FACTORS INFLUENCING DAMAGE TO CEREBRAL WHITE MATTER IN THE ELDERLY

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"We must welcome the future, remembering that soon it will be the past; and we must respect the past, remembering that it was once all that was humanly possible."
-George Santayana

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ABSTRACT

BACKGROUND: White matter hyperintensities (WMH) appearing on magnetic resonance images (MRI) occur in the non-demented and demented elderly. Age is an established risk factor associated with WMH. Other possible risk factors include sex, smoking, hypertension, biochemical markers and genotypes associated with B vitamin status, and *APOE* ε4 status. WMH have been associated with an increased risk of depression and cognitive dysfunction.

OBJECTIVES: To investigate factors associated with WMH 2 to 5 years later in healthy elderly. Measures of cognitive impairment, depression, and brain volume were also evaluated in relation to WMH.

METHODS: 155 healthy volunteers (60 to 90 years) were included (49% women). Participants completed approximate yearly examinations including a medical history, cognitive assessment, depression survey, T1 and T2-weighted MRI, blood samples measuring markers and genotypes associated with B vitamin deficiencies, and *APOE* ε4 status. Associations were investigated by Pearson correlations, followed by linear regression, adjusted for age and sex.

RESULTS: Age was positively associated with WMH. Other significant prospective risk factors included systolic blood pressure (SBP) and diastolic blood pressure (DBP). DBP and total WMH were positively associated in hypertensive, but not normotensive participants. Elevated homocysteine was positively associated with WMH within the top tertile. No other factors were significantly associated with WMH. Baseline WMH were negatively associated with follow-up (3 to 5 years) cognitive assessments of executive function and processing speed, and positively associated with depressive symptoms. Furthermore, WMH were positively associated with ventricular cerebrospinal fluid volume and negatively associated with percent brain volume change and grey matter volume, but not white matter volume.

CONCLUSIONS: Age is the most striking risk factor associated with WMH; other significant factors include SBP and DBP. Baseline WMH were associated with decreased performance on tests of executive function, processing speed, and increased depressive symptoms. These data suggest that WMH is not a benign process of aging considering the positive associations to hypertension, cognitive impairment, and depression.

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GLOSSARY OF ABBREVIATIONS

AD	*Alzheimer's Disease*
ANOVA	*Analysis of Variance*
APOE	*Gene for Apolipoprotein E*
ARWMC	*Age Related White Matter Changes*
BGH	*Basal Ganglia Hyperintensities*
BMI	*Body Mass Index*
CANTAB	*The Cambridge Automated Testing Battery*
CAMCOG	*The Cambridge Cognitive Examination*
CAMDEX	*The Cambridge Examination for Mental Disorders of the Elderly*
CERAD	*The Consortium to Establish a Registry for Alzheimer's Disease*
CI	*Confidence Interval*
CT	*Computed Tomography*
DBP	*Diastolic Blood Pressure*
DWMH	*Deep White Matter Hyperintensities*
FLAIR	*Fluid-Attenuated Inversion Recovery*
GDS	*Geriatric Depression Scale*
GNT	*Graded Naming Test*
HoloTC	*Holotranscobalamin*
ITFH	*Infra-Tentorial Foci of Hyperintensity*
MMA	*Methylmalonic Acid*
MMSE	*Mini Mental State Examination*
MR	*Magnetic Resonance*
MTHFR	*Gene for Methylenetetrahydrofolate Reductase*
MTRR	*Gene for Methionine Synthase Reductase*
NS	*Not Significant*
OPTIMA	*Oxford Project to Investigate Memory and Aging*
PAL	*Paired Associate Learning*
PBVL	*Percentage of Whole Brain Volume Loss*
PP	*Pulse Pressure*
PRM	*Pattern Recognition Memory Task*
PVH	*Periventricular Hyperintensities*
SBP	*Systolic Blood Pressure*
SIENA	*Structural Image Evaluation Using Normalization of Atrophy*
SDMT	*Symbol Digit Modalities Test*
SRM	*Spatial Recognition Memory Task*
tHcy	*Plasma Total Homocysteine*
TC	*Transcobalamin*
TCN	*Gene for Transcobalamin*
TPT	*The Placing Test*
WMH	*White Matter Hyperintensities*
WLM	*Word List Memory Test*

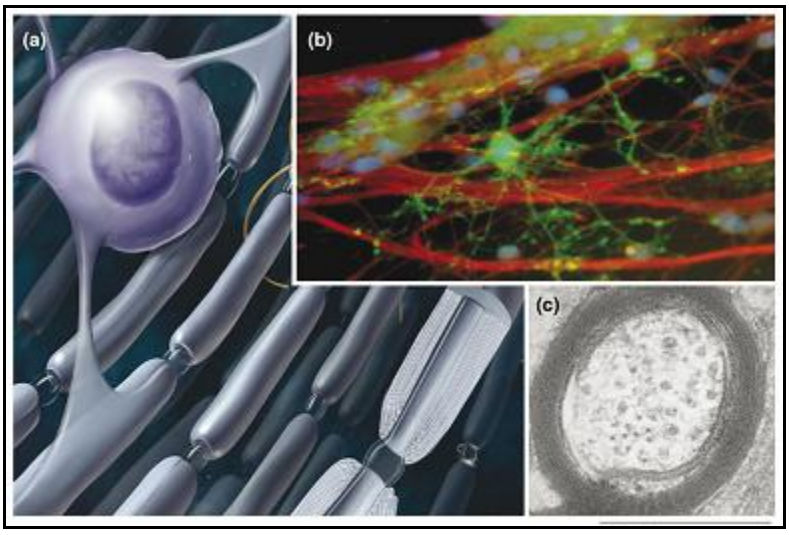
CHAPTER ONE
BACKGROUND TO THE RESEARCH

I. MYELIN, WHITE MATTER, AND LEUKOARAIOSIS

White matter comprises approximately 40-50% of the brain's total volume and plays a vital role in human physical and cognitive functioning (1, 2). It is made up of large bundles of axons insulated with myelin, a protein and lipid rich substance, essential for efficiently conducting nerve impulses between various cortical regions in the brain (1). **Figure 1** illustrates myelin wrapped around axons at a microscopic level.

Figure 1 MYELIN AND AXONS

(a) *Illustrates myelin wrapped around axons,* ***(b)*** *the initial stage of an oligodendrocyte (green) wrapping myelin around several axons (red) and* ***(c)*** *a cross-sectional view of an axon and multiple layers of myelin in a rat brain. Re-printed from (4).*



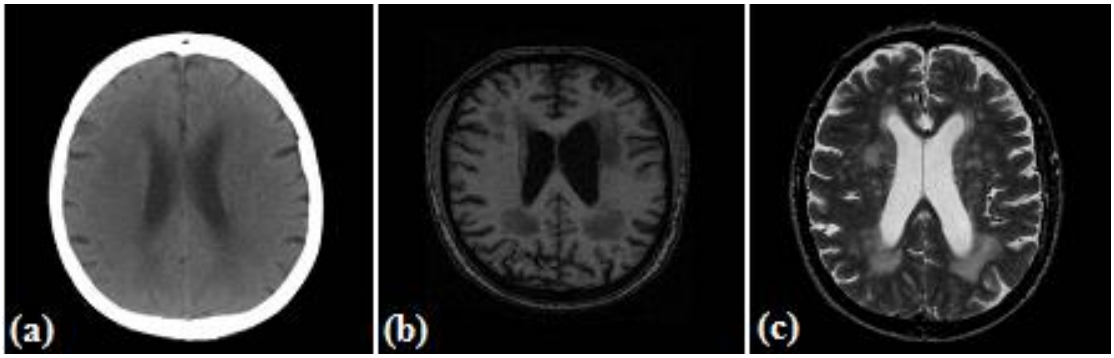
The destruction or dysfunction of myelin can have devastating consequences to the nervous system. Dysmyelinating diseases are associated with defects in white matter production and/or function, while demyelinating diseases are associated with the loss of previously acquired normal myelin (2). Known dysmyelinating disorders include leukodystrophies

such as Canavan's disease and Alexander's disease; while demyelinating diseases include Multiple Sclerosis and vitamin B_{12} deficiency (2). There are many other disorders associated with alterations in white matter tracts or myelin including Alzheimer's disease (AD) and autism (1). Psychiatric disorders such as depression and schizophrenia have also been linked with white matter disorders (1).

Changes in white matter have been studied even before Hachinski coined the term 'leuko-araiosis' in 1987. The term was derived from 'leuko,' meaning 'white,' and referenced the white matter in the brain, while 'araiosis' referred to the 'refraction' of the white matter. These Greek-inspired routes combine together to form leukoaraiosis which was initially studied on computed tomography (CT) scans and later on magnetic resonance (MR) images. Leukoaraiosis emerges on CT scans and T1-weighted MR images as areas of hypodensity and areas of hyperintensity on T2-weighted MR images **(figure 2)**. These areas appear this way due to the degeneration of myelin and the re-organization of water molecules which, in turn, changes the relaxation rate of molecules and appears brighter on T2-weighted images (3, 4). These signals are associated with cerebral white matter degeneration and potential brain volume loss (2, 5-7). This paper will focus on leukoaraiosis appearing on T2-weighted images, also referred to as white matter hyperintensities (WMH).

Figure 2 LEUKOARAIOSIS DISPLAYED ON IMAGES

*Leukoaraiosis on a **(a)** computed tomography **(b)** T1-weighted magnetic resonance (MR) image **(c)** T2-weighted MR image.*



II. VIEWING AND RATING WMH ON T2-WEIGHTED MR IMAGES

In comparison to alternative imaging modalities, T2-weighted MR imaging is the preferred method for viewing and evaluating WMH due to its noninvasive nature and sensitivity to abnormalities in white matter (2). Rating scales designed to measure WMH from MR imaging are abundant; however they vary significantly in sensitivity, specificity, and intent (7). At present, a standardized rating scale designed to quantify and/or qualify the severity of white matter damage has not yet been established, thus making study comparisons difficult. Nonetheless, there are some visual rating scales that are more commonly seen in publications than others. These include the Fazekas Scale (8), the Scheltens Semiquantitative Rating Scale (9), the Age Related White Matter Changes (ARWMC) scale (10) and the Rotterdam Scan Study Scale (11). Van Straaten and colleagues evaluated the differences in inter-rater reliability between Fazekas, Scheltens, and ARWMC scales and found all to be reliable – with no one rating scale more significantly reliable than another (12). Additional studies concur that these rating scales are valid and reliable (8, 12-15).

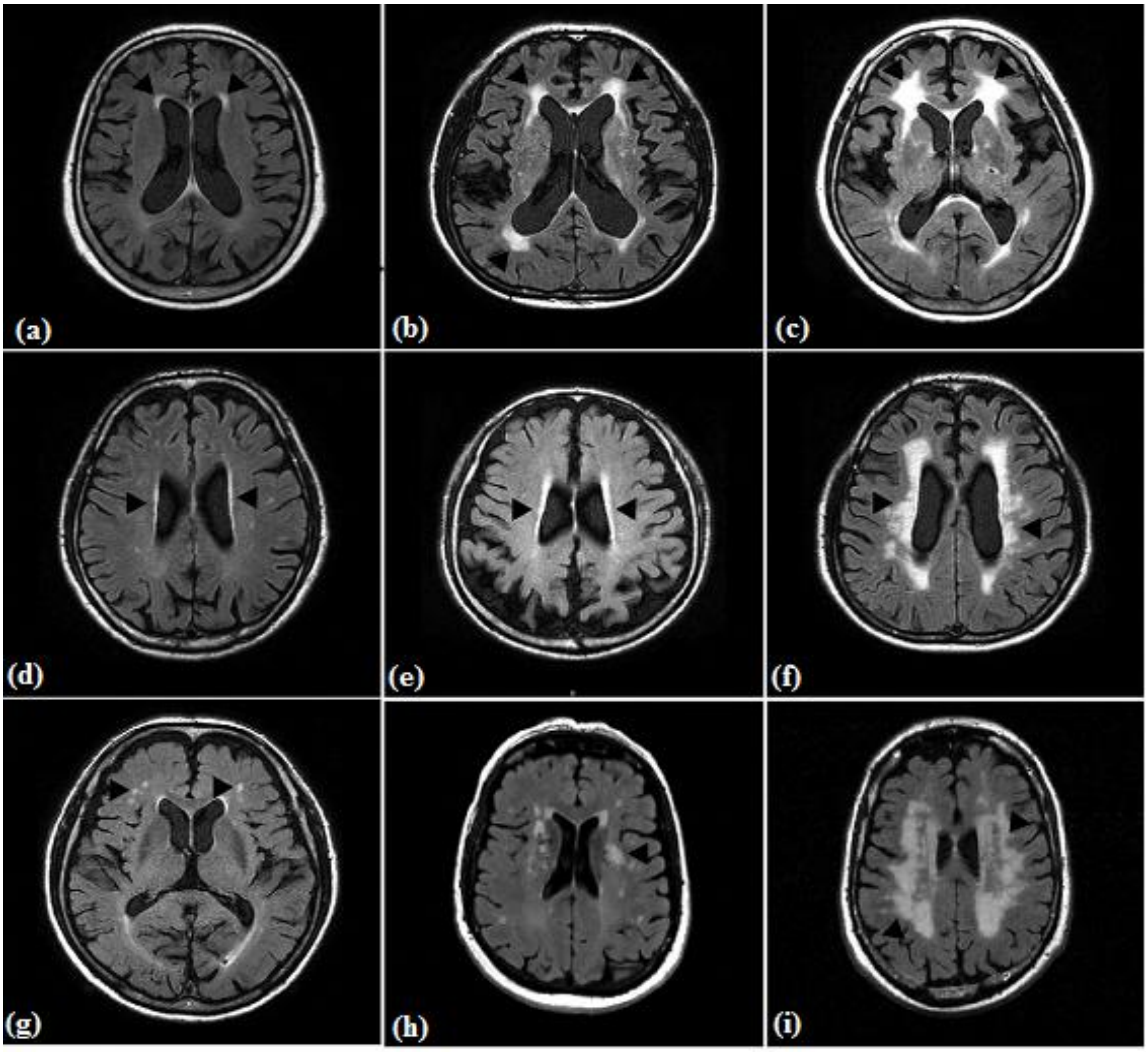
Differentiating between Periventricular and Deep White Matter Hyperintensities

Research studies often classify WMH in terms of severity and anatomical location, with a common distinction between periventricular hyperintensities (PVH) and deep white matter hyperintensities (DWMH) (9, 10, 16). Kim and colleagues credit Fazekas (16) as the first to establish a rating system that distinguished between these two regions (6). Since then, most rating scales have defined PVH by their contiguous proximity to the lateral ventricles while the classification of DMWH has been qualified by the patchy areas of hyperintensities in the subcortical white matter (6, 8). Fazekas and colleagues attest that most scales classify PVH as extending into the subcortical white matter when PVH are greater than 1 cm (8).

Common terms used to describe damage in the periventricular region include ‘caps,’ ‘halo,’ ‘bands,’ ‘pencil-thin lining,’ or ‘irregular lining.’ Periventricular ‘caps’ are sometimes found on the frontal or occipital horns of the lateral ventricles, while a ‘halo,’ ‘bands,’ or ‘lining,’ lie parallel and adjacent to the ventricle surface. In terms of severity, PVH range from small caps and/or bands to irregular periventricular bands extending into the deep white matter. Common terms used to describe DWMH, with respect to increasing severity, include ‘punctuate foci,’ ‘beginning confluence,’ or ‘confluence.’ Further possible classifications of DWMH may also be made based on the size, number, and shape of the hyperintensities (6). In more severe cases, classifying the two regions becomes more complex when WMH from the two regions merge together (6, 8). A visual representation of these classifications is elegantly demonstrated by Kim and colleagues (6) in **figure 3.**

Figure 3 CLASSIFICATIONS OF WHITE MATTER HYPERTENSITIES

Periventricular hyperintensity (PVH) classification: ***(a)*** *small caps (****b****) large caps (****c****) extending caps* ***(d)*** *thin lining of the lateral ventricles* ***(e)*** *smooth halo* ***(f)*** *irregular PVH extending into the deep white matter. Deep white matter hyperintensity (DWMH) classification:* ***(g)*** *punctuate DWMH* ***(h)*** *DWMH beginning confluence* ***(i)*** *confluent DWMH. Adapted from (6).*



Some studies have noted that the clinical manifestations of WMH are more strongly associated with one region than another. For example, the risk of dementia and cognitive decline has been more strongly associated with PVH than DWMH (11, 17-19) and depression has been more strongly associated with DWMH than PVH (20-22). Some studies show that specific cognitive domains may be diversely associated with regional WMH. For example, processing speed may be more strongly associated with PVH (23) and some memory tests were more strongly associated with DWMH (24). Vascular risk factors and aging have also been more strongly associated with PVH than DWMH (25).

Despite some convincing evidence, there is some debate about whether clinical manifestations of white matter disease are truly correlated with the anatomical location of WMH (25). Some WMH rating scales implement arbitrary and anatomically irrelevant scoring criteria (6, 25) which complicates the interpretation of results. Moreover, PVH and DWMH are highly correlated with each other and the overall score of WMH (25, 26). This suggests that the stratification of WMH by anatomical region may be unnecessary (27). Therefore, methods implementing an anatomically amalgamated score of WMH may be sufficient for attaining a general representation of white matter damage and for establishing relationships between variables and WMH (27). This debate is perpetuated by varying definitions of PVH and DWMH. Fazekas and colleagues argue that a justifiable comparison of study results is practically impossible until rating systems are standardized (8). Since many researchers focus on simply generating an overall representation of WMH, further investigations that distinguish between regions are needed before researchers can overlook the importance of anatomically specific rating scales.

Selecting a Rating Scale

Although there are many rating scales designed to assess WMH, this study required one that was reliable, had clear rating criteria, and specified between regional WMH. The

author also sought a commonly used rating system to allow for later study comparison. Based on extensive literature reviews, the Scheltens semiquantitative rating scale and Fazekas rating scale appeared to be the most appropriate for this study. Both scales have demonstrated good inter-rater reliability with no significant difference between them (14, 28). While both scales distinguish between periventricular and subcortical WMH, the Scheltens semiquantitative rating scale also accounts for WMH in the basal ganglia and 13 subcortical regions over all (9, 16). Although this study does not use all 13 regions in analyses, the option was left available for future investigations. Due to limited time and resources automated methods were not considered.

Magnetic Resonance Imaging Limitations

MR imaging may be the preferred method for viewing WMH, but there are still some prevalent issues associated with quantifying WMH with rating scales. MR imaging allows raters to detect the most minute white matter lesions, however some believe that MR imaging may actually display lesions that are of little pathological importance (29, 30), while another group argues that WMH are underestimated in relation to corresponding histopathological examinations (31). Moreover, MR images limit our ability to generate an accurate representation of the general population. For example, patients with pacemakers or suffering from claustrophobia cannot be included in studies that implement MR images in the study protocol. Fortunately, imaging is still an evolving field, and as technology progresses, our abilities to measure the progression of WMH will certainly improve.

Automated methods for quantifying WMH provide some advantages to visual rating scales (32). In comparison to automated methods, visual rating scales are not as reliable for determining progressive WMH (28). Nonetheless, visual rating scales still maintain some advantages over automated methods. Automated methods require two high quality images for comparison, which may be impossible to attain retrospectively. Alternatively, visual

rating scales may be adapted to account for discrepancies between baseline and follow-up image quality. Automated rating scales often quantify WMH by volume, while visual rating scales can account for irregularities in size and shape (8). Despite the differences between the two rating methods, automated methods are still limited to studies with the financial resources and without time constraints (8).

III. WHITE MATTER AND COGNITIVE IMPAIRMENT

Cognitive impairment often results as a clinical manifestation of white matter disorders such as genetic disorders, infectious disorders, metabolic disorders, vascular disease, and trauma (2). Convincing evidence has also demonstrated that individuals with severe WMH are more cognitively impaired than those individuals with little or no WMH (17, 27, 33-35). These studies are not limited to the elderly, nor are they limited to demented study populations, thereby suggesting that WMH may precede dementia (27). Healthy elders exhibit decreased performance on cognitive tests compared with younger healthy controls, suggesting that age also plays a significant role in cognitive impairment (36).

Studies have also identified that WMH are associated with specific cognitive domains (24, 27, 34-38). White matter is believed to play a vital role in the speed in which messages can be transmitted, so it seems appropriate that tests associated with processing speed are strongly correlated to WMH (34). Some studies found that severe WMH were significantly associated with memory impairment (24, 39) while Kramer and colleagues found no significant relationship between WMH and memory (36). However, they did find a significant correlation between WMH and executive function (36). Others found no significant relationship between WMH and outcomes measures of intelligence (35) or verbal memory (27).

Some studies have investigated the relationship between specific anatomical locations of WMH and various cognitive domains (34). In 2000, de Groot and colleagues were the first

group to analyze the difference between PVH and DWMH. They found that PVH were statistically associated with cognitive decline, but DWMH were not (34). These studies are still limited, as many researchers use rating scales that generate a general representation of WMH instead of anatomically specific rating scales. The association between regional presence of WMH and cognitive domain is still controversial (25, 27). Further research is required to elucidate whether or not the anatomical location of WMH has a significant effect on specific cognitive domains.

IV.WHITE MATTER AND DEPRESSION

Depression is the most common neuropsychiatric symptom of white matter disorders (2) and is associated with increased WMH (1, 21, 40-44). While not all participants with WMH show signs of depression or vice versa, depressed subjects demonstrated more severe WMH than healthy, age-matched controls (45). Depression in the elderly is often associated with impaired cognitive function, particularly processing speed, attention, memory, and executive function – cognitive domains that are also strongly associated with increased WMH (21, 46, 47). On the other hand, some researchers have found no significant relationship between WMH severity and depression (48).

In some studies, depression is associated with either early-onset depression or late-onset depression, however one group found that there is no statistical difference between depression onset and WMH (45). Clearly, more information is required to understand the complex relationship between WMH and depression.

V. RISK FACTORS AND WHITE MATTER HYPERINTENSITIES

Aging

The reported prevalence of white matter hyperintensities (WMH) in the elderly varies from 5% to 90% (11) and appear in both the diseased and relatively healthy geriatric population (2, 11, 49, 50). Aging has been associated with the degeneration of white matter, loss of myelinated fibers, and malformation of myelin sheaths (7). A large number of studies have cited aging as the most prevalent and established risk factor associated with WMH with little, if any, discordance (11, 29, 33, 51, 52). Although, aging is inextricably linked with increased WMH, researchers have yet to ascertain why.

Sex

When it comes to general age-related changes in the brain, men have been identified as being at greater risk than women (35). This is believed to be due to male susceptibility to factors such as hypertension, stroke, elevated blood concentrations of total homocysteine (tHcy) and heart disease (53). However, many studies show that when it comes to WMH prevalence, women are at greater risk (17, 33, 53-55). In a recent cross-sectional study Sachdev and colleagues compared men and women with congruent medical histories, including a corresponding incidence of hypertension, heart disease, elevated concentrations of tHcy, smoking, and folate deficiency. They found that women have more WMH than men (53). Various studies support these findings (11, 33, 54), while some researchers challenge them (56). Further research is required to explore this relationship and determine why women might be more vulnerable to WMH than men. Longstreth and colleagues demonstrated one possible explanation. They reported that women taking estrogen had more severe WMH than those women who were not taking estrogen (57).

Hypertension

Stage I hypertension is defined by a systolic blood pressure (SBP) from 140 to 159 mmHg or a diastolic blood pressure (DBP) from 90 to 99 mmHg, and stage II hypertension is

defined by a SBP ≥160 mmHg or a DBP ≥100 mmHg (**table 1)** (58). SBP increases with age, while DBP peaks around the age of 50 years (**figure 4**). Therefore, it is important to note that hypertension and age may interact significantly and must be accounted for in statistical analysis (58).

Table 1 CLASSIFICATION OF HYPERTENSION

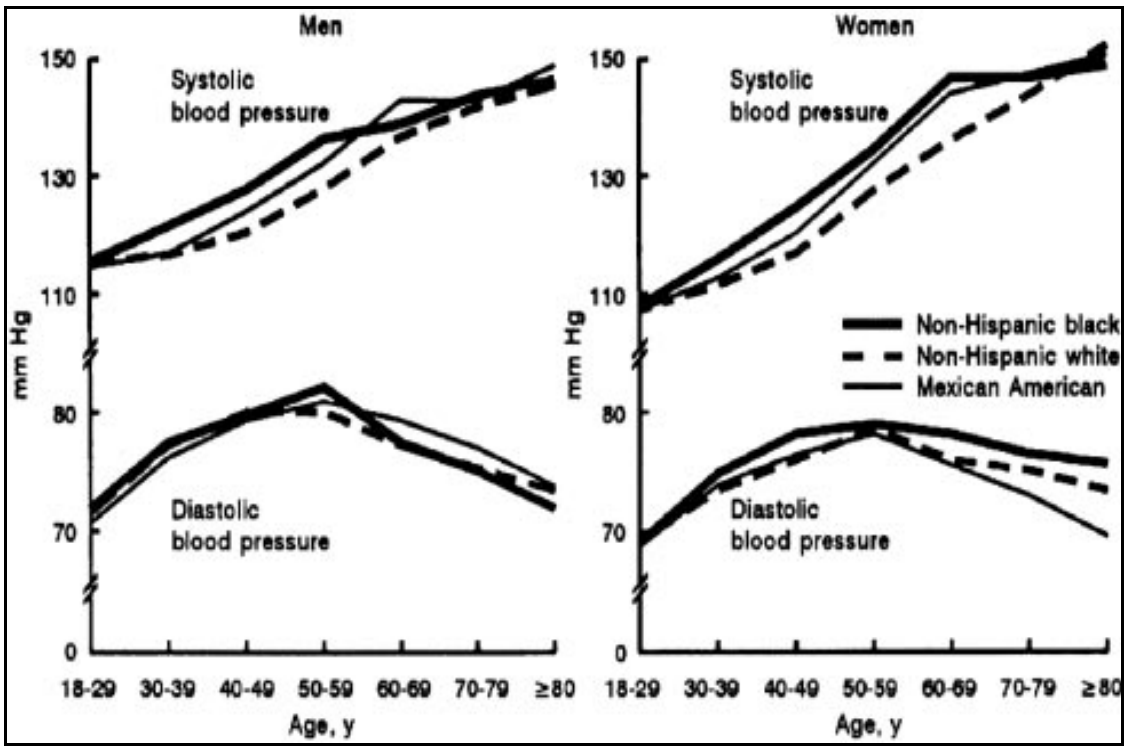
Stages of hypertension defined by systolic and diastolic blood pressure measurements. Table adapted from (58).

CLASSIFICATION	SBP (mmHg)	DBP (mmHg)
NORMAL	< 120	< 80
PREHYPERTENSION	120 -139 OR	80-89
STAGE I HYPERTENSION	140-159 OR	90-99
STAGE II HYPERTENSION	≥ 160 OR	≥ 100

Abbreviations – SBP (Systolic Blood Pressure); DBP (Diastolic Blood Pressure)

Figure 4 BLOOD PRESSURE IN RELATION TO AGE, SEX, AND RACE

Systolic and diastolic blood pressure in relation to age, sex, and race. Figure re-printed from (58).



Convincing evidence has demonstrated that hypertension is statistically associated with severe WMH (33, 59-61). Studies evaluating the association between blood pressure and

WMH are abundant, however prospective studies are relatively few and studies considering the anatomical location of WMH on MR images are scarce (62-64). A recently published, 32-year longitudinal study by Guo and colleagues evaluated the association of SBP, DBP, pulse pressure (PP), and mean arterial pressure with white matter lesions on CT. They found that the presence of white matter lesions was associated with SBP and mean arterial pressure, but not DBP or PP (62). In an MR imaging study of 1625 people, Van Dijk and colleagues found that both DBP and SBP were associated with severe WMH (63). More specifically, they found that both increases and decreases in DBP were associated with severe PVH, while an increase in SBP was associated with severe subcortical WMH and PVH (63).

Dufouil and colleagues found that hypertension, defined as SBP $\geq$ 160 mmHg, DBP $\geq$ 95 mmHg, or use of antihypertensive medication, was a major risk factor for WMH at both baseline and at a 4-year follow-up (65). Additional studies support that participants with hypertension at baseline are at greater risk for developing severe WML than normotensive and/or hypertensive controlled participants (61, 63, 65).

Notably, hypertension has also been associated with dementia (61, 66). A study following participants for 15-years indicated that participants that developed dementia had higher SBP and DBP than those participants that did not develop dementia (61). Another study demonstrated that regulating hypertension over time reduced the incidence of dementia (67). The relationship between dementia, hypertension, and WMH is complex, but intriguing. Monitoring and treating blood pressure could play a vital role in reducing severe WMH and subsequent dementia; especially considering the high prevalence of hypertension in the geriatric population (63).

Diabetes

Both type I and type II diabetes mellitus are risk factors that have been associated with changes in the brain, particularly severe WMH (16, 68-70); however other investigators found no significant association between the two (33, 59, 71, 72). These discrepancies are most likely explained by study design, which use varying WMH rating scales, definitions of diabetes (self-reported, type I, and/or type II), and study samples with controlling medications or concomitant disease. Diabetes also interacts with hypertension and various other cerebrovascular risk factors, making it difficult to isolate diabetes from other comorbid conditions (71).

Elevated Homocysteine

Cognitive impairment and dementia are associated with many risk factors, including elevated blood concentrations of total plasma homocysteine (tHcy) (2, 73-77). Although many researchers concur with these findings, others have found no significant relationship between tHcy level and impaired cognition, particularly after controlling for additional factors (78-81). It should be noted that various cut-off points were used to identify elevated tHcy as well as different methods for attaining the blood samples. Measurements of cognitive impairment vary, ranging from measures of global cognitive functioning to specific cognitive domains or individual tests. In one longitudinal study, investigators found that participants with tHcy levels $\geq$ 15 μmol/L were 2.8 times more likely to experience cognitive decline than those participants with tHcy levels < 10 μmol/L. These findings remained significant when controlling for WMH severity (74).

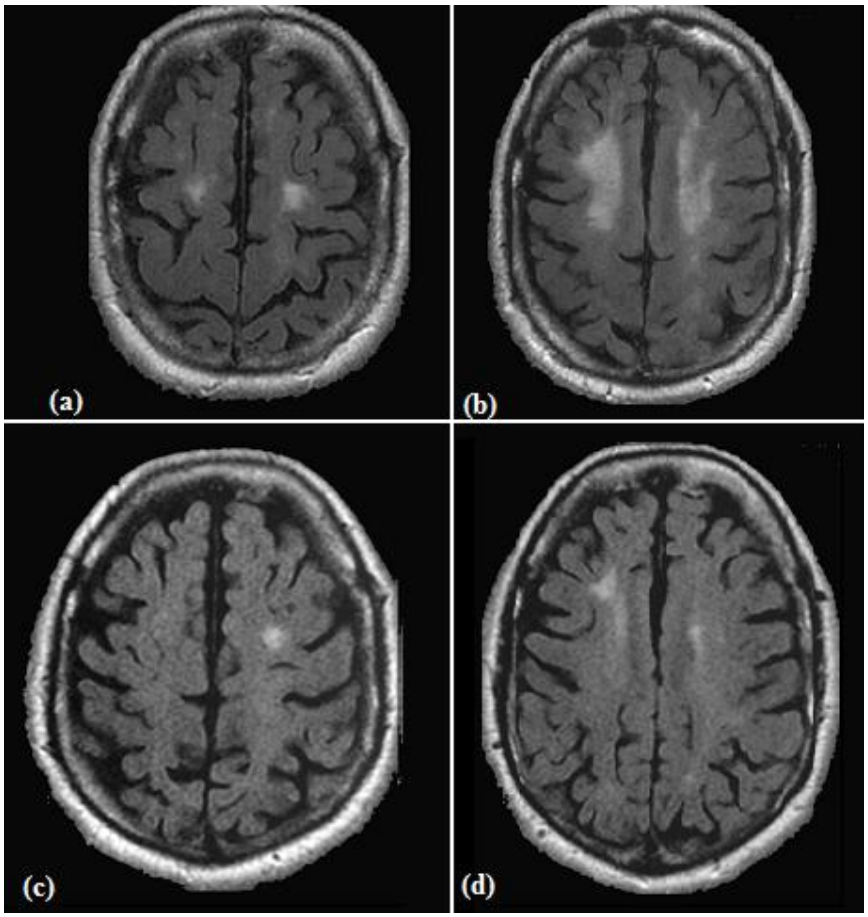
Elevated blood concentrations of tHcy have been identified as a risk factor for cognitive impairment and severe WMH in various studies (79, 80, 82-85). These results indicate that elevated concentrations of tHcy may be one factor connected to the underlying processes of WMH, although some investigators have reported no significant relationship between tHcy and WMH (86).

Interestingly, tHcy has been implicated in relation to AD (87). Clarke and colleagues found a significant relationship between participants with *post mortem* confirmed AD and elevated (≥ 15μmol/L) blood concentrations of tHcy (85). The rate of cognitive impairment in AD patients has also been correlated to levels of tHcy; the higher the blood concentrations of tHcy, the faster the cognitive decline (88).

Since elevated blood concentrations of tHcy are often the result of vitamin B_{12} and folate deficiencies (89) these findings would be best supported with intervention studies in which participants were treated with B vitamin supplementation (77, 88). In one case study, a 42 year old male patient presented with extremely elevated tHcy (230 μmol/L) and cognitive impairment despite normal levels of both plasma vitamin B_{12} and folate. The elevated tHcy was believed to be caused by a vitamin B_{12} metabolic defect. In an attempt to lower tHcy levels the patient was administered a combination of vitamin B_{12}, B_6, and folate supplements over 18 months. **Figure 5** illustrates images taken 4 months prior to tHcy-lowering therapy (tHcy = 230 μmol/L) and 18 months into the treatment (tHcy = 90 μmol/L); a marked reduction in WMH was noted in conjunction with improved cognitive function (90).

Figure 5 REDUCED WHITE MATTER HYPERINTENSITIES WITH HOMOCYSTEINE LOWERING THERAPY

*FLAIR images of a patient with late onset vitamin B_{12} deficiency **(a/b)** 4 months prior to tHcy lowering therapy **(c/d)** and after 18 months of tHcy lowering therapy. Adapted from (90).*



Vitamin B_{12} (Cobalamin) Deficiency

Vitamin B_{12} deficiency is a common health problem in the elderly population often due to insufficient consumption of vitamin B_{12} foodstuffs or poor absorption (2, 91, 92). Vitamin B_{12} can be acquired through the ingestion of most dairy, seafood, and meat products or through dietary supplementation. Since vitamin B_{12} is important for much of the body's cellular processes, a deficiency can sometimes result in severe effects (91). The most recognized cause is pernicious anemia which could result in memory impairment, but may be reversible through vitamin B_{12} supplementation (2). Vitamin B_{12} is also essential for the formation of myelin, which inevitably compromises the nervous system if left untreated

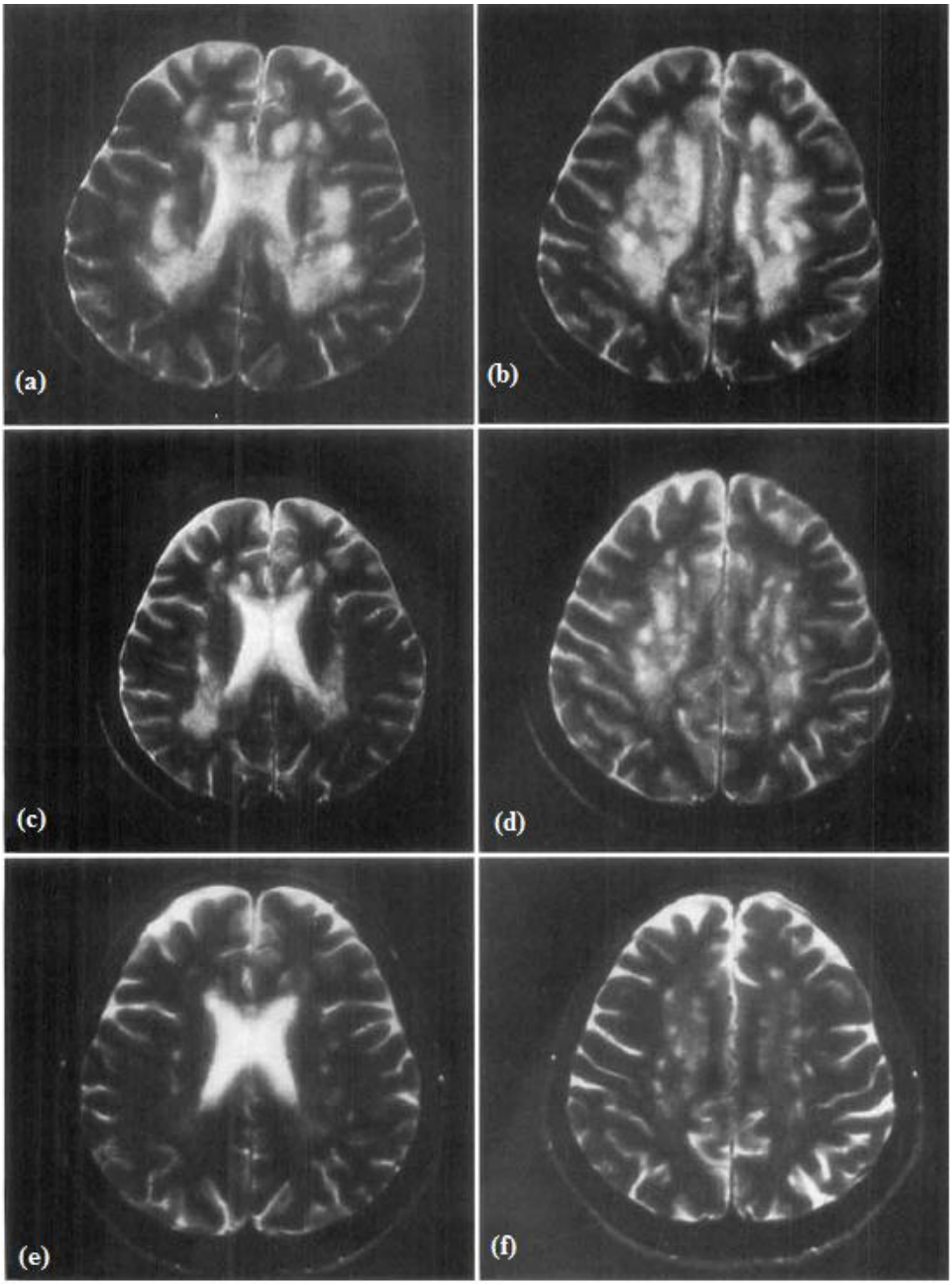
(91). Consequences of vitamin B_{12} deficiency include cognitive impairment (50, 93-96), increased WMH (97-100), brain volume loss (101), AD (87, 88), and depression (50, 102).

Studies show that plasma vitamin B_{12} is not the most accurate representation of the available vitamin B_{12} in the body, but is still used by a substantial number of studies to ascertain whether or not participants are vitamin B_{12} deficient (103). In addition to divergent vitamin B_{12} assays, indefinite cut-off points also lead to controversial findings. Alternatively, researchers suggest that vitamin B_{12} status is best represented by blood concentration levels of tHcy and methylmalonic acid (MMA) (92). Newer methods include measuring holotranscobalamin (holoTC) or total transcobalamin (TC) saturation which correspond to the total vitamin B_{12} available for cellular use; however some researchers suggest combining methods for improved accuracy (104).

A few studies have reported positive neurological changes in response to vitamin B_{12} treatment, including improved cognition and/or visibly decreased WMH (99, 100, 105, 106). One remarkable case study, reported by Stojsavljevic and colleagues, demonstrated MR images with a significant reduction in WMH over a 44-month period of vitamin B_{12} treatment **(figure 6)**. The patient also displayed improved cognitive function concurrent with the reduction of WMH (99).

Figure 6 REDUCED WHITE MATTER HYPERINTENSITIES WITH VITAMIN B12 REPLACEMENT THERAPY

*A patient undergoing vitamin B_{12} replacement therapy **(a/b)** brain before treatment **(c/d)** reduced WMH 2 months into treatment **(e/f)** significantly reduced WMH 22 months into treatment. Image re-printed from (99).*



Further investigations are required to truly understand the relationship between vitamin B_{12} deficiency, WMH, and cognitive dysfunction. Intervention studies involving MR imaging and vitamin B_{12} replacement therapy on significantly large samples may be particularly useful.

Folate Deficiency

Folate is obtained through the diet through dark leafy vegetables, legumes, and some fruits or through fortified products, such as breads and cereals (107). Like vitamin $B_{12,}$ folate is vital to the nervous system at all stages of life and may play a vital role in the aging process (108). Folate deficiency commonly afflicts alcoholics, pregnant women, individuals taking anticonvulsant drugs, individuals with a congenital inability to metabolize folate, and the elderly (2). Research demonstrates that patients with folate deficiency are at greater risk for developing brain atrophy (109), AD (87, 108), dementia (50), cognitive impairment (108, 110), and depression (108, 111); however study findings are reportedly inconsistent (77, 103). To our knowledge, few studies have investigated the direct relationship between folate and WMH (110). Intervention methods involving folate replacement have not been as successful as vitamin B_{12} therapy (2).

Genetic Risk

Thus far, I have discussed environmental factors associated with WMH, however genetic risk factors or the interaction between the two must also be considered. Twin studies have demonstrated that the presence of severe WMH is more strongly associated with monozygotic twins (68% concordance) than dizygotic twins (38% concordance) (112) leading scientists to explore the genetic influence on WMH. This section considers the relevance of the *Apolipoprotein E (APOE)* alleles (ε2, ε3, ε4) and polymorphisms associated with vitamin $B_{12,}$ folate, and tHcy status *methylenetetrahydrofolate reductase (MTHFR) 677C→T; methionine synthase reductase (MTRR) 66A→G;* and *transcobalamin (TCN) 776C→G.*

The *APOE* allele ε4 in relation to WMH has been studied in great depth. It has been statistically associated with risk factors associated with WMH (113, 114), its clinical manifestations (115, 116), and WMH themselves (113). On the other hand, some researchers have come up with null findings (117), suggesting that more research must be

completed to better understand this relationship. The *MTHFR 677C→T* polymorphism is associated with elevated blood concentrations of tHcy and low plasma folate (118); so for the reasons discussed above it is not surprising that this genetic risk factor has been associated with WMH (119, 120). The *MTHFR 677C→T* polymorphism has also been associated with an increased risk of dementia (77), cardiovascular disease, and depression (121); however other studies contradict these findings (122). *MTRR* is an important enzyme involved in tHcy metabolism through the maintenance of sufficient vitamin B_{12} levels (123). The polymorphism *MTRR 66A→G* is therefore associated with elevated blood concentrations of tHcy and consequently risk factors associated with WMH (123), though there do not seem to be any reports of the polymorphism's association with WMH. The *TCN* polymorphism 776C→G affects transcobalamin, which is responsible for the binding, transport, and availability of vitamin B_{12} to cells. As a result, it has been associated in relation to elevated blood concentration levels of tHcy and MMA (124, 125). Genetic studies that investigate genotypes that influence vitamin B_{12} and folate status in relation to other factors often have a limited number of subjects, that prevents significant conclusions regarding the effect of the genotype (77).

VI.STUDY AIMS

Despite the controversies underlying the risk factors associated with WMH, most scientists will agree; cerebral WMH are not benign. More research must be conducted to better understand the pathological processes underlying cerebral white matter damage. The overarching goal of this study is to identify risk factors associated with the progression of WMH between baseline and follow-up. Those risk factors included in this study include:

(i) Demographic risk factors (age and sex).

(ii) Cerebrovascular risk factors (smoking, diabetes, and hypertension).

(iii) Biochemical markers associated with B vitamin and folate deficiency (tHcy, MMA, plasma vitamin B_{12}, holoTC and TC, serum and red cell folate)

(iv) Genetic risk factors (*APOE ε4*, *MTRR 66A→G*, *MTHFR 677C→T*, and *TCN 766C→G*).

(v) Additionally, this study aims to identify if WMH are associated with cognitive functioning and if so, which cognitive domains are most associated with WMH and which neuropsychological assessments are most sensitive to increased WMH.

(vi) This study will also investigate the association between increased WMH and late-onset depression.

(vii) Finally, the association between visually rated WMH, volumetric brain measurements, and *post mortem* results will be evaluated.

The key original aspect of this study is that changes in WMH over time are assessed in relation to the above factors, as well as simple cross-sectional correlations in a healthy elderly population.

CHAPTER TWO
METHODOLOGY

I. STUDY PARTICIPANTS

The Oxford Project to Investigate Memory and Aging (OPTIMA) was founded in 1988 with the overarching goal of identifying risk factors and developing prevention strategies for AD and other forms of dementia. In 1997 OPTIMA recruited a new cohort, Challenge, with the aim of identifying early biochemical and radiological markers for cognitive impairment as well as sensitive neuropsychological tests for early detection of cognitive impairment. These participants were recruited through talks and radio advertisements intended for the elderly population (aged 60 years or older) who believed their memory and health were good in comparison to their peers (126).

Advertisement respondents were screened using the Cambridge Cognitive Examination (CAMCOG) (127) and Mini Mental State Examination (MMSE) (128). Subjects scoring less than 80 on the CAMCOG; 24 on the MMSE; or with major depressive disorder were excluded from the study. The baseline average CAMCOG and MMSE scores of the 154 enrolled participants were 97.8 (range, 84 to 105) and 28.5 (range, 24 to 30) respectively. Participants with diabetes mellitus, coronary artery disease, hypertension, prior ischemic attack/minor cerebrovascular accident (> 1 year previously), and subjects receiving vitamin B_{12} supplements or injections were not excluded (129).

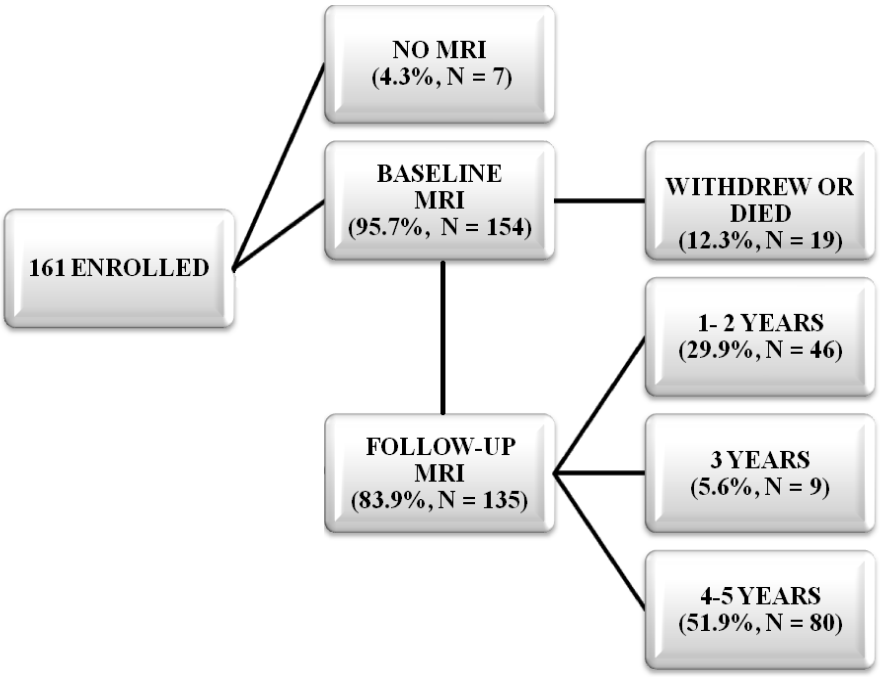
A baseline medical examination was performed for all participants in conjunction with blood tests, a cognitive assessment, and cerebral MRI. Medical history, medication, smoking, alcohol intake, and the use of vitamin supplements were also documented. These

measures were repeated annually from 1997 to 2000 and continued after re-consent from 2002 to 2003.

Of the 161 participants enrolled in the study, 154 participants completed baseline cerebral MRI scans. For the purposes of this study, the 7 participants without baseline MRI scans were excluded. Of the 154 participants with baseline MRI scans, 83.9% (n = 135) of the sample completed at least one follow-up MRI scan. The total time between baseline and final follow-up scans differed due to participant death or study withdrawal. 51.9% of participants had 4 to 5 years between their baseline and follow-up MR image, while 5.6% had 3 years and 29.9% had 1 to 2 years between baseline and follow-up MR images. 12.3% withdrew or died before completing a follow-up scan **(figure 7).**

Figure 7 STUDY POPULATION MAGNETIC RESONANCE IMAGES

The figure shows when participants had baseline and follow-up magnetic resonance images.



To date, 46 Challenge participants have died and 43 participants have donated their brains to OPTIMA for *post-mortem* examination. At present, 24 histopathological reports are complete, 11 have not yet been reported, and 8 are still awaiting *post-mortem* examination.

Ethical approval for these studies was granted by the Central Oxford Research Ethics Committee. Written informed consent was obtained for all participants and testing, including the initial study screening and *post-mortem* examination.

II. MR IMAGING PROTOCOL AND ANALYSES

All cerebral MR images were acquired using the same Siemens 1.5-tesla Magnetom Vision MRI scanner. Study participants were administered a T1-weighted brain scan of the full head (2.00mm contiguous axial slices, in-slice resolution 0.86 x 0.86mm, three repeats). T2-weighted brain scans from 1997 to 2000 and 2002 to 2003 were axial views of the full head (256 x 256 x 23 slices, 5.00mm) and (256 x 256 x 50 slices, 3.00mm) respectively.

Structural image evaluation using normalization of atrophy (SIENA) was used on T1-weighted MR images to calculate the percentage of whole brain volume change (PBVC) over time. First, SIENA uses an automated 'brain extraction tool' to separate the brain from non-brain structures. Then it automatically registers the two images from different time points and measures the change based on 'movement' of the structural edges in the brain. This method has proven to be robust and accurate (0.2% brain volume change error) (130). An adapted version of SIENA, called SIENAX, is used to evaluate cross-sectional brain volume measurements. Instead of registering two brains together, a single image is aligned to a standard brain space. Finally, cross-sectional brain volume estimates are produced (130).

WMH were rated on T2-weighted cerebral MR images using the Scheltens semiquantitative rating scale (9). All T2-weighted scans were viewed using OsiriX Imaging Software (version 3.3.2), a DICOM viewer designed for Macintosh platforms and MacOS X operating systems. WMH were measured contiguously in the axial plane using the ruler tool in OsiriX (131). The Scheltens semiquantitative rating scale is a detailed method for assessing WMH on MRI; advantages include reliability and anatomic

specificity. The presence and severity of the lesions are measured based on the size, number, and anatomical location. The overall score, ranging from 0 to 84, represents increasing cerebral white matter damage and is computed by summing the four subgroups (periventricular, deep white matter, basal ganglia, and infra-tentorial foci hyperintensities) defined by anatomical region (9).

The periventricular subscale (0 to 6) accounts for the presence and severity of high signal bands and capping of the frontal and occipital horns of the lateral ventricles. The DWMH subscale is equally represented by lobe (frontal, temporal, parietal, and occipital) and ranges from 0 to 24. A score for basal ganglia hyperintensities (BGH) (0 to 30) is derived from five regions: the caudate nucleus, putamen, globus pallidus, thalamus, and internal capsule. Similarly, the scale for infra-tentorial foci (0 to 24) is divided by region (cerebellum, mesencephalon, pons, and medulla) using the same criteria to measure the BGH and DWMH. Scoring criteria (9) are shown in **table 2** and **figure 8** illustrates an example of how the WMH were measured using the ruler tool in OsiriX. **Figure 9** shows a healthy, 78 year old, female brain (Scheltens total score = 2) and an abnormal, 87 year old, male brain (Scheltens total score = 48).

The rater assessed the earliest (baseline) and latest (follow-up) available MR images separately to avoid bias. Additional blinded information included cognitive test results, medical exam notes, blood measurements, demographic information, and previous MR images.

Table 2 SCHELTENS SEMIQUANTITATIVE RATING SCALE

The following criteria, based on the Scheltens rating scale, were used to rate cerebral white matter hyperintensities (9).

RATING CRITERIA	SCHELTENS SUBSCALES	MIN – MAX
0 = NO ABNORMALITIES	**PERIVENTRICULAR HYPERINTENSITIES (PVH)**	**0 – 6**
1 = ≤ 5 mm	CAPS OCCIPITAL	0 – 2
	CAPS FRONTAL	0 – 2
2 = > 5 AND < 10 mm	BANDS	0 – 2
0 = NO ABNORMALITIES	**DEEP WHITE MATTER HYPERINTENSITIES (DWMH)**	**0 – 24**
1 = < 3 mm, LESIONS ≤ 5	FRONTAL	0 – 6
	TEMPORAL	0 – 6
2 = < 3 mm, LESIONS > 5	PARIETAL	0 – 6
	OCCIPITAL	0 – 6
3 = 4-10 mm, LESIONS ≤ 5	**BASAL GANGLIA HYPERINTENSITIES (BGH)**	**0 – 30**
4 = 4-10 mm, LESIONS > 5	CAUDATE NUCLEUS	0 – 6
	PUTAMEN	0 – 6
5 = > 11 mm, LESIONS ≥ 1	GLOBUS PALLIDUS	0 – 6
6 = CONFLUENT	THALAMUS	0 – 6
	INTERNAL CAPSULE	0 – 6
	INFRA-TENTORIAL FOCI OF HYPERINTENSITY (ITFH)	**0 – 24**
	CEREBELLUM	0 – 6
	MESENCEPHALON	0 – 6
	PONS	0 – 6
	MEDULLA	0 – 6
	SCHELTENS TOTAL SCORE	**0 – 84**

Figure 8 MEASURING WHITE MATTER HYPERINTENSITIES

The OsiriX ruler tool was used to measure cerebral white matter lesions.

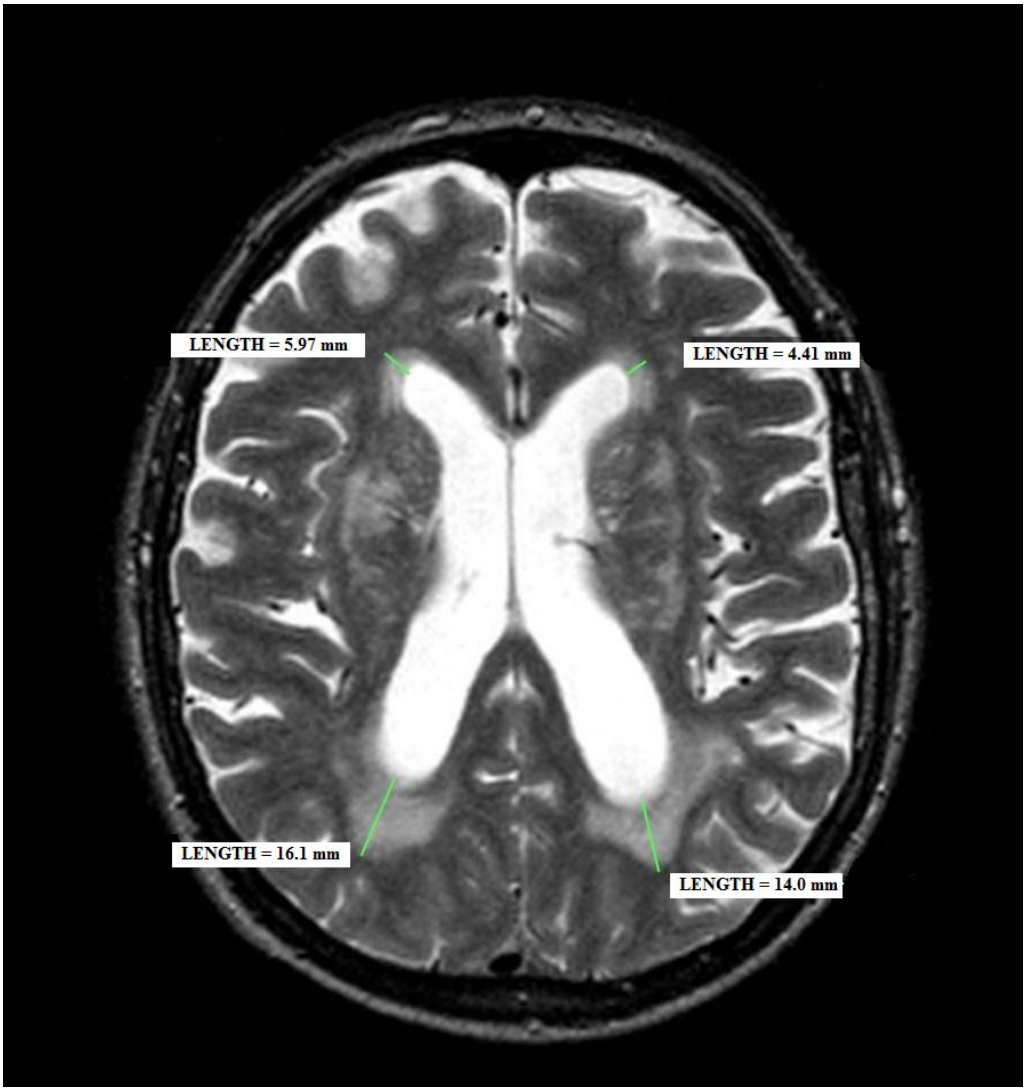
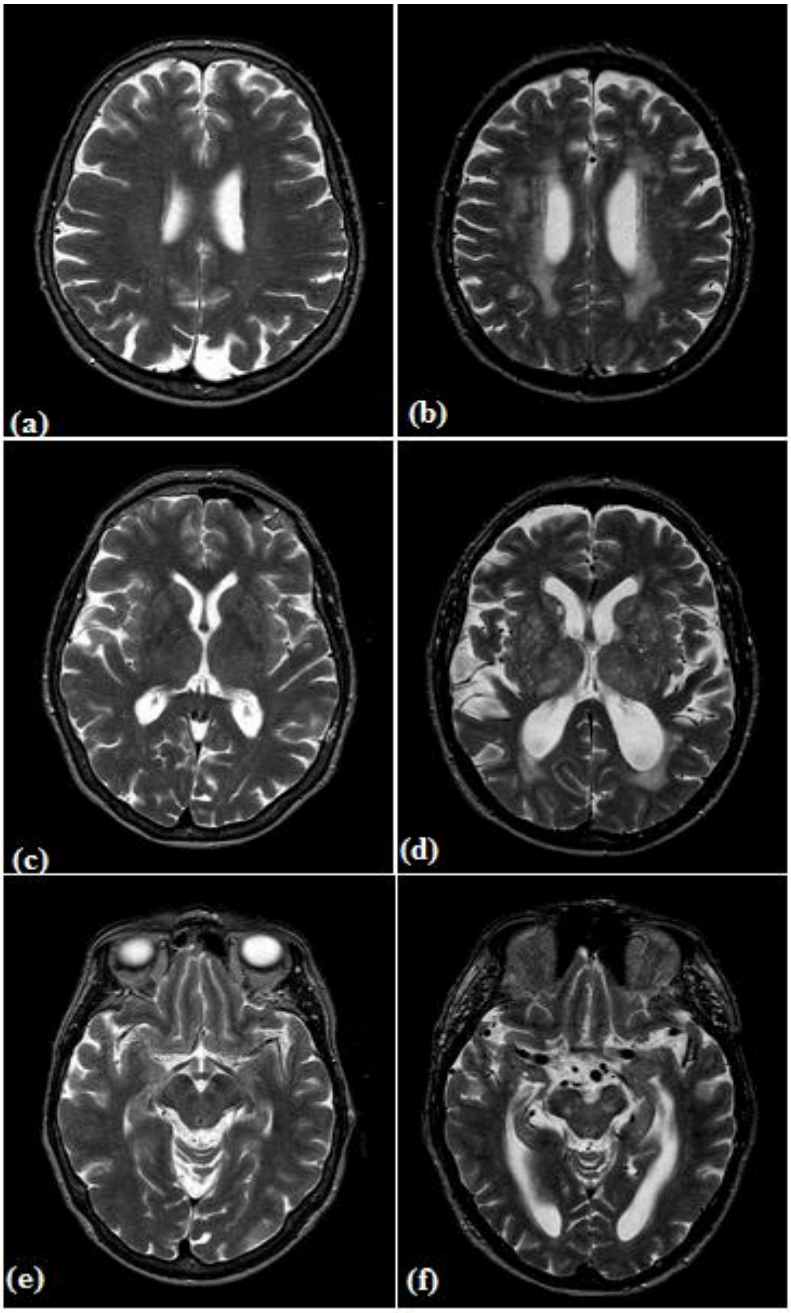


Figure 9 HEALTHY VS. ABNORMAL BRAIN

A series of T2-weighted magnetic resonance images from a healthy and abnormal brain. ***(a/c/e)*** *are examples of a relatively healthy, 78 year old, female brain (Scheltens total score = 2) with little white matter hyperintensities in the periventricular, subcortical, or basal ganglia regions while* ***(b/d/e)*** *are examples of an abnormal 87 year old, male brain with extensive hyperintensities in the periventricular, deep white matter, and basal ganglia regions (Scheltens total score = 48).*



III. BLOOD PRESSURE, BIOCHEMISTRY, AND GENETICS

Blood pressure was taken manually using a sphygmomanometer (mercury manometer) with the left arm positioned at the same level as the heart. Measurements were recorded after 5 minutes of resting while the participant was lying down. Blood pressure measurements were available at baseline and episode 5 (2 to 3 years later). All measurements were completed by a certified research nurse.

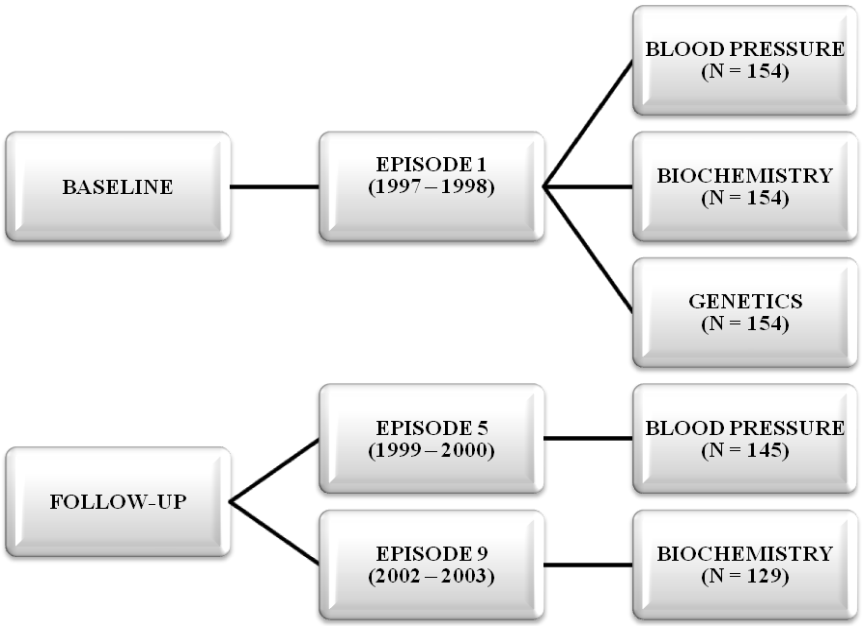
Serum and EDTA plasma were collected from all participants. Samples measured levels of creatinine, MMA, tHcy, plasma vitamin B_{12}, total TC, holoTC, red cell folate, and serum folate. For the purposes of statistical analysis measurements from the first and last available episode will be used. **Figure 10** illustrates when baseline and follow-up measures were obtained for the study population. All samples were analyzed by a biomarker research assistant using the following protocol:

> Briefly, plasma concentrations of vitamin B_{12} were determined with microbiologic assays using a colistin sulfate-resistant strain of Lactobacillus leichmanii. The vitamin B_{12} assays were adapted to a microtiter plate format and performed by a robotic workstation (Perkin Elmer MultiProbe 11). Plasma holoTC and TC concentrations were measured using magnetic beads (microspheres) with immobilized monoclonal antibody specific for human TC to isolate TC, followed by a conventional microbiologic assay for vitamin B_{12} as above. Plama TC was measured after saturating TC with added vitamin B_{12}. The coefficient of variation for total plasma vitamin B_{12} was 5%. The coefficient variation for holoTC and TC was 5% to 8%. Plasma MMA was analyzed by a modified gas chromatography-mass spectrometry method base on ethylchloroformate derivatizations (132). The coefficient variation for MMA was <5%. Plasma tHcy was measured by fluorescence polarization immunoassay analyzer (coefficient variation approximately 3%). Serum folate was measured by the Abott Architect binding assay (coefficient variation < 10%) (101).

Genotypes were also determined for: *APOE* ε4, *MTHFR* 677 C→T, *MTRR* 66 A→G, and *TCN* 776 C→G.

Figure 10 STUDY POPULATION BLOOD MARKERS

Episode dates and the number of samples obtained from the study population at baseline and follow-up.

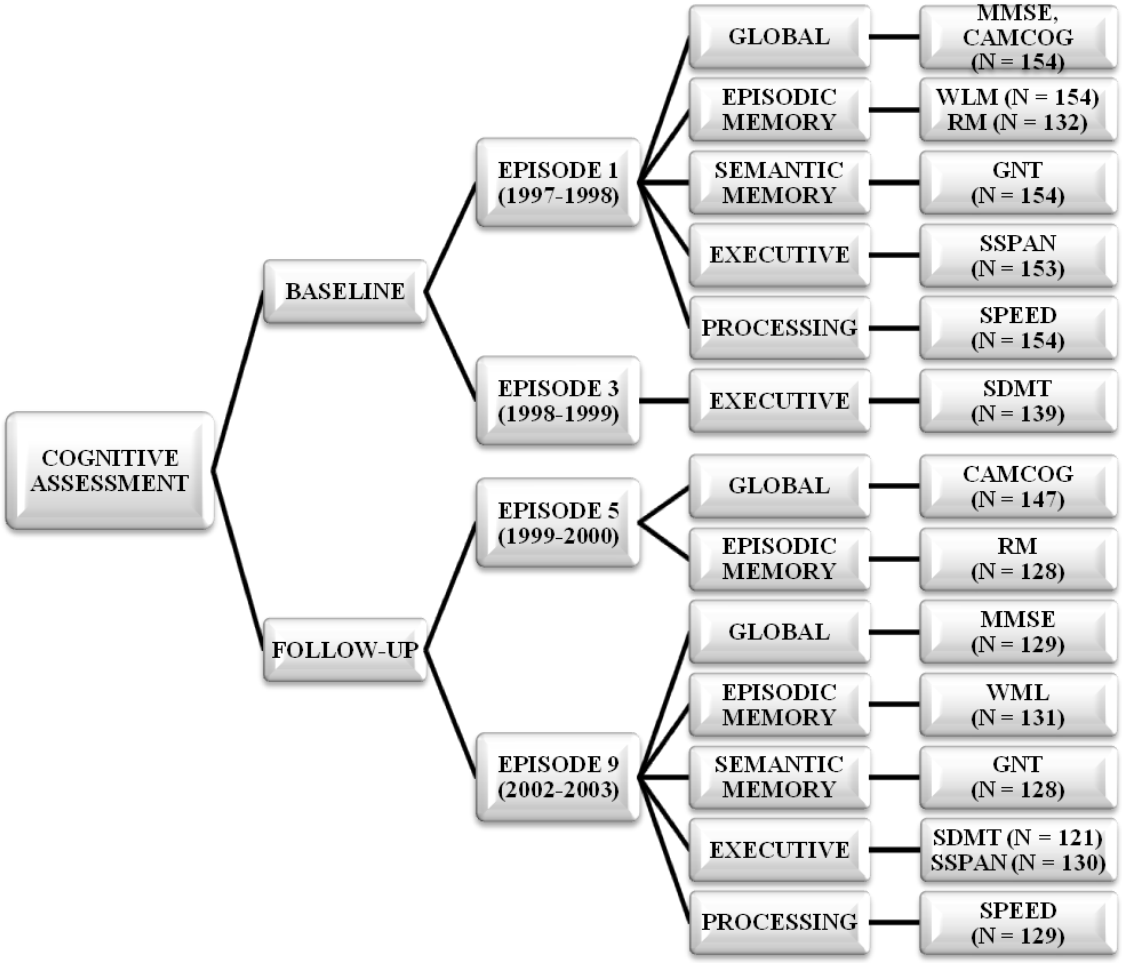


IV.COGNITIVE ASSESSMENT

Participants were administered a comprehensive cognitive assessment designed to test global cognitive function, memory (episodic and semantic), executive function, and processing speed (126). The entire test battery was completed in less than one hour. The cognitive assessments have varying baseline and follow-up dates due to the addition and retraction of tests throughout the duration of the study. **Figure 11** illustrates the baseline and follow-up dates of relevant cognitive assessments used later in analyses.

Figure 11 STUDY POPULATION COGNITIVE ASSESSMENTS

The following illustrates when baseline and follow-up cognitive assessments were obtained from the study population and the number of data points collected for each cognitive domain.



Abbreviations – MMSE (Mini Mental State Examination); CAMCOG (Cambridge Cognitive Examination); WLM (Word List Memory); RM (Recognition Memory); GNT (Graded Naming Test); SSPAN (Spatial Span); SDMT (Symbol Digit Modalities Test)

Global Cognitive Function

The Mini Mental State Examination (MMSE) is one of the most widely used neuropsychological tests designed to assess general cognitive function. The first section of the test evaluates the participant's orientation to the time and place, memory, and attention. Next the participant is asked to follow verbal and written commands, write a sentence, copy a complex design, and draw a clock. The maximum score is 30 points, with a score less than 24 points indicating some cognitive impairment (128).

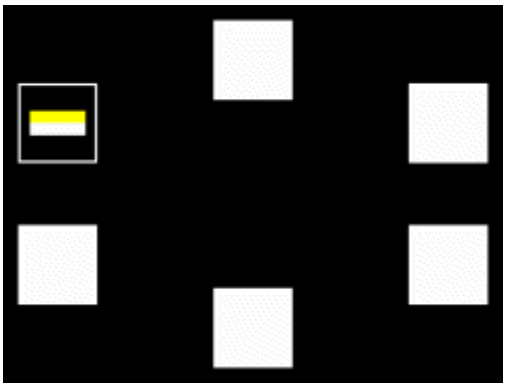
The Cambridge Cognitive Examination (CAMCOG) is another common test used to assess global cognitive function. It has 11 subscales including orientation, language (comprehension and expression), memory (remote, recent, and learning), attention, praxis, calculation, abstract thinking, and perception. The maximum score (107 points) comprises a sum of the subscales; a score of 80 points or less indicates some form of cognitive impairment (127).

Episodic Memory

The Paired Associate Learning (PAL)Test is a computerized test from the Cambridge Automated Testing Battery *(CANTAB) (133)* designed to measure visual-spatial memory. To start, the participant is asked to attend to a screen on which six boxes are displayed. The boxes then 'open' one by one revealing patterns of various shapes and colors, as displayed in **figure 12.** At the end of the sequence, the participant is asked to recall which box contained the cued pattern. The sequence is repeated 6 times or until the participant can identify the correct pattern location. In total, 3 sets of patterns are presented in ascending degrees of difficulty (with displays of 2, 4, and 6 patterns). Scores include the number of trials to completion and the number of errors extracted from the 6 pattern sequence (134).

Figure 12 THE PAIRED ASSOCIATES LEARNING TEST

An example of an 'open' box displaying a pattern in the Paired Associates Learning Test. Re-printed from (133).



The Pattern Recognition Memory Test (PRM) is also a computerized test from the CANTAB (133) that is usually administered in conjunction with the *Spatial Recognition Memory Test (SRM)* and the PAL. The pattern and spatial recognition memory tests are designed to assess visual memory; the former is a visual recognition memory task while the latter is visual-spatial recognition memory task. The PRM begins by displaying a series of 12 patterns of varying colors and indescribable patterns. Following this display, the participant is presented with two patterns and asked to identify the displayed pattern from a novel pattern **(figure 13)**. In the SRM test boxes appear one by one in sporadic locations on the screen. Participants are told to concentrate on the location of the boxes rather than the order of appearance. In the recognition phase, participants are asked to discriminate between the location of two boxes **(figure 14)**. Outcome measures for both tests are based on the number and percent correct as well as latency speed (135).

Figure 13 THE PATTERN RECOGNITION MEMORY TASK

An example of the Pattern Recognition Memory discrimination task where the participant must identify a familiar pattern from a novel one. Re-printed from (133).

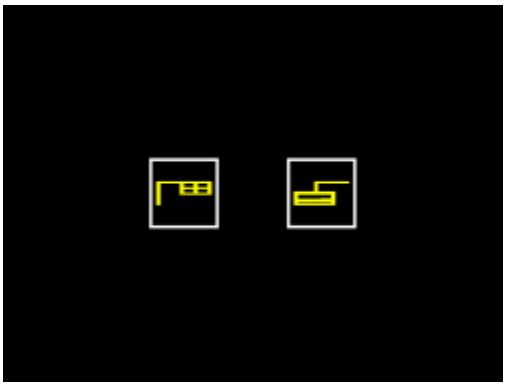
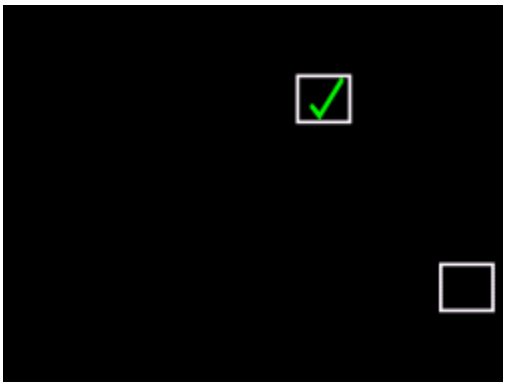


Figure 14 THE SPATIAL RECOGNITION MEMORY TASK

An example from the Spatial Recognition Memory discrimination task. In this instance, the participant correctly identified the location of the familiar box (indicated by the tick mark). Re-printed from (133).

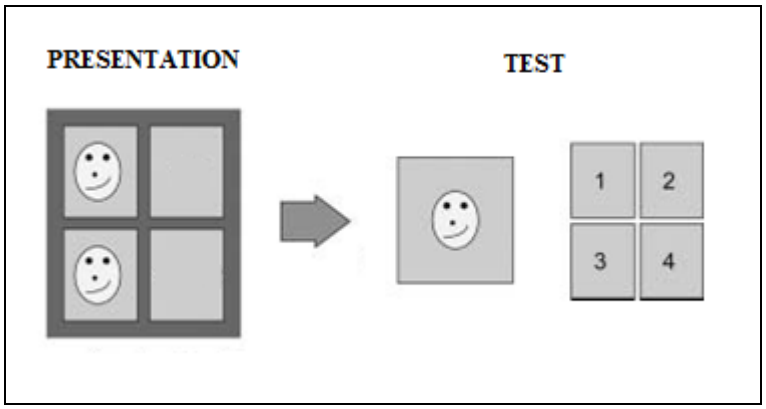


The Placing Test (TPT) (136) is an associative location learning test designed to assess memory impairment. In this test, participants are presented with a series of 10 cards containing 2 objects (set 1) or 2 faces (set 2) and 2 blank squares randomly divided between four equal quadrants. In order to maintain the participants' attention, they are asked to distinguish whether the objects or the facial expressions are pleasant, unpleasant, or neutral. Participants are then shown the same series of 10 objects or faces in a random order. Following each card presentation the participant is asked to identify the location based on quadrant number **(figure 15)**. The score represents the number of items located

correctly. This test is advantageous because it is not confounded by age, gender, or education level (137).

Figure 15 THE PLACING TEST

An example of The Placing Test in which the participant must correctly identify the location of the familiar face. Adapted from (136).



The Word List Memory (WLM) Test is part of the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) (138) and is designed to assess learning ability and the retention of new verbal information. This test is divided into recall, delayed recall, and recognition segments. In the recall section participants are asked to read a 10 word list aloud and then immediately recall as many words from the list as possible. This is repeated three times with a different word order each time. After this test, participants were administered 10 minutes of distracter tasks (including non memory cognitive tasks). Following this participants are asked to recite as many words as they can remember; the number of correct responses comprises the delayed recall score (138).

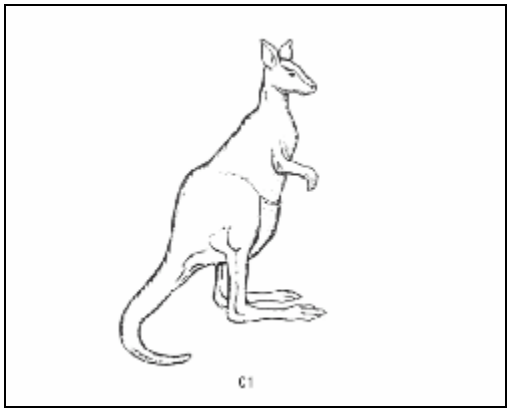
Semantic Memory

The Graded Naming Test (139) is a semantic memory test that indicates impaired language functioning and word-finding difficulty. For this test, examiners present participants 30 black and white drawings, like **figure 16,** in ascending degrees of supposed familiarity. The score is quantified by the number of correctly identified images. Advantages of this

test include resistance to ceiling effects and sensitivity to small cognitive changes that may not be detected by global cognitive tests (139).

Figure 16 THE GRADED NAMING TEST

An example of a familiar item from the Graded Naming Test (139).



Working Memory

Digit Span and Digit Score (140) is a commonly administered working memory test. Examiners begin the test by reciting a 3 digit numerical string to the participant and then asking the participant to recall the string in its entirety. As the participant answers correctly, the examiner recites another string with an additional digit. If incorrect, the examiner gives the participant a second chance by reciting a different numerical string of the same length. If the participant answers incorrectly again, the test is discontinued. If correct, the test continues until the participant makes two consecutive errors or when reaching and recalling the 9 digit string. Outcome measures include the highest correctly recalled digit string (digit span) and the total number of correctly recalled strings (digit score, maximum 14 points) (141).

Executive Function

The Symbol Digit Modalities Test (SDMT) is a test of executive function and is sensitive to changes in cognitive function over time. For this task, participants are presented with a table of corresponding geometric symbols and digits (1 to 9) and asked to complete a set as

practice. Next, using the table as a guide, the participants must match as many geometric symbols to digits as possible (142). The outcome measure is calculated by subtracting the total number of errors from the total completed (134).

Processing Speed

The Pattern and Letter Comparison Test (PCS & LCS) (143) is a measure of simple processing speed. This test is often used to control for the effects of speed on test performance and to determine its association with age. The test is divided into two parts, a pattern and letter comparison, with three levels of complexity. In the pattern comparison, participants are asked to dichotomize 30 pattern pairs as the same or different. The letter comparison is administered in the same way, but in the form of letter strings. The participant has 20 seconds to complete each task and is scored based on the number of correct responses. Only one of the two pages was used in this test battery (137).

Measure of Depression

The Geriatric Depression Scale is a basic yes-no questionnaire designed to screen for depression in the geriatric population. The examiner reads the questions to the participant and marks the response. Responses are either negatively or positively indicative of depression. The outcome measure is a sum of the total depressed answers; a score from 0 to 10 is considered normal to mild; 11to 20 is moderate; and 21to 30 is severe (144).

V. STATISTICAL ANALYSES

All statistical analyses were performed using SPSS for Windows (16^{th} ed.; Chicago, IL). The entire population was evaluated as a whole irrespective of the time between MR images due to a limited sample size; however the subset of participants with 4 to 5 years between measures (n = 80) were also analyzed as a separate entity. Normality distribution tests were done using Skewness and Kurtosis tests and Q-Q plots. The logarithmic value was used for all variables with a skewed distribution. Student t-tests

were used to evaluate whether the difference between baseline and follow-up variables was significant. Pearson rank correlations were performed to assess simple correlations. Univariate analysis of variance (ANOVA), controlling for age and sex, was used to identify significant effects on groups. Furthermore, linear regression analyses, also controlling for age and sex, were conducted to assess relationships between variables and Scheltens total score and subscales. Stepwise linear regression analysis was conducted for all variables that were borderline significant ($p < 0.10$) in adjusted linear regression analysis. A p-value < 0.05 was considered statistically significant for general analyses and a p-value of < 0.10 was considered statistically significant when evaluating interaction effects.

CHAPTER THREE
POTENTIAL RISK FACTORS: THE FINDINGS

The results will be described for the following subjects:

(i) up to 154 subjects at baseline;

(ii) prospectively for up to 135 subjects for whom there was a follow-up scan, running from 2 to 5 years;

(iii) prospectively for up to 80 subjects with a follow-up scan 4 to 5 years after their baseline scan.

I. THE PRESENCE OF WHITE MATTER HYPERINTENSITIES

MR images used to measure WMH were collected from 154 participants at baseline and 135 participants at follow-up. A positive Scheltens total score (≥ 1) was present in 94.5% of participants at baseline and 96.9% at follow-up. The study population had a mean Scheltens total score of 11.5 (range, 0 to 39) at baseline and 16.9 (range, 0 to 48) at follow-up. The baseline and follow-up Scheltens total score and subscales (DWMH, PVH, and BGH) for the entire study population are outlined in **table 3;** the same results are shown in **table 3.b** for those participants with 4 to 5 years between baseline and follow-up MR images (n = 80). Student t-tests demonstrate that Scheltens total score and subscale scores (DWMH, PVH, and BGH) increased significantly between baseline and follow-up. There was no significant difference between baseline and follow-up white matter damage for the Scheltens ITFH subscale. This was expected as very few subjects exhibited white matter damage in this region. Due to this, the Scheltens ITFH subscale will be omitted in future analyses.

Table 3 WHITE MATTER HYPERINTENSITIES OVER TIME

Student t-tests for Scheltens scores in baseline and follow-up measures of white matter hyperintensities for the entire study cohort.

SCHELTENS SCORE	N	BASELINE MEAN (SD)	FOLLOW-UP MEAN (SD)	P-VALUE (STUDENT T)
TOTAL SCORE	135	11.0 (8.6)	16.9 (11.4)	< 0.001
DWMH SUBSCALE	135	5.9 (5.3)	8.7 (6.8)	< 0.001
PVH SUBSCALE	135	2.3 (1.7)	2.6 (1.9)	< 0.001
BGH SUBSCALE	135	2.8 (2.5)	4.8 (4.2)	< 0.001

Table 3.b *Student t-tests for Scheltens scores in baseline and follow-up measures of white matter hyperintensities for the longer-term subgroup with 4 to 5 years between images.*

SCHELTENS SCORE	N	BASELINE MEAN (SD)	FOLLOW-UP MEAN (SD)	P-VALUE (STUDENT T)
TOTAL SCORE	80	10.6 (8.5)	18.0 (12.4)	< 0.001
DWMH SUBSCALE	80	5.3(5.1)	9.3 (7.2)	< 0.001
PVH SUBSCALE	80	2.1 (1.8)	2.5 (2.0)	0.001
BGH SUBSCALE	80	2.6 (2.4)	5.6 (4.8)	< 0.001

Abbreviations – SD (Standard Deviation); DWMH (Deep White Matter Hyperintensities); PVH (Periventricular Hyperintensities); BGH (Basal Ganglia Hyperintensities).

II. AGE IS ASSOCIATED WITH WMH AND OTHER FACTORS

The study population consisted of 154 participants at baseline with a mean age of 73.7 years (range, 60 to 90 years) at baseline and 77.4 years (range, 64 to 90 years) at follow-up; 49.4% (n = 76) participants were women. The total years of education were computed by combining years of schooling education with years of higher learning. On average, the study population completed a total of 13.9 years (range, 10 to 21 years) of academic learning per person. The study population was slightly overweight with a mean BMI of 25.8 (normal range, 18.5 to 24.9); overall 72 (47.1%) participants were classified as being overweight. Sixty-four (41.8%) participants were a healthy weight, 17 (11.1%) participants qualified as obese (BMI > 30), and none of the participants were underweight. Baseline

study population demographics, blood results, and selected neuropsychological tests are outlined in **table 4** with respect to age.

The study population was dichotomized into two groups of participants, those < 74 and those ≥ 74 years of age at baseline. Student t-tests were performed on baseline study population characteristics to evaluate whether or not the study variables were significantly different between age groups, logarithmic values were used for all skewed variables. There was a significant difference between age groups for CAMCOG scores, blood pressure, tHcy, MMA, and creatinine. Notably, there was also a significant difference between age groups for baseline Scheltens total scores and subscales (**table 4)**. Additional baseline study population characteristics (sex, education, smoking, diabetes, and *APOE ε4* status) are also shown in **table 4.**

Table 4 STUDY POPULATION CHARACTERISTICS IN RELATION TO AGE

Student t-tests for mean baseline characteristics split by the study population median age. Logarithmic values were used for all skewed variables. The mean baseline value is also shown for the entire study cohort (n = 154).*

BASELINE VARIABLE	ALL	< 74 YEARS	≥ 74 YEARS	P-VALUE (STUDENT T)
AGE (years)	73.7	68.4	78.6	0.05
EDUCATION (years)	12.4	12.3	12.4	NS
WOMEN, N (%)	76	37 (49.3)	39 (48.8)	NA
SMOKING (+), N (%)	88	39 (52.7)	49 (62.1)	NA
DIABETES (+), N (%)	6	2 (2.7)	4 (5.1)	NA
APOE ε4, N (%)	30	13 (17.3)	17 (21.2)	NA
BMI (kg/m^2)	25.8	25.8	25.8	NS
WEIGHT (kg)	73.8	75.0	73.1	NS
SCHELTENS TOTAL	11.5	8.0	14.8	0.05
DWMH SUBSCALE	5.9	3.7	8.0	0.05
PVH SUBSCALE	2.6	2.1	3.2	0.05
BGH SUBSCALE	4.8	4.4	5.2	0.10
MMSE	28.5	28.6	28.4	NS
CAMCOG	97.8	98.5	97.2	*0.10
GDS	4.6	4.1	5.1	NS
DBP (mmHg)	82	82	81	NS
SBP (mmHg)	154	152	155	NS
HOLOTC (pmol/L)	64.6	71.0	59.3	*0.05
TOTAL TC (pmol/L)	939	920	957	*NS
VITAMIN B12 (pmol/L)	355	370	340	*0.10
THCY (µmol/L)	12.7	11.4	13.9	*0.05
MMA (µmol/L)	0.23	0.19	0.27	*0.05
SERUM FOLATE (nmol/L)	12.4	12.6	12.2	*NS
RED CELL FOLATE (nmol/L)	463	477	449	*NS
CREATININE (µmol/L)	105	102	108	*0.10

*Abbreviations – APOE (Apolipoprotein) BMI (Body Mass Index); DWMH (Deep White Matter Hyperintensities); PVH (Periventricular Hyperintensities); BGH (Basal Ganglia Hyperintensities); MMSE (Mini Mental Status Examination); CAMCOG (Cambridge Cognitive Examination); GDS (Geriatric Depression Scale); DBP (Diastolic Blood Pressure); SBP (Systolic Blood Pressure); TC (Transcobalamin); tHcy (Total Homocysteine); MMA (Methylmalonic Acid); NS (Not Significant); NA (Not Applicable); * = Logarithmic value used.*

Therefore, since age interacts with many of the study variables it will be adjusted for in further analyses. **Table 5** indicates that the study population with baseline and follow-up MR images (n = 135) incurred a significant increase in WMH over time even when separating the study population by age group, **figure 17** illustrates these findings. Only 3 participants showed improvements in WMH over time; the remainder of the population incurred increases in WMH between baseline and follow-up MR images.

Table 5 WHITE MATTER HYPERINTENSITIES IN RELATION TO AGE

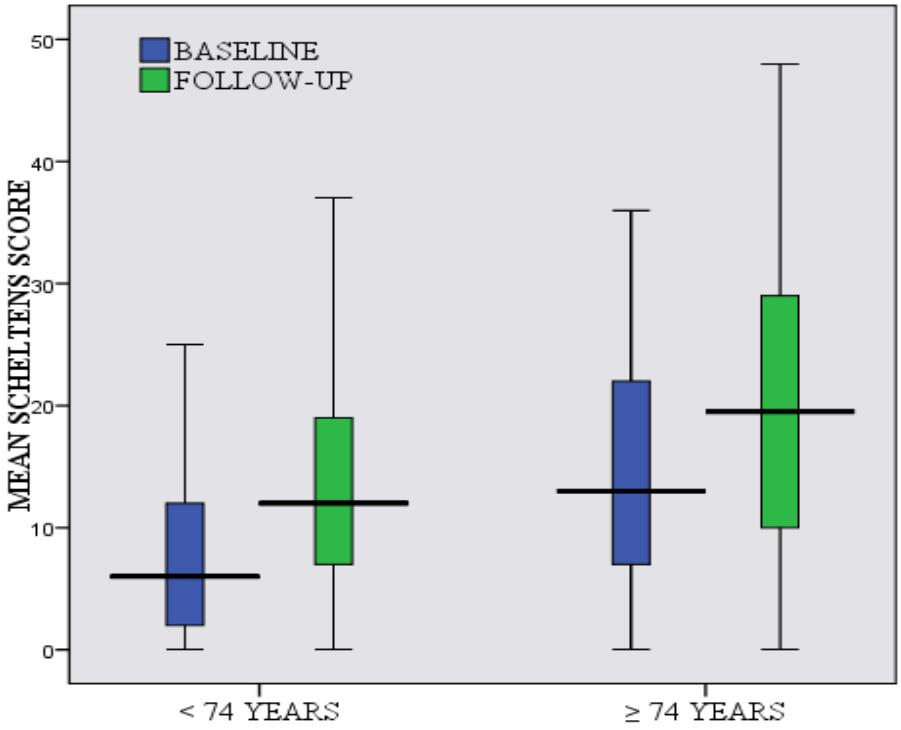
Mean baseline and follow-up Scheltens scores (Total, DWMH, BGH, PVH) with respect to baseline age groups. Student t-tests indicate whether white matter hyperintensities increased significantly between baseline and follow-up.

	< 74 YEARS OF AGE				≥ 74 YEARS OF AGE			
	N	B	F	P-VALUE (STUDENT T)	N	B	F	P –VALUE (STUDENT T)
TOTAL SCORE	69	7.9	13.9	< 0.001	66	14.2	19.9	< 0.001
DWMH SUBSCALE	69	3.6	6.8	< 0.001	66	7.4	10.7	0.001
PVH SUBSCALE	69	1.7	2.1	< 0.001	66	2.8	3.2	< 0.001
BGH SUBSCALE	69	2.2	4.4	< 0.001	66	3.1	5.2	< 0.001

Abbreviations – B (Baseline); F (Follow-up); DWMH (Deep White Matter Hyperintensities); PVH (Periventricular Hyperintensities); BGH (Basal Ganglia Hyperintensities); N (number of participants)

Figure 17 SCHELTENS TOTAL SCORE IN RELATION TO AGE

Baseline and follow-up Scheltens total scores in relation to the median split age for the entire study cohort (n = 135).



Linear regression analyses indicate that baseline age was positively associated with follow-up Scheltens total score ($r = 0.28$, $p < 0.001$), DWMH ($r = 0.30$, $p < 0.001$), and PVH ($r = 0.34$, $p < 0.001$) subscales. Baseline age was not statistically associated with the follow-up Scheltens BGH subscale. Based on the R^2 value, baseline age explains 8% of the variance in the follow-up Scheltens total score **(figure 19)**; while age may be an important factor associated with WMH, this indicates that additional factors must be associated with WMH.

Linear regression analyses were then performed for the longer-term subgroup of participants with 4 to 5 years between baseline and follow-up (n = 80). Results indicated that baseline age was strongly associated with follow-up Scheltens total score ($r = 0.30$, $p < 0.01$) **(figure 20)**, DWMH ($r = 0.32$, $p < 0.01$), PVH ($r = 0.26$, $p < 0.001$), and BGH subscales ($r = 0.23$, $p < 0.05$).

Figure 18 illustrates the cross-sectional relationship between baseline age and baseline Scheltens total score ($r = 0.38$, $p < 0.001$) for 154 participants, **figure 19** illustrates the prospective relationship between baseline age and follow-up Scheltens total score ($n = 135$) and **figure 20** demonstrates the association between baseline age and follow-up Scheltens total score for the longer-term subgroup ($n = 80$) with 4 to 5 years between baseline and follow-up.

Figure 18 AGE AND WHITE MATTER HYPERINTENSITIES

Linear regression with 95% confidence interval (CI) lines is shown for baseline age in relation to baseline Scheltens total score for the entire study cohort ($r = 0.38$, $p < 0.001$) ($n = 154$).

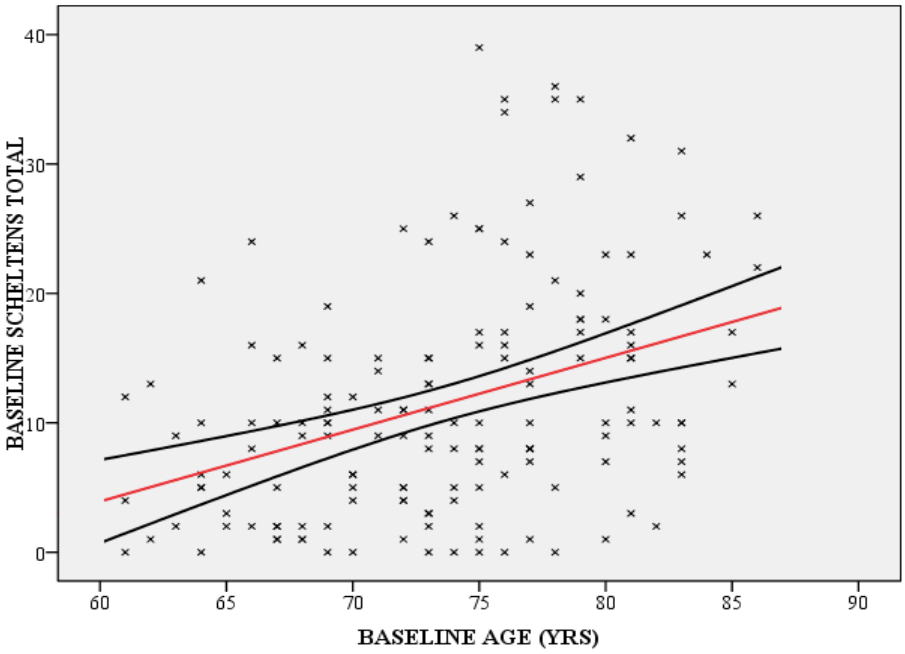


Figure 19 AGE AND WHITE MATTER HYPERINTENSITIES

Linear regression with 95% CI lines is shown for baseline age in relation to follow-up Scheltens total score assessed 2 to 5 years later (r = 0.28, p < 0.001) (n = 135).

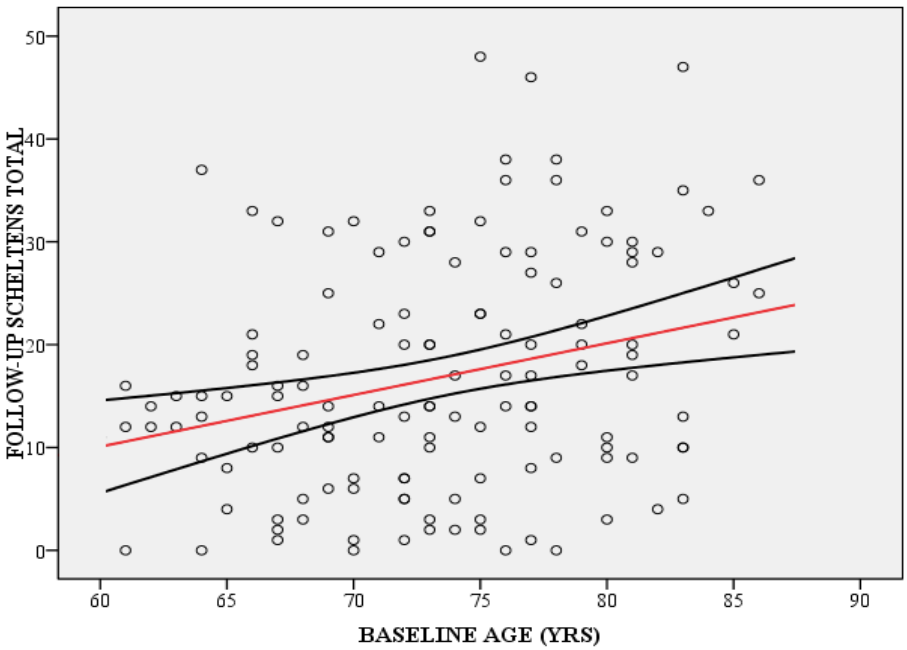
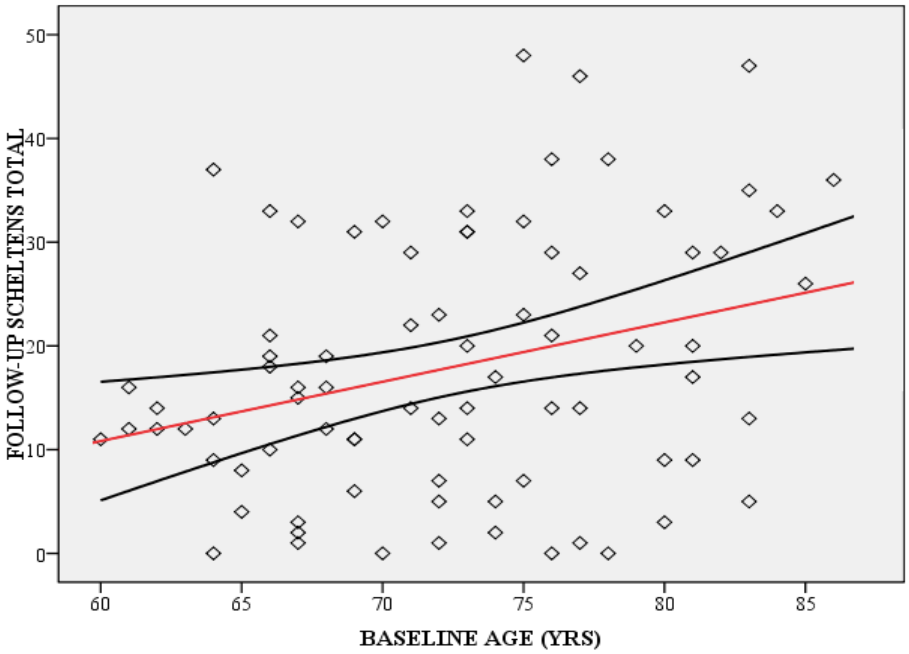


Figure 20 PROSPECTIVE AGE AND WHITE MATTER HYPERINTENSITIES

Linear regression with 95% CI lines is shown for baseline age in relation to follow-up Scheltens total score assessed in the longer-term subgroup 4 to 5 years later (r = 0.30, p < 0.01) (n = 80).



III. MALE AND FEMALE SEX

The study population was comprised equally of males (n = 78, 50.6%) and females (n = 76, 49.3%) at baseline. Student t-tests were performed to evaluate whether or not baseline study variables were significantly different for males and females. There was a significant difference between men and women for weight, creatinine, MMA, vitamin B_{12}, and serum folate. Notably, there was no significant difference between men and women for Scheltens total scores or subscales **(table 6)**. Since sex was significantly associated with some variables that are likely to influence WMH, future analyses will be adjusted by sex in addition to age.

Table 6 STUDY POPULATION CHARACTERISTICS IN RELATION TO SEX

Student t-tests show significant differences between the sexes for baseline study characteristics. Logarithmic values were used for all variables that were not normally distributed (n = 154).*

BASELINE	ALL	FEMALE	MALE	P-VALUE (STUDENT T)
AGE (YEARS)	73.7	73.7	73.6	NS
EDUCATION (YEARS)	12.4	12.5	12.3	NS
BMI (kg/m^2**)**	25.8	25.5	26.0	NS
WEIGHT (kg)	73.8	66.9	79.6	0.05
SCHELTENS TOTAL SCORE	11.5	11.3	11.7	NS
DWMH SUBSCALE	5.9	5.8	6.00	NS
PVH SUBSCALE	2.6	2.8	2.49	NS
BGH SUBSCALE	4.8	4.9	4.65	NS
MMSE	28.5	28.4	28.6	NS
CAMCOG	97.8	97.8	97.8	*NS
GDS	4.6	4.9	4.3	NS
DBP (mmHg)	82	82	82	NS
SBP (mmHg)	154	154	153	NS
VITAMIN B_{12} (pmol/L)	355	382	329	*0.05
HOLOTC (pmol/L)	64.6	67.6	62.4	*NS
TOTAL TC (pmol/L)	939	956	924	*NS
tHCY (µmol/L)	12.7	12.0	13.4	*0.05
MMA (µmol/L)	0.23	0.23	0.22	*NS
SERUM FOLATE (nmol/L)	12.4	13.2	11.6	*0.05
RED CELL FOLATE (nmol/L)	463	481	445	*NS
CREATININE (µmol/L)	105	97.1	113	*0.05

*Abbreviations – BMI (Body Mass Index); DWMH (White Matter Hyperintensities); PVH (Periventricular Hyperintensities); BGH (Basal Ganglia Hyperintensities); MMSE (Mini Mental Status Examination); CAMCOG (Cambridge Cognitive Examination); GDS (Geriatric Depression Scale); DBP (Diastolic Blood Pressure); SBP (Systolic Blood Pressure); TC (Transcobalamin); tHcy (Total Homocysteine); MMA (Methylmalonic Acid); NS (Not Significant); * = Logarithmic value used.*

Linear regression analyses indicated that sex was not significantly associated with follow-up Scheltens total score or subscales (DWMH, PVH, or BGH) when adjusting for age.

IV.DIABETES

Diabetes was defined by participants taking oral anti-diabetics or insulin. Using these criteria, only 4.4% (n = 6) of the study population were classified as diabetic. All six diabetic participants survived through the last follow-up exam 5 years later. Univariate ANOVA, controlling for age and sex, revealed that a baseline diagnosis of diabetes does not have a significant effect on follow-up Scheltens total score **(table 7)** or subscales.

Table 7 DIABETES AND WHITE MATTER HYPERINTENSITIES

Univariate ANOVA for follow-up Scheltens total score in relation to diabetes when adjusted for age and sex for the entire study cohort with follow-up MR images (n = 135).

DIABETES	N	FOLLOW-UP SCHELTENS TOTAL SCORE	STD. ERROR	95% CONFIDENCE INTERVAL	P-VALUE (ANOVA)
YES	6	13.5	4.58	(4.5, 22.6)	NS
NO	129	17.0	0.97	(15.1, 19.0)	

Abbreviations – NS (Not Significant)

V. SMOKING HISTORY

The study population with follow-up MR images (n = 135) contained 4.5% (n = 6) current smokers, 52.2% (n = 70) ex-smokers, and 43.3% (n = 58) non-smokers, the smoking history from one participant was unavailable. In order to evaluate the potential effects of smoking those participants with an affirmative smoking history (n = 76, 56.7%) were compared with those participants with a non-smoking history (n = 58, 43.3%). Univariate ANOVA, controlling for age and sex, revealed that smoking history did not have a significant effect on follow-up Scheltens total score **(table 8)** or subscales.

Table 8 SMOKING STATUS AND WHITE MATTER HYPERINTENSITIES

Univariate ANOVA for follow-up Scheltens total score in relation to smoking history recorded at baseline when adjusting for age and sex for the entire study cohort with follow-up MR images (n = 134).

SMOKING HISTORY	N	SCHELTENS TOTAL FOLLOW-UP	STD. ERROR	95% CONFIDENCE INTERVAL	P-VALUE (ANOVA)
NEVER SMOKED	58	16.3	1.5	(13.4, 19.3)	NS
EVER/CURRENT SMOKED	76	17.3	1.3	(14.7, 19.9)	

In addition, dose-response linear regression analyses indicated no association between smoking history and Scheltens total scores or subscales when controlling for age and sex.

VI.BLOOD PRESSURE

The study population had a mean baseline DBP of 81.6 mmHg (range, 54 to 110 mmHg), and a mean baseline SBP of 154 mmHg (range, 108 to 212 mmHg). **Table 9** demonstrates partial r correlations (adjusted for age and sex) for baseline and follow-up blood pressure measurements and baseline and follow-up Scheltens total score and subscales; **table 9.b** shows the same analyses for the longer-term subgroup (n = 80) with 4 to 5 years between the baseline blood pressure measurements and follow-up MR images.

Table 9 BLOOD PRESSURE AND WHITE MATTER HYPERINTENSITIES

Partial r correlations are shown for baseline and follow-up blood pressure measurements and baseline and follow-up Scheltens scores (Total, DWMH, PVH, and BGH) for the entire study cohort (n = 133) when controlling for age and sex.

			SCHELTENS TOTAL		DWMH SUBSCALE		PVH SUBSCALE		BGH SUBSCALE	
	TIME	N	B	F	B	F	B	F	B	F
DBP	**B**	133	**0.14***	**0.18****	NS	NS	**0.15***	NS	NS	**0.21*****
DBP	**F**	133	**0.24*****	**0.24*****	**0.22*****	**0.17****	NS	NS	**0.15***	**0.28*****
SBP	**B**	133	**0.18****	**0.17****	NS	NS	**0.19****	NS	**0.17****	**0.27*****
SBP	**F**	133	**0.19****	**0.17****	**0.15***	NS	**0.17****	NS	**0.19****	**0.20****

Table 9.b *Partial r correlations are shown for baseline and follow-up blood pressure measurements and baseline and follow-up Scheltens scores (Total, DWMH, PVH, and BGH) for the longer-term subgroup (n = 80) with 4 to 5 years between baseline blood pressure and the follow-up MR image when controlling for age and sex.*

			SCHELTENS TOTAL		DWMH SUBSCALE		PVH SUBSCALE		BGH SUBSCALE	
	TIME	N	B	F	B	F	B	F	B	F
DBP	**B**	80	NS	**0.23****	**0.20***	NS	NS	NS	NS	**0.28*****
DBP	**F**	80	**0.26****	**0.26****	**0.27****	**0.20***	NS	NS	NS	**0.29*****
SBP	**B**	80	NS	**0.20***	NS	NS	NS	NS	NS	**0.30*****
SBP	**F**	80	NS	**0.25****	NS	**0.23****	NS	NS	NS	**0.25****

Abbreviations – N (Number of Participants); B (Baseline); F(Follow-up); DBP (Diastolic Blood Pressure); SBP (Systolic Blood Pressure); PP (Pulse Pressure); DWMH (Deep White Matter Hyperintensities); PVH (Periventricular Hyperintensities); BGH (Basal Ganglia Hyperintensities)
p < 0.01; **p <0 .05; p < 0.10*; NS (Not Significant)

Cross-Sectional Analyses

The cross-sectional analyses of DBP and SBP in relation to Scheltens total score and subscales (DWMH, PVH, and BGH) were completed for baseline and follow-up measures. Results indicate that baseline SBP was positively associated with baseline Scheltens total score, PVH, BGH subscales. In cross-sectional analyses evaluated at follow-up, results indicate that follow-up SBP was no longer significantly associated with Scheltens follow-up PVH subscale, but remained significant for follow-up

Scheltens total score **(figure 21)** and follow-up BGH subscale. Interestingly, baseline cross-sectional analyses for DBP and baseline Scheltens total score and subscales were not significant; however follow-up DBP was positively associated with follow-up Scheltens total score **(figure 22),** DWMH, and BGH subscales.

Cross-sectional analyses for the longer-term subgroup for baseline DBP and SBP were no longer associated with baseline Scheltens total score or subscales. Follow-up SBP was positively associated with follow-up Scheltens total score, DWMH, and BGH subscales.

Figure 21 SYSTOLIC BLOOD PRESSURE AND WHITE MATTER HYPERINTENSITIES

Linear regression with 95% CI lines is shown for the cross-sectional relationship between follow-up logarithmic value of SBP and follow-up Scheltens total score for the entire study cohort when adjusted for age and sex (partial r = 0.17, p < 0.05) (n = 133).

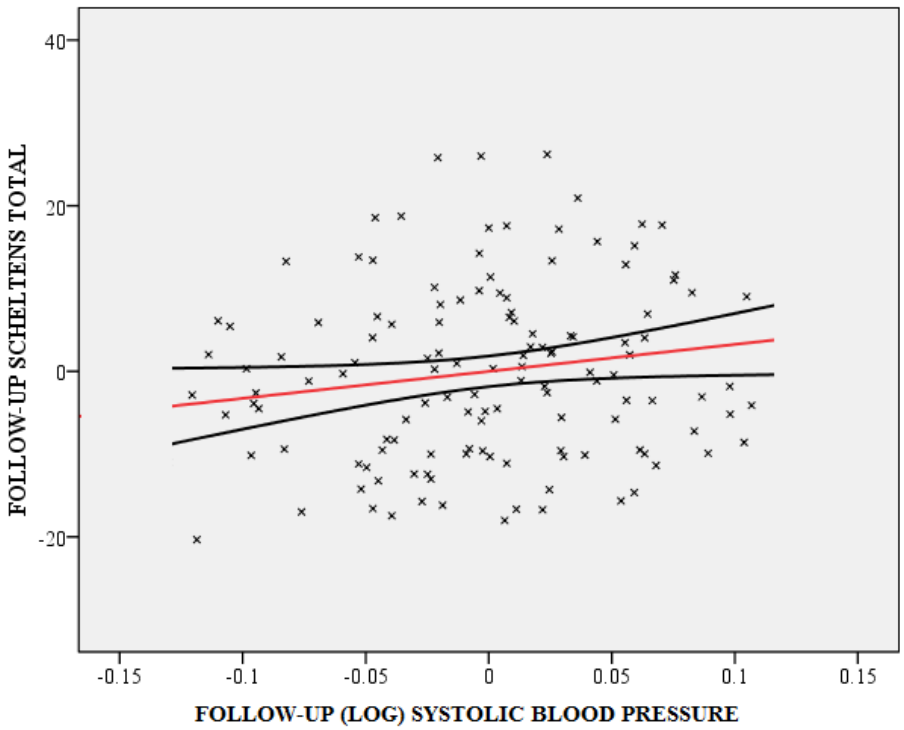
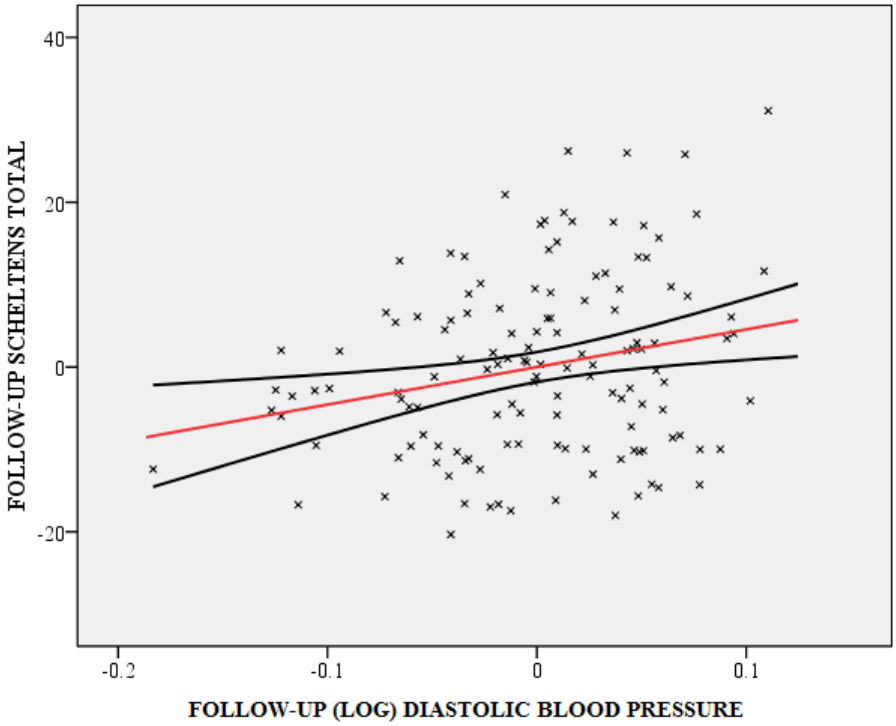


Figure 22 DIASTOLIC BLOOD PRESSURE AND WHITE MATTER HYPERINTENSITIES

Linear regression with 95% CI lines is shown for the cross-sectional association between follow-up logarithmic value of DBP and follow-up Scheltens total score for the entire study cohort when adjusted for age and sex (partial r = 0.24, p < 0.01) (n = 133).



Prospective Analyses

Linear regression analyses revealed that baseline SBP was positively associated with follow-up Scheltens total score **(figure 23)** and the follow-up BGH subscale. Baseline DBP was positively associated with follow-up Scheltens total scores **(figure 24)** and follow-up BGH subscale when adjusting for age and sex.

The same analyses for the longer-term subgroup revealed similar findings. Baseline SBP was approaching significance for follow-up Scheltens total score and positively associated with the follow-up BGH subscale. Baseline DBP was positively associated with follow-up Scheltens total score and BGH subscale.

Figure 23 SYSTOLIC BLOOD PRESSURE AND WHITE MATTER HYPERINTENSITIES

Linear regression with 95% CI lines is shown for the prospective association between baseline logarithmic value of SBP and follow-up Scheltens total score for the entire study cohort when adjusted for age and sex (partial r = 0.17, p < 0.05) (n = 133).

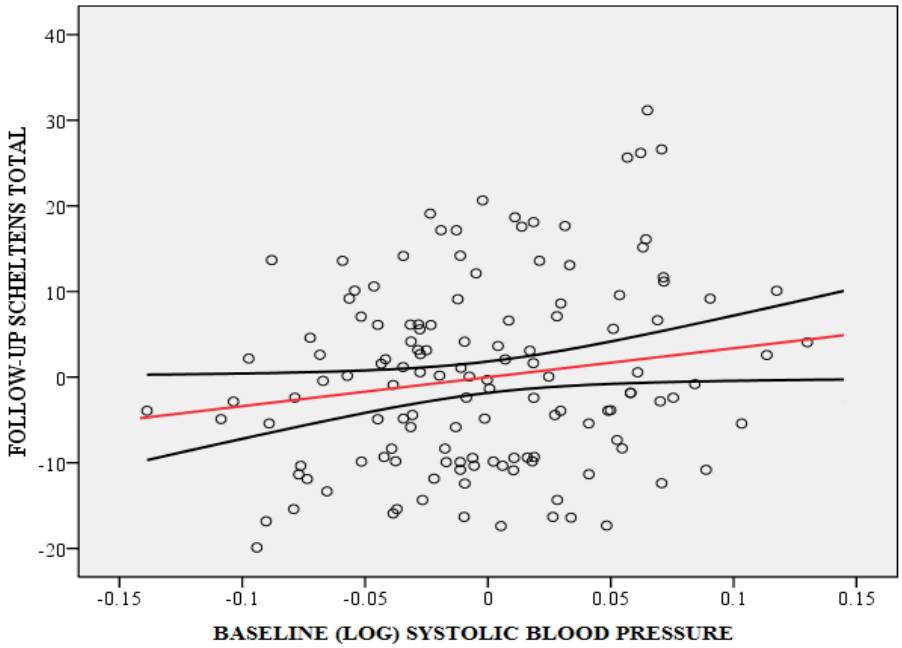
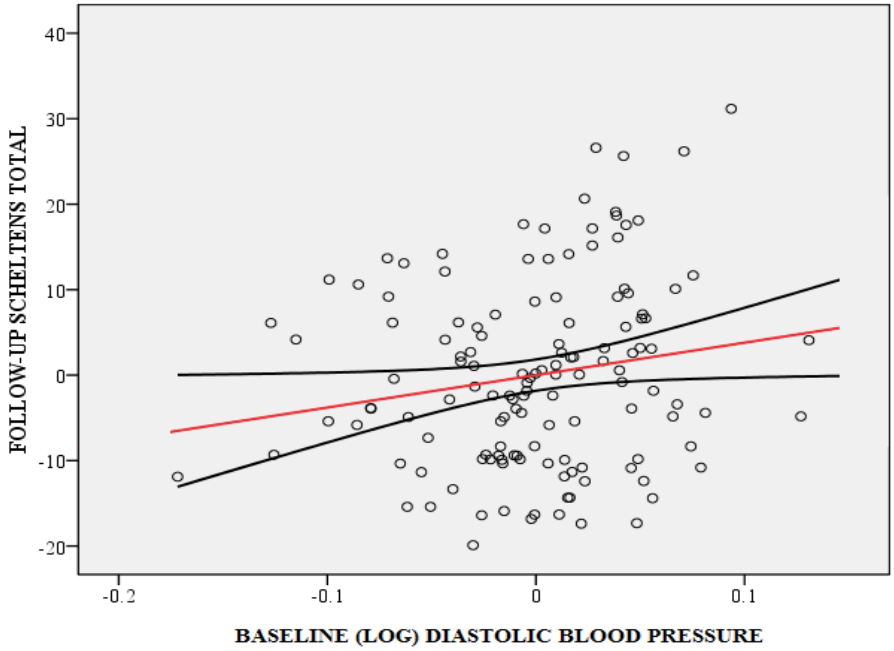


Figure 24 DIASTOLIC BLOOD PRESSURE AND WHITE MATTER HYPERINTENSITIES

Linear regression with 95% CI lines is shown for the prospective association between baseline logarithmic value of DBP and follow-up Scheltens total score when adjusted for age and sex for the entire study cohort (partial r = 0.18, p < 0.05) (n = 133).



Student t-tests were conducted to evaluate whether or not the study population incurred a significant change in blood pressure over time. SBP showed significant decreases over time and tests for DBP showed significant decreases for hypertensive, but not normotensive participants **(table 10)**. Notably, a significant proportion of the study population (n = 72, 54.1%) were either prescribed antihypertensive medications by their respective doctors or had hypertension defined by a SBP ≥ 160 mmHg or DBP ≥ 100 mmHg at the time of the baseline interview. In order to investigate the effect of hypertension, univariate ANOVA, controlling for age and sex, was conducted for the entire study population with baseline and follow-up measurements **(table 10).** Results indicate that there was a significant difference between normotensive and hypertensive participants for baseline and follow-up SBP and baseline DBP, but not at follow-up.

Table 10 NORMOTENSIVE VS. HYPERTENSIVE PARTICIPANTS

Normotensive and hypertensive groups were analyzed by univariate ANOVA (controlling for age and sex). Student t-tests reveal whether there was a significant difference between baseline and follow-up blood pressure measurements (n = 133).

	N	BASELINE (mmHg)	FOLLOW-UP (mmHg)	P-VALUE (STUDENT T)
DIASTOLIC BLOOD PRESSURE				
HYPERTENSIVE	72	83	80	0.004
NORMOTENSIVE	61	79	78	NS
P-VALUE (ANOVA)		***0.002***	***NS***	
SYSTOLIC BLOOD PRESSURE				
HYPERTENSIVE	72	164	149	< 0.001
NORMOTENSIVE	61	141	141	0.001
P-VALUE (ANOVA)		***<0.001***	***< 0.001***	

Abbreviations – NS (Not Significant)

Univariate ANOVA was also conducted to evaluate the effect of baseline hypertension on follow-up Scheltens total score and subscales. Results indicate a significant difference between normotensive and hypertensive groups for follow-up Scheltens total score and follow-up BGH subscale, but no significant difference for follow-up DWMH or PVH subscales **(table 11).** Further analyses of those participants in the longer-term subgroup

were not significant; however baseline hypertensive participants tended to have higher follow-up Scheltens total scores and subscales **(table 11.b).**

Table 11 HYPERTENSION AND WHITE MATTER HYPERINTENSITIES

Univariate ANOVA for follow-up Scheltens scores (Total, DWMH, PVH, and BGH) in relation to baseline hypertension for the entire study cohort when adjusted for age and sex (n = 135)

	N	FOLLOW-UP SCHELTENS SCORE		STD. ERROR	95% CONFIDENCE INTERVAL	P-VALUE (ANOVA)
HYPERTENSIVE	73	TOTAL SCORE	18.7	1.2	(16.2, 21.3)	0.04
NORMOTENSIVE	62		14.6	1.4	(11.9, 17.4)	
HYPERTENSIVE	73	DWMH SUBSCALE	9.4	0.78	(7.8, 10.9)	NS
NORMOTENSIVE	62		7.9	0.84	(6.2, 9.5)	
HYPERTENSIVE	73	PVH SUBSCALE	2.8	0.21	(2.4, 3.2)	NS
NORMOTENSIVE	62		2.4	0.23	(1.9, 2.9)	
HYPERTENSIVE	73	BGH SUBSCALE	5.6	0.49	(4.6, 6.6)	0.02
NORMOTENSIVE	62		3.8	0.53	(2.8, 4.9)	

Table 11.b *Univariate ANOVA for follow-up Scheltens scores (Total, DWMH, PVH, and BGH) in relation to baseline hypertension for the longer-term subgroup with 4 to 5 years between baseline hypertension assessments and follow-up MR images (n = 80). All analyses were adjusted for age and sex.*

	N	FOLLOW-UP SCHELTENS SCORE		STD. ERROR	95% CONFIDENCE INTERVAL	P-VALUE (ANOVA)
HYPERTENSIVE	41	TOTAL SCORE	19.7	1.9	(15.9, 23.6)	NS
NORMOTENSIVE	39		16.1	2.0	(12.1, 20.0)	
HYPERTENSIVE	41	DWMH SUBSCALE	9.9	1.1	(7.7, 12.2)	NS
NORMOTENSIVE	39		8.5	1.1	(6.3, 10.8)	
HYPERTENSIVE	41	PVH SUBSCALE	2.6	0.32	(2.0, 3.2)	NS
NORMOTENSIVE	39		2.3	0.31	(1.7, 2.9)	
HYPERTENSIVE	41	BGH SUBSCALE	6.5	0.76	(5.0, 8.0)	NS
NORMOTENSIVE	39		4.7	0.78	(3.1, 6.2)	

Abbreviations - N (Number of Participants); DWMH (Deep White Matter Hyperintensities); PVH (Periventricular Hyperintensities); BGH (Basal Ganglia Hyperintensities); STD Error (Standard Error); NS (Not Significant)

In order to evaluate the effects of hypertension on WMH, linear regression analyses were conducted for both normotensive and hypertensive groups. **Table 12** demonstrates partial r correlations for normotensive participants for both baseline and

follow-up blood pressure measurements and baseline and follow-up Scheltens total score and subscales (n = 62). For normotensive participants, baseline DBP was not significantly associated with follow-up WMH 2 to 5 years later. Baseline SBP was approaching significance to follow-up Scheltens total score and follow-up BGH subscale after adjusting for age and sex. Results for the longer-term subgroup **(table 12.b)** indicated that there was no significant association between baseline blood pressure measurements and WMH for normotensive participants (n = 39).

Table 12 BLOOD PRESSURE AND WHITE MATTER HYPERINTENSITIES FOR NORMOTENSIVE PARTICIPANTS

Partial r correlations are shown for baseline and follow-up blood pressure measurements and Scheltens scores for normotensive participants from the entire study cohort when adjusted for age and sex (n = 62).

			SCHELTENS TOTAL		DWMH SUBSCALE		PVH SUBSCALE		BGH SUBSCALE	
	TIME	N	B	F	B	F	B	F	B	F
DIASTOLIC BLOOD PRESSURE	B	62	NS	NS	NS	NS	**0.27****	NS	NS	NS
	F	62	NS	NS	NS	NS	NS	NS	NS	NS
SYSTOLIC BLOOD PRESSURE	B	62	NS	**0.22***	NS	NS	**0.21***	NS	NS	**0.23***
	F	62	NS	NS	NS	NS	NS	NS	NS	NS

Table 12.b *Partial r correlations are shown for baseline and follow-up blood pressure measurements and Scheltens scores for normotensive participants in the longer-term subgroup with 4 to 5 years between measures. All analyses were adjusted for age and sex (n = 39).*

			SCHELTENS TOTAL		DWMH SUBSCALE		PVH SUBSCALE		BGH SUBSCALE	
	TIME	N	B	F	B	F	B	F	B	F
DIASTOLIC BLOOD PRESSURE	B	39	NS	NS	NS	NS	NS	NS	NS	NS
	F	39	NS	NS	NS	NS	NS	NS	NS	NS
SYSTOLIC BLOOD PRESSURE	B	39	NS	NS	NS	NS	NS	NS	NS	NS
	F	39	NS	NS	NS	NS	NS	NS	NS	NS

Abbreviations – B (Baseline); F (Follow-up); DWMH (Deep White Matter Hyperintensities); PVH (Periventricular Hyperintensities); BGH (Basal Ganglia Hyperintensities)

p <0 .05; p < 0.10*; NS (Not Significant)

Interestingly, these associations changed quite remarkably when evaluating the same associations for hypertensive participants. **Table 13** outlines the partial r correlations for the entire hypertensive population (n = 73) and **table 13.b** outlines partial r correlations for those hypertensive participants in the longer-term subgroup (n = 41). Prospective analyses indicated that baseline DBP was not significantly associated with follow-up Scheltens total score or subscales, however follow-up cross-sectional analyses were highly significant for Scheltens total score **(figure 25)**, DWMH subscale and BGH subscale, but not the PVH subscale. Baseline SBP on the other hand approached significance to follow-up BGH subscale, but no other measure of WMH. Cross-sectional analyses for SBP were not

significant. When analyzing the longer-term subgroup, the relationship between DBP and WMH was attenuated and the relationship between SBP and WMH disappeared completely.

Table 13 BLOOD PRESSURE AND WHITE MATTER HYPERINTENSITIES FOR HYPERTENSIVE PARTICIPANTS

Partial r correlations are shown for baseline and follow-up blood pressure measurements and Scheltens scores for participants from the entire study cohort with baseline hypertension (n = 73). All analyses were adjusted for age and sex.

			SCHELTENS TOTAL		DWMH SUBSCALE		PVH SUBSCALE		BGH SUBSCALE	
	TIME	N	B	F	B	F	B	F	B	F
DIASTOLIC BLOOD PRESSURE	**B**	73	NS	NS	NS	NS	NS	NS	NS	NS
	F	73	**0.30*** **	**0.31*** **	**0.31*** **	**0.24** **	NS	NS	NS	**0.32*** **
SYSTOLIC BLOOD PRESSURE	**B**	73	NS	NS	NS	NS	NS	NS	NS	**0.21***
	F	73	**0.19***	NS	NS	NS	NS	NS	NS	NS

Table 13.b *Partial r correlations are shown for baseline and follow-up blood pressure measurements and Scheltens scores for participants with baseline hypertension in the longer-term subgroup with 4 to 5 years between measures (n = 41). All analyses were adjusted for age and sex.*

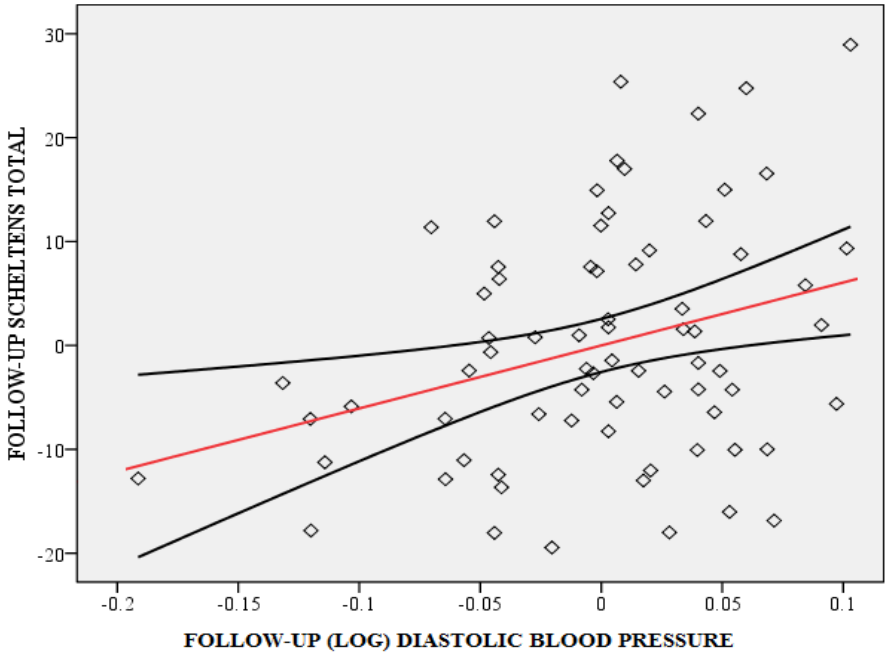
			SCHELTENS TOTAL		DWMH SUBSCALE		PVH SUBSCALE		BGH SUBSCALE	
	TIME	N	B	F	B	F	B	F	B	F
DIASTOLIC BLOOD PRESSURE	**B**	41	NS	NS	NS	NS	NS	NS	NS	**0.32** **
	F	41	**0.32** **	**0.30***	**0.30***	NS	NS	NS	**0.28***	**0.36** **
SYSTOLIC BLOOD PRESSURE	**B**	41	NS	NS	NS	NS	NS	NS	NS	NS
	F	41	NS	NS	NS	NS	NS	NS	NS	NS

Abbreviations – DWMH (Deep White Matter Hyperintensities); PVH (Periventricular Hyperintensities); BGH (Basal Ganglia Hyperintensities); B (Baseline); F (Follow-up)

***p < 0.01; **p <0 .05; p < 0.10*; NS (Not Significant)**

Figure 25 DIASTOLIC BLOOD PRESSURE AND WHITE MATTER HYPERINTENSITIES FOR PARTICIPANTS WITH HYPERTENSION

Linear regression with 95% CI lines is shown for the cross-sectional relationship between follow-up logarithmic value of diastolic blood pressure and follow-up Scheltens total score for participants with hypertension when adjusted for age and sex (partial r = 0.31, p < 0.008) (n = 41).



VII. MEASURES OF VITAMIN B_{12} AND FOLATE

Univariate ANOVA, adjusting for age and sex, was conducted for baseline MMA and tHcy comparing the bottom three quartiles with the top quartile and vice versa for baseline vitamin B_{12}, holoTC, total TC, serum folate and red cell folate to see if extreme values significantly influenced WMH at follow-up. Analyses were conducted for the entire population and also for the longer-term subgroup with 4 to 5 years between baseline markers and follow-up MR images.

Univariate ANOVA for the entire study population indicated that baseline tHcy was borderline significant when comparing the bottom three quartiles (≤ 14.8 µmol/L) with the top quartile (> 14.8 µmol/L) for follow-up Scheltens total score and significant for the follow-up BGH subscale **(table 14).** However, univariate ANOVA for the longer-term subgroup indicated that the tHcy level was positively associated to follow-up Scheltens

total score, and follow-up DWMH, PVH, and BGH subscales **(table 14.b)**. Thus, results show a trend for increased WMH with higher blood concentrations of tHcy. All other markers demonstrated insufficient evidence of a relationship with WMH.

Table 14 HOMOCYSTEINE AND WHITE MATTER HYPERINTENSITIES

Univariate ANOVA for follow-up Scheltens scores in relation to total plasma homocysteine when adjusted for age and sex (n =135) .

BASELINE tHCY (µmol/L)	N	FOLLOW-UP SCHELTENS		STD. ERROR	95% CONFIDENCE INTERVAL	P-VALUE (ANOVA)
< 14.8	104	**TOTAL SCORE**	15.9	1.1	(13.7, 18.0)	0.07
≥ 14.8	31		20.2	2.0	(16.2, 24.2)	
< 14.8	104	**BGH SUBSCALE**	4.4	0.41	(3.6, 5.2)	0.05
≥ 14.8	31		6.1	0.77	(4.6, 7.6)	

Table 14.b *Univariate ANOVA for follow-up Scheltens scores in relation to baseline total plasma homocysteine for participants in the longer-term subgroup adjusted for age and sex (n=80).*

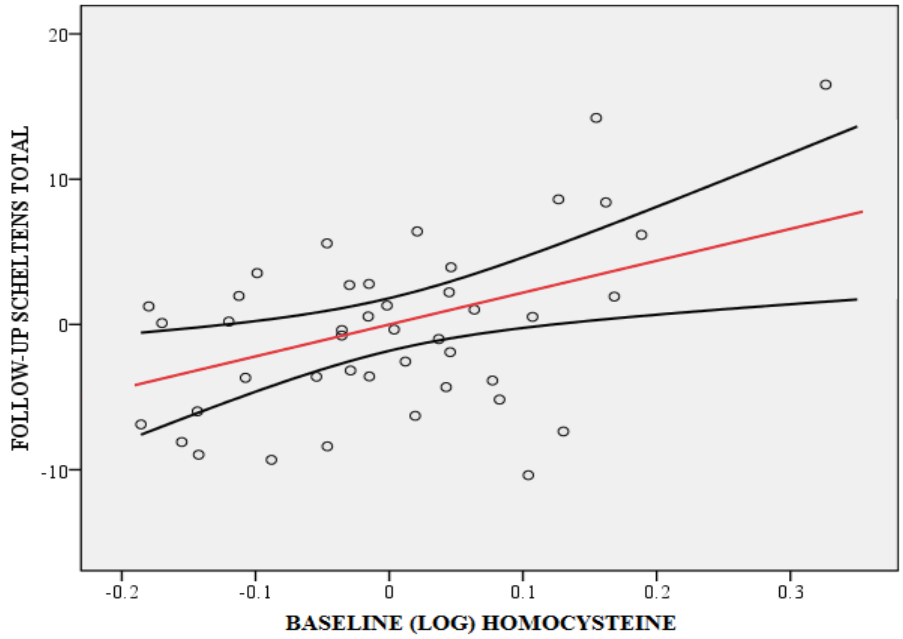
BASELINE tHCY (µmol/L)	N	SCHELTENS TOTAL SCORE		STD. ERROR	95% CONFIDENCE INTERVAL	P-VALUE (ANOVA)
< 14.8	64	**TOTAL SCORE**	16.0	1.4	(13.1, 18.9)	0.01
≥ 14.8	16		25.6	3.0	(19.6, 31.5)	
< 14.8	64	**DWMH SUBSCALE**	8.4	0.9	(6.7, 10.1)	0.03
≥ 14.8	16		12.7	1.8	(9.2, 16.2)	
< 14.8	64	**PVH SUBSCALE**	2.2	0.2	(1.8, 2.7)	0.03
≥ 14.8	16		3.4	0.5	(2.4, 4.4)	
< 14.8	64	**BGH SUBSCALE**	4.8	0.31	(3.7, 6.0)	0.01
≥ 14.8	16		8.7	0.31	(6.3, 11.1)	

Abbreviations – tHcy (Homocysteine); DWMH (Deep White Matter Hyperintensities); PVH (Periventricular Hyperintensities); BGH (Basal Ganglia Hyperintensities)

Linear regression analyses, controlling for age and sex, for those participants with follow-up Scheltens scores in the top tertile (n = 44) were conducted for the entire study population. Results indicated that baseline tHcy levels were positively associated with follow-up Scheltens total score (partial r = 0.39, p < 0.01) and BGH subscale (partial r = 0.47, p < 0.006), but not for follow-up DWMH or PVH subscales. This relationship was not conducted for the longer-term subgroup due to study size limitations. **Figure 26** illustrates the relationship between baseline tHcy and follow-up Scheltens total score 2 to 5 years later.

Figure 26 HOMOCYSTEINE AND WHITE MATTER HYPERINTENSITIES

Linear regression with 95% CI lines is shown for baseline logarithmic total plasma homocysteine in relation to follow-up Scheltens total score in the top tertile for the entire study cohort (n = 44) when adjusted for age and sex (partial r = 0.39, p < 0.01).



Linear regression analyses were completed for baseline creatinine, tHcy, MMA, vitamin B_{12}, holoTC, total TC, red cell folate, and serum folate. All tests were adjusted for age and sex and logarithmic values were used for variables that were not normally distributed. None of the variables was found to be associated with follow-up Scheltens total score or subscales. Moreover, when assessing the longer-term subgroup there were no significant associations between baseline and follow-up markers and baseline and follow-up Scheltens total or subscales.

VIII. GENETIC RISK

Of the study population, 19.4% (n = 30) had the *APOE* ε4 allele. Univariate ANOVA, controlling for age and sex, was conducted for follow-up Scheltens total score and subscales to distinguish whether there was a significant difference between carriers and non-carriers of the *APOE* ε4 allele. Results indicated that there was no significant effect on follow-up Scheltens total score or subscales when separated by *APOE ε4* polymorphisms **(table 15).**

Table 15 *APOE ε4* STATUS AND WHITE MATTER HYPERINTENSITIES

Univariate ANOVA, adjusting for age and sex, for follow-up Scheltens total score in relation to APOE genotypes.

APOE STATUS	N	FOLLOW-UP SCHELTENS TOTAL SCORE	STD. ERROR	95% CONFIDENCE INTERVAL	P-VALUE (ANOVA)
NON ε4	105	16.2	1.1	(14.1, 18.4)	NS
ε4 POSITIVE	30	19.5	2.3	(15.0, 24.0)	

Abbreviations – N (Number of Participants); STD. Error (Standard Error); NS (Not Significant)

The study population was comprised of 42.6% (n = 66) *MTHFR 677CC* homozygous; 43.7% (n = 68) *MTHFR 677CT* heterozygous; and 13.5% (n = 21) *MTHFR 677TT* homozygous. Univariate ANOVA compared the effect of these genotypes on follow-up Scheltens total score and subscales. Results indicated that there was no significant difference between genotypes **(table 16).**

Table 16 THE *MTHFR 677C →T* POLYMORPHISM AND WHITE MATTER HYPERINTENSITIES

Univariate ANOVA, adjusting for age and sex, for follow-up Scheltens total score in relation to MTHFR 677C →T genotypes.

MTHFR	**N**	**FOLLOW-UP SCHELTENS TOTAL SCORE**	**STD. ERROR**	**95% CONFIDENCE INTERVAL**	**P-VALUE (ANOVA)**
CC	66	16.3	1.5	(13.4, 19.3)	
CT	68	17.0	1.5	(14.0, 20.0)	NS
TT	21	18.2	2.8	(12.7, 23.7)	

Abbreviations – N (Number of Participants); STD. Error (Standard Error); NS (Not Significant)

The study population was comprised 19.3% (n = 30) *MTRR 66AA* homozygous; 52.9% (n = 82) *MTRR 66AG* heterozygous; and 27.7% (n= 43) *MTRR 66GG* homozygous. Univariate ANOVA, controlling for age and sex, indicated that *MTRR* genotypes had no significant effect on follow-up Scheltens total score or subscales **(table 17).**

Table 17 THE *MTRR 66A→G* POLYMORPHISM AND WHITE MATTER HYPERINTENSITIES

Univariate ANOVA, adjusting for age and sex, for follow-up Scheltens total score in relation to MTRR 66A→G genotypes.

MTRR	N	FOLLOW-UP SCHELTENS TOTAL SCORE	STD. ERROR	95% CONFIDENCE INTERVAL	P-VALUE (ANOVA)
AA	30	16.6	2.3	(12.1, 21.1)	
AG	82	16.8	1.4	(14.1, 19.4)	NS
GG	43	17.2	1.9	(13.5, 20.9)	

Abbreviations – N (Number of Participants); STD. Error (Standard Error); NS (Not Significant)

The study population was 23.2% (n = 36) *TCN 776CC* homozygous; 54.8% (n = 85) *TCN 776CG* heterozygous; and 21.9% (n = 34) *TCN* 776GG homozygous. Univariate ANOVA, controlling for age and sex, revealed that *TCN* genotypes had no significant effect on follow-up Scheltens total score or subscales **(table 18).**

Table 18 THE *TCN 776C→G* POLYMORPHISM WHITE MATTER HYPERINTENSITIES

Univariate ANOVA, adjusting for age and sex, for follow-up Scheltens total score in relation to TCN 776C→G genotypes.

TCN	N	FOLLOW-UP SCHELTENS TOTAL SCORE	STD. ERROR	95% CONFIDENCE INTERVAL	P-VALUE (ANOVA)
CC	36	14.9	2.1	(10.7, 19.1)	
CG	85	17.9	1.3	(15.3, 20.5)	NS
GG	34	16.3	1.9	(12.4, 20.2)	

Abbreviations – N (Number of Participants); STD. Error (Standard Error); NS (Not Significant)

Dose respnse relationships between *APOE ε4*, *MTHFR 677C→T*, *MTRR 66A→G*, and *TCN 776C→G* polymorphisms and follow-up Scheltens total score and subscales were not significant in unadjusted or adjusted analyses.

IX.FURTHER ANALYSIS OF RISK FACTORS

According to the above results age, tHcy, DBP, and SBP appear to be the most prominent risk factors associated with WMH. In order to evaluate these risk factors further, stepwise linear regression models were performed. Sex was also included in the model.

Linear stepwise regression revealed that age and baseline DBP were positively associated with Scheltens follow-up total score **(table 19)** in the total population. Analyses for the longer-term subgroup indicated that age and baseline DBP were significantly associated with follow-up Scheltens total score **(table 19.b).**

Table 19 AGE AND DIASTOLIC BLOOD PRESSURE

Linear stepwise regression model for follow-up Scheltens total score (included age, sex, systolic blood pressure, diastolic blood pressure, logarithmic values for total homocysteine) for the entire study cohort (n = 135).

MODEL, SCHELTENS FOLLOW-UP	BETA	SIGN.	UNADJUSTED CORRELATION	PARTIAL CORRELATION
AGE	0.29	0.001	0.28	0.30
BASELINE DBP	0.18	0.04	0.16	0.18

Table 19.b *Linear stepwise regression model for follow-up Scheltens total score (included age, sex, systolic blood pressure, diastolic blood pressure, logarithmic values for total homocysteine) for the longer-term subgroup with 4 to 5 years between baseline and follow-up measures (n = 80).*

MODEL, SCHELTENS FOLLOW-UP	BETA	SIGN.	UNADJUSTED CORRELATION	PARTIAL CORRELATION
AGE	0.31	0.004	0.30	0.32
BASELINE DBP	0.22	0.04	0.20	0.23

Abbreviations – Sign. (Significance); DBP (Diastolic Blood Pressure)

Stepwise linear regression revealed that age was positively associated with follow-up Scheltens DWMH subscale (partial $r = 0.30$, $p < 0.001$), PVH subscale (partial $r = 0.26$, $p < 0.001$), and BGH subscale (partial $r = 0.28$, $p < 0.001$).

Stepwise linear regression analyses for the longer-term subgroup indicated that baseline age was associated to follow-up Scheltens subscales DWMH (partial $r = 0.32$, $p < 0.003$), PVH (partial $r = 0.26$, $p < 0.02$), and BGH (partial $r = 0.32$, $p < 0.04$).

CHAPTER FOUR
COGNITIVE ASSESSMENTS: THE FINDINGS

I. DEPRESSION

Depression was assessed using the GDS score; only 1.1% showed severe depressive symptoms; 14.1% moderate; and 84.8% showed no signs of depression at follow-up. All participants with major depressive disorder were excluded. Only 92 participants were administered the GDS at follow-up. Univariate ANOVA, controlling for age and sex, indicated that when the study population was dichotomized by the median split of the baseline Scheltens total score, there was a borderline significant difference between follow-up GDS scores; however there was a significant difference between GDS scores when the baseline Scheltens DWMH subscale was dichotomized by the median split value **(table 20).**

The same analyses for the longer term subgroup indicated that baseline Scheltens total score had a significant effect on the follow-up GDS score. The baseline DMWH subscale was the only subscale that showed a significant effect on the follow-up GDS score **(table 20.b).**

Table 20 GERIATRIC DEPRESSION SCALE IN RELATION TO WHITE MATTER HYPERINTENSITIES

Univariate ANOVA, adjusting for age and sex, for follow-up GDS scores in relation to the baseline median split of Scheltens total score and DWMH subscale in the entire cohort.

	N	GDS FOLLOW-UP	STD. ERROR	95% CONFIDENCE INTERVAL	P-VALUE (ANOVA)
BASELINE SCHELTENS TOTAL < 10	46	4.1	0.66	(2.8, 5.4)	0.07
BASELINE SCHELTENS TOTAL ≥ 10	46	5.9	0.66	(4.5, 7.2)	
BASELINE DWMH SUBSCALE ≤ 5	46	4.1	0.60	(2.9, 5.3)	0.03
BASELINE DWMH SUBSCALE > 5	46	6.2	0.73	(4.8, 7.7)	

Table 20.b *Univariate ANOVA, adjusting for age and sex, for follow-up GDS scores in relation to the baseline median split of Scheltens total score and DWMH subscale for the longer-term subgroup.*

	N	GDS FOLLOW-UP	STD. ERROR	95% CONFIDENCE INTERVAL	P-VALUE (ANOVA)
BASELINE SCHELTENS TOTAL < 10	22	2.7	0.78	(1.1, 4.2)	0.03
BASELINE SCHELTENS TOTAL ≥ 10	23	5.2	0.76	(4.0, 6.8)	
BASELINE DWMH SUBSCALE ≤ 5	22	2.7	0.69	(1.3, 4.1)	0.01
BASELINE DWMH SUBSCALE > 5	23	5.8	0.82	(4.1, 7.4)	

Abbreviations – GDS (Geriatric Depression Scale); DWMH (Deep White Matter Hyperintensities)

Prospective linear regression analyses, adjusting for age and sex, indicated that baseline Scheltens total score **(figure 27)** and DWMH subscale were positively associated with follow-up GDS scores 2 to 3 years later (n = 92). Follow-up Scheltens total score and DWMH subscale were also positively associated to follow-up GDS scores; however baseline cross-sectional analyses were not significant **(table 21)**.

Prospective linear regression analyses for the longer-term subgroup (n = 45) indicated that baseline Scheltens total score and DWMH, PVH, and BGH subscales were positively associated with follow-up GDS scores. Baseline and follow-up cross-sectional analyses for the longer-term subgroup followed the same trends as the entire study cohort **(table 21b)**.

Table 21 GERIATRIC DEPRESSION SCALE AND WHITE MATTER HYPERINTENSITIES

Partial r correlations are shown for baseline and follow-up GDS scores and baseline and follow-up Scheltens scores (Total, DWMH, PVH, and BGH) for the entire study cohort with follow-up GDS scores (n = 92) when adjusted for age and sex.

		GDS	
	TIME	**B**	**F**
	N	92	92
SCHELTENS TOTAL	**B**	NS	**0.27*** **
SCHELTENS TOTAL	**F**	NS	**0.23** **
DWMH SUBSCALE	**B**	NS	**0.33*** **
DWMH SUBSCALE	**F**	NS	**0.30*** **
PVH SUBSCALE	**B**	NS	NS
PVH SUBSCALE	**F**	NS	NS
BGH SUBSCALE	**B**	NS	NS
BGH SUBCALE	**F**	NS	NS

Table 21.b *Partial r correlations are shown for baseline and follow-up GDS scores and baseline and follow-up Scheltens scores (Total, DWMH, PVH, and BGH) for the longer-term subgroup (n = 45) when adjusted for age and sex.*

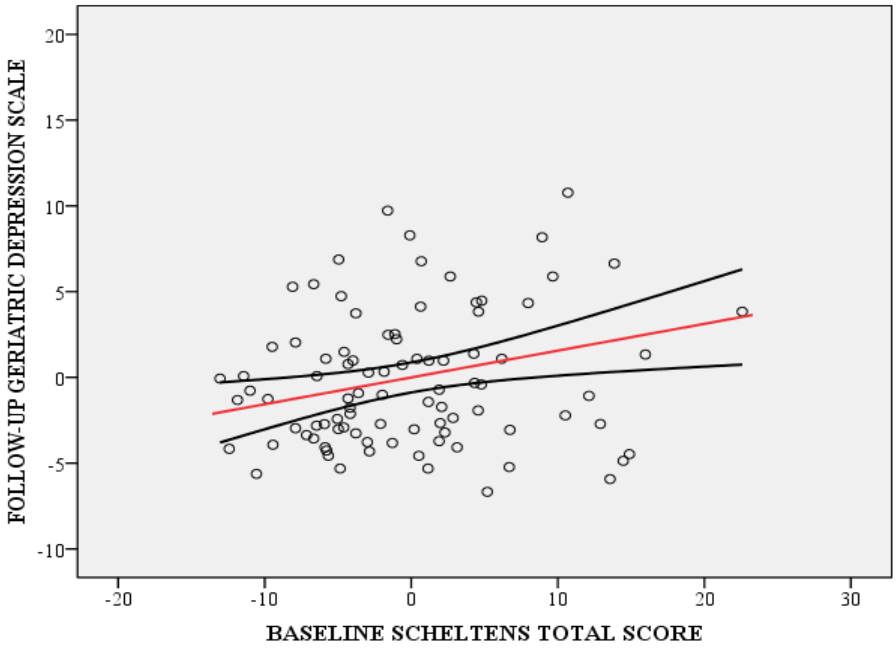
		GDS	
	TIME	**B**	**F**
	N	45	45
SCHELTENS TOTAL	**B**	NS	**0.35** **
SCHELTENS TOTAL	**F**	NS	**0.46*** **
DWMH SUBSCALE	**B**	NS	**0.49*** **
DWMH SUBSCALE	**F**	NS	**0.30*** **
PVH SUBSCALE	**B**	NS	**0.43*** **
PVH SUBSCALE	**F**	NS	NS
BGH SUBSCALE	**B**	NS	**0.31** **
BGH SUBSCALE	**F**	NS	NS

Abbreviations – B (Baseline); F (Follow-up); N (Number of Participants); GDS (Geriatric Depression Scale); DWMH (Deep White Matter Hyperintensities); PVH (Periventricular Hyperintensities); BGH (Basal Ganglia Hyperintensities)

p < 0.01; **p < 0.05; NS (Not Significant)

Figure 27 GERIATRIC DEPRESSION SCALE AND WHITE MATTER HYPERINTENSITIES

Linear regression with 95% CI lines is shown for baseline Scheltens total score in relation to the follow-up Geriatric Depression Scale 2 to 3 years later for the entire study population (partial r = 0.27, p < 0.01) (n = 92).



II. COGNITIVE ASSESSMENTS

All participants underwent a variety of tests designed to detect cognitive impairment in various cognitive domains. The most sensitive test measures indicated that 30.9% (n = 47) of the study sample had some form of cognitive impairment at baseline. Mean MMSE scores were 28.3 for the entire study population and did not vary significantly across baseline age groups. None of the participants scored below 24 at baseline, which is used as a cut-off for identifying dementia. The mean CAMCOG score was 97.8, none of the participants scored below the cut-off score of 80 for cognitive impairment at baseline.

Models controlling for baseline age, sex, and years of education were evaluated for all tests; the logarithm for all tests with a skewed distribution was used to normalize the distribution. The aim was to see if baseline WMH were associated with performance on follow-up cognitive tests 2 to 5 years later.

Global Function

Linear regression analyses, adjusting for age, sex, and years of education, showed that follow-up CAMCOG and MMSE were not associated with baseline Scheltens total score or subscales in any analyses.

Episodic Memory

Several tests of episodic memory (Paired Associates Learning Test, Pattern and Spatial Recognition Memory (RM) Tests, The Placing Test (TPT), and Delayed and Free Recall Word List Memory Test) were evaluated in relation to Scheltens total score and subscales. Only the RM and WLM tests revealed significant correlations in adjusted analyses; partial r correlations, adjusted for age, sex, and years of education are listed in **table 22** for the entire cohort and in **table 22b** for the longer-term subgroup.

In prospective analyses, baseline Scheltens total score **(figure 28)** and DWMH and PVH subscales were inversely associated with the follow-up free-recall task. This indicates that WMH at baseline negatively impacted performance on the WLM Test. The BGH subscale was not significantly associated with the test. When adjusting for baseline test performance in addition to age, sex and years of education, baseline Scheltens total score (partial $r = -0.19$, $p < 0.03$) and DWMH subscale (partial $r = -0.26$, $p < 0.003$) remained associated to the follow-up free-recall task. Prospective analyses for the delayed recall portion of the WLM test indicated that performance at follow-up approached significance to baseline Scheltens total score and PVH subscale and was associated with the Scheltens BGH subscale. The baseline DWMH subscale was not significantly associated with follow-up delayed WLM outcome measures.

The same analyses for the longer-term subgroup 4 to 5 years later indicated that only the follow-up free-recall portion of the WLM test was significantly associated with baseline Scheltens total score and the DWMH, PVH and BGH subscales. Follow-up outcome

measures for the SRM, PRM, PAL and TPT were not significantly associated with baseline Scheltens total score or subscales for either set of analyses.

Table 22 EPISODIC MEMORY AND WHITE MATTER HYPERINTENSITIES

Partial r correlations are shown for Scheltens scores (Total, DWMH, PVH, and BGH) and episodic memory tests for the entire study cohort when controlling for age, sex, and years of education.

		TESTS OF EPISODIC MEMORY							
		DWLM		FWLM		PRM		SRM	
	TIME	B	F	B	F	B	F	B	F
	N	123	123	123	123	120	120	121	121
SCHELTENS TOTAL	B	**-0.16***	**-0.17***	**-0.22*****	**-0.26*****	**-0.18****	NS	NS	NS
SCHELTENS TOTAL	F	NS	**-0.16***	NS	**-0.26*****	NS	NS	**-0.17***	NS
DWMH SUBSCALE	B	**-0.15***	NS	**-0.17****	**-0.29*****	**-0.25*****	NS	NS	NS
DWMH SUBSCALE	F	NS	NS	NS	**-0.32*****	NS	NS	NS	NS
PVH SUBSCALE	B	NS	**-0.16***	**-0.17****	**-0.19****	NS	NS	**-0.19****	NS
PVH SUBSCALE	F	NS	**-0.17***	NS	**-0.20****	NS	NS	**-0.17***	NS
BGH SUBSCALE	B	**-0.16****	**-0.24***	**-0.19****	NS	NS	NS	**-0.15***	NS
BGH SUBSCALE	F	NS	**-0.17***	NS	NS	NS	NS	**-0.19****	NS

Table 22.b *Partial r correlations are shown for Scheltens scores (Total, DWMH, PVH, and BGH) and episodic memory tests for the longer-term subgroup when adjusted for age, sex, and years of education.*

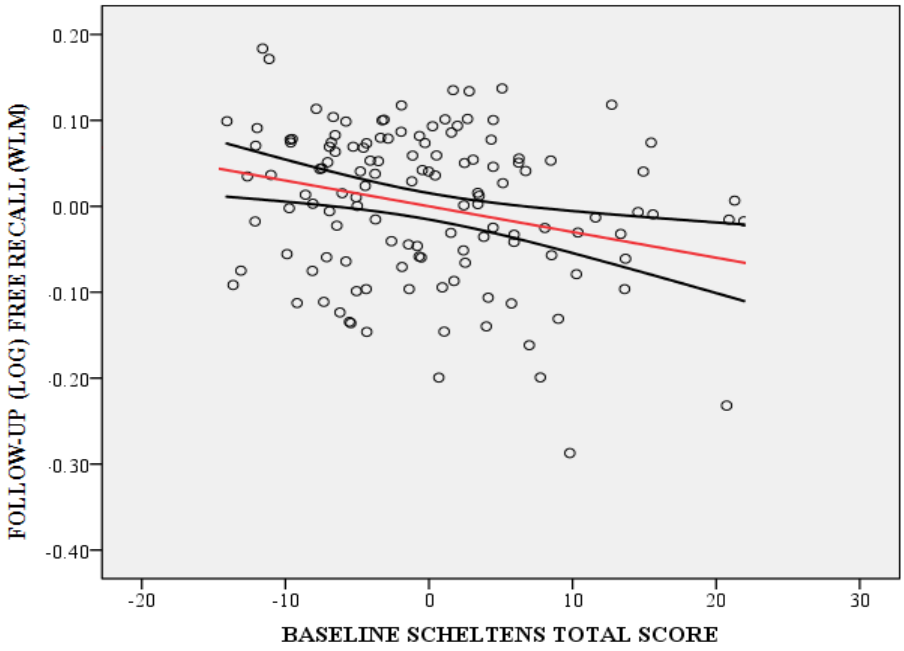
		FWLM	
	TIME	B	F
	N	79	79
SCHELTENS TOTAL	B	NS	**-0.36*****
SCHELTENS TOTAL	F	NS	**-0.33****
DWMH SUBSCALE	B	NS	**-0.37*****
DWMH SUBSCALE	F	NS	**-0.39*****
PVH SUBSCALE	B	NS	**-0.33****
PVH SUBSCALE	F	NS	**-0.35****
BGH SUBSCALE	B	NS	**-0.26****
BGH SUBSCALE	F	NS	NS

Abbreviations – B (Baseline); F (Follow-up); N (Number of Participants); DWML (Delayed Word List Memory); FWML (Free Recall Word List Memory); PRM (Pattern Recognition Memory); SRM (Spatial Recognition Memory)

******p < 0.01; **p < 0.05;*p < 0.10; NS (Not Significant)***

Figure 28 WHITE MATTER HYPERINTENSITIES AND EPISODIC MEMORY

Linear regression with 95% CI lines is shown for the prospective relationship between baseline Scheltens total score and the follow-up logarithm of the free-recall portion of the WLM Test 2 to 5 years later when adjusted for age, sex, and years of education (partial r = -0.27, p < 0.003) (n = 123).



Semantic Memory

Prospective linear regression analyses, adjusted for age, sex, and years of education, revealed that the baseline Scheltens DWMH subscale was inversely associated with the GNT at follow-up; however additional measures of baseline WMH were not associated to performance on the GNT **(table 23).** This association remained significant ($p < 0.03$) when also controlling for baseline GNT performance. Cross-sectional analyses indicated that follow-up Scheltens DWMH subscale was inversely associated to performance on the GNT at follow-up; however baseline cross-sectional analyses were not significant. Therefore, hyperintensities in the subcortical white matter were most strongly associated with performance on this semantic memory test, but the associations did not hold for the longer-term subgroup when adjusted for age, sex, and years of education. It is also noteworthy that associations between WMH and working memory were not significant.

Table 23 SEMANTIC MEMORY AND WHITE MATTER HYPERINTENSITIES

Partial r correlations are shown for the GNT and Scheltens scores (Total, DWMH, PVH, BGH) for the entire study cohort (n = 128) when adjusted for age, sex, and years of education.

		GNT	
	TIME	**B**	**F**
	N	128	128
SCHELTENS TOTAL	**B**	NS	NS
SCHELTENS TOTAL	**F**	NS	NS
DWMH SUBSCALE	**B**	NS	**-0.21****
DWMH SUBSCALE	**F**	NS	**-0.22*****
PVH SUBSCALE	**B**	NS	NS
PVH SUBSCALE	**F**	NS	NS
BGH SUBSCALE	**B**	NS	NS
BGH SUBSCALE	**F**	NS	NS

Abbreviations – B (Baseline); F (Follow-up); GNT (Graded Naming Test); DWMH (Deep White Matter Hyperintensities); PVH (Periventricular Hyperintensities); BGH (Basal Ganglia Hyperintensities)
****** p < 0.01; **p < 0.05; NS (Not Significant)***

Executive Function

Some assessments of executive function demonstrated strong significant relationships to baseline Scheltens scores. Prospective associations between follow-up measures for the Spatial Span Test were nearly associated with baseline Scheltens total score and were inversely associated to baseline subscales PVH and BGH subscales in adjusted analyses for age, sex, and years of education. The baseline Scheltens DWMH subscale was not significantly associated with test performance on the follow-up Spatial Span Test **(table 24)**.

The same analyses for the longer-term subgroup indicated that baseline Scheltens total score and PVH and BGH subscales were all associated with decreased performance on the follow-up Spatial Span Test 4 to 5 years later; but not the DWMH subscale. Cross-sectional analyses at follow-up also indicated that the Scheltens total score, PVH, and BGH

subscales were associated with decreased performance on the Spatial Span Test at follow-up; however baseline cross-sectional analyses were not statistically significant **(table 24b)**.

Prospective analyses, adjusted for age, sex, and years of education, for baseline Scheltens total score **(figure 28)**, DWMH, PVH, and BGH subscales were associated with decreased performance on the SDMT at follow-up. Baseline Scheltens total score (partial $r = -0.17$, $p < 0.06$) and DWMH subscale (partial $r = -0.19$, $p < 0.05$) remained significant to the SDMT at follow-up when also controlling for baseline performance on the SDMT. Baseline and follow-up cross-sectional analyses for the Scheltens total score and subscales were also associated with decreased performance on the SDMT **(table 24)**.

The same analyses for the longer-term subgroup indicated that baseline Scheltens total score and the Scheltens subscales DWMH, PVH, and BGH were also associated with decreased performance on the SDMT. Cross-sectional analyses at baseline and follow-up also indicated that markers of WMH were inversely associated with performance on the SDMT **(table 24b)**.

Table 24 EXECUTIVE FUNCTION AND WHITE MATTER HYPERINTENSITIES

Partial r correlations are shown for Scheltens scores (Total, DWMH, PVH, BGH) and tests of executive function for the entire study cohort when adjusted for age, sex, and years of education.

	TESTS OF EXECUTIVE FUNCTION				
		SDMT		SSPAN	
	TIME	B	F	B	F
	N	121	121	130	130
SCHELTENS TOTAL	B	-0.32***	-0.30***	NS	-0.16*
SCHELTENS TOTAL	F	-0.28***	-0.37***	NS	NS
DWMH SUBSCALE	B	-0.28***	-0.26***	NS	NS
DWMH SUBSCALE	F	-0.25***	-0.37***	NS	NS
PVH SUBSCALE	B	-0.25***	-0.25***	NS	-0.19**
PVH SUBSCALE	F	-0.27***	-0.30***	NS	-0.16*
BGH SUBSCALE	B	-0.28***	-0.27***	NS	-0.23***
BGH SUBSCALE	F	-0.19**	-0.25*	NS	-0.21*

Table 24.b *Partial r correlations are shown for Scheltens scores (Total, DWMH, PVH, BGH) and tests of executive function for the longer-term subgroup when adjusted for age, sex, and years of education.*

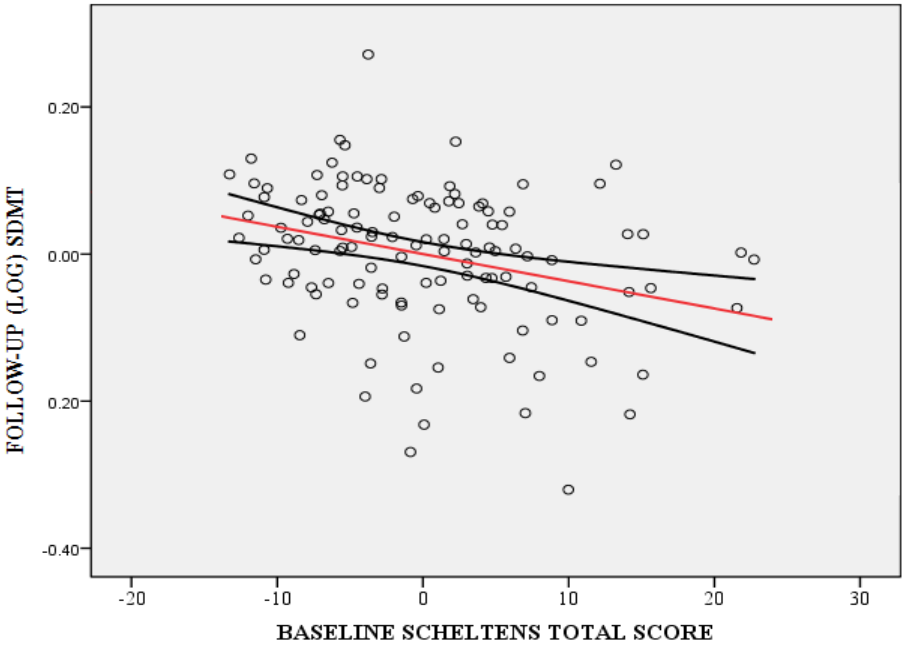
		SDMT		SSPAN	
	TIME	B	F	B	F
	N	79	79	79	79
SCHELTENS TOTAL	B	-0.22**	-0.41***	NS	-0.30**
SCHELTENS TOTAL	F	-0.27**	-0.45***	NS	-0.27**
DWMH SUBSCALE	B	-0.20*	-0.38***	NS	NS
DWMH SUBSCALE	F	-0.28**	-0.47***	NS	-0.20*
PVH SUBSCALE	B	-0.20**	-0.34**	NS	-0.23**
PVH SUBSCALE	F	-0.23**	-0.36**	NS	-0.24**
BGH SUBSCALE	B	-0.20*	-0.35**	NS	-0.41***
BGH SUBSCALE	F	-0.20*	-0.31**	NS	-0.27**

Abbreviations – B(Baseline); F (Follow-up); SDMT (Symbol Digit Modalities Test); Trails (Trails A and B); SSPAN (Spatial Span); DWMH (Deep White Matter Hyperintensities); PVH (Periventricular Hyperintensities); BGH (Basal Ganglia Hyperintensities)

****** p < 0. 01; **p < 0.05; *p < 0.10; NS (Not Significant)***

Figure 28 EXECUTIVE FUNCTION AND WHITE MATTER HYPERINTENSITIES

Linear regression with 95% CI lines is shown for the prospective relationship between baseline Scheltens total score and the follow-up logarithmic value of the SDMT for the entire study cohort when adjusted for age, sex, and years of education (partial r = -0.30, p < 0.001) (n = 121).



Processing Speed

The Pattern (PCS) and Letter (LCS) Comparison Speed Tests that measure processing speed were both individually significant; however the outcome measure that combines both PCS and LCS measures was used in analyses. The adjusted associations between WMH load and the outcome measure of processing speed are displayed for the entire study population in **table 25.** Prospective analyses indicated that baseline Scheltens total score **(figure 29)**, DWMH, PVH, and BGH subscales were inversely associated with the outcome measure of processing speed when adjusted for age, sex, and years of education. Baseline Scheltens total score (partial r = -0.20, p < 0.03) and DWMH subscale (partial r = -0.24, p < 0.007) remained associated to follow-up processing speed when also adjusted for baseline test performance on the same test.

Linear regression analyses for the longer-term subgroup 4 to 5 years later indicated that baseline Scheltens total score and DWMH subscale were associated to processing speed at

follow-up. Baseline cross-sectional analyses revealed similar associations; however follow-up associations indicated an additional association between follow-up PVH and follow-up outcome measures of processing speed **(table 25b).** .

Table 25 PROCESSING SPEED AND WHITE MATTER HYPERINTENSITIES

Partial r correlations are shown for the combined pattern and letter comparison speed outcome measure and Scheltens scores (Total, DWMH, PVH, BGH) for the whole cohort when adjusted for age, sex, and years of education (n = 129).

		SPEED	
	TIME	B	F
	N	129	129
SCHELTENS TOTAL	B	-0.26***	-0.29***
SCHELTENS TOTAL	F	-0.21**	-0.29***
DWMH SUBSCALE	B	-0.23***	-0.29***
DWMH SUBSCALE	F	-0.19***	-0.33***
PVH SUBSCALE	B	-0.20**	-0.19**
PVH SUBSCALE	F	-0.18**	-0.24***
BGH SUBSCALE	B	-0.21***	-0.20**
BGH SUBSCALE	F	-0.18**	NS

Abbreviations – B(Baseline); F (Follow-up); SPEED (Pattern + Letter Comparison Speed); DWMH (Deep White Matter Hyperintensities); PVH (Periventricular Hyperintensities); BGH (Basal Ganglia Hyperintensities)

****** p < 0.01; **p < 0.05; *p < 0.10; NS (Not Significant)***

Table 25.b *Partial r correlations are shown for the logarithmic value of the combined pattern and letter comparison speed outcome measure and Scheltens scores (Total, DWMH, PVH, BGH) for the longer-term subgroup when adjusted for age, sex, and years of education (n = 79).*

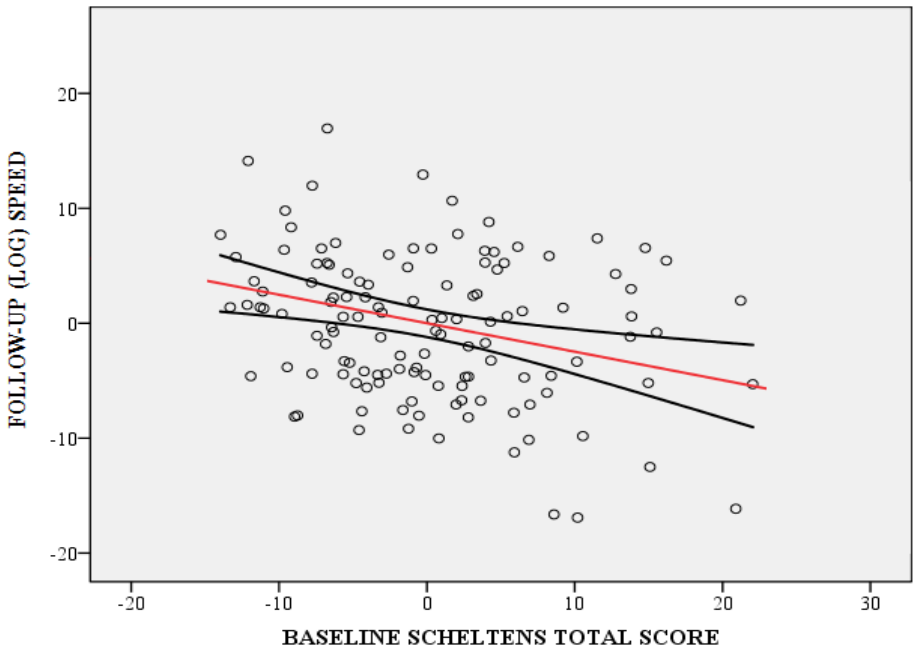
		SPEED	
	TIME	B	F
	N	79	79
SCHELTENS TOTAL	B	-0.24**	-0.46***
SCHELTENS TOTAL	F	-0.27**	-0.34**
DWMH SUBSCALE	B	-0.26***	-0.39***
DWMH SUBSCALE	F	-0.29**	-0.40***
PVH SUBSCALE	B	NS	-0.24**
PVH SUBSCALE	F	NS	-0.34**
BGH SUBSCALE	B	-0.21*	-0.28**
BGH SUBSCALE	F	NS	NS

Abbreviations – B(Baseline); F (Follow-up); SPEED (Pattern + Letter Comparison Speed); DWMH (Deep White Matter Hyperintensities); PVH (Periventricular Hyperintensities); BGH (Basal Ganglia Hyperintensities)

****** p < 0.01; **p < 0.05; *p < 0.10; NS (Not Significant)***

Figure 29 PROCESSING SPEED AND WHITE MATTER HYPERINTENSITIES

Linear regression with 95% CI lines is shown for the prospective association between baseline Scheltens total score and logarithmic value of the follow-up letter and pattern comparison speed test total for the entire study cohort when adjusted for age, sex, and years of education (partial r = -0.29, p < 0.001) (n = 129).



CHAPTER FIVE
AUTOMATED BRAIN VOLUME MEASURES

This study also explored the relationship between WMH and brain volume measures. Linear regression analyses were conducted to see if baseline WMH were associated with follow-up brain volume measures 2 to 5 years later **(table 26)** and also for the longer-term subgroup with 4 to 5 years between baseline and follow-up MR images **(table 26.b).** All analyses were adjusted for age and diastolic blood pressure; however, since previous analyses from this study indicate that sex is not associated with WMH, it was not adjusted for in these analyses.

Results indicated that baseline Scheltens total score **(figure 30)** and DWMH were significantly inversely related to follow-up measures of grey matter volume; the baseline PVH subscale was borderline significant. These associations remained significant for analyses of the longer-term subgroup. Surprisingly, baseline representations of WMH were not associated with white matter volume in any analyses.

Baseline Scheltens total score and PVH subscale approached significance to follow-up measures of VCSF volume, while the baseline DWMH subscale was positively associated with follow-up VCSF. These associations were comparable to analyses for the longer-term subgroup.

Percent brain volume change was assessed by subtracting the total brain volume at baseline from the total brain volume at follow-up. Scheltens total score and DWMH subscale were negatively associated with measures of brain volume change assessed 2 to 5 years later, but not for the longer-term subgroup.

Table 26 BRAIN VOLUME MEASURES AND WHITE MATTER HYPERINTENSITIES

Partial r correlations are shown for baseline and follow-up for brain volume measures and Scheltens scores (Total, DWMH, PVH, BGH) for the entire study cohort (n = 135). All analyses adjusted for age and diastolic blood pressure.

		GREY MATTER		WHITE MATTER		VCSF		PBVC
	TIME	B	F	B	F	B	F	F-B
	N	135	135	135	135	135	135	135
SCHELTENS TOTAL	B	NS	**-0.17****	NS	NS	NS	**0.15***	**-0.14***
SCHELTENS TOTAL	F	NS	**-0.16***	NS	NS	NS	**0.19****	**-0.16***
DWMH SUBSCALE	B	NS	**-0.18****	NS	NS	NS	**0.19****	**-0.16****
DWMH SUBSCALE	F	NS	**-0.17***	NS	NS	NS	**0.23*****	**-0.17****
PVH SUBSCALE	B	**-0.17****	**-0.15***	NS	NS	NS	**0.17***	NS
PVH SUBSCALE	F	NS	**-0.15***	NS	NS	NS	**0.19****	NS
BGH SUBSCALE	B	NS	NS	NS	NS	NS	NS	NS
BGH SUBSCALE	F	NS	NS	NS	NS	NS	NS	**-0.16***

Table 26.b *Partial r correlations are shown for baseline and follow-up for brain volume measures and Scheltens scores (Total, DWMH, PVH, BGH) for the longer-term subgroup with 4 to 5 years between measures (n = 80) when adjusted for age and diastolic pressure.*

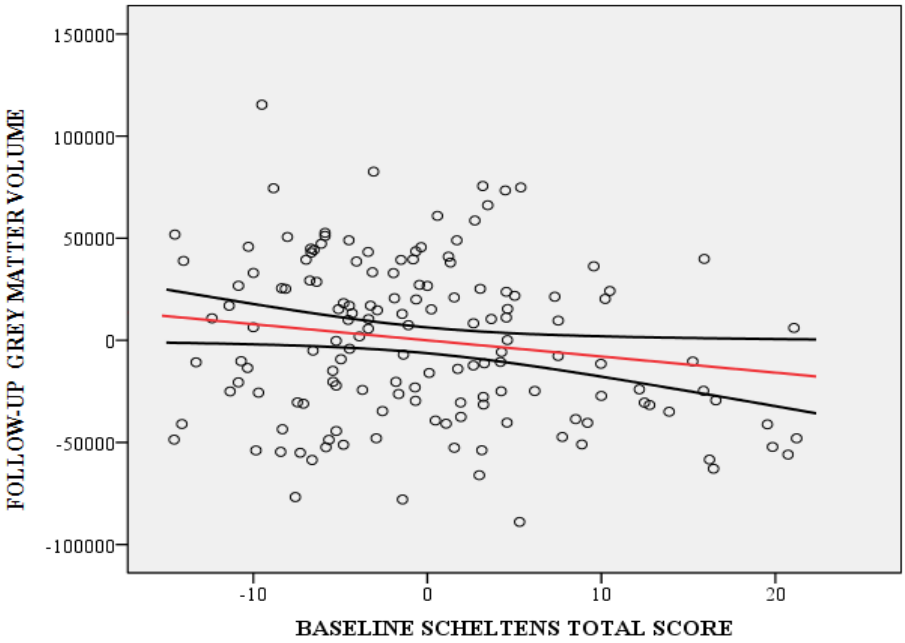
		GREY MATTER		WHITE MATTER		VCSF		PBVC
	TIME	B	F	B	F	B	F	F-B
	N	80	80	80	80	80	80	80
SCHELTENS TOTAL	B	NS	**-0.26****	NS	NS	NS	NS	NS
SCHELTENS TOTAL	F	NS	NS	NS	NS	NS	**0.25****	NS
DWMH SUBSCALE	B	NS	**-0.23****	NS	NS	NS	**0.19***	NS
DWMH SUBSCALE	F	NS	NS	NS	NS	NS	**0.26****	NS
PVH SUBSCALE	B	NS	**-0.23****	NS	NS	NS	**0.21****	NS
PVH SUBSCALE	F	NS	NS	NS	NS	NS	**0.24****	NS
BGH SUBSCALE	B	NS	**-0.23****	NS	NS	NS	NS	**-0.21***
BGH SUBSCALE	F	NS	NS	NS	NS	NS	NS	NS

Abbreviations – B(Baseline); F(Follow-up); N (Number of Participants); PG (Peripheral Grey Matter Volume), VCSF (Ventricular Cerebral Spinal Fluid), and PBVC (Percent Brain Volume Change); DWMH (Deep White Matter Hyperintensities); PVH (Periventricular Hyperintensities); BGH (Basal Ganglia Hyperintensities)

******p < 0.01; **p < 0.05; *p <0 .10; NS (Not Significant)***

Figure 30 WHITE MATTER HYPERINTENSITIES AND GREY MATTER VOLUME

Linear regression with 95% CI lines is shown for the prospective relationship for Scheltens baseline total score and follow-up measures of grey matter volume for the entire study cohort (n = 135) when adjusted for age and diastolic blood pressure (partial r = -0.17, p< 0.04).



CHAPTER SIX
POST MORTEM DIAGNOSIS

There is insufficient evidence from our study population to evaluate the relationship between *post mortem* diagnosis and white matter damage in depth. Of the participants with available *post mortem* reports (n = 24), 12.5% (n = 3) exhibited abnormal pathology **(table 27)**; one participant (4.2%) was diagnosed with AD; and the remaining 83.3% (n = 20) were normal controls without pathology. The participant diagnosed with AD had a baseline Scheltens total score of 32, notably higher than the baseline mean Scheltens total score for control subjects (16.7), moreover the participant with AD died before completing his follow-up exam. On average, the 3 participants demonstrating some pathology had higher Scheltens total scores at both baseline and follow-up.

Table 27 WHITE MATTER HYPERINTENSITIES AND POST MORTEM RESULTS

The study population post mortem diagnoses and mean baseline and follow-up Scheltens total score. The average age for each group is also outlined.

POST MORTEM DIAGNOSIS	N	MEAN BASELINE AGE (SD)	SCHELTENS BASELINE	SCHELTENS FOLLOW
ALZHEIMER'S DISEASE	**1**	**81**	**32**	**N/A**
CONTROL WITH PATHOLOGY	**3**	**75 (5.2)**	**20**	**25.3**
C1008 (POSSIBLE AD)	1		36	36
C1077 (CEREBROVASCULAR DISEASE, WITH SEVERE AMYLOID ANGIOPATHY, INFARCT)	1		19	31
C1007 (PARKINSON'S DISEASE)	1		5	9
CONTROL, NO PATHOLOGY	**20**	**79 (3.5)**	**16.7**	**19.9**

Abbreviations: SD (Standard Deviation)

CHAPTER SEVEN
DISCUSSION

The strengths of this study include the broad number of factors evaluated prospectively in relation to WMH. Moreover, this study investigated the regional influence of WMH, which is often overlooked by other studies. The study population consisted of a healthy elderly population aged 60 to 90 years, reducing the effect of potentially confounding illnesses. On the other hand, the study population's good overall health most likely influenced the sparse number of participants that smoke or have diabetes, thus limiting sufficient numbers for the credible interpretation of analyses. Other limitations include the racial and socioeconomic homogeneity of the study population, comprised entirely of Caucasian, primarily middle-class participants. Due to the time constraints of this thesis, WMH were only assessed by the student. Future analyses involving this data would require an additional rater to confirm the author's reliability.

The main findings in this healthy elderly population is that older age, higher diastolic blood pressure, and elevated blood concentration levels of tHcy at baseline were all positively associated with WMH at follow-up 2 to 5 years later. These associations will be discussed in greater detail in this section. Among the risk factors that were not significant were sex, the concentrations of creatinine, folate, MMA, transcobalmin, the presence of the *APOE* ε4 allele, and polymorphisms of *MTRR 66A→G, MTHFR 677C→T,* and *TCN677C→G*. Insufficient evidence for smoking status and diabetes led to inconclusive findings.

I. AGE

The findings of this study corroborate previously documented evidence (7, 11, 29, 33) indicating that age is a significant risk factor associated with overall WMH. Prospective analyses from this study indicate that age was also positively associated with the WMH in the deep white matter and periventricular regions; however age was not significantly associated with WMH in the basal ganglia region. Although many studies evaluate the regional differences between DMWH and PVH, no known studies have investigated WMH in the basal ganglia regions in relation to risk factors. The null association between BGH and age therefore suggests that the BGH region may differ in terms of etiology in comparison to WMH in the subcortical and periventricular regions.

Since old age is a well established risk factor associated with WMH, it would be gainful for future studies to investigate younger sample populations. One recent study published by Wen and colleagues, identified that small punctuate foci in the subcortical region were common (50.9%) in a study sample of 428 healthy individuals aged 44 to 48 years (145). Hopkins and colleagues studied a cohort aged 16 to 65 years. They identified WMH in 5.3% of their study population and noted that individuals over 55 years of age were 10 times more likely to have WMH (56). Studies such as these should help researchers identify potential modifiable risk factors before WMH affect cognition or become irreversible. In concordance with these findings, a review by Sachdev *et al* suggests that researchers focus their efforts on subjects in their 40s, since WMH are a rarity in young adults and subjects in their 30s (146). Prospective studies following middle-aged subjects into their elder years would be particularly beneficial for identifying modifiable risk factors associated with WMH.

II. SEX

This study was comprised of an equally balanced population (49.0% women) with relatively congruent medical histories. Results indicate that neither men nor women are at greater risk for WMH when controlling for age in all cerebral regions (deep white matter, periventricular, and basal ganglia). Two studies that include younger study participants confirm this finding (56, 145). A two year study also stated that sex was not an associated risk factor for WMH (147) and de Groot and colleagues only found the difference marginally significant (34). Conversely, others cite significant differences between the sexes, with a tendency for women to be at greater risk (11, 53, 54). All three of these studies were much larger (range, 492 to 1920 participants) than the present study's population, but comparable in age and health. Therefore, it could be that this study is too small to identify a difference between men and women.

III. BLOOD PRESSURE

Linear stepwise analyses indicated that age and baseline DBP are positively associated with WMH over time and explained 11% of variation in WMH. This relationship remained significant for participants with 4 to 5 years between measures and explained 14% of variation in WMH. Veldink and colleagues (148) were the first to publish a positive association between increased DBP and WMH in 1998 and subsequent studies add that DBP is more strongly associated with WMH than SBP (148-151) as found in this study.

Just over half (54.9%) of the study population was hypertensive (SBP ≥ 160 mmHg or DBP ≥ 100 mmHg) and/or prescribed antihypertensive medications. For the purposes of this study these participants were defined as hypertensive and the others were considered normotensive. In adjusted analyses for normotensive participants, baseline SBP and DBP were not associated with follow-up WMH assessed 2 to 5 years later (n = 62) or in longer-term analyses 4 to 5 years later (n = 39) for cross-sectional or prospective analyses. As

expected, these relationships were dramatically different in participants with hypertension (n = 73). In these participants, follow-up DBP was positively associated with follow-up measures of overall WMH and WMH in the subcortical and basal ganglia regions, but not the periventricular region at follow-up when adjusting for age and sex. Relationships between SBP and WMH were not significant. Therefore, these results indicate that hypertension, particularly elevated diastolic blood pressure, may be a risk factor for WMH. These results may be biased because hypertensive individuals were not distinguished by the duration of hypertension or by the success of the antihypertensive drugs. Moreover, antihypertensive drugs were prescribed using distinct parameters by the participants' respective doctors. On the other hand, it is likely that a participant with any history of hypertension, successfully treated or not, would be at a greater risk for developing WMH in relation to a participant with no history of hypertension. This study worked under this assumption, as others have done (52).

Other studies confirm that hypertension is associated with WMH (64, 65), despite varying definitions. De Leeuw and colleagues found that a lower threshold of hypertension (SBP ≥ 140 mmHg or DBP ≥ 90 mmHg) was associated with increased WMH over time, indicating that hypertension does not need to be severe to have a negative effect on the brain (64). One study identified that subjects taking antihypertensive drugs with controlled hypertension had a reduced risk of developing WMH (52).

The main explanation for the association between hypertension and WMH is the affect hypertension has on cerebral blood flow. People with hypertension more often suffer from general atherosclerotic disease responsible for the narrowing and stiffening of the blood vessels in the brain which decreases the circulation of blood throughout the brain (152). As a result, the oxygen supply to the brain is reduced, resulting in ischemia. Ischemia consequentially results in cell death and damage to cerebral white matter (30). Notably, if

cell death occurred in the white matter, one would expect white matter volume to be reduced, which did not fit with our findings (see section on **brain volumes**). Therefore, treatment with antihypertensive drugs might prevent further damage, but would not likely reverse existing damage. Studies have shown that subjects with untreated hypertension are at greater risk of WMH than those participants with treated hypertension (52, 153), and one 3 year, placebo-controlled, longitudinal found that a blood pressure lowering regimen stopped or slowed the progression of WMH in stroke patients, but did not improve existing WMH (60). Therefore, if hypertension can be detected in the early stages, it is likely that WMH can be prevented.

In 2009, Dufouil and colleagues attested that most studies that have evaluated blood pressure and WMH have been limited to cross-sectional analyses (52). Therefore, this study is valuable because it contributes prospective findings about the relationship between blood pressure measurements and hypertension and WMH 2 to 5 years later.

IV.HOMOCYSTEINE

Plasma tHcy was not associated with WMH in linear regression after adjusting for age and sex in the whole cohort. On the other hand, baseline tHcy levels in the upper quartile (≥ 14.8 μmol/L) were positively associated with follow-up Scheltens total score and subscales (DWMH, PVH, and BGH) evaluated from MR images 4 to 5 years later. Similarly, Longstreth and colleagues did not find a significant association between tHcy and WMH, but identified some evidence that suggests that elevated tHcy may be related to WMH in further analyses (86).

When examining the population further, results for participants with severe WMH (n = 43) indicated that baseline tHcy was significantly associated with follow-up Scheltens total score and BGH subscale in linear regression analyses after controlling for age and

sex. Due to sample size restrictions this relationship was not explored for the longer-term subgroup. Previous studies have reported using similar methods to evaluate the association of tHcy and WMH (82). A larger study sample would be expected to generate significant findings with the inclusion of less severe WMH as others have found (82, 84, 154, 155) indicating that elevated blood concentrations of tHcy are associated with WMH. Elevated tHcy has been linked to cognitive impairment (77), vascular dementia (88), and AD (87, 156) suggesting that tHcy does play a critical role in mechanisms that negatively influence the brain.

Elevated tHcy is a well established and sensitive marker for vitamin B_{12} and folate deficiency (89). It was therefore unexpected that other measures associated with B vitamin status such as serum folate, plasma vitamin B_{12}, transcobalamin, MMA, and creatinine, were not correlated to WMH independent of age and sex. These findings are in line with others who noted that WMH could not be explained by vitamin B_{12} status, folate levels, or renal function (79, 82). Then again, other studies such as de Lau and colleagues have identified low-normal vitamin B_{12} status (155) or low-normal serum folate status as risk factors for WMH (110).

Elevated tHcy is inextricably linked to B vitamin status and low folate (89), which are also involved in processes involving myelin production. The production of tHcy occurs during the metabolism of methionine, an essential amino acid, which plays an important role in brain function. tHcy is metabolized either by remethylation, which converts tHcy back to methionine, or transsulfuration which converts tHcy to cysteine. These processes rely heavily on vitamin B_{12} and folate, so a deficiency in either would result in an excess of tHcy and a shortage of methionine (157). Methionine is important because it is converted to S-adenosylmethionine (SAM), which is ultimately

responsible for the methylation and stabilization of proteins, including myelin basic protein (158). This is believed to be one of the mechanisms that underlie white matter damage. The myelin basic protein makes up 30% of the protein in myelin, so a deficit in this protein would have a detrimental effect on the formation and maintenance of myelin in the brain (159). Therefore, it is possible that WMH may reflect the deficit in myelin basic protein. This may also explain why some studies have found that WMH decrease with vitamin B_{12} replacement therapy. The studies found that as vitamin $B_{12,}$ increased tHcy levels decreased which would revitalize the production of methionine, SAM, and ultimately the myelin basic protein (90, 99).

Some have also hypothesized that elevated tHcy may contribute to atherosclerosis by damaging the vascular wall (160) which fits with the idea that elevated tHcy compromises cerebrovascular circulation which may result in ischemia and ultimately white matter damage (77). When controlling for blood pressure in linear regression analyses, tHcy was no longer significantly related to WMH. This fits with the idea that tHcy is linked to vascular mechanisms that later contribute to white matter damage. Others have suggested that tHcy has a neurotoxic effect on nerve cells (161), however the present study's findings suggest that white matter volume is not lost so this theory seems less plausible. Prospective intervention studies aimed at lowering tHcy and evaluating white matter change in conjunction with cognitive improvement would clarify the role tHcy plays in cerebral processes involving white matter.

V. DEPRESSION

Although the GDS is a valid measure of depression symptoms and correlated with diagnoses of depression (162), it is not a clinical diagnosis so these results should be interpreted with caution. It should also be noted that OPTIMA recruited this Challenge

cohort through volunteer mechanisms, suggesting that the study population may have been more motivated than the general population, which is uncharacteristic of individuals with depression. Moreover, participants with major depressive disorder were excluded from the study. This study found that baseline WMH were positively associated to measures of probable depression 2 to 3 years later; however not all participants with WMH showed probable signs of depression. Unfortunately, information regarding time of onset or duration of depression was unavailable.

While previous studies concur that white matter damage is associated to depression (42, 45, 46, 163), it is still unclear why. The main hypothesis, according to Herrmann *et al,* is that WMH reflect an underlying condition that predisposes individuals to the development of late life depression (45). The vascular depression hypothesis is based on the idea that cerebrovascular disease can make an individual vulnerable to or exacerbate depression in adults (164). Moreover, individuals with WMH are expected to have persistent depressive symptoms, remission, poorer prognoses, and increased risk of dementia (165, 166). Studies of late onset depression have also been associated with vascular risk factors such as elevated tHcy (50, 122), *MTHFR 677C→T* polymorphisms (121, 167), and vitamin B_{12} or folate deficiency (46, 108) which correspond both with the vascular depression hypothesis and the association of WMH with depression.

WMH in the subcortical region were more strongly associated to depression than WMH in the periventricular region in this study. The regional influence of WMH on depression is not well understood; however previous studies support these findings (21, 41, 168) and Tupler and colleagues noted that this finding 'agrees with most' (21). Previous studies, such as one by Hickie and colleagues, note that subcortical WMH are associated with increased age and late onset of depression (42, 46, 163). Taylor and colleagues suggest that subcortical ischemia is associated with depression, which may explain the relationship

to depression and subcortical WMH (166). Neuropathological studies confer that subcortical WMH are associated with atherosclerosis and vascular changes consistent with the vascular depression hypothesis (26, 169, 170). It therefore seems plausible that this study found that baseline subcortical WMH were most strongly associated with increased depressive symptoms.

VI.COGNITIVE ASSESSMENTS

The present study aimed to identify if WMH at baseline influence cognitive test performance 2 to 5 years later and for the longer-term subgroup 4 to 5 years later. This study also considered the association of regional WMH on specific cognitive tests. Decreased performance on tests of processing speed and executive function were most strongly associated with increased WMH based on a comprehensive cognitive assessment comprised of tests designed to measure global cognitive function, executive function, memory (episodic and semantic), and processing speed.

The CAMCOG and MMSE are both common tests used to assess global cognitive function however both have failed to detect milder cases of cognitive impairment (126). Therefore, it is not surprising that decreased performance on the MMSE and CAMCOG were not associated with WMH. Findings by Au *et al* concur that there was no significant association between WMH and measures of global cognitive function (27). De Groot and colleagues, however, identified that decreased performance on the MMSE was significantly correlated with an increased presence of WMH in the periventricular region (34). These discrepancies may be due to methodological differences used to rate WMH.

The present study found that baseline WMH negatively influenced test performance on the free-recall task from the WLM test 2 to 3 years later, but was only weakly

associated to other tests designed to assess episodic and semantic memory. There appeared to be no regional effect of WMH on tests of memory since test performance was significantly influenced by baseline WMH in the subcortical and periventricular regions. Silbert and colleagues also found that baseline DWMH significantly influenced memory impairment over time in a similarly sized (n = 104), cognitively intact study population; however they did not find that PVH were associated with memory impairment (24). They implemented an automated volumetric WMH rating system which may account for this difference. Au and colleagues noted that WMH significantly influenced visuospatial memory, but not verbal memory (27). The present study found the opposite; WMH were not associated to visuospatial memory, but are associated to verbal memory. This is most likely due to methodologic differences used to identify visuospatial and verbal memory deficits. This also highlights a common issue; most studies cannot be compared directly. In general, studies often report associations between memory deficits and WMH; however many emphasize that other cognitive domains, such as executive function and processing speed are more strongly associated with WMH (24, 27).

Baseline WMH were also negatively associated with decreased performance on executive function tasks 2 to 5 years later and in the longer-term group 4 to 5 years later. Baseline WMH in all regions (DWMH, PVH, and BGH) were associated with impaired test performance on the SDMT; however baseline WMH in the subcortical region were not associated with impaired performance on the Spatial Span Test. Other studies agree that tests of executive function are significantly associated with WMH (171, 172) however the assessments used differ from those in the present study.

This study supports overwhelming evidence that baseline WMH are strongly associated with performance on tests of processing speed (27, 29, 33, 34) over time. All measures of WMH were negatively associated to measures of processing speed acquired 4 to 5 years later. Subcortical WMH were more strongly associated to measures of processing speed than periventricular WMH. Some studies that distinguished between regional WMH support these findings (173) while others tend to disagree (29, 34). These discrepancies are most likely due to study protocol including distinct WMH rating systems and varying cognitive assessments.

In summary, this study finds baseline WMH are associated with decreased cognitive function across multiple cognitive domains independent of age, sex, and educational level. Moreover, the study identifies that baseline WMH most strongly associated to impaired performance on tests designed to evaluate executive function and processing speed.

Van de Heuvel *et al* suggest that there is specific order in which cognitive domains are affected by WMH. Interestingly, processing speed is thought to deteriorate prior to memory function (174). This may explain why the present study found that baseline WMH were most strongly associated with processing speed. Since the study population was cognitively intact at baseline it may explain why the relationship between WMH and memory function was less apparent at follow-up. It is also biologically plausible that WMH would affect cognitive functioning. Since white matter tracts are responsible for connecting various cortical regions of the brain and efficiently transmitting signals, it would make sense that damage to these tracts would significantly slow cognitive processes (175). De Groot *et al* theorize that cognitive dysfunction, particularly impaired processing speed, may be more strongly associated

with damage to the long association fibers that connect cortical regions, as opposed to damage to the shorter U-fibers that connect adjacent gyri. This is based on the assumption that cognitive test performance requires various cortical regions to successfully complete tasks (34). This would support previous studies that suggest that WMH in the periventricular region rather than in the subcortical region are more strongly associated with impaired cognitive function (29, 34) . The results from this study did show that WMH in the periventricular region were associated to cognitive function; however results would also suggest that damage to the shorter U-fibers in the subcortical region may be just as detrimental to cognitive function. Further research investigating specific types of white matter tracts would be particularly useful for understanding the processes that underlie cognitive impairment.

VII. GENETIC DETERMINANTS

This study did not find any significant correlations between WMH and genetic determinants in dose-response linear regression analyses controlling for age and sex. Although the associations were not significant, there were apparent trends for the *APOE ε4* and *MTHFR 677C→T* genotypes. In both cases analyses were limited to a small number of participants with the rarefied genotype, which may explain why the findings were not significant.

In the present study, *APOE ε4* carriers (n = 30) appeared to have more severe WMH than non-carriers (n = 105). Analyses were limited to *APOE ε4* allele-containing genotypes and not specific to the additional variants of the *APOE* genotype; however this is not an uncommon practice (176). It is well-established that the *APOE ε4* allele is involved in the mechanisms underlying AD and has also been implicated as a risk factor for WMH (177). Studies have also shown that the *APOE ε4* allele is associated

with decreased cerebral blood flow due to increased atherosclerosis which may explain why the *APOE ε4* allele has been associated with WMH (178). Two recent studies conducted thorough meta-analyses of the association between *APOE ε4* and WMH. Despite the fact that some studies found positive associations between *APOE ε4* carriers and WMH, both studies concluded that there was insufficient evidence confirm the association (176, 177). Since methodologic discrepancies are currently unavoidable this association remains contested.

The present study also identified a trend for Scheltens scores to increase with the number of T alleles in the *MTHFR 677C→T* genotypes. Since only 21 participants carried the *MTHFR 677TT* genotype, it is not surprising that we had insufficient power to detect a significant association with WMH. Previous studies have identified that the *MTHFR 677TT* genotype is associated with elevated tHcy, low vitamin B_{12}, and low folate (118, 179). Since elevated tHcy, low vitamin B_{12}, and folate deficiencies have been associated with WMH (155), it is therefore possible that the *MTHFR 677C→T* genotype is also a genetic risk factor for WMH (119, 120).

Kohara and colleagues found that the *MTHFR 677TT* genotype was independently associated with moderately advanced WMH in an elderly Japanese study population (n = 209) (119). These findings remain controversial, however, since one recent meta-analysis of three studies (n = 2796) that compared the influence of the *MTHFR 677TT* genotype with the *677TC* and *677CC* genotypes and WMH indicated that there was only a possible association between *MTHFR* and WMH (176). Furthermore, one study found that the interaction of *APOE ε4* allele, *MTHFR 677TT* polymorphism, and the angiotensin-converting enzyme DD was also associated with the risk of developing

WMH (120) suggesting that future studies should consider the effect of multiple genes on WMH. Further research with much larger studies will be required to confirm the association between the *MTHFR 677C→T* genotypes and WMH and also consider other genetic risk factors such as *APOE ε4*.

VIII. BRAIN VOLUME MEASURES

This study also investigated the relationship between baseline WMH and brain volume measures of grey matter, white matter, ventricular cerebral spinal fluid, and percent brain volume change 2 to 5 years later. The results from this study indicate that WMH are associated with measures of grey matter atrophy and increased VCSF. A recent review (Appelman, 2009) of 48 articles pertaining to WMH and global brain atrophy indicates that the majority of studies agree with these findings (180). Notably, only 10 of these studies controlled for age and other potential risk factors; 90% of these studies found that WMH were independently associated with measures of brain atrophy (180). The present study found that follow-up measures of grey matter atrophy and increased VCSF were associated with WMH independent of age and diastolic blood pressure.

Despite these findings, WMH were remarkably unassociated with measures of white matter volume. Only 2 of the 48 articles identified by Appelman and colleagues evaluated white matter independently of other brain volume measurements. Contrary to our findings, the Rotterdam Scan Study found that WMH were associated with volumetric measures of white matter, but not grey matter in a general study population (181). Their study was significantly larger (n = 490); however they did not control for additional risk factors such as blood pressure. This discrepancy may also be due to the fact that they assessed WMH using a volumetric method, whereas this study used a visual rating scale. Bigler and colleagues also reported a significant relationship

between PVH and decreased white matter volume (182). Their study sample size (n = 192) was comparable to this study sample (n = 135); however their population consisted of individuals with cognitive impairment. Moreover, they controlled for age, but did not consider the effect of additional risk factors. It is therefore possible that if these studies adjusted for additional risk factors the said association between WMH and white matter volume may be null.

While the findings of Bigler and Ikram are commonsensical, additional deliberation may suggest otherwise. Convincing evidence has demonstrated that WMH and cognitive dysfunction improve with vitamin B_{12} replacement therapy (90, 99), allowing us to surmise that WMH displayed on MR images are not due to the destruction of white matter, but rather reversible damage, probably to myelin. WMH likely represent the detection of the reorientation of water inside white matter (2) but not the *loss* of white matter. This may suggest why this study does not find an association between WMH and white matter volume.

This study is the only known study that has evaluated the relationship between WMH and white matter volume and controlled for age and blood pressure over time. Only 2 other studies have evaluated WMH in relation to white matter volume independently of other brain volume measurements. Therefore, further research is required to elucidate these conflicting findings.

I. SUMMARY AND PERSPECTIVES

This thesis sought to identify risk factors associated with cerebral white matter damage in 154 healthy elderly participants. Of the risk factors assessed, the following baseline risk factors were associated with WMH 2 to 5 years later:

- **Age –** Data from 135 elderly participants indicated that age was positively associated with WMH assessed 2 to 5 years later. Analyses from the longer-term subgroup (n = 80) also indicated that age was positively associated with WMH assessed 4 to 5 years later.

- **Hypertension –** Baseline SBP and DBP were positively associated with WMH 2 to 3 years later (n = 133) when adjusting for age and sex; however the association between DBP and WMH was stronger. Further analyses indicated that DBP, but not SBP, were positively associated with WMH in hypertensive participants. These findings suggest that efforts to prevent hypertension may inhibit the development of WMH later in life.

- **Homocysteine –** Baseline tHcy was positively associated with severe WMH in the top tertile (n = 44) 2 to 5 years later when adjusting for age and sex. It is hypothesized that tHcy ultimately interferes with the production of the myelin basic protein and consequently the integrity of myelin. Therefore, these findings suggest that if tHcy can be easily modified with vitamin B_{12} replacement therapy, the integrity of white matter may be preserved and therefore fewer WMH would appear on MR images.

- ***APOE ε4*** **–** Although the association between *APOE* and WMH was not significant when adjusting for age and sex, there was a trend that suggested that *APOE ε4* carriers (n = 30) may be at greater risk for developing WMH than non-carriers (n = 105).

- ***MTHFR 677C→T*** **–** The association between *MTHFR 677C→T* genotypes and WMH was not significant when adjusting for age and sex, however there was

an emerging trend that indicated that participants with the *MTHFR 677TT* (n = 21) genotype may more likely to develop WMH than the *MTHFR 677TC* (n = 68) and *677CC* genotype (n = 66).

Among the risk factors that were not associated with WMH in this study population were sex, smoking, creatinine, MMA, plasma vitamin B_{12}, serum folate, red cell folate, *MTRR 66A→G* genotypes, and *TCN 766C→G* genotypes.

This thesis also aimed to identify if baseline WMH had an effect on cognitive test performance at follow-up when adjusting for age, sex, and level of education. Baseline WMH were most strongly associated with decreased performance on tests of executive function and processing speed 4 to 5 years later and less strongly associated with tests of memory function 2 to 3 years later. Baseline WMH were not significantly associated to tests of global cognitive function 2 to 3 years later (n = 134).

Baseline WMH were positively associated with depressive symptoms at follow-up 2 to 3 years later. Moreover, baseline WMH in the subcortical region were associated with depressive symptoms, but WMH in the periventricular region 2 to 3 years later were not. This regional association has been found in previous studies, however the mechanisms underlying this difference are still unclear.

Finally, this thesis evaluated the relationship between automated brain volume measurements and WMH in 135 participants over time. Baseline WMH were associated with decreased grey matter volume over 2 to 5 years and in the longer-term subgroup 4 to 5 years later. As expected, WMH were associated with VCSF volume. WMH were associated with decreased brain volume, but not in the longer-term subgroup 4 to 5 years later. Surprisingly, WMH were not associated with white matter

volume which may suggest that the white matter is remains intact; however the integrity of the white matter is damaged. This is consistent with studies that report the reversibility of white matter damage, at least for some etiologies such as vitamin B_{12} deficiency. Our data suggest that the association between WMH and white matter volume should be further investigated.

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