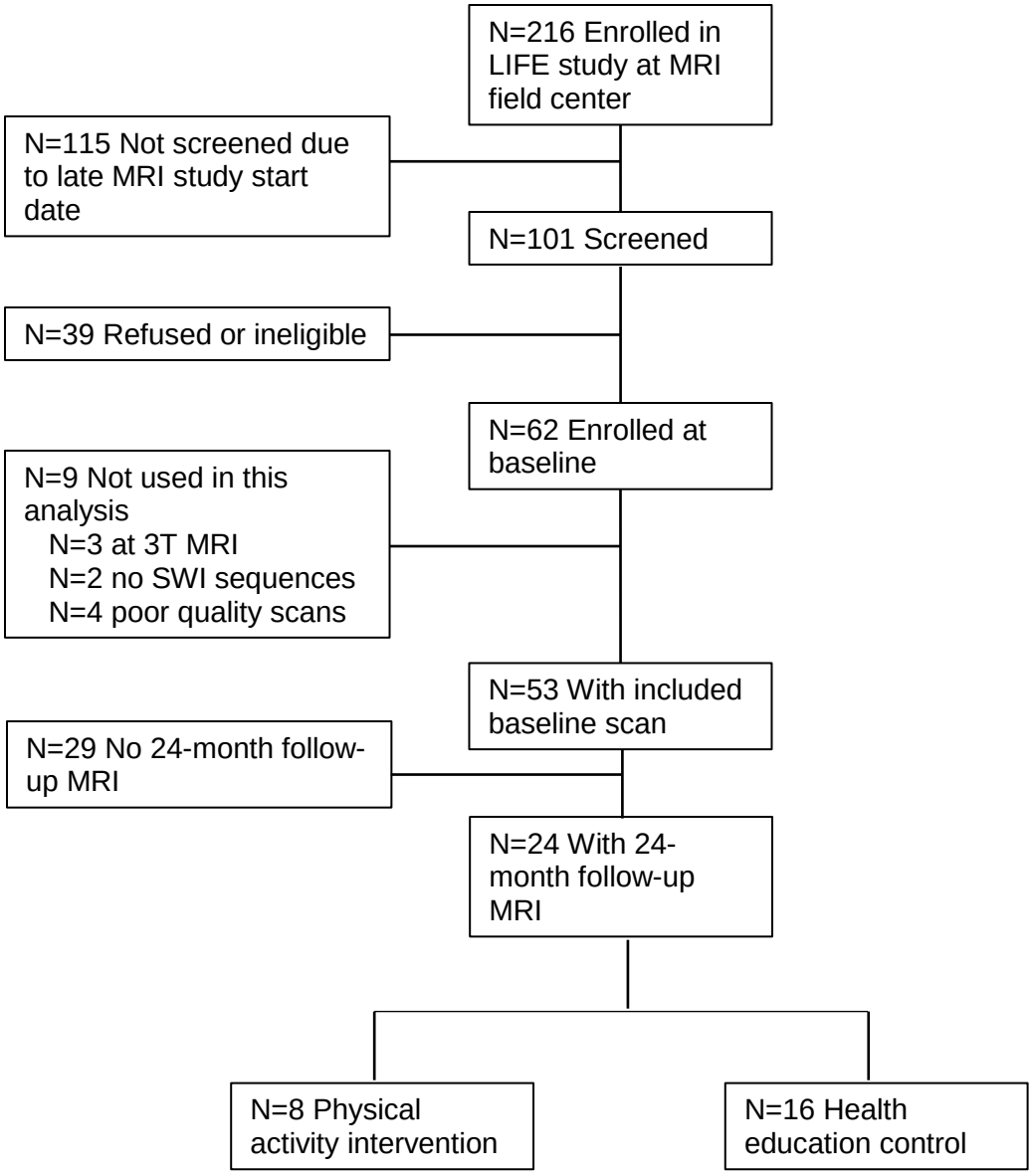

Figure 9-1. Study participant flow diagram

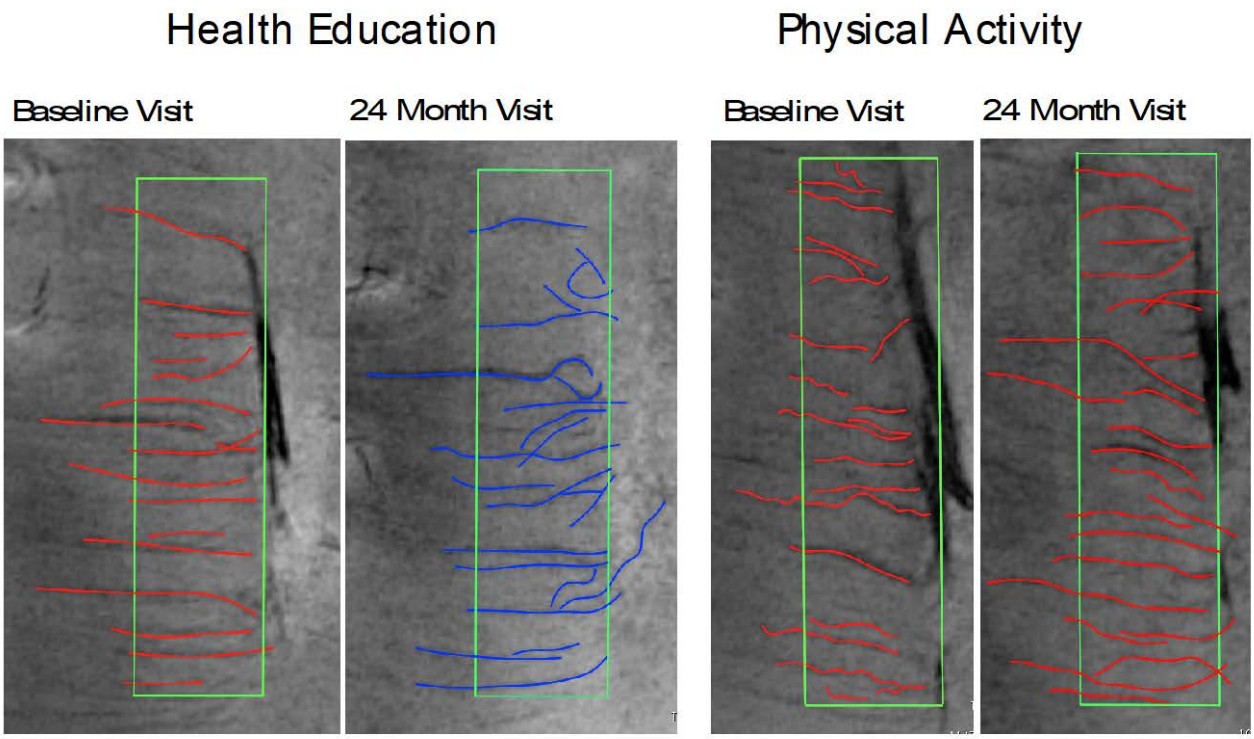

Figure 9-2. Sample vein tracings on 7T susceptibility-weighted MRI at baseline and 24-month follow-up in the Health Education and Physical Activity groups

Caption: The participant in the health education group had the following percent changes in their vein outcomes: straight vein length, 9.3%; tortuous vein length, 57.2%; and tortuosity ratio, 43.9%. The participant in the physical activity intervention had the following percent changes in their vein outcomes: straight vein length, 25.0%; tortuous vein length, -22.5%; and tortuosity ratio, -38.1%. Positive percent change indicates an increase from baseline to follow-up while negative percent change indicates a decrease from baseline to follow-up.

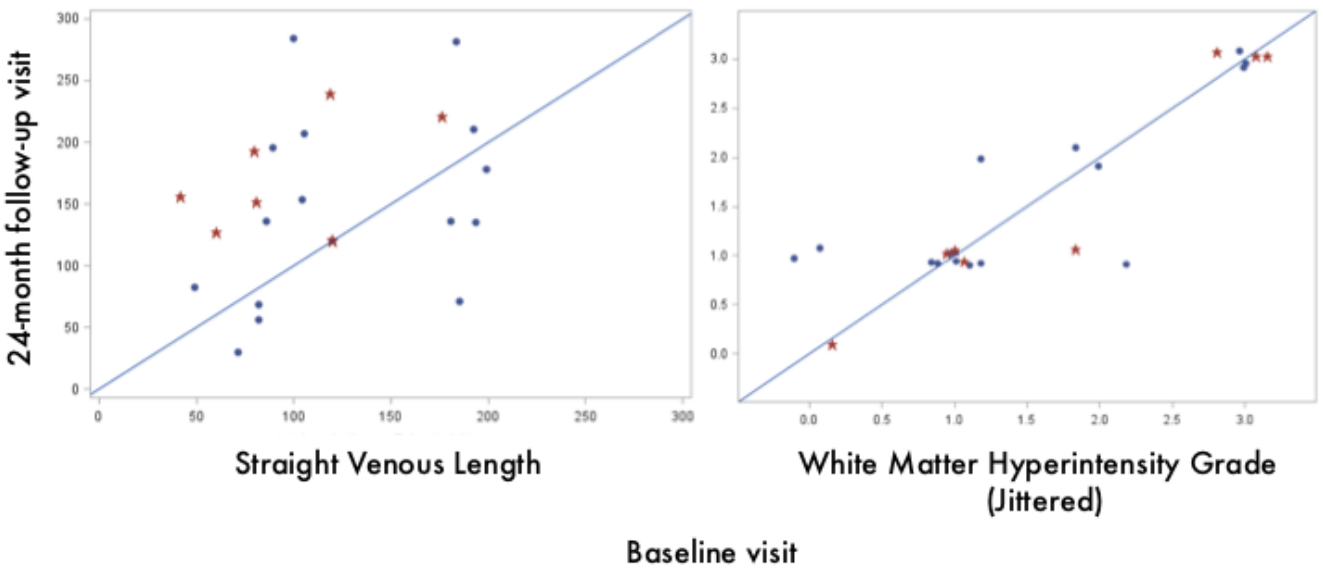

Figure 9-3. Change in straight venous length and white matter hyperintensity grade from baseline to 24-month follow-up by intervention group

Red stars=Physical activity group; blue circles=health education control group. The diagonal line represents no change from baseline to 24-month follow-up. Above the line=increase; below the line=decrease. Note: The white matter hyperintensity grade figure is jittered to make all points visible because many were layered on top of one another. N=3 from the health education group worsened in white matter hyperintensity grade, while N=0 from the physical activity group worsened.

10.0 PAPER 3: INTERACTIONS OF VASCULAR AND CARDIOMETABOLIC RISK FACTORS AND NON-MODIFIABLE FACTORS FOR RISK OF MCI, ALL-CAUSE DEMENTIA, AD DEMENTIA, AND COGNITIVE PERFORMANCE

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10.1 ABSTRACT

Increasingly, vascular and cardiometabolic risk factors (VCMRF) are recognized risk factors for cognitive impairment and dementia, including Alzheimer's disease (AD). In an era of increasing multimorbidity with increasing age, the interactions of these risk factors and non-modifiable factors are critical to evaluate and to inform prevention and treatment efforts. Existing studies have been limited in that they have not evaluated multiple VCMRF and interactions in the same model. The primary aim of this work was to identify VCMRF interactions related to incident all-cause dementia. We used an algorithm to select interactions of interest based on a review of the literature, univariable associations of variables with our outcomes, and focused on those variables with impacts on vessel health and cerebral blood flow. Our secondary aim was to repeat these analyses with incident mild cognitive impairment (MCI), AD dementia, and overall cognitive performance. Finally, we explored for new important interactions. The Monongahela-Youghiogheny Healthy Aging Team (MYHAT) Study is an observational cohort study of cognitive impairment with 10 cycles of study visits (N=1982, median (IQR) age, 78 (12), 61.1% female, 94.7% white). Over the study follow-up time, 373 participants developed MCI, 109 developed dementia (90 of whom developed AD), and 881 remained cognitively normal. Time dependent Cox regression for incident all-cause dementia, MCI, and AD dementia and linear mixed modeling for overall cognitive performance were used to test the interactions of interest first in minimally adjusted models and then in models adjusted for non-modifiable factors and confounders. For the exploratory analyses, classification and regression trees (CART) with 10-fold cross-validation were used to screen for interactions using PROC HPSPLIT in SAS in a training set that was 50% of the

full dataset. The candidate interactions were tested in the test set, the remaining 50% of the full dataset, using Cox proportional hazards (PH) regression. The risk of all-cause dementia conferred by stroke was even greater among those with congestive heart failure (CHF) (p for interaction=0.04). Among those who ever drank alcohol, history of stroke reduced cognitive performance nearly 1/10th of a standard deviation, coefficient (95% CI): -0.085 (-0.135, -0.035), but there was no significant effect of stroke on cognitive performance among those who never drank alcohol, 0.046 (-0.071, 0.162). These results did not survive adjustment for multiple comparisons. None of our candidate interactions were significantly associated with incident AD dementia or MCI. In our exploratory CART modeling, we found that greater age was significantly associated with increased risk of AD dementia among those walking less per week, Hazard Ratio (HR) [95% CI], 31.19 [4.24, 229.70], but not among those walking more per week, 1.22 [0.22, 6.93]. Physical activity and prevention of multimorbidity should be prioritized for prevention of cognitive impairment and dementia.

10.2 INTRODUCTION

Increasingly, vascular and cardiometabolic risk factors (VCMRF) are recognized risk factors for cognitive impairment, and this is embodied in the relatively new term “vascular contributions to cognitive impairment and dementia (VCID)”. Co-occurrence of cerebrovascular lesions with neurodegenerative Alzheimer’s disease (AD) pathology nearly doubles dementia prevalence,[235] and in vivo neuroimaging confirms that presence of VCMRF is associated with reduced glucose metabolism by FDG-PET and reduced cortical thickness in brain regions implicated in AD.[236]

Not only are large vessel lesions such as stroke implicated in cognitive impairment and dementia,[237] but cerebral small vessel disease (SVD), pathology of the cerebral small arteries, veins, and capillaries,[3] is as well[238,239] and is garnering increased attention and priority from researchers and funders. One new initiative demonstrating this priority is the MarkVCID consortium, which calls itself a “consortium of US academic medical centers whose mission is to identify and validate biomarkers for the small vessel diseases of the brain that produce vascular contributions to cognitive impairment and dementia (VCID).”[240] Studies examining VCMRF have found that blood pressure related markers,[101-112,115,117-120,122,123,126-133] subclinical vascular disease markers,[123,151-159] clinical cardiovascular disease,[128] smoking,[104,112,113,119,121,126,133,149,150] inflammation,[140-142,144] dyslipidemia,[109,135,136] and visceral fat accumulation[138,145] are associated with poorer cerebral small vessel health. Neuroimaging markers of SVD are known risk factors for cognitive impairment and dementia.[175,241,242]

As we have undergone the epidemiologic transition, mortality rates have declined and chronic diseases have become commonplace.[164] We are living longer, but with

multiple chronic conditions. Given how commonplace such multimorbidity is among older adults, the impact of VCMRF co-occurrence and the impact of sub-group specific VCMRF are critical to evaluate. An analysis of Medicare claims data showed that the prevalence of multimorbidity, defined as the presence of 2 or more chronic health conditions, ranged from 50.3% of beneficiaries under 65 years of age up to 81.5% of beneficiaries 85 and older.[165] Thus, most older adults present to their primary care practitioners with multiple chronic conditions. Understanding which interactions of VCMRF contribute to cognitive impairment and dementia would allow us to understand risk of these outcomes in individuals with overlapping risk factors as well as to tailor preventions and treatments. Examining differences in risk conferred by VCMRF in different sub-groups and in the context of different behavioral and lifestyle factors is critical for the promise of precision medicine. Despite the importance of examining interactions of VCMRF and other non-modifiable factors, we recently reviewed the literature and found that studies of VCMRF impacts on cerebral small vessel health have been limited in that they have tended not to evaluate multiple interactions of VCMRF with one another and other non-modifiable risk factors.[100] Traditional statistical modeling approaches cannot examine all possible interactions and thus require the subjective input of the modeler to select which interactions to evaluate.

Our review identified interactions which are important for SVD in adults 65 and older. For example, in a study evaluating the relationship of *APOE*4* carrier status and hypertension with white matter lesion volume, *APOE*4* carriers with hypertension had the greatest lesion volumes.[103] In addition to interactions with non-modifiable factors such as *APOE*4*, VCMRF may interact with one another. For example, diabetes may interact with

SBP to increase risk of infarcts.[114] Understanding whether such interactions extend to risk of dementia is critically important.

Thus, our primary aim was to evaluate interactions of VCMRF with one another and with non-modifiable factors for associations with incident all-cause dementia. Our secondary aim was to repeat the primary analysis with incident AD dementia, incident mild cognitive impairment (MCI), and global cognitive performance. For these two aims, interactions were carefully selected based on an algorithm incorporating our literature review described above and univariable associations of VCMRF with the outcome of interest. Our exploratory aim was to explore for new important interactions using a novel, data-driven approach. We evaluated interactions for incident all-cause dementia, AD dementia, MCI, and global cognitive performance in a population-based cohort study with 10 years of follow-up.

10.3 METHODS

10.3.1 Study participants

The Monongahela-Youghiogheny Healthy Aging Team (MYHAT) Study, is an observational cohort study of cognitive impairment. Participants were selected from voter rolls to be representative of older adults of the area, a group of contiguous former steel towns in southwestern Pennsylvania. Participants were seen either in their home or in the study field office and had up to 10 yearly study visits. Study visits examined here took place between 3/20/2006 (first date of study visit 1) and 9/8/2017 (last date of study visit 10). The University of Pittsburgh Institutional Review Board reviewed and approved the study, and written informed consent was received from all participants before initiation of any study procedures.

10.3.2 Demographics

Participants self-reported age, sex, race, and education level at the baseline visit.

10.3.3 VCMRF

Four categories of VCMRF were collected: physical exam measures, laboratory tests, chronic health conditions, and behavioral factors.

10.3.3.1 Physical exam measures

Blood pressure, apical pulse, and height, waist, and hip circumference in inches were measured by the examiner during the physical exam. Weight was self-reported by participants until visit 9 when it was measured by the examiner. Mean arterial pressure (MAP), a measure of organ perfusion was estimated as: $MAP \cong \frac{SBP+(2xDBP)}{3}$. Pulse pressure (PP), a measure of pulsatility, was calculated as systolic blood pressure (SBP)-diastolic blood pressure (DBP). Body mass index (BMI) was calculated as: $\frac{\text{weight in pounds}}{(\text{height in inches}^2 * 703)}$. Waist to hip ratio (WHR) was calculated and used as a measure of central obesity.

10.3.3.2 Laboratory tests

Participants were invited to participate in blood tests. Among those who consented, the blood markers that follow were included. ApoA1 and ApoB were measured, and the ratio of ApoB to ApoA1 was calculated. ApoB:ApoA1 is a measure of atherogenic to anti-atherogenic lipoprotein particles, and is a risk factor for cardiovascular events.[243] Hemoglobin A1C is a running average measure of blood glucose within the past 3 months, and 6.5% is used as the cutoff for diabetes.[244] Cystatin C is a marker of glomerular filtration that is also related to cardiovascular disease.[245] Homocysteine, an amino acid produced through methionine metabolism, has been correlated with SVD.[246,247] C-reactive protein was included as an inflammatory marker which may be related to SVD.[141] *APOE* genotyping was carried out. The presence or absence of *APOE*4* was coded as *APOE*4* carrier or non-carrier. Finally, non-fasting lipids were also tested. Total

cholesterol and high-density lipoprotein cholesterol (HDL-C) were measured, and low-density lipoprotein cholesterol (LDL-C) was calculated as total cholesterol-HDL-C.

The Chemistry and Nutrition Lab at the University of Pittsburgh Graduate School of Public Health completed the assays.

10.3.3.3 Chronic health conditions and behavioral factors

At study baseline, participants were asked whether they had *ever* been told by a doctor or nurse that they have or had: a stroke, transient ischemic attack (TIA), diabetes, hypertension (HTN), myocardial infarction (MI), high cholesterol, congestive heart failure (CHF), irregular heartbeat (including atrial fibrillation or arrhythmia), depression, anxiety ("nerves" or "nervousness"), or sleep apnea. At each follow-up visit, they were asked whether a doctor or nurse told them they have or had any of these conditions *since they were last seen* by the study. Study staff administered the modified centers for epidemiologic studies-depression (mCESD) inventory at each study visit.[248] The score ranges from 0-20, and a higher score indicates more depressive symptoms. Medications for diabetes and hypertension were self-reported by participants. We defined uncontrolled hypertension as those with SBP≥130 or DBP≥80[249] among those on antihypertensive medications. Ever and current smoking and drinking alcohol were self-reported by participants. Number of days per week and minutes per day walking for exercise was obtained by participant self-report, and the minutes per week spent walking for exercise was calculated.

10.3.4 Cognitive assessments and cognitive outcomes

Incident all-cause dementia and MCI diagnoses were based on clinical dementia rating (CDR) scores,[250] without an etiological determination. The CDR is an assessment of impairment severity due to declines in cognitive functioning. In this study, it is completed based on interview questions and observations made by the interviewer during the evaluation. For these analyses, dementia is defined as CDR≥1 and MCI as CDR=0.5. Later in the conduct of the study, an online consensus adjudication system was used to determine etiology in incident dementia cases. Study clinicians reviewed subjective concerns, activities of daily living (ADL) and instrumental activities of daily living (IADL), depression data, medical history and medication use, physical and neurological exam, neuropsychological data, and any available medical records including neuroimaging reports. They determined whether the dementia etiology was AD, vascular, mixed AD and vascular, or a variety of other conditions including medication, depression, and other dementias. Clinicians could review each other's etiology ratings and edit their own ratings until consensus was achieved.

We combined cases of incident dementia due to AD or mixed vascular and AD presentation to create the incident AD dementia events for this analysis. The risk set for incident all-cause dementia and AD dementia included all participants without prevalent dementia at baseline. The risk set for incident MCI included all study participants without prevalent MCI or dementia at baseline. If a participant's cognitive outcomes fluctuated from visit to visit, the first occurrence of CDR=0.5 or CDR≥1 was used to define the incident event. We censored those without an event at their last available study visit. For those who had an event, the visit at which they were diagnosed with the event was used

as the event visit. For the AD dementia risk set, the date of last follow-up was cut off at 1/27/2016, the date of last follow-up of the incident dementia cases with etiological determination. By that date, the first 100/109 incident dementia cases had been adjudicated for etiology. Thus, participants without the event were censored at the last visit prior to 1/27/2016, and those who developed incident dementia after the cut-off date were censored at the last visit prior to 1/27/2016.

Trained study staff administered cognitive tests at each study visit. The Mini-Mental State Examination (MMSE) was administered as a measure of global cognition.[251] Several neuropsychological tests were administered in each cognitive domain (Table 10-1). We standardized each test score to a z score by centering at the baseline sample mean and dividing by the baseline sample standard deviation. Then we created domain z scores by averaging the z scores of each cognitive test within the domain, and an overall cognitive z score, which was the average of all available domain z-scores. This overall cognitive domain z score was used as the outcome of interest in the global cognitive performance analyses.

10.3.5 Statistical analyses

10.3.5.1 Criteria to select the interaction terms of interest

We selected the interactions of interest using an algorithm based on the following criteria. First, based on the interactions identified in our literature review of risk factors for SVD, we selected the following interactions of interest which focused on blood flow and vessel health and which could be tested in our population: *APOE*4**hypertension and diabetes*SBP. In order for the candidate interaction to be tested, the component variables

had to be significantly related to incident all-cause dementia in univariable analysis. Second, we measured the univariable associations between VCMRF and incident all-cause dementia; those variables significantly associated with the outcome at $p<0.05$ were ordered by effect size. If the variable was protective, the hazard ratio (HR) was inverted (1/HR) to format all effect sizes in the same direction. The VCMRF with the top three largest effect sizes were selected as candidate factors impacting vessel health or blood flow. Thus, our final list of interactions consisted of the following: any of our literature review-based candidates in which the component variables were also significantly associated with incident all-cause dementia at $p<0.05$ and two-way interactions formed by the three variables with the largest effect sizes. We used the same approach to select interactions of interest for incident MCI, AD dementia, and global cognitive performance.

10.3.5.2 Modeling the interactions

To evaluate the relationship of VCMRF with incident all-cause dementia, MCI, and AD dementia, we used time dependent Cox regression models using the counting process to deal with time dependent covariates. The counting process converts the data into intervals of time, for example the interval from visit 1 to visit 2, with covariates for that interval. This allows for covariates to change at each interval. This continues up to the visit with the incident event or censoring from the study. To test the interactions of interest, we fit three models: 1) a minimally adjusted time dependent Cox model with the main and interaction variables of interest, 2) a model further adjusted for non-modifiable variables including age, sex, race, education, and *APOE*4*, and 3) a model further adjusted for confounders. Confounders are any variables significantly related to both the predictor and outcome, but not on the causal path between the predictor and outcome. We corrected

for multiple comparisons for our primary and secondary aims using Sidak's correction. This modeling was carried out using the survival package in R.

To evaluate the relationships of these interactions with global cognitive performance, we used linear mixed modeling. The relationship of each variable of interest with the overall average cognitive z-score was tested first in a minimally adjusted model 1 that included study visit as the time variable and the variables of interest. We included fixed effects of study visit and the variables of interest. We allowed the intercept and visit to vary by participant by including a random intercept and a random slope for visit. Model 2 included model 1 further adjusted for fixed effects of the non-modifiable characteristics. This was further adjusted for fixed effects of confounders in model 3. The linear relationship of visit with cognitive performance was assessed by visual inspection of a plot of average cognitive performance at each visit, and quantile-quantile plots of residuals were reviewed to assess normality. These tests were carried out using the lme4, lmertest, and psycholing packages in R.

10.3.5.3 Exploring new interactions

New interactions were explored via a two-step process. The first step consisted of using classification and regression trees (CART). CART uses recursive partitioning to split the sample into terminal leaves composed of as homogenous a sample of the outcome of interest as possible. For example, for all-cause dementia, CART will try split the sample of those in the risk set by the variables included for consideration in the model such that each terminal leaf is either primarily dementia or primarily non-dementia. In this way, the algorithm learns to predict dementia.

The baseline datasets for the all-cause dementia, AD dementia, and MCI risk sets were each randomly split into a 50% training set and a 50% test set using the package caTools in R with a seed set to 123. Setting a seed allows re-creation of the same data split thus facilitating replication. For each of these incident outcomes, a classification tree was built without including the laboratory tests in order to maximize sample size. Baseline data and incident event indicators only were used, given that it would become quite complicated for CART to deal with repeated measures. Time to the event was not incorporated into these CART models. The classification trees were run with 10-fold cross validation on each training set using PROC HPSPLIT in SAS 9.4. The splitting criterion used was entropy. Based on this metric, splits were made such that the purest groups possible were created. Splits of continuous variables suggest the best cutpoints of those variables. The algorithm splits the data into so many leaves that it will overfit the data. Therefore, the classification trees were also pruned. This allows the model to generalize more easily to other datasets. If the proportion of those with and without the outcome in leaves split on variable B are dependent on variable A, this represents an interaction. We selected the first three relevant splitting variables that made up the interactions in CART (a three-way interaction) to test in the second step. For more background about CART and why and how it was used, see Appendix 3.

In the second step, these candidate interactions were modeled in Cox PH regression models using only the baseline covariates just as the CART modeling did. Binary variables were created from continuous variables based on the best cutpoints selected by the CART models. The proportional hazards assumption was tested by running models including interactions of the variables of interest with time and log(time).

If proportional hazards was violated, a Cox PH model stratified on the offending variable was used. The interactions were then tested in a minimally adjusted model of the interaction(s) and component variables and in a model further adjusted for the non-modifiable variables. If the model with the three-way interaction could not run, the component two-way interactions were tested in separate models.

10.4 RESULTS

The original full study sample included 1,982 participants (see Figure 10-1 for disposition of all participants). A summary of number of participants with each study visit is shown in Table 10-2. Baseline descriptive statistics are shown in Table 10-3. Overall, participants were a median (IQR) age of 78.0 (12.0) years old, 61.1% female, 94.7% white, and 86.2% had at least a high school education. VCMRF chronic health condition prevalence/history ranged from <5% for stroke to approximately 65% of participants with a history of hypertension. Even more were on an antihypertensive medication (72.2%), but among those on antihypertensive medication, hypertension was uncontrolled in about 2/3. This is within the range of previously reported uncontrolled hypertension prevalence estimates for older adults (50.6%-87.6%).[252,253]

Table 10-4 presents baseline characteristics by cognitive outcome. Out of N=1,413 who were cognitively normal at baseline, N=881 remained cognitively normal throughout their time in the study while N=373 developed incident MCI. Out of N=1,959 free from prevalent dementia at baseline, N=109 developed incident all-cause dementia, and out of the first 100 incident dementia cases, N=90 were adjudicated to be due to AD (see Figures 10-2 and 10-3 for study flow for the all-cause dementia, AD dementia, and MCI risk sets). As expected, older age, lower education, *APOE*4* carrier status, and lower MMSE were found in those who developed MCI, all-cause dementia, and AD dementia vs. those who remained cognitively normal. In addition, they had lower baseline cholesterol and BMI levels; greater prevalence of hypertension and stroke; and lower prevalence of ever or current drinking.

10.4.1 Primary Outcome: Incident all-cause dementia

Hazard ratios (HR) and 95% confidence intervals (CI) for univariable relationships with incident all-cause dementia are shown in Table 10-5. All of the non-modifiable variables, except sex, were significantly related to incident all-cause dementia in the expected direction. Among the VCMRF and behavioral and factors, the three with the largest effect sizes were stroke HR[95% CI]: 9.90 [4.76, 20.58]; current drinking: 0.30 [0.19, 0.46]; and CHF: 2.91 [1.60, 5.31]. Hypertension, cardiac arrhythmia, and depression were associated with increased risk of incident all-cause dementia, while larger BMI, self-reported high cholesterol, greater minutes walked for exercise, and ever smoking were associated with decreased risk.

The interactions that made it through our selection algorithm were: *APOE*4**hypertension, stroke*current drinking, stroke*CHF, and CHF*drank alcohol in the past year. Despite the component variables being strongly related to incident all-cause dementia in univariable time dependent Cox models, none of their interactions were significantly associated with dementia in models 1 or 2 (Table 10-6). The stroke*CHF interaction term became significant in the model adjusted for confounders (p for interaction =0.04) indicating that the risk of all-cause dementia associated with stroke history is greater among those with CHF. However, this did not withstand correction for multiple comparisons (n=4 comparisons; all p>0.013 (Sidak corrected p-value)).

10.4.2 Secondary Outcomes: Incident AD dementia and MCI

Univariable associations with incident AD dementia were similar to incident all-cause dementia (data not shown). The interactions examined with this outcome were the same as with incident all-cause dementia. For the models with stroke*current drinking, a warning was generated indicating that the coefficient may be infinite. In these cases, the author of the "survival" package in R advises that the standard errors and Wald-test p-values should not be trusted, but that the Likelihood ratio test (LRT) is still accurate.[254] Thus, the p-value for the interaction term in these models is the LRT p-value comparing the model with the interaction term to the model without the interaction term. None of these interactions of interest were significantly associated with incident AD dementia (Table 10-7).

Univariable associations with incident MCI were overall similar to incident all-cause dementia and AD dementia. However, with MCI, females had a four-fold increased risk of MCI, HR: 4.05 [3.22, 5.10], and DBP, PP, HbA1C, cystatin C, TIA, and MI were also significant risk factors (data not shown). The interactions selected by our algorithm include *APOE*4**hypertension, MI*TIA, TIA*stroke, MI*stroke. Stroke and TIA both violated PH, so the relevant models were stratified on those variables. For models 1 and 2 of the myocardial infarction*TIA interaction, a warning was generated indicating that the beta may be infinite. Thus, the LRT p-value comparing the model with the interaction term to the model without the interaction term is used. Model 3 of that interaction could not run due to small sample size for the interaction of interest once all confounders were entered into the model. The model with TIA*stroke could not run at all, perhaps because it is the

model examining the interaction of two stratified variables. None of the interactions of interest were significantly associated with incident MCI (Table 10-8).

10.4.3 Secondary Outcome: Global cognitive performance

Univariable analyses with global cognitive performance indicated that higher cystatin C and homocysteine levels were associated with poorer global cognitive performance as were stroke, MI, HTN, arrhythmia and depression (data not shown). Interestingly, *APOE*4* carrier status was not significantly related to global cognitive performance. Greater BMI, more minutes walked for exercise, and drinking were associated with better cognitive performance. The interactions of interest were determined to be ever drank alcohol*stroke, ever drank alcohol*cystatin C, and stroke*cystatin C. The interaction term for ever drank alcohol*stroke became significant in the model adjusted for non-modifiable factors and the model further adjusted for confounders (Table 10-9). This did not survive adjustment for multiple comparisons. We repeated the analysis stratified by history of ever drinking. Among those who never drank alcohol, stroke was not significantly associated with cognitive performance, coefficient (95% CI): 0.046 (-0.071, 0.162). However, among those who ever drank alcohol, stroke was associated with worse cognitive performance, -0.085 (-0.135, -0.035; Figure 10-4). This indicates among those who ever drank alcohol, history of stroke reduces cognitive performance nearly 1/10th of a standard deviation, but there is no significant effect of stroke on cognitive performance among those who never drank alcohol. Put another way, those with a history of stroke do not receive as much benefit from history of drinking as

those who have not had a stroke. None of the other interactions of interest were significantly associated with global cognitive performance.

10.4.4 CART modeling

The CART models using the training sets generated 41-63 leaves when grown, and after pruning ranged from 6-9 leaves. The three-way interactions suggested based on CART modeling were: age*SBP*waist to hip ratio for the primary outcome of incident all-cause dementia; age*DBP*BMI for incident MCI; and age*waist to hip ratio*minutes walked weekly for exercise for incident AD dementia.

These interactions were tested in Cox PH regression models using the test sets to attempt to confirm these results. For incident all-cause dementia, both models 1 and 2 of the three-way interaction of age, SBP, and waist to hip ratio, the significance of the three-way interaction term was tested with the Likelihood Ratio Test due to the instability of the Wald test. Neither the three-way interaction term nor any of the component two-way interaction terms were significant in the dementia models. The coefficient for the three-way interaction term for age*DBP*BMI for the MCI model could not be generated, perhaps due to small sample size in the groups of interest. Thus, the lower order interaction models were run separately. No interactions were significant in these models. Finally, for model of the interaction of age*waist to hip ratio*minutes walked weekly for exercise for incident AD dementia, none of the coefficients could be estimated for terms involving waist to hip ratio. When we tested the component interaction models separately, age*minutes walked was significantly associated with lower risk of incident AD dementia (p for interaction=0.02; Table 10-10). When we stratified by walking status, age was not

significantly associated with AD dementia among those walking more per week: 1.22 [0.22, 6.93]. However, greater age (here ≥ 76.2 years) was significantly associated with increased risk of AD dementia among those walking less per week: 31.19 [4.24, 229.70]. Using the cutpoints selected by the CART modeling, this indicates that the effect of age on AD dementia risk is mitigated among those who completed ≥ 33.6 minutes of walking weekly.

10.5 DISCUSSION

10.5.1 Primary outcome: Incident all-cause dementia

We selected interactions of VCMRF with non-modifiable variables and with other VCMRF through an algorithm incorporating a review of the literature, model based large effect sizes, and biological plausibility for impacts on vessel health and blood flow. We tested these interactions for relationships with our primary outcome of incident all-cause dementia and found that the risk of dementia among those with a stroke history is even greater among those who have CHF than among those who do not, although this did not survive adjustment for multiple comparisons. Stroke was the greatest risk factor for dementia in our study, with nearly a 10-fold increased risk among those with vs. without stroke history. Most prior population-based studies report a doubling of risk of dementia among those with stroke,[255-257]. Our results are more in line with a medical records linkage analysis, which demonstrated up to a 9-fold increased risk of dementia in those with vs. without stroke.[258]

In our univariable model of incident all-cause dementia, CHF was associated with nearly a 3-fold increased risk. Results from several European population cohort studies suggest a link between CHF and dementia.[259-261] There is good biological plausibility for the interaction of stroke and CHF in dementia risk. CHF is cross-sectionally associated with lower cerebral blood flow.[262,263] Adding poor oxygen and nutrient delivery on top of parenchymal death from stroke is more likely to overburden the brain's compensation abilities. CHF may be secondary to both myocardial infarction and hypertension. Long-term exposure to hypertension causes left-ventricular hypertrophy, and poorer pumping

capability. Hypertension, but not myocardial infarction was associated with greater risk of incident all-cause dementia in our univariable modeling.

Despite strong univariable associations of the component variables, we did not confirm the presence of any of our other algorithm-based selected interactions. Others have reported such an interaction in relation to SVD[103] and cognitive trajectories[264] such that *APOE*4* carriers with hypertension have greater WMH burden and more rapid cognitive decline than non-carriers, but we did not find a significant *APOE*4**hypertension interaction for all-cause dementia. It is possible that this interaction is more relevant for mid-life hypertension and *APOE*4*. Indeed, such a relationship has been reported in relation to poor late-life cognitive function.[265] Our youngest participants began the study at age 65, and we do not have data on the duration of hypertension, so we were unable to study the effect of mid-life hypertension in combination with *APOE*4* carrier status. We also did not find a significant interaction of history of stroke or CHF and current drinking.

10.5.2 Secondary outcomes: Incident AD dementia, incident MCI, and overall cognitive performance

None of our candidate interactions in models of incident AD dementia or MCI were significant. We found strong and consistent beneficial effects of ever drinking alcohol across all of our outcomes, and this is consistent with others' results,[266,267] and when examining overall cognitive performance, we found a significant interaction of ever drinking alcohol with stroke such that a stroke history lessened the beneficial effect of ever drinking. However, this did not survive adjustment for multiple comparisons. Clearly parenchymal death outweighs the benefits associated with light to moderate drinking.

10.5.3 Exploratory outcomes: Interactions suggested by CART

Our CART modeling suggested an interaction of age with minutes of walking for exercise. We confirmed the presence of this interaction by testing it in a Cox PH model. The interaction indicates either that walking mitigates the effect of age on AD dementia risk, or, given the long and intertwined trajectories of cognitive decline and physical function, those who are less likely to get AD dementia also happen to be functioning well enough to keep walking. We cannot clarify possible reverse causality with this modeling approach. Nevertheless, many have reported beneficial effects of physical activity on AD-spectrum changes.[29-34,97,215]

The interaction of age with minutes walking was the only CART-generated interaction supported by our confirmatory testing with Cox PH models. At least one component of our ability to detect this interaction has to do with AUC of each model. The AUC for the dementia and MCI models was fair, while that for the AD dementia model was good (data not shown). Having greater prediction accuracy in this classification tree increased our chances of confirming the result with our Cox PH modeling.

10.5.4 Strengths and limitations

It is important to keep some limitations in mind when considering these results. When we corrected our analyses of interactions in incident all-cause dementia for multiple comparisons, the interaction of stroke with CHF was no longer significant. Nevertheless, given the strong relationship of both of these variables with incident all-cause dementia, testing this interaction in additional studies may be worthwhile. In a similar vein, the

interaction of stroke with ever drinking in relation to overall cognitive performance did not survive correction for multiple comparisons. Additionally, it is unclear if and how one should address this with our CART modeling given that the algorithm is essentially comparing all possible variables for splits. Therefore, we view the CART results as hypothesis generating.

Our study also has many strengths. First, we have a well-characterized cohort who has yearly follow-up data for up to 10 years. This allowed us to test relationships of many VCMRF and non-modifiable factors, adjust for potential confounding, and test longitudinal associations. Second, we tested interactions that were selected based on a combination of evidence in the literature, effect sizes in univariable associations, and biological plausibility. We also applied a machine learning technique that allows for examination of all possible splits within the data to generate additional candidate interactions. This is helpful as researchers cannot include all possible interactions within regression frameworks, but the CART algorithm can. In addition, this removes subjectivity in interaction selection on the part of the modeler. Finally, we tested these CART-suggested interactions in a test set of the data using Cox PH modeling in order to confirm them.

10.5.5 Future directions

There are other interactions of VCMRF with one another as well as with non-modifiable factors, which we found were important for SVD, but which could not be tested here. For example, mid-life hypertension is associated with greater risk of poor brain health as opposed to late-life hypertension,[102,104,105,121] and higher risk of silent brain infarcts/lacunes than in men may be found in older, but not younger, pre-menopausal

women.[119,128,150,168 130,268] We began following our participants when they were age 65 or older, thus we could not address interactions that split age between mid- and late-life. *APOE*4* may also interact with cholesterol. Willey, et al. found that among *APOE*4* carriers, those with total cholesterol ≥200 mg/dL trended toward having lower white matter hyperintensity volume than those with lower total cholesterol.[135] We did not test this interaction because of our particular interest in VCMRF associated with vessel health and blood flow and cholesterols stronger connection to myelin, synapse formation and maturation, and cellular membrane. These interactions should be tested in appropriate cohorts (for example mid-life cohorts that are now evolving into studies of aging). Our results, especially those based on CART, should be confirmed in other cohorts.

10.5.6 Conclusions

Physical activity and prevention of multimorbidity should be prioritized for prevention of cognitive impairment and dementia.

10.6 ACKNOWLEDGMENTS

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10.7 TABLES AND FIGURES

Table 10-1. Cognitive domains and tests used in the MYHAT study

Cognitive domain	Tests
attention/processing speed	Trailmaking Test A Digit span
executive function	Trailmaking Test B Initial letter fluency Clock drawing
language	Boston Naming Test Category fluency Modified Token Test
memory	Immediate and delayed logical memory Visual reproduction
visuospatial skill	Block design

Table 10-2. MYHAT study participants at each visit

Visit	N
1	1982
2	1697
3	1497
4	1320
5	1161
6	1022
7	922
8	834
9	745
10	623

Table 10-3. Baseline characteristics of all MYHAT study participants

	Total N	Median (IQR) or N (%)
Non-modifiable factors		
Age strata 65-74 75-84 85+	1982	 680 (34.31) 916 (46.22) 386 (19.48)
Female Sex	1982	1210 (61.05)
White Race	1982	1877 (94.70)
≥ HS education	1982	1709 (86.22)
*APOE*4* carrier	1778	372 (20.92)
Vascular and cardiometabolic factors		
Physical exam measures		
BMI	1934	27.0 (6.6)
Waist to hip ratio	1863	0.9 (0.1)
Pulse	1967	68.0 (12.0)
SBP	1969	132.0 (20.0)
DBP	1967	74.0 (12.0)
MAP	1967	93.3 (12.7)
PP	1967	58.0 (18.0)
Laboratory tests		
HbA1C	960	6.2 (1.0)
Total Cholesterol	1037	187.0 (59.0)
HDL-C	1037	45.5 (8.2)
LDL-C	1037	140.1 (53.4)
ApoB:ApoA1 ratio	1023	0.7 (1.2)
Homocysteine	1019	11.7 (4.9)
Cystatin C	1023	1.1 (0.4)
C-reactive protein	1023	2.0 (3.1)
Chronic health conditions		
History of stroke	1978	98 (4.95)
History of TIA	1975	187 (9.47)
History of MI	1977	292 (14.77)
History of hypertension	1977	1282 (64.85)
Current antihypertensive medication use	1982	1430 (72.15)
Hypertension uncontrolled with medication	1420	951 (66.97)
History of diabetes	1979	432 (21.83)
Antidiabetic medication	1982	255 (12.87)
History of high cholesterol	1972	1188 (60.24)
History of congestive heart failure	1977	188 (9.51)
History of cardiac arrhythmia	1978	595 (30.08)
Behavioral factors		
Walking for exercise (min/week)	1981	0.0 (90.0)
History of smoking (ever)	1977	1049 (53.06)

Table 10-3 Continued

Smoked in the past year	1975	145 (7.34)
Smoke now	1975	137 (6.94)
History of alcohol use (ever)	1978	1697 (85.79)
Current alcohol use (within past year)	1978	1298 (65.62)
History of Depression	1979	283 (14.30)
mCESD	1975	0.0 (1.0)
History of Anxiety	1979	211 (10.66)
Sleep apnea	1971	164 (8.32)
Cognition and functioning		
CDR=0	1982	1413 (71.29)
MMSE	1982	27.0 (3.0)
Attention	963	0.5 (0.7)
Executive	1007	0.5 (0.6)
Language	1116	0.5 (0.5)
Memory	1011	0.5 (0.6)
Visuospatial	904	0.5 (1.0)

Note: BMI=body mass index; SBP=systolic blood pressure; DBP=diastolic blood pressure; MAP=mean arterial pressure; PP=pulse pressure; CRP=c-reactive protein; TIA=temporary ischemic attack; MI: myocardial infarction; HTN=hypertension; DM=diabetes mellitus; CHF=congestive heart failure; mCESD= modified centers for epidemiologic studies-depression scale; CDR=clinical dementia rating scale; MMSE=Mini-Mental State Examination

Table 10-4. Baseline characteristics by cognitive outcome among those with at least one follow-up visit

Median (IQR) or N (%)	Cognitive Outcome							
Baseline Measures	**Cognitively Normal N=881**	**N**	**Incident MCI N=373**	**N**	**Incident All-cause Dementia N=109**	**N**	**Incident AD Dementia N=90**	**N**
Non-modifiable factors								
Age by strata 65-74 75-84 85+	 419 (47.56) 471 (42.11) 91 (10.33)	881	 64 (17.16) 209 (56.03) 100 (26.81)	373	 12 (11.01) 55 (50.46) 42 (38.53)	109	 5 (5.56) 45 (50.00) 40 (44.44)	100
Female Sex	547 (62.09)	881	258 (69.17)	373	71 (65.14)	109	59 (65.56)	90
White Race	851(96.59)	881	352 (94.37)	373	96 (88.07)	109	80 (88.89)	90
>= HS education	808 (91.71)	881	306 (82.04)	373	81 (74.31)	109	67 (74.44)	90
*APOE*4* carrier	156 (19.16)	814	85 (24.64)	345	34 (34.00)	100	27 (32.53)	83
Vascular and cardiometabolic factors								
Physical exam measures								
BMI	27.6 (6.8)	863	27.1 (6.5)	368	26.3 (7.0)	108	26.3 (6.2)	89
Waist to hip ratio	0.9 (0.1)	834	0.9 (0.1)	359	0.9 (0.1)	106	0.9 (0.1)	89
Pulse	68.0 (14.0)	877	68.0 (12.0)	371	68.0 (13.5)	108	68.0 (12.0)	89
SBP	132.0 (20.0)	875	132.0 (20.0)	371	132.0 (16.0)	109	132.0 (14.0)	90
DBP	76.0 (10.0)	874	72.0 (12.0)	371	72.0 (12.0)	109	72.0 (12.0)	90
MAP	94.5 (12.7)	874	92.7 (12.0)	371	93.3 (11.3)	109	93.3 (11.3)	90
PP	58.0 (14.0)	874	58.0 (18.0)	371	58.0 (18.0)	109	58.0 (18.0)	90
Laboratory Tests								
HbA1C	6.2 (0.9)	454	6.3 (1.0)	182	6.2 (0.9)	48	6.2 (0.9)	36
Total Cholesterol	192.0 (58.0)	487	185.5 (59.0)	190	179.0 (34.0)	51	177.0 (39.0)	39
HDL-C	46.2 (17.8)	487	45.6 (17.7)	190	45.2 (15.1)	51	45.2 (12.3)	39
LDL-C	143.4 (52.7)	487	134.1 (58.8)	190	134.5 (40.1)	51	131.8 (41.7)	39
ApoB:ApoA1 ratio	0.7 (0.2)	480	0.7 (0.3)	188	0.7 (0.2)	51	0.7 (0.3)	39
Homocysteine	11.3 (4.4)	478	11.7 (5.0)	188	11.7 (4.5)	51	11.7 (5.3)	39

Table 10-4 Continued

Cystatin C	1.0 (0.3)	480	1.1 (0.4)	188	1.1 (0.3)	51	1.0 (0.3)	39
C-reactive protein	2.0 (3.1)	480	1.8 (2.4)	188	1.8 (2.1)	51	1.9 (2.2)	39
Chronic Health Conditions								
History of stroke	20 (2.27)	880	14 (3.76)	372	6 (5.50)	109	4 (4.44)	90
History of TIA	58 (6.59)	880	35 (9.41)	372	13 (11.93)	109	10 (11.11)	90
History of MI (heart attack)	118 (13.41)	880	55 (14.78)	372	14 (12.84)	109	11 (12.22)	90
History of hypertension	540 (61.43)	879	247 (66.40)	372	74 (67.89)	109	62 (68.89)	90
Current antihypertensive medication use	594 (67.42)	881	277 (74.26)	373	85 (77.98)	109	73 (81.11)	90
Hypertension uncontrolled with medication	399 (67.86)	588	191 (69.20)	276	56 (65.88)	85	47 (64.38)	73
History of diabetes	180 (20.43)	881	75 (20.16)	372	23 (21.10)	109	14 (15.56)	90
Antidiabetic medication	97 (11.01)	881	48 (12.87)	373	14 (12.84)	109	8 (8.89)	90
History of high cholesterol	539 (61.32)	879	227 (61.52)	369	52 (48.15)	108	40 (44.94)	89
History of congestive heart failure	66 (7.49)	881	38 (10.22)	372	8 (7.34)	109	4 (4.44)	90
History of cardiac arrhythmia	225 (25.54)	881	110 (29.57)	372	34 (31.19)	109	26 (28.89)	90
Behavioral factors								
Walking for exercise (min/week)	0.0 (90.0)	881	0.0 (90.0)	373	0.0 (60.0),	108	0.0 (30.0)	90
History of smoking (ever)	469 (53.30)	880	166 (44.62)	372	43 (39.45)	109	34 (37.78)	90
Smoked in the past year	75 (8.53)	879	21 (5.65)	372	6 (5.50)	109	4 (4.44)	90
Smoke now	71 (8.08)	879	19 (5.11)	372	6 (5.50)	109	4 (4.44)	90
History of alcohol use	778 (88.31)	881	295 (79.30)	372	87 (50.46)	109	71 (78.89)	90
Current alcohol use (within past year)	632 (71.74)	881	218 (58.60)	372	55 (50.46)	109	46 (51.11)	90
History of Depression	110 (12.49)	881	44 (11.83)	372	16 (14.68)	109	10 (11.11)	90
mCESD	0.0 (0.0)	881	0.0 (1.0), 371	371	0.0 (1.0)	109	0.0 (1.0)	90
History of Anxiety	73 (8.29)	881	35 (9.41)	372	13 (11.93)	109	8 (8.89)	90
Sleep apnea	69 (7.88)	876	30 (8.09)	371	10 (9.17)	109	8 (8.89)	90

Table 10-4 Continued

Cognition and functioning								
CDR=0	881 (100.0)	881	373 (100.00)	373	43 (39.45)	109	34 (37.78)	90
MMSE	28.0 (2.0)	881	27.0 (4.0)	373	25.0 (5.0)	109	26.0 (5.0)	90

Note: BMI=body mass index; SBP=systolic blood pressure; DBP=diastolic blood pressure; MAP=mean arterial pressure; PP=pulse pressure; CRP=c-reactive protein; TIA=temporary ischemic attack; MI: myocardial infarction; HTN=hypertension; DM=diabetes mellitus; CHF=congestive heart failure; mCESD= modified centers for epidemiologic studies-depression scale; CDR=clinical dementia rating scale; MMSE=Mini-Mental State Examination

Table 10-5. Univariable relationship of variables with incident all-cause dementia

Variables	HR	lower .95	upper .95	z	Pr(>|z|)
Baseline age (≥ 78 y)	**5.82**	**3.58**	**9.48**	**7.09**	**<0.001**
Sex, female	1.05	0.71	1.55	0.23	0.82
Race, white	**0.38**	**0.21**	**0.67**	**-3.30**	**<0.01**
Education					
<HS (ref)	--	--	--	--	--
HS	**0.42**	**0.26**	**0.68**	**-3.59**	**<0.001**
>HS	**0.31**	**0.19**	**0.50**	**-4.65**	**<0.001**
***APOE*4* carrier**	**2.02**	**1.33**	**3.05**	**3.32**	**<0.001**
BMI	**0.58**	**0.43**	**0.77**	**-3.73**	**<0.001**
Waist to Hip Ratio	0.84	0.67	1.04	-1.60	0.11
Pulse	1.22	1.00	1.48	1.98	0.05
SBP	0.92	0.75	1.12	-0.85	0.39
DBP	0.83	0.68	1.01	-1.91	0.06
MAP	0.85	0.69	1.03	-1.66	0.10
PP	1.02	0.84	1.25	0.25	0.81
HbA1c	0.99	0.74	1.34	-0.04	0.97
Total cholesterol	0.79	0.59	1.07	-1.51	0.13
HDL-C	0.85	0.63	1.16	-1.02	0.31
LDL-C	0.83	0.62	1.12	-1.22	0.22
ApoB:ApoA1	0.97	0.70	1.35	-0.16	0.87
Homocysteine	1.03	0.80	1.32	0.23	0.81
Cystatin C	1.13	0.84	1.52	0.80	0.42
CRP	0.93	0.63	1.37	-0.37	0.71
Stroke	**9.90**	**4.76**	**20.58**	**6.13**	**<0.001**
TIA	1.78	0.44	7.23	0.81	0.41
MI	0.80	0.11	5.71	-0.23	0.82
HTN	**1.64**	**1.04**	**2.58**	**2.12**	**0.03**
DM	1.09	0.71	1.69	0.40	0.69

Table 10-5 Continued

High cholesterol	**0.61**	**0.42**	**0.89**	**-2.54**	**0.01**
CHF	**2.91**	**1.60**	**5.31**	**3.49**	**<0.001**
Cardiac arrhythmia	1.52	1.01	2.30	2.00	0.05
Walking for exercise (minutes per week)	**0.65**	**0.47**	**0.90**	**-2.64**	**0.01**
Ever smoked	**0.62**	**0.42**	**0.91**	**-2.47**	**0.01**
Smoked in the past year	0.88	0.36	2.16	-0.27	0.78
Smoke now	0.95	0.39	2.34	-0.10	0.92
History of alcohol use (ever)	**0.58**	**0.36**	**0.93**	**-2.27**	**0.02**
Current alcohol use (within past year)	**0.30**	**0.19**	**0.46**	**-5.45**	**<0.001**
Depression	**1.81**	**1.08**	**3.04**	**2.25**	**0.02**
mCESD	**1.39**	**1.26**	**1.53**	**6.62**	**<0.001**
Anxiety	1.24	0.64	2.37	0.64	0.52

Note: BMI=body mass index; SBP=systolic blood pressure; DBP=diastolic blood pressure; MAP=mean arterial pressure; PP=pulse pressure; CRP=c-reactive protein; TIA=temporary ischemic attack; MI: myocardial infarction; HTN=hypertension; DM=diabetes mellitus; CHF=congestive heart failure; mCESD= modified centers for epidemiologic studies-depression scale

Table 10-6. Relationship of interactions of interest with incident all-cause dementia

	Model 1				Model 2				Model 3			
Variables	HR	95% CI		p	HR	95% CI		p	HR	95% CI		p
***APOE*4*HTN**[a]												
*APOE*4*	2.04	0.86	4.86	0.10	2.50	**1.04**	**6.00**	**0.04**	2.64	0.76	9.14	0.13
HTN	1.65	0.91	2.98	0.10	1.41	0.77	2.55	0.26	2.32	1.00	5.36	0.05
*APOE*4**HTN	1.00	0.37	2.69	>0.99	1.04	0.39	2.81	0.93	1.08	0.28	4.23	0.9102
Stroke*current drinking[b]												
stroke	**10.65**	**4.81**	**23.56**	**<0.001**	**10.48**	**4.09**	**26.86**	**<0.001**	11.45	**4.40**	**29.77**	**<0.001**
current drinking	**0.31**	**0.20**	**0.49**	**<0.001**	**0.43**	**0.26**	**0.69**	**<0.001**	0.50	**0.31**	**0.82**	**<0.01**
stroke*current drinking	0.48	0.06	4.11	0.50	0.57	0.06	5.20	0.62	0.46	0.05	4.24	0.50
Stroke*CHF[c]												
stroke	**6.93**	**2.78**	**17.27**	**<0.001**	**5.47**	**1.70**	**17.59**	**<0.01**	**4.54**	**1.40**	**14.75**	**0.01**
CHF	**2.31**	**1.17**	**4.59**	**0.02**	1.83	0.91	3.66	0.09	1.65	0.80	3.39	0.18
stroke*CHF	3.35	0.67	16.80	0.14	4.40	0.73	26.42	0.11	**6.46**	**1.06**	**39.35**	**0.04**
CHF*current drinking[d]												
CHF	**2.57**	**1.33**	**4.99**	**<0.01**	**2.25**	**1.15**	**4.40**	**0.02**	**2.41**	**1.18**	**4.91**	**0.02**
current drinking	**0.31**	**0.19**	**0.48**	**<0.001**	**0.43**	**0.26**	**0.70**	**<0.001**	**0.53**	**0.32**	**0.87**	**0.01**
CHF*current drinking	0.98	0.20	4.76	0.98	0.85	0.17	4.18	0.84	0.71	0.14	3.51	0.67

Model 1: variables and interaction of interest.

Model 2: Model 1 + non-modifiable factors (baseline age, sex, race, education, *APOE*4*)

Model 3: Model 2 + vascular and cardiometabolic risk factors and behavioral factors that are confounders as follows:

[a] high cholesterol, BMI, minutes per week walking for exercise, current drinking, history of depression

[b] hypertension, pulse, minutes per week walking for exercise, ever smoked

[c] hypertension, arrhythmia, high cholesterol, minutes per week walking for exercise, history of depression

[d] hypertension, arrhythmia, high cholesterol, pulse, minutes per week walking for exercise, ever smoked, history of depression

Note: HTN=hypertension; CHF=congestive heart failure;

Table 10-7. Relationship of interactions of interest with incident Alzheimer's disease dementia

	Model 1				Model 2				Model 3			
Variables	HR	95% CI		p	HR	95% CI		p	HR	95% CI		p
***APOE*4*HTN**[a]												
*APOE*4*	1.95	0.78	4.89	0.15	**2.66**	**1.05**	**6.75**	**0.04**	2.96	0.85	10.29	0.09
HTN	1.47	0.79	2.74	0.22	1.21	0.65	2.27	0.55	1.91	0.81	4.53	0.14
*APOE*4**HTN	0.97	0.33	2.79	0.95	1.02	0.35	2.96	0.97	0.98	0.24	3.99	0.97
Stroke*current drinking**[b]												
stroke	**9.02**	**3.57**	**22.80**	**<0.001**	**10.00**	**3.46**	**28.87**	**<0.001**	**11.48**	**3.84**	**34.37**	**<0.001**
current drinking	**0.28**	**0.17**	**0.46**	**<0.001**	**0.38**	**0.22**	**0.65**	**<0.001**	**0.38**	**0.21**	**0.68**	**<0.001**
stroke*current drinking	$1.37*10^{-7}$	--	--	0.12	$1.32*10^{-7}$	--	--	0.15	$1.17*10^{-7}$	--	--	0.16
Stroke*CHF[c]												
stroke	**4.99**	**1.56**	**15.95**	**<0.01**	**4.55**	**1.10**	**18.86**	**0.04**	3.48	0.82	14.83	0.09
CHF	**2.47**	**1.19**	**5.13**	**0.01**	1.80	0.86	3.76	0.12	1.59	0.71	3.59	0.26
Stroke*CHF	3.31	0.47	23.27	0.23	3.41	0.40	29.16	0.26	5.53	0.63	48.22	0.12
CHF*current drinking[d]												
CHF	**2.41**	**1.16**	**5.05**	**0.02**	1.96	0.93	4.14	0.08	2.07	0.90	4.78	0.09
Current drinking	**0.26**	**0.15**	**0.44**	**<0.001**	**0.36**	**0.20**	**0.63**	**<0.001**	**0.37**	**0.20**	**0.70**	**<0.01**
CHF*current drinking	1.45	0.28	7.45	0.66	1.23	0.23	6.40	0.81	1.37	0.25	7.43	0.72

Model 1: variables and interaction of interest.

Model 2: Model 1 + non-modifiable factors (baseline age, sex, race, education, *APOE*4*)

Model 3: Model 2 + vascular and cardiometabolic risk factors and behavioral factors that are confounders as follows:

[a] high cholesterol, BMI, waist to hip ratio, minutes per week walking for exercise, drank in the past year

[b] waist to hip ratio, minutes per week walking for exercise, ever smoked

[c] arrhythmia, high cholesterol, mCESD

[d] arrhythmia, high cholesterol, waist to hip ratio, minutes per week walking for exercise, ever smoked, mCESD

**Note: For these models, a warning was generated indicating that the beta may be infinite. In these cases, the author of the "survival" package in R advises that the standard errors and Wald-test p-values should not be trusted, but that the Likelihood ratio test is still accurate. Thus, the p-value for the interaction term here is the LRT p-value comparing the model with the interaction term to the model without the interaction term. HTN=hypertension; CHF=congestive heart failure

Table 10-8. Relationship of interactions of interest with incident mild cognitive impairment

	Model 1				Model 2				Model 3			
Variables	HR	95% CI		p	HR	95% CI		p	HR	95% CI		p
***APOE*4*HTN**[a]												
*APOE*4*	1.46	0.98	2.17	0.06	1.50	1.00	2.23	0.05	1.70	0.94	3.07	0.08
HTN	1.19	0.91	1.54	0.20	0.92	0.70	1.20	0.53	1.11	0.75	1.66	0.59
*APOE*4**HTN	0.86	0.52	1.42	0.55	0.95	0.57	1.58	0.84	0.83	0.39	1.77	0.63
MI*TIA**[b]												
MI	2.97	1.63	5.41	<0.001	2.40	1.31	4.42	<0.01	1.78	0.69	4.61	0.23
MI*TIA	0.00	--	--	0.22	0.00	--	--	0.32	NA	NA	NA	NA
MI*Stroke[c]												
MI	2.77	1.48	5.20	<0.01	2.30	1.22	4.35	0.01	2.11	0.78	5.72	0.14
MI*stroke	0.73	0.04	12.62	0.83	0.49	0.03	6.98	0.60	0.41	0.02	8.44	0.56

Model 1: variables and interaction of interest.
Model 2: Model 1 + non-modifiable factors (baseline age, sex, race, education, APOE4)
Model 3: Model 2 + vascular and cardiometabolic risk factors and behavioral factors that are confounders as follows:

[a] Pulse pressure, BMI, Cystatin C, minutes per week walking for exercise, drank in the past year, mCESD

[b] NA

[c] DBP, Cystatin C, ever smoke

**Note: For models 1 and 2 of this interaction, a warning was generated indicating that the beta may be infinite. In these cases, the author of the "survival" package in R advises that the standard errors and Wald-test p-values should not be trusted, but that the Likelihood ratio test is still accurate. Thus, the p-value for the interaction term here is the LRT p-value comparing the model with the interaction term to the model without the interaction term. NA: This model was not able to run due to small sample size for the interaction of interest once all of the covariates were entered into the model. HTN=hypertension; MI=myocardial infarction; TIA=temporary ischemic attack.

Table 10-9. Relationship of interactions of interest with overall cognitive performance

	Model 1					Model 2					Model 3				
Variables	Estimate	95% CI		SE	t	Estimate	95% CI		SE	t	Estimate	95% CI		SE	t
Ever drank* Stroke[a]															
ever drank	**0.095**	**0.054**	**0.135**	**0.021**	**4.60**	**0.050**	**0.011**	**0.089**	**0.020**	**2.51**	**0.049**	**0.010**	**0.088**	**0.020**	**2.48**
stroke	0.023	-0.089	0.135	0.057	0.40	0.038	-0.076	0.151	0.058	0.65	0.039	-0.074	0.153	0.058	0.68
ever drank* stroke	-0.106	-0.229	0.017	0.063	-1.69	-0.125	**-0.249**	**-0.001**	**0.063**	**-1.97**	**-0.125**	**-0.249**	**-0.482 * 10^{-3}**	**0.063**	**-1.97**
Ever drank* Cystatin C[b]															
ever drank	**0.062**	**0.006**	**0.118**	**0.029**	**2.18**	0.015	-0.036	0.067	0.026	0.59	0.017	-0.034	0.069	0.026	0.66
cystatin C	-0.029	-0.079	0.022	0.026	-1.11	-0.009	-0.055	0.037	0.024	-0.39	-0.004	-0.050	0.042	0.024	-0.17
ever drank* cystatin C	-0.027	-0.080	0.027	0.027	-0.98	-0.007	-0.055	0.042	0.025	-0.27	-0.008	-0.056	0.041	0.025	-0.30
Stroke* Cystatin C[c]															
stroke	-0.043	-0.115	0.030	0.037	-1.16	-0.032	-0.104	0.039	0.037	-0.89	-0.028	-0.100	0.044	0.037	-0.77
cystatin C	**-0.052**	**-0.069**	**-0.036**	**0.008**	**-6.26**	-0.015	-0.030	0.001	0.008	-1.80	-0.010	-0.026	0.007	0.008	-1.18
stroke* cystatin C	-0.009	-0.061	0.043	0.027	-0.34	-0.015	-0.067	0.036	0.026	-0.58	-0.019	-0.071	0.034	0.027	-0.70

Model 1: variables and interaction of interest.
Model 2: Model 1 + non-modifiable factors (baseline age, sex, race, education, *APOE*4*)
Model 3: Model 2 + vascular and cardiometabolic risk factors and behavioral factors that are confounders as follows:

[a] Hypertension, minutes per week walking for exercise, mCESD
[b] Hypertension, myocardial infarction, arrhythmia, homocysteine, minutes per week walking for exercise, mCESD
[c] Hypertension, myocardial infarction, arrhythmia, homocysteine, minutes per week walking for exercise, drank in the past year, mCESD

Table 10-10. Cox proportional hazards model of CART generated candidate interaction of age and walking for incident AD dementia

	Model 1				Model 2			
Variables	HR	lower .95	upper .95	p	HR	lower .95	upper .95	p
age	**33.89**	**4.65**	**247.26**	**<0.001**	**31.69**	**4.30**	**233.26**	**<0.001**
walking minutes	4.22	0.44	40.59	0.21	4.46	0.46	42.84	0.20
age*walking minutes	**0.06**	**$0.05*10^{-1}$**	**0.67**	**0.02**	**0.04**	**$0.03*10^{-1}$**	**0.55**	**0.02**

Model 1: variables and interaction of interest.
Model 2: Model 1 + non-modifiable factors (baseline age, sex, race, education, *APOE*4*)
Note: The three-way interaction of age*walking minutes*waist to hip ratio and two-way interactions involving waist to hip ratio were also tested. However, the models involving waist to hip ratio would not run, likely due to small sample size.

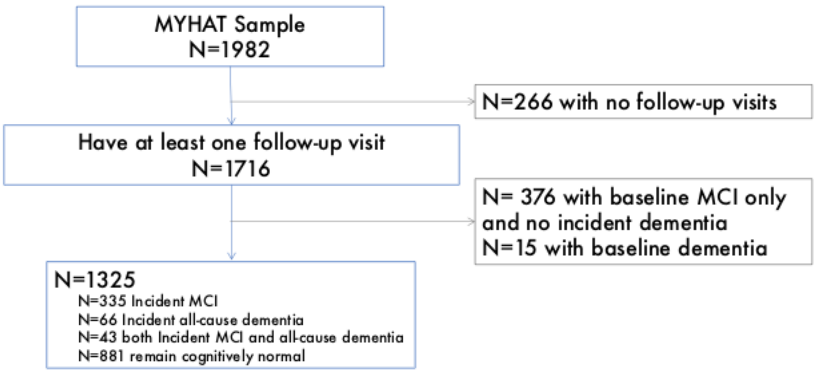

Figure 10-1. Study flow for all MYHAT study participants

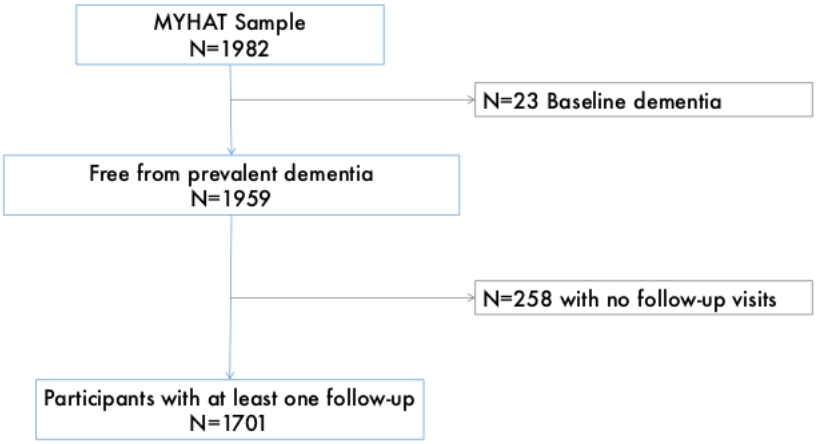

Figure 10-2. Creation of the all-cause dementia and AD dementia risk set

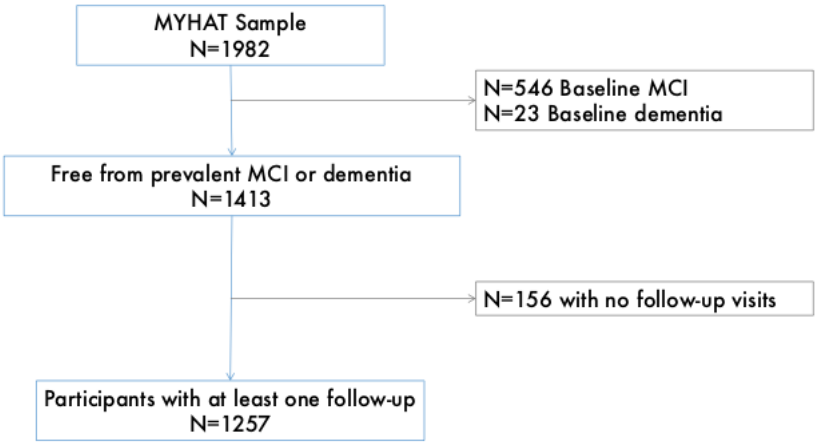

Figure 10-3. Creation of the MCI risk set

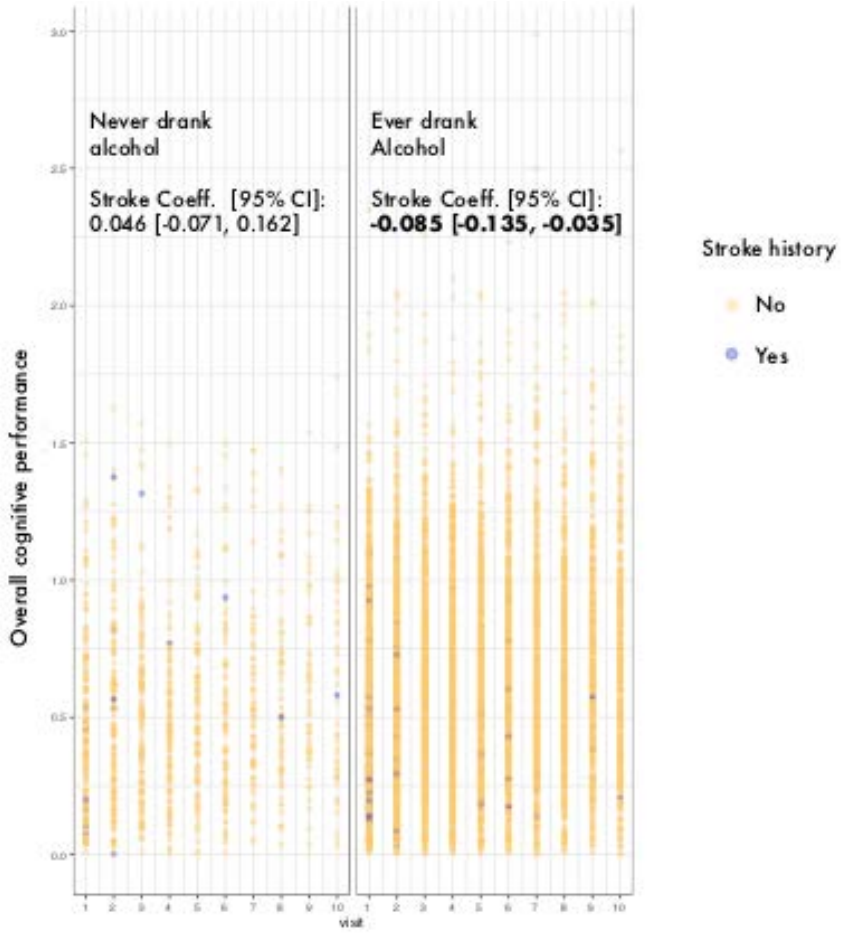

Figure 10-4. Relationship of stroke with overall cognitive performance by history of drinking

11.0 DISCUSSION

With no good disease modifying treatments for AD and related disorders, determining whether the most promising candidates actually promote cerebral small vessel integrity and thereby prevent cognitive disorders can point to new intervention strategies, a critical public health goal. Therefore, in this dissertation, I have used a population neuroscience approach to examine whether PA, growth factors, and VCMRF reduction are plausible pathways to small vessel integrity and cognitive health.

11.1 RESULTS SUMMARY

I wanted to know whether PA and growth factors could promote integrity of the cerebral small vessels themselves, rather than neuroimaging markers of later stage disease. Before I could answer this question, I needed to find a way to measure the vessels directly in vivo. In paper 1, I developed a method to image deep medullary veins in older adults using 7T SWI. This approach was feasible to apply in older, community-dwelling adults. In cross-sectional analysis, I found that *APOE*4* allele presence, lower VEGF, and male sex were associated with greater tortuosity ratio. The relationship of *APOE*4* with tortuosity ratio was a robust result that survived adjustment for confounders and multiple comparisons.

Confident in this method, I could thus wade into my main question. In a randomized controlled trial in paper 2, I discovered that indeed, PA appeared to be a promoter of

cerebral small vessel integrity. I also found an indication that greater percent increases in BDNF were positively associated with percent changes in straight venous length.

Next, I wanted to know whether VCMRF interact with one another and non-modifiable factors to confer differential risk of cognitive disorders. I found that the risk of all-cause dementia conferred by stroke was even greater among those with CHF; the beneficial effects of alcohol consumption on overall cognitive performance varied by stroke history; and in exploratory analyses I found that the detrimental effect of age on AD dementia risk was lower among those who walked more.

11.2 DISCUSSION & FUTURE DIRECTIONS

Next, I will discuss the implications of these results and future directions, ending with a proposal of a research program to address my next questions. I successfully adapted a method to image the cerebral deep medullary veins in older adults. This represents a major advance in methodology that could significantly benefit research into cerebral SVD. Traditional neuroimaging markers of SVD do not directly image the vessels, but instead image markers more distal in the pathophysiological process. SWI at ultra-high field strength provides a window to the cerebral small vessels in living humans. Future multimodal studies of SVD should incorporate markers of parenchymal health (gray matter volume, white matter volume, cortical thickness), traditional neuroimaging markers of SVD (WMH, SBI/lacunes, microbleeds) and novel markers of SVD visualizing both structure (7T SWI, TOF, FA, MD) and function (CBF, CVR). The

application of such modalities across the lifespan could allow this research field to make substantive progress in understanding the pathophysiological natural history of SVD.

In addition, 7T SWI provides a way to study the venous side small vasculature, which has remained generally unstudied until now. The cerebral venous circulation is important for three key reasons: it is critical for healthy blood flow, it is the site of initial inflammatory response, and it is implicated in deposition and clearance of aβ. The cerebral veins are unique. Unlike veins in the limbs, they have no valves, and unlike arteries they have less muscle and thus thinner walls. This makes them susceptible to loss of elasticity, damage, and venous congestion, and perhaps in a process similar to the development of varicose veins, this leads to tortuosity. When blood pools due to venous congestion, the pressure on the venous walls is increased. This can damage the vein, making it more tortuous. Whether this is the same process impacting cerebral small veins needs to be tested more mechanistically, perhaps in studies in which venous tissue is exposed to various pressures to assess impact on tortuosity. Evaluating changing tortuosity over a lifespan could also help to rule this in or out. Blood flow in the veins is low and slow. This allows for leukocyte capture and transmigration. Interestingly, both the increased pressure from venous congestion and the inflammatory process will push fluid through the vessel walls and into the interstitial space. Whether this can explain WMH, which are essentially marking fluid in the brain, should be determined. I found no association of tortuosity ratio with WMH in paper 1. Finally, if the venous side is the site of initial aβ deposition as has been suggested by animal work,[269] early deposits in the veins potentially block effective clearance via perivenular spaces. In addition, anything blocking bulk flow, which is proposed as a key mechanism of glymphatic clearance,[270]

could be implicated in AD-spectrum pathophysiology. For all of these reasons, 7T SWI of cerebral small veins may have utility as a biomarker of small vessel VCID. A quest to identify and validate biomarkers of small vessel VCID for use in clinical trials is currently underway.[240] In addition, while my work so far has focused on the deep medullary veins, this imaging approach could be extended throughout the brain. Of particular interest to me would be using this approach to study the venous system of the hippocampus in vivo as a way to extend multimodal research regarding mechanisms of PA's beneficial effects on the hippocampus as discussed in section 2.1.1. This approach would allow me to continue to assess the importance of vascularization to PA effects on hippocampal volume.

In my cross-sectional analysis, I found that *APOE*4* allele presence was associated with a larger tortuosity ratio, while it was not associated with percent change in venous markers. Therefore, it seems that *APOE*4* is related to where one starts with regard to straight and tortuous venous length and tortuosity ratio, but not to changes over time. Confirming these relationships of the *APOE*4* allele with small vein morphology in younger, healthy individuals would strengthen this conclusion. Therefore, future studies should extend the use of 7T SWI into mid-life, young adulthood, and childhood.

I found that lower VEGF was cross-sectionally associated with a greater tortuosity ratio, but that percent increases in BDNF were associated with percent increases in straight venous length in my PA intervention analysis. This result with VEGF did not survive adjustment but is worth investigating in a larger study to confirm whether this is the true relationship or due to lack of power. I found that my measures of BDNF and VEGF were very noisy, and this difficulty was compounded by my small sample size. My

future studies incorporating peripheral makers like this will need to take this into account when determining sample size.

Male sex was associated with greater tortuosity ratio at study baseline in paper 1. Why this is the case when female sex has been associated with SBI/lacunes[119,150,168] and WMH progression,[113,119] especially for black females,[113] is important to explore further. It is possible that women are at greater risk of cerebral SVD because they are more likely than men to have coronary heart disease due to microvascular disease,[271] but research evaluating the relationship of coronary microvascular disease with cerebral SVD and shared risk and protective factors is lacking. Understanding sex-differences in VCID could suggest tailored intervention strategies for cognitive disorders. Interestingly, sex-differences are also found in AD dementia, and it may be that some of these AD dementia sex differences can be explained by SVD sex differences. Although others have found that females have a greater risk of AD dementia that is not explained by longevity alone,[272] I did not find an association of female sex with incident all-cause dementia, AD dementia, or overall cognitive performance in univariable analyses in paper 3. I did find a univariable association of female sex with greater risk of MCI. Our study participants were all 65 or older, so I could not evaluate a potential interaction of interest in which women 65 or older may be at greater risk of SBI/lacunes than men, but not women <65.[119,126,128-130,150] Interestingly, CART, which can explore all possible variable combinations and also determine best cut-points, also did not suggest sex-based interactions.

There are many outstanding questions that this research has suggested. For example, are the promoters of vessel integrity and pathophysiological factors different for straight and tortuous veins? I discovered that only straight veins only seemed capable of

increasing over time in response to PA and BDNF. Confirming that cerebral small veins are truly malleable is a perfect question for back-translation into experimental animal models in which conditions can be tightly controlled and PA and growth factors precisely administered and measured. The effect of wheel-running, administration of BDNF and VEGF, and blockade of BDNF and VEGF receptors on straight and tortuous veins of mice or rats would help researchers understand whether it is biologically plausible that these promoters of small vessel integrity can alter straight but not tortuous veins.

There are many opportunities for this neuroimaging method to grow and improve. Our method of tracing the deep medullary veins was manual and two dimensional. A way for this method to continue to evolve will be to develop automated, three dimensional approaches. During the course of my dissertation, others have been pushing this neuroimaging modality forward in these ways.[178,185] This group has implemented a three-dimensional method of venous tracing. This is advantageous because it reduces bias associated with veins that may run out of plane in two dimensional images. Their metric of tortuosity places the full vein length over the straight-line length drawn from end to end of the vein. This approach to tortuosity is appealing in that no subjective human judgement on length or degree of curvature is required. This allows more reliable measurement which can be automated. Finally, this approach to measuring the veins via SWI could likely be implemented at a lower field strength. This is important to reduce selection bias that occurs with neuroimaging studies at higher field strength due to their more stringent exclusion criteria.

I found several interactions with VCMRF including stroke*congestive heart failure in relation to incident all-cause dementia, age*minutes walking for incident AD dementia,

and ever drinking alcohol*stroke in relation to overall cognitive performance. That older adults who exercise more have a lower risk of AD dementia is consistent both with my own hypothesis that PA is a promoter of cerebral small vessel integrity which in turn promotes cognitive function and with the extant literature. For example, I discovered that the total length of small straight vessels increases in response to PA even among very old adults (70-89 years old). Our research group has also shown that hippocampal volume was beneficially impacted by PA in this age group.[35] In addition, the parent study of this neuroimaging study found a beneficial effect of PA on executive function only among those 80 and older.[273]

Most of my analyses of interactions in paper 3 were hypothesis generating or did not survive corrections for multiple comparison. As such, they should be replicated. One option for such replication testing would be within the Monongahela Valley Independent Elders Study (MoVIES; PI: Ganguli), a similar cohort study of dementia epidemiology. As opposed to training in 50% of the MYHAT datasets and testing in 50%, this would afford the opportunity to retrain the CART models on 100% of the MYHAT dataset, and then validate in the MoVIES cohort.

The approach I used for modeling exposure to variables of interest over time in survival analyses employed time dependent covariates in Cox regression. However, this approach may be biased when the time dependent covariates are endogenous—internal variables in which the current value of the covariate is dependent on a prior value of the covariate. For example, current BMI or HbA1c are internal variables in which current value is dependent on past values. Time dependent Cox models can underestimate the association of the evolution of the covariate with the survival outcome. Therefore, when

the covariates of interest are endogenous, the joint modeling framework is a better approach.[274] In this application, joint modeling uses linear or generalized linear mixed modeling to create an evolution of the covariate of interest. The strength of association of this evolution with a survival outcome can be assessed by using this evolution as an exposure of interest in Cox regression. While I attempted this approach using the JMBayes package in R, the models would not converge. Nevertheless, this is a promising approach that incorporates a whole pattern of exposure to a factor over many years. Attempting to implement joint modeling in these analyses using SAS or some custom code may be worthwhile. The joint modeling framework also allows for dynamic prediction—given an individual's exposure evolution up to a certain time at which they had not yet had the event, what is their probability of survival after that time. The prediction is dynamic because the survival probabilities can be updated as each new measure of the exposure variable is taken. Such dynamic individual prediction is a tool that can advance precision medicine.

There were several non-modifiable factors that I was unable to study in paper 3. The MYHAT study population is majority white, and thus racial differences could not be evaluated. Many important interactions reported in the literature relate to mid-life VCMRF effects on late-life small vessel integrity and cognitive health. I could not assess any of these relationships since we began following our sample at age 65. Based on my review of the literature regarding risk factors for SVD,[100] the importance of pushing the study of SVD earlier in the life course is clear. We must push back to mid-life or earlier to truly understand the pathogenesis of SVD. Better assessment of critical periods of exposure to risk factors for development of SVD as well as duration of exposure must be

incorporated into these studies. Without these measures, whether these associations of VCMRF in mid-life are due to onset or duration of exposure cannot be clarified.

One way to assess the impact of duration of exposure with risk of cognitive disorders that can be implemented immediately is the use of a simulation. In the MYHAT study, durations of hypertension, diabetes, and other VCMRF are not known. From a publicly available dataset in which duration of chronic diseases is known, durations of individuals similar to MYHAT study participants could be selected and assigned to the respective MYHAT participant. The association of duration of exposure with cognitive disorders could then be tested. A likely source of this data is the Health and Retirement Survey,[275] a population representative survey of 20,000 older U.S. adults. This study has information on year of diagnosis for diabetes, congestive heart failure, and cardiac arrhythmia.

As researchers we often apply stringent exclusion criteria to both descriptive and interventional epidemiologic studies. Individuals with multiple chronic conditions may be excluded, or we as researchers may disregard the possible complexities of comorbidities and evaluate these conditions one at a time. But I have shown that there are important interactions of VCMRF. My study results, and the real-world experience of clinicians who see that older adults do not simply present with one chronic illness, demonstrate that reductive analyses focusing only on individual risk or protective factors are both failing to harness population heterogeneity to answer research questions and condemning clinicians to apply population average care. Instead, we must harness heterogeneity to find the groups that are differentially at risk of SVD and cognitive disorders and those who

will preferentially benefit from specific interventions. Next, I detail more of my vision of a research program to answer some of the questions I have identified above.

11.3 RESEARCH PROGRAM PROPOSAL

Given all of these remaining questions, I would like to propose a research program that could be used to answer them. The program would use a population neuroscience approach. Basic science and human research (both observational and interventional) would be integrated and allow for translation of basic science findings to humans *and* back-translation from humans to basic science. This process is not constrained to be linear, but rather represents an iterative cycle of questions, discovery, surprise, and investigation. In this paradigm, unexpected or unexplained clinical or research-based observations in humans generate new tests of mechanism in basic science research. The team would be multidisciplinary, incorporating vascular biologists, physiologists, basic scientists, neuroscientists, psychologists, epidemiologists, neuroimaging specialists, biostatisticians, and clinicians.

A key place to start would be with a large, racially / ethnically diverse population-based cohort of men and women. Ideally, I would follow individuals across the entire lifespan to distinguish the influence of maximal development of brain and cognitive health (which may be seen as the starting point in a study starting at mid- or late-life) from declines with age and morbidity. An alternative approach to recruiting a new birth cohort would be to add additional measures to or combine existing cohorts. Combining cohorts may be the most resource efficient approach and is already in progress for studies of

VCID in late-life.[276] This approach should be extended to studies of adolescence and mid-life including the Adolescent Brain Cognitive Development Study,[277] Adult Health and Behavior project, phase II (AHAB-II)[278], the Pittsburgh Imaging Project (PIP)[279], Study of Women's Health Across the Nation (SWAN), Ms. Heart.[280], and the UK Biobank, a very large (N=500,000) prospective study of mid- and late-life.[281] Such cohorts could be followed through late-life.

A life course approach would increase our understanding of the natural history of SVD pathophysiology and identify potential time dependent processes and critical / vulnerable periods. Participants would agree to donate their brains and be eligible and willing to undergo serial exams, until as close as possible to death, for multimodal neuroimaging scans, medical examination, interviews on lifestyle, blood draws, and behavioral assessment. Primary measures of interest would include neuroimaging, serum biomarkers, genotype, behavior and function, postmortem neuroimaging, and histopathology. Neuroimaging measures should be multimodal and integrative including the modalities listed in section 11.2 as well as PET imaging of aβ and tau. Integrating modalities could yield new insights, and such integration is beginning to occur (see Bangen, et al. [282] for an example of such a study). Assessing the functional and clinical relevance of such changes would be achieved by including measures of cognitive and physical functioning and following participants for critical endpoints such as cognitive decline, dementia, and death. The size of the samples must be large enough to conduct well-powered sex- and race-stratified analyses of data thus obtained, with a focus on interactions between multimorbidities.

Tools to handle large amounts of data should be used. For example, machine learning and decision tree algorithms can combine high dimensional neuroimaging data, demographics, and clinical variables obtained at repeated time points to produce predictive models of clinically relevant outcomes.[283] This approach can be effective if integrated with an understanding of the underlying conditions and principles of rigorous study design. Simulations can also be used to model possible influence of variables not currently contained in the datasets.

11.4 PUBLIC HEALTH SIGNIFICANCE

Taken together, my results suggest that PA, growth factors, and VCMRF reduction are promoters of cerebral small vessel integrity. The rates of meeting weekly PA guidelines among older adults are very low. Evidence-based approaches to increase PA among older adults should be prioritized. Whether the benefits of PA may be conferred on those who are too frail for PA, potentially through administration of growth factors, should be tested. Multimodal interventions to prevent multimorbidity and thus increased risk of cognitive disorders should be tested among individuals who already have one VCMRF chronic condition. These approaches could yield reductions in late-life cognitive disorders through promotion of cerebral small vessel integrity.

APPENDIX A: SVD RISK FACTORS LITERATURE REVIEW METHODS

A PubMed search was carried out to assess the current state of knowledge regarding VCMRF for SVD in healthy adults. Using the search terms described below, the search was carried out on June 20, 2017.

Search terms. We searched for “cerebral small vessel disease” in conjunction with the following terms:

- “leukoaraiosis” or “white matter hyperintensities” or “white matter lesions” or “white matter changes”
- “lacunes”
- “silent brain infarcts”
- “cerebrovascular reactivity”
- “cerebral blood flow”

All of those were combined with AND with the following added with NOT:

- “cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy” or “CADASIL”
- “acute stroke”

APPENDIX B: RESEARCH INVESTIGATORS FOR THE LIFE STUDY, PAPERS 1 & 2

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APPENDIX C: BACKGROUND ON MACHINE LEARNING WITH A FOCUS ON CLASSIFICATION AND REGRESSION TREES

Classification and regression trees (CART) comprise a machine learning approach to prediction of a categorical outcome in the case of a classification tree or a continuous outcome in the case of a regression tree. Both approaches use recursive partitioning of the data in order to predict an outcome. When a continuous outcome is predicted by a regression tree, the prediction will be the mean outcome value for that partition. Alternately, when a categorical outcome is predicted by a classification tree, the outcome value is the outcome with the greatest proportion for that partition. For example, if a classification tree is attempting to predict dementia vs. no dementia and the dataset is split into three terminal leaves (endpoints of the decision tree), the outcome assigned to each terminal leaf is based on the proportion of dementia and no dementia contained within it. If terminal leaf A has 75% dementia and 25% no dementia, that terminal leaf predicts an outcome of dementia.

While a plethora of machine learning approaches exist, paper 3 uses CART because it is ideal to solve a very specific problem: screen for multiple possible interactions simultaneously and predict a categorical outcome. With typical regression approaches, when multiple main effects and interactions are of interest, the researcher cannot test all possible combinations, especially when sample sizes are limited. On the other hand, with a classification tree, the investigator need only run one test and all possible splits are tested with the best selected. In this way, it is certainly testing more possible interactions than a researcher can in a typical regression framework.[284] A

classification tree proceeds in a branching pattern from the top downward. Interactions can be of two types. First, the proportion of the outcome of interest in terminal daughter nodes split on variable A may vary by variable B.[284] This is a symmetric branching pattern. Alternately, a split on variable A may result in a terminal leaf on one branch, but be split again by variable B on the other branch.[284] This is an asymmetric branching pattern.

The recursive partitioning criterion we used was based on reducing entropy—a splitting criterion designed to reduce impurity and create the most homogenous groups. After this growing phase, the will have many terminal leaves, and this is likely to overfit the data. Thus, the tree must also be pruned, and we used cost-complexity pruning. This approach tries to balance correct classification with tree complexity to reduce the number of leaves. We also used 10-fold cross validation with our classification trees. This approach randomly splits the sample into 10 equally sized groups and develops a decision tree in 10 groups—each one holding out one fold. An error term for that classification tree is calculated on the held-out fold. The cost-complexity parameter (the parameter that penalizes complexity and misclassification) with the minimum error is then selected to grow the final tree.

One limitation of CART is that it is highly dependent on the dataset and can produce unstable results.[285] A natural machine learning extension of this classification tree approach which can address this limitation is an ensemble learning approach known as random forests. Here, bootstrapping is used to select multiple sub-samples with replacement from the data. The subset of variables in each tree generated can also be randomly sampled. The predicted probability an observation has of being in a certain outcome category is based on the proportion of times it is assigned to that outcome

category during the bootstrap sampling. That case is then assigned to the category for which it has the largest probability of being assigned. By essentially averaging over many selected trees (the forest in “random forests” and ensemble in “ensemble learning”), the random forests approach thus generates results with lower variance.[285] Its drawback is that a *single* tree is not produced, thus making interpretation more complicated.

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