White Matter Hyperintensities Are Under Strong Genetic Influence

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Background and Purpose—The genetic basis of white matter hyperintensities (WMH) is still unknown. This study examines the heritability of WMH in both sexes and in different brain regions, and the influence of age.

Methods—Participants from the Older Australian Twins Study were recruited (n=320; 92 monozygotic and 68 dizygotic pairs) who volunteered for magnetic resonance imaging scans and medical assessments. Heritability, that is, the ratio of the additive genetic variance to the total phenotypic variance, was estimated using the twin design.

Results—Heritability was high for total WMH volume (0.76), and for periventricular WMH (0.64) and deep WMH (0.77), and varied from 0.18 for the cerebellum to 0.76 for the occipital lobe. The genetic correlation between deep and periventricular WMH regions was 0.85, with one additive genetics factor accounting for most of the shared variance. Heritability was consistently higher in women in the cerebral regions. Heritability in deep but not periventricular WMH declined with age, in particular after the age of 75.

Conclusions—WMH have a strong genetic influence but this is not uniform through the brain, being higher for deep than periventricular WMH and in the cerebral regions. The genetic influence is higher in women, and there is an age-related decline, most markedly for deep WMH. The data suggest some heterogeneity in the pathogenesis of WMH for different brain regions and for men and women. **(*Stroke*. 2016;47:1422-1428. DOI: 10.1161/STROKEAHA.116.012532.)**

Key Words: aging ■ epidemiology ■ heritable quantitative trait ■ magnetic resonance imaging ■ white matter

White matter hyperintensities (WMH) are signal changes in the white matter seen on T2-weighted magnetic resonance imaging which are frequent incidental findings in otherwise healthy middle-aged and elderly individuals.[1–3] WMH do not have a specific pathogenesis, but those seen in asymptomatic older individuals are likely to be ischemic in origin and are associated with arteriosclerosis and vascular risk factors (eg, hypertension, diabetes mellitus, coronary artery disease, high homocysteine levels).[4] These vascular risk factors, however, account for only a proportion of the variance in WMH, and studies have shown that genetic factors possibly make a major contribution.[5]

Evidence for genetic contributions to WMH comes from various sources. Several gene mutations have been described leading to monogenic disorders manifesting with WMH, such as Fabry disease and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy,[5] but these are rare. Single nucleotide polymorphisms as genetic risk markers for WMH, either directly or through their interactions with medical risk factors, have been investigated using candidate gene[5] and genome-wide association studies,[6,7] but there have been few independent replications.

Considering that the genetic basis of WMH is still unclear, it is important to quantify what proportion of variance in these lesions is genetically determined. Heritability studies quantify the degree to which a trait is passed from parent to offspring and is expressed as the ratio of the additive genetic variance to the total phenotypic variance.[8] High WMH heritability was reported in a study of older male twins, comprising 74 monozygotic (MZ) and 71 dizygotic (DZ) pairs (0.73)[9] and in 2 family studies.[10,11] However, several deficiencies in the literature remain. Although the classic twin method is the best technique for examining heritability of a trait,[8] only 1 prior twin study has estimated WMH heritability but in men only.[9] In fact, there is evidence of sexual dimorphism in WMH.[12] It is arguable that WMH are not a uniform category, and at

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least 2 subcategories—periventricular (PWMH) and deep (DWMH)—have been identified which may have different but overlapping pathogenic mechanisms.[13] The present study was performed to address some of these concerns.

Methods

Participants

Participants were drawn from the Older Australian Twins Study (OATS), a study of elderly MZ–DZ twin pairs living in the 3 Eastern states of Australia and registered with the Australian Twin Registry. Detailed OATS methodology has been previously published.[14] This study was approved by the institutional ethics committees and participants provided written informed consent. Inclusion criteria were as follows: age ≥65 years, having a consenting cotwin and sufficient English proficiency to undertake a neuropsychological assessment. Exclusion criteria were as follows: current diagnosis of malignancy, life-threatening illness, acute psychiatric disorder, or intellectual handicap. Participants underwent comprehensive assessments and those who consented and had no contraindications (n=412) underwent magnetic resonance imaging scans. Because the heritability analysis used only twin pairs without missing data, the final sample comprised 92 MZ (59 women and 33 men) and 68 DZ (35 women, 8 men, and 25 opposite sex) pairs (n=320).

Assessment

Participants were interviewed by trained research assistants for baseline demographic information, including assessment of vascular risk factors. Two readings of sitting blood pressure (BP) were taken 2 hours apart and averaged. Hypertension was diagnosed as follows: definite if participant was on treatment for hypertension, or had a mean systolic BP ≥160 mm Hg, or a mean diastolic BP ≥95 mm Hg; borderline hypertension if self-reported hypertension but with no treatment or systolic BP 140 to 159 mm Hg or diastolic BP 90 to 94 mm Hg; or normotensive. Diabetes mellitus was considered present if the participant was physician-diagnosed and on current treatment has a diabetic diet, hypoglycemic tablets, or insulin, or if fasting blood glucose level was ≥7 mmol/L. Obesity was measured using the body mass index. History of previous myocardial infarction, atrial fibrillation, and stroke was noted.

Overnight fasting bloods were collected and fractionated and plasma was stored at −70°C for future measurements. Plasma total homocysteine level was measured by reverse phase high-performance liquid chromatography (Shimadzu Scientific Instruments, Sydney) with fluorometric detection after derivatization with 4-(aminosulfonyl)-7-fluorobenzo-2-oxa-1, 3-diazole (reference range, women: <12 μmol/L; men: <15 μmol/L) using a homocysteine kit (Bio-Rad Laboratories, CA.).

Zygosity was confirmed using the genotypes from a high-density single nucleotide polymorphism array (Illumina Omni Express).[14]

Magnetic Resonance Imaging Scanning and WMH Quantification

Magnetic resonance imaging data were obtained from 3 different centers. In Sydney, a Phillips 1.5T Gyroscan scanner (Philips Medical Systems, Best, The Netherlands) was initially used (n=118) and was later replaced by a Philips 3T Achieva Quasar Dual scanner (n=35). In Melbourne, a 1.5T Siemens Magnetom Avanto scanner (n=152) and in Brisbane, a 1.5T Siemens Sonata (n=107; Siemens Medical Solutions, Malvern, PA) of similar upgrades and years of manufacture were used. Acquisition protocols of resolution and slice thickness were matched between the centers. Five volunteers were scanned at the 3 centers and a 3D phantom was used to correct for geometric distortions.[14] Twin pairs were always scanned on the same scanner and were scanned either on the same day or within a few weeks of each other.

Three dimensional T1-weighted scans and T2-weighted fluid-attenuated inversion recovery sequence scans were used for data analysis and an automated extraction of WMH was performed (Materials in the online-only Data Supplement). Extracted WMH was then classified into different brain regions and DWMH, PWMH, and false WMH clusters.

Preparation of WMH Images for Voxel-Wise Analysis

Individual WMH maps were normalized into a standard space. The deformation field was computed using 3D T1-weighed image of each participant, and binary WMH images were used, that is, the images had the information for each voxel being either WMH or non-WMH.

Statistical Analysis

T tests were used to test the equality of means between the 2 zygosity groups for continuous measurements and the χ^2 test for equality of proportions. Because of dependent observations in twin pairs, *P* values were computed using a permutation procedure.[15]

Structural equation modeling was used to estimate the heritability and genetic correlations. Phenotypic covariance between twin pairs was modeled as a function of additive genetic (A), shared environmental (C), and unique environmental (E) components (Figure I in the online-only Data Supplement; without the moderator effect). Under the structural equation modeling, the model containing the 3 parameters (A, C, and E), known as the ACE model, was fitted and compared with models containing the variance components A and E (AE), C and E (CE), and E.[16] Twin structural equation modeling analysis was performed using the openMx (2.0.1) R package.[17] The trivariate ACE Cholesky model[18] was used to estimate the genetic correlations among total WMH, DWMH, and PWMH. In this model, a Cholesky factorization was applied to the covariance matrix composed of the phenotypes and shared and unique environmental factors (Figure 1). Because the distribution of WMH at the regions of interests was highly skewed, the data were inversely normal transformed before the analysis.

To model the differences in the genetic parameters between the 2 sexes, a general heterogeneity model was considered, which is similar to the homogeneity model (Figure I in the online-only Data Supplement), but uses different sets of parameters for male and female samples. In addition, the DZ opposite twin pairs are modeled with both male and female path coefficients and there is an additional parameter representing the genetic correlation between male and female samples (Figure II in the online-only Data Supplement). The complete likelihood is the sum of MZ and DZ pairs separately for both sexes (4 components) and DZ opposite sex pairs. The homogeneity test between the 2 sexes is examined through the likelihood ratio test comparing the heterogeneity model against a constrained model with the same set of parameters for male and female samples.

To examine the variation of heritability as a function of age and sex, a gene-environment interaction model[19] was considered (Figure I in the online-only Data Supplement).

For voxel-wise analysis, WMH at each voxel were binarized as described above. Heritability for the binary WMH was estimated using the structural equation model described above, applying the liability threshold.[20]

Results

Sample Characteristics

Mean age of the sample (n=320) was 70.07 years, SD=4.91 (range 65–85 years; 213 women and 107 men). Table 1 compares the clinical characteristics of MZ (92 pairs; 59 female and 33 male pairs) and DZ (68 pairs; 35 women, 8 men, and 25 opposite sex pairs) twins. The only 2 variables on which the 2 groups differed were hypertension (*P*=0.022) and atrial fibrillation (*P*=0.011). The number with atrial fibrillation was, however, small (11/320=3.4%). Summary statistics of the risk factors grouped by sex are presented in Table I in the online-only Data Supplement. There are significant differences in age, hypertension, and systolic BP between the 2 sexes.

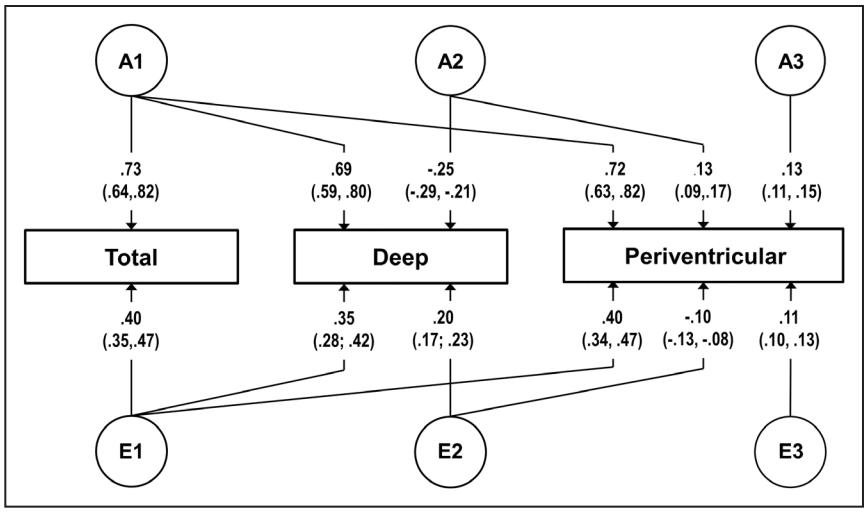

Figure 1. Multivariate Cholesky model for genetic and environmental relation between periventricular, deep, and whole brain (total) white matter hyperintensities. On the basis of model parsimony (ACE vs AE, *P*=0.695; difference in log likelihood=3.88 for 6 degrees of freedom), estimates (non-standardized) were obtained under AE model. Confidence intervals are presented in brackets. A indicates genetic, C, shared environmental, and E, unique environmental components.

Phenotypic correlations between WMH volumes in different brain regions are presented in Table II in the online-only Data Supplement, with high correlations observed between most regions except for the cerebellum and brain stem.

Heritability Estimates

A full ACE model was initially fitted with covariates: age, sex, scanners, intracranial volume (ICV), hypertension, systolic BP, diastolic BP, BMI, homocysteine, heart attack, atrial fibrillation and stroke (Table III in the online-only Data Supplement). Putative risk factors were nonsignificant. Table 2 shows the heritability estimates under a reduced model with age, sex, scanner, and ICV. Heritability estimates were high for total WMH volume (0.76), and for both PWMH (0.64) and DWMH (0.77). Heritability for different brain regions varied from low for the cerebellum (0.18) to high (0.76) for the occipital lobe.

Genetic Correlations

For total volume, DWMH, and PWMH, the AE Cholesky model was fitted, controlling for age, sex, scanner, and ICV. Figure 1 shows the estimated path coefficients. One common factor (A1) accounted for the majority of the variance in both DWMH (88%) and PWMH (93%). Unique environmental variances were similar for all WMH regions of interests (0.23 for total volume and deep; 0.25 periventricular). Genetic (environmental) correlations of total volume with PWMH and DWMH were 0.94 and 0.97 (0.87 and 0.93), respectively. The genetic correlation between DWMH and PWMH regions was higher (0.85) compared with the corresponding environmental correlation (0.68).

Sex Differences and Age and Sex Moderation Model

A sex heterogeneity model was used to contrast the differences in heritability between male and female samples. Heritability (adjusted for age, scanner, and ICV) was consistently higher in women in the cerebral regions and for DWMH and PWMH (Table IV in the online-only Data Supplement). A comparison of the models with separate versus same set of parameters for both sexes (test of homogeneity) was nonsignificant, indicating that both the models were

Table 1. Sample Characteristics

Covariate	n Missing	DZ (n=136)	MZ (n=184)	Stat	*P* Value
Age, y	0	69.92 (4.60)	70.18 (5.14)	–0.49	0.627
Sex (women)	0	95 (69.85%)	118 (64.13%)	0.91	0.132
Hypertension	3	98 (72.06%)	117 (63.57%)	2.18	0.022
BP systolic, mm Hg	9	139.04 (16.86)	137.08 (18.00)	1.00	0.317
BP diastolic, mm Hg	9	81.75 (10.33)	79.61 (9.60)	1.89	0.067
BMI	36	27.60 (3.94)	27.33 (4.41)	0.58	0.556
Homocysteine, μmol/L	81	13.32 (3.47)	12.81 (3.35)	1.29	0.206
Heart attack	0	5 (3.68%)	10 (5.43%)	0.22	0.293
Atrial fibrillation	11	2 (1.47%)	10 (5.43%)	2.40	0.011
Stroke	0	4 (2.94%)	7 (3.80%)	0.12	0.544

For continuous measures, means (SD) are presented. For categorical measures, n (%) is presented. *T* tests for continuous variables and χ^2 tests for all other measures were used to compare MZ and DZ pairs. *P* values were obtained using 10 000 permutations of zygosity of the samples. BMI indicates body mass index; BP, blood pressure; DZ, dizygotic; and MZ, monozygotic.

Table 2. Heritability of WMH Volumes in Whole Brain (Total) and Different Brain Regions: Estimates and Model Summary

WMH ROI	ICC MZ (95% CI)	ICC DZ (95% CI)	A (95% CI)	C (95% CI)	E (95% CI)	*P*-AE	*P*-CE	*P*-E	Covariates
Total	0.76 (0.66–0.83)	0.38 (0.33–0.53)	0.76 (0.44–0.83)	0.00 (0.00–0.29)	0.24 (0.17–0.34)	1	<E–05	<1E–16	1010
Periventricular	0.74 (0.63–0.81)	0.42 (0.32–0.57)	0.64 (0.30–0.81)	0.10 (0.00–0.40)	0.26 (0.19–0.37)	0.58	<E–03	<1E–16	1010
Deep	0.77 (0.67–0.84)	0.39 (0.33–0.47)	0.77 (0.58–0.84)	0.00 (0.00–0.17)	0.23 (0.16–0.33)	1	<E–06	<1E–16	1010
Frontal	0.61 (0.47–0.72)	0.32 (0.24–0.49)	0.59 (0.16–0.72)	0.02 (0.00–0.39)	0.39 (0.28–0.53)	0.91	0.007	<1E–11	1011
Temporal	0.63 (0.48–0.73)	0.36 (0.25–0.53)	0.53 (0.12–0.73)	0.10 (0.00–0.44)	0.37 (0.27–0.52)	0.61	0.011	<E–11	1010
Parietal	0.70 (0.58–0.79)	0.35 (0.29–0.45)	0.70 (0.46–0.79)	0.00 (0.00–0.20)	0.30 (0.21–0.42)	1	<E–04	<E–12	1010
Occipital	0.76 (0.65–0.83)	0.38 (0.32–0.46)	0.76 (0.59–0.83)	0.00 (0.00–0.14)	0.24 (0.17–0.35)	1	<E–07	<1E–16	1010
Cerebellum	0.51 (0.35–0.65)	0.42 (0.22–0.57)	0.18 (0.00–0.61)	0.33 (0.00–0.57)	0.49 (0.36–0.65)	0.14	0.422	<E–08	1010
Brain stem	0.70 (0.59–0.79)	0.43 (0.30–0.61)	0.54 (0.16–0.78)	0.16 (0.00–0.51)	0.30 (0.22–0.41)	0.46	0.005	<1E–16	0010

Standardized additive genetic (A=heritability), shared environment (C) and unique environment (E) variance components (95% CI) of WMH for different ROIs obtained using the ACE model. The columns *P*-AE, *P*-CE, and *P*-E, respectively, denote the *P* values from the likelihood ratio test comparing ACE model vs AE, CE, and E models. *P*-CE is also the *P* value for heritability because testing the component A=0 is equivalent to testing heritability is zero. Last column indicates the significance of covariates. Significance of the *P* value ($P<0.05$) for any of the covariates age, sex, scanners, and intracranial volume in that order is indicated as a string; 1= significant; 0= not significant. CI indicates confidence interval; DZ, dizygotic; ICC, intraclass correlation coefficient; MZ, monozygotic; ROI, region of interests; and WMH, white matter hyperintensities.

comparable. For model parsimony with a liberal threshold ($P<0.5$), there was substantial difference in heritability between the sexes for PWMH, frontal, temporal, brain stem, and cerebellar WMHs.

To understand the variation in heritability of WMH with age and sex, an ACE age, sex moderation model was fitted (Figure I in the online-only Data Supplement). For total, DWMH, and PWMH volumes, heritability for men and women as a function of age is plotted in Figure 2. Generally, the heritability in DWMH but not PWMH is seen to decline with age, particularly after 75 years of age. For all brain regions considered here, the heritabilities and their confidence intervals for male and female samples are summarized in Table V in the online-only Data Supplement.

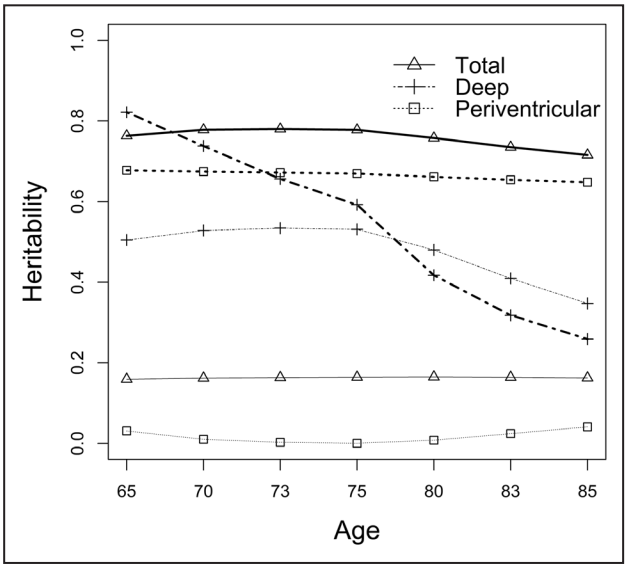

Figure 2. Heritability of white matter hyperintensities (WMH) as a function of age (years) and sex. Heritability of total, deep, and periventricular WMH for men (thin lines) and women (thick lines) were obtained using the ACE age, sex, and age-sex interaction models. A indicates genetic, C, shared environmental, and E, unique environmental components. See Figure I in the online-only Data Supplement for further information.

Voxel-Wise Heritability Estimates

Heritabilities were estimated using both ACE and AE models. The comparison of log likelihood of ACE and AE models showed that the AE model was as good as the ACE model for the majority of voxels ($P>0.5$ for 76% and $P<0.05$ for <1% of voxels). WMH heritability estimated using the AE model for each voxel is presented in Figure 3. Of the 2 122 945 (121×145×121) total voxels, only ≈157 000 voxels had WMH present in at least 1 individual. However, heritability can only be measured for voxels with enough individuals with nonzero values. Hence, heritability estimates were available only for 42 334 voxels. We observed that the WMH heritability in the areas directly around lateral ventricles was somewhat lower than those further afield.

Discussion

This study shows that WMH are highly heritable in a sample of female and male older adult twins, suggesting a strong genetic influence. Heritability is high in both deep and periventricular regions and the majority of cerebral lobes but is much lower in the cerebellum and brain stem. Heritability is higher in women compared with men in all cerebral lobes, particularly for the periventricular region, for which heritability in men was low. Heritability of deep WMH in both sexes decreased with age, especially after 75 years of age.

Our findings are similar to those from previous studies but extend the results by examining heritability of cerebral regions as well. In a study of male twins with a mean age of 72 years (range 68–79), the heritability estimate of total WMH volumes was 71% (95% confidence interval, 0.66–0.76).[9] A large family cohort of the Framingham Heart Study and their offspring[21] with a mean age of 61 years (range 34–88) estimated the heritability of total WMH volumes at 0.55. In the San Antonio Family Heart Study,[10] with a mean age of 47.9 years (range 19–85), whole brain (or total WMH), subcortical (DWMH), and ependymal (PWMH) volumes were found to have heritabilities of 0.72, 0.66, and 0.73, respectively.

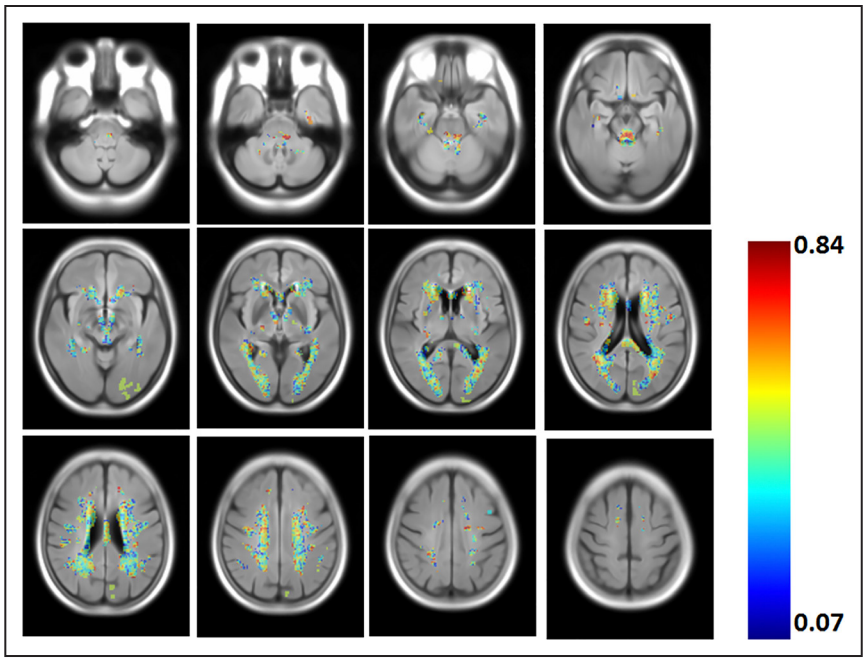

Figure 3. Voxel-wise heritability of white matter hyperintensities using the binary AE model. The bar on the right presents the color scale for heritability estimates for each voxel. A indicates genetic, C, shared environmental, and E, unique environmental components. A 3D movie of heritability has been provided (Movie I in the online-only Data Supplement).

Our finding of higher heritability in women is similar to the Framingham Study,[21] which found higher heritability for total WMH for women (0.78) compared with men (0.05). Other heritability studies have not examined sex differences. A previous study showed that after accounting for brain volume, women have more WMH than men[2,3] and one study reported a more rapid rate of progression in women.[22] This sex difference is not accounted for by known risk factors of small-vessel disease, and in fact men have higher rates of cerebrovascular risk factors.[23] Thus, women may be more vulnerable to developing WMH, which may be independent of small-vessel disease risk factors; our study suggests that this vulnerability may be genetically based. Higher heritability in women relative to men has also been reported for ischemic stroke[24] and body mass index.[25] There are many possible explanations for this observation which need to be further explored. It is unknown whether there is a mother-to-daughter transmission for WMH. Mitochondrial transmission with greater penetrance in women is possible. Epigenetic mechanisms could also account for the sex differences.[24]

Reduction of WMH heritability with age in our study is also somewhat consistent with the Framingham Study,[21] where heritability for total WMH declined with age in the entire sample and for women after 50 to 60 years of age. We also observed a similar sex difference in age-related decline of heritability but for deep WMH, with a more marked decline for women. Because WMH are multifactorial in origin and there are several identified nongenetic factors that are likely to be become more prominent in individual ages, it is not surprising that the genetic contribution is less in the old. In our study, the change was most obvious after 75 years of age, especially for deep WMH, which had higher genetic influence. It is well recognized that the genetic influences on complex traits are dynamic during the life course. Untreated hypertension decreased the heritability of cognition in late middle age as previously reported.[26]

In the San Antonio Family Study,[10] the shared genetic variability between subcortical (DWMH) and ependymal (PWMH) volumes was 21%, indicating significant pleiotropy. We found greater shared genetic variance between DWMH and PWMH (68%), than the San Antonio Study, although the methodology used to define PWMH was somewhat different in the 2 studies. We also observed high shared genetic variance between WMHs located in the cerebral regions, which to our knowledge has not been considered previously.

The differences in genetic influences on DWMH and PWMH are worthy of comment. Most studies, like ours, show a high correlation between WMH in these 2 regions. However, risk factors, pathogenic mechanisms, and functional consequences of DWMH and PWMH are overlapping but not identical.[13] The vascularization pattern of the periventricular region is different from that of the deep white matter, making the periventricular region a distal irrigation field.[4] This region is also vulnerable to changes in pressure in the ventricles.[27] Neuropathological differences between DWMH and PWMH have been described.[28] The observation that deep WMH are more heritable suggests that PWMH are influenced by more varied environmental factors and are therefore more multifactorial in their determination.

The genetic variants identified to date by linkage and association studies have failed to explain the majority of WMH phenotypic variability. The candidate genes examined include those involved in hypertension and its pathways, cholesterol regulation and atherosclerosis, oxidative stress pathways, neuronal repair, homocysteine levels, and nitric oxide signaling.[4,26] The candidate gene approach has supported the role of apolipoprotein E, the renin–angiotensin system, and the Notch3 signaling pathway in the development of WMH.[21] Several genome-wide linkage studies[10,29–32] have shown linkages to chromosomes 4, 1, 5, and 11, respectively, but independent replication has not occurred. A large genome-wide association study from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE)

consortium identified 6 novel risk-associated genes, including a novel locus on chromosome 17, which together accounted for 4% to 8% of the WMH burden.[6] A more recent large multiethnic genome-wide association study confirmed the chromosome 17 locus and identified 4 novel genes implicated in inflammatory and glial proliferative pathways.[7] From these studies, it is evident that the associated genes still account for only a small proportion of the genetic contribution suggested by the heritability studies. Whether the missing heritability for WMH is caused by other heritable factors, such as copy number variants or epigenetic factors, remains to be elucidated.

This study has some limitations. Because the sample largely comprised volunteers participating in a twins register, it is unclear how representative this population is. However, the sample generally had characteristics similar to those of the overall Australian population in this age group.[14] This is a cross-sectional study and it is not possible to investigate the relative contribution of genetics and environment to the rate of WMH development. The age range of the sample (65–88 years), while covering much of old age, is somewhat restrictive because it is known that WMH commonly become manifested in the 40s and 50s,[15] and it is in the earlier ages that genetic factors may be even more prominent. In addition, this study had a smaller sample of men than women and the heritability estimates thus require verification in a larger sample. Monogenic disorders, such as cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy and Fabry disease, have the potential to bias heritability estimates but are unlikely to significantly influence our estimates, given their low prevalence in the general population. There are also methodological constraints in identifying WMH. Although the use of fluid-attenuated inversion recovery and T1-weighted images tends to identify most small infarcts and perivascular spaces, not all may be excluded from our WMH volume estimates. Furthermore, WMH do not capture all subinfarct pathology in the white matter, and normal-appearing white matter on fluid-attenuated inversion recovery may show abnormality if other imaging techniques such as diffusion tensor imaging are used. Our study was performed on 1.5T scanners. Although 3.0T scanners are preferred for their higher signal to noise ratio and possibly better detection of small WMH (1–5 mm), the overall consensus is that 1.5T scanners are acceptable,[33] the overall differences are minor and not always consistent.[34] Moreover, for heritability estimates, of greater import was the use of the same scanner for imaging both twins. Finally, the attempt to examine the heritability of WMH at a finer resolution through estimation heritability of binarized WMH at each voxel was limited by small sample size at the voxel level resolution.

Despite these limitations, this study presents an intriguing picture of the heritability of WMH and suggests that studies that focus on identifying specific genes associated with the risk for WMH may be best served by examining the sexes separately and paying attention to the age of the participants and the anatomic location of the lesions.

Appendix

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Disclosures

None.

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White Matter Hyperintensities Are Under Strong Genetic Influence

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SUPPLEMENTAL MATERIAL

WHITE MATTER HYPERINTENSITIES ARE UNDER STRONG GENETIC INFLUENCE

Cover title: Heritability of white matter hyperintensities

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MRI parameters and quantitation of WMH

Both 3D T1-weighted scans and T2-weighted fluid-attenuated inversion recovery (FLAIR) sequence scans were used for data analysis. The following protocol was used for T1-weighted MRI scans on the 1.5T scanners in all three centres: in-plane resolution 1×1 mm with slice thickness of 1.5 mm, contiguous slices, TR/TE/TI = 1530/3.24/780 ms, and flip angle = 8. FLAIR scans were acquired axially with the same acquisition parameters on the 1.5T scanners in all three centres, i.e. TR/TE/TI = 10000/120/2800 ms, with slice thickness 3.5 mm and in-plane resolution 0.898×0.898 mm^2. On the 3T scanner in centre 1, we had spatial resolution of 1×1×1 mm^3, TR/TE = 6.39/2.9 ms for T1-weighted scans, and TR/TE/TI = 10000/110/2800 ms, with slice thickness 3.5 mm and in-plane resolution 0.898×0.898 mm^2 for FLAIR scans. Intracranial volume (ICV), the sum of grey matter, white matter and cerebrospinal fluid was calculated using SPM8 (Wellcome Department of Cognitive Neurology, Institute of Neurology, London, UK. http://www.fil.ion.ucl.ac.uk/spm/software/spm8/).

The contrast properties of FLAIR facilitate the possibility of automated segmentation and classification of WMH. The method has been previously described[1]. A parametric method[1] was adapted and applied to the initial WMH detection. The extracted candidate WMH clusters were further investigated using a non-parametric kNN rule and then classified into different brain regions and deep (DWMH), periventricular (PWMH), and false WMH clusters.

The automated classification of WMHs employed in this study was carried out in the native space of the T1-weighted images. Five pre-processing steps were taken to prepare the images for the analysis, as described previously [1]: (i) the FLAIR and T1 images of the same subject were co-registered using mutual information method [2]; (ii) segmentation [3] of T1-weighted images into three separate tissue components; (iii) removal of non-brain tissue from both T1-weighted and co-registered FLAIR images using the brain mask transformed from the average mask originally defined in the standard space by inverting the deformation matrix [4]; (iv) inverting the spatial normalization transformation to produce the brain masks and white matter probability maps in the native space for the WMH detection and non-brain tissue removal; (v) intensity correction [5] of both FLAIR and T1-weighted images after the removal of non-brain tissues. Some other smaller steps such as removal of the bright areas observed in the FLAIR sequence ventricles caused by choroid plexus and partial voluming were also carried out. SPM8 was used with Matlab R2013b (MathWorks, Natick, MA, U.S.A.) for these pre-processing steps.

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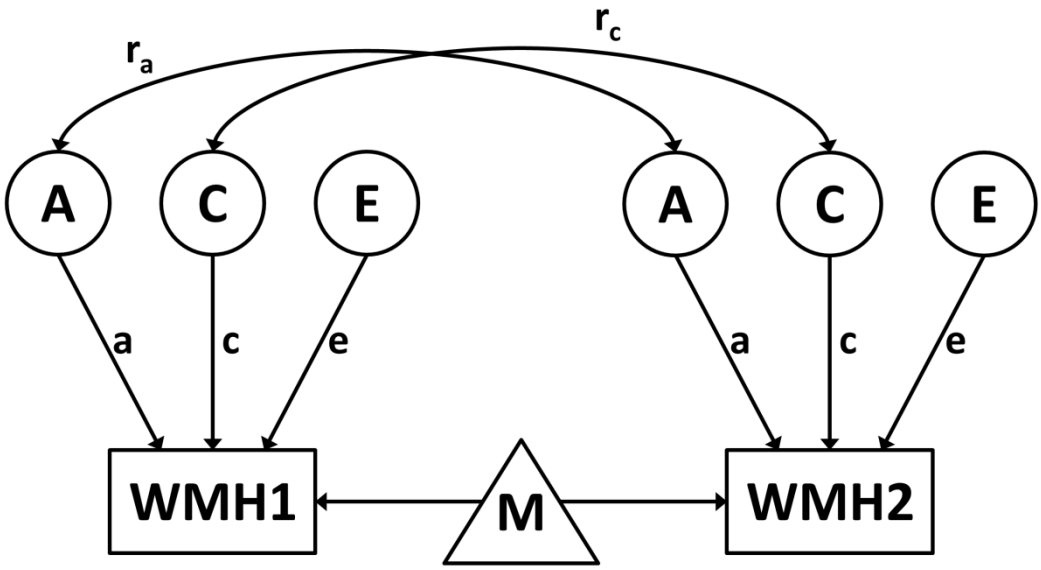

Figure I. Path diagram for the age and sex moderated ACE twin model. WMH of twin 1 (WMH1) and 2 (WMH2) are modelled as the function of the mean parameter (M) and the latent additive (A), shared environment (C) and environment (E) factors. The mean is further modelled as a function of the *k* covariates $M = \mu + \beta_1 X_1 + \cdots + \beta_k X_k$, where μ is the overall mean of the phenotypes and X_1, X_2,…,X_k are the k covariates (such as age, sex, scanners and ICV) and β_1, β_2,…, β_k are the regression parameters of the model. The path coefficients a, c and e are the estimated loadings of the latent factors, which are further decomposed as $a=a_0+a_1age+a_2sex$; $c=c_0+c_1age+c_2sex$; $e=e_0+e_1age+e_2sex$ to accommodate the moderating effects of age and sex. The parameter r_a (r_a=1 for MZ twin pairs and r_a=0.5 for DZ twin pairs) and r_c ($r_{c=1}$ for both MZ and DZ twin pairs) respectively denote the additive genetic and shared environmental correlations between the twin pairs.

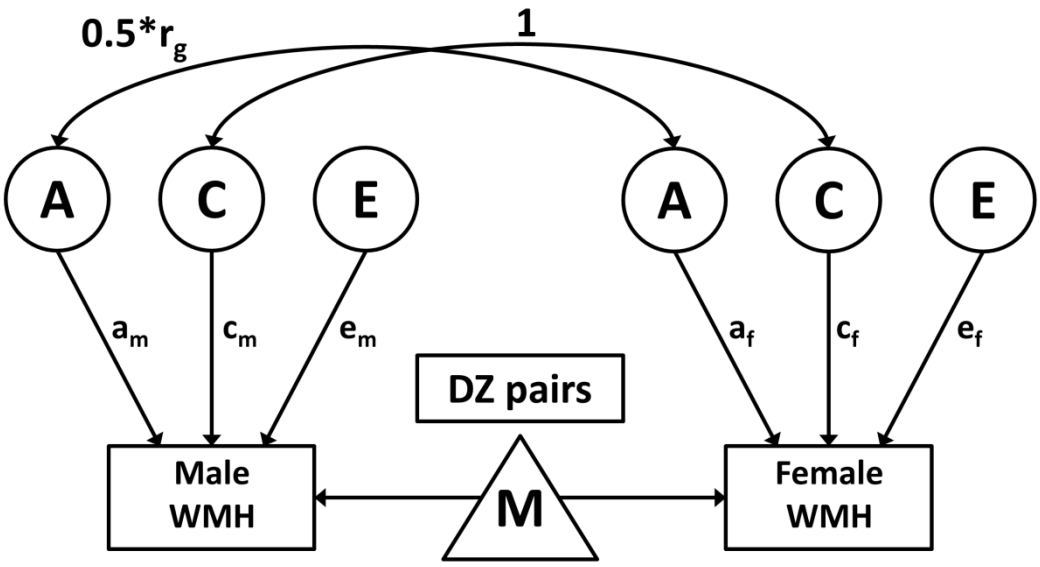

Figure II. The path diagram for the opposite sex DZ twin pairs in the general sex heterogeneity model. For opposite DZ twin pairs, the path coefficients for the male samples a_m, c_m, e_m are same as the path coefficients for the male MZ and DZ pairs. Similarly the path coefficients for the female samples a_f, c_f and e_f are also the same as the path coefficients for female MZ and DZ pairs. The correlation between the additive genetic components in opposite sex pairs is half of the genetic correlation between male-female genetic correlation r_g.

TABLE I. Sample characteristics by sex

Covariate	Female (N=213)	Male (N=107)	Stat	p
Age (years)	69.54 (4.75)	71.14 (5.08)	-2.72	0.005
Hypertension	136 (63.84%)	79 (73.83%)	2.78	0.017
BP systolic (mmHg)	135.66 (17.11)	142.40 (17.55)	-3.27	0.001
BP diastolic (mm Hg)	79.97 (9.77)	81.61 (10.26)	-1.37	0.174
BMI	27.33 (4.5)	27.69 (3.6)	-0.79	0.436
Homocysteine (µmol/L)	12.84 (3.52)	13.41 (3.16)	-1.48	0.141
Heart attack	8 (3.76%)	7 (6.54%)	0.69	0.131
Artrial fibrillation	7 (3.29%)	5 (4.67%)	0.09	0.416
Stroke	6 (2.81%)	5 (4.67%)	0.29	0.247

For continuous measures, means (SD) are presented. For categorical measures, N (%) is presented. T-tests for continuous variables and chi-square tests for all other measures were used to compare between the two sexes. All the p-values were obtained using 10000 permutations.

TABLE II. Phenotypic correlations across white matter hyperintensity (WMH) ROIs

	Total	Periventricular	Deep	Frontal	Temporal	Parietal	Occipital	Cerebellum	Brainstem
Total	1.00	0.96	0.94	0.83	0.72	0.92	0.69	0.40	0.39
Periventricular	0.96	1.00	0.85	0.76	0.64	0.85	0.58	0.30	0.25
Deep	0.94	0.85	1.00	0.85	0.76	0.95	0.76	0.43	0.42
Frontal	0.83	0.76	0.85	1.00	0.65	0.77	0.51	0.34	0.33
Temporal	0.72	0.64	0.76	0.65	1.00	0.68	0.57	0.41	0.43
Parietal	0.92	0.85	0.95	0.77	0.68	1.00	0.67	0.39	0.32
Occipital	0.69	0.58	0.76	0.51	0.57	0.67	1.00	0.48	0.53
Cerebellum	0.40	0.30	0.43	0.34	0.41	0.39	0.48	1.00	0.58
Brainstem	0.39	0.25	0.42	0.33	0.43	0.32	0.53	0.58	1.00

Pearson correlation coefficients are presented ignoring the relationship between the zygotic twin pairs.

TABLE III. Heritability of white matter hyperintensities (WMH) volumes in whole brain (total) and different brain regions: estimates and model summary

WMH ROI	ICC MZ (95 % CI)	ICC DZ (95 % CI)	A (95% CI)	C (95% CI)	E (95% CI)	P-AE	P-CE	P-E	Covariates
Total	0.78 (0.68,0.84)	0.39 (0.34,0.56)	0.77 (0.42,0.84)	0.01 (0.00,0.33)	0.22 (0.16,0.32)	0.97	<1E-04	<1E-16	111000000000
Periventricular	0.76 (0.66,0.83)	0.44 (0.34,0.60)	0.63 (0.29,0.83)	0.13 (0.00,0.43)	0.24 (0.17,0.34)	0.49	<1E-06	<1E-16	111000001100
Deep	0.78 (0.69,0.85)	0.39 (0.34,0.49)	0.78 (0.57,0.85)	0.00 (0.00,0.2)	0.22 (0.15,0.31)	1	0.014	<1E-16	111000000000
Frontal	0.63 (0.48,0.74)	0.36 (0.25,0.53)	0.53 (0.11,0.74)	0.10 (0.00,0.45)	0.37 (0.26,0.52)	0.63	0.026	<1E-10	111101000000
Temporal	0.66 (0.52,0.75)	0.44 (0.29,0.60)	0.44 (0.05,0.75)	0.22 (0.00,0.54)	0.34 (0.25,0.48)	0.27	<1E-04	<1E-12	111000001100
Parietal	0.71 (0.59,0.80)	0.35 (0.29,0.47)	0.71 (0.43,0.80)	0.00 (0.00,0.24)	0.29 (0.20,0.41)	1	<1E-07	<1E-13	111000000000
Occipital	0.79 (0.68,0.86)	0.39 (0.34,0.47)	0.79 (0.63,0.86)	0.00 (0.00,0.13)	0.21 (0.14,0.32)	1	<1E-07	<1E-16	101000000001
Cerebellum	0.51 (0.36,0.64)	0.47 (0.27,0.60)	0.07 (0.00,0.55)	0.43 (0.01,0.60)	0.49 (0.36,0.64)	0.04	0.738	<17E-9	101010010000
Brainstem	0.71 (0.60,0.80)	0.45 (0.31,0.62)	0.53 (0.16,0.79)	0.18 (0.00,0.52)	0.29 (0.20,0.40)	0.41	0.004	<1E-16	001000000000

Standardised additive genetic (A=heritability), shared environment (C) and unique environment (E) variance components (95% confidence intervals) of WMH for different ROIs obtained using ACE model. Missing values for the covariates were imputed using the multiple imputation procedure as implemented in the R-package “mice” (van Buuren S, Groothuis-Oudshoorn K: Mice: Multivariate Imputation by Chained Equations in R. J Stat Softw 2011;45:1-67).The columns P-AE, P-CE and P-E respectively denote the p-values from the likelihood ratio test comparing ACE model vs AE, CE and E models. P-CE is also the p-value for heritability because testing the component A=0 is equivalent to testing heritability is zero. Last column indicates the significance of covariates. Significance of the p-value ($p<0.05$) for any of the covariates age, sex, scanners, ICV, hypertension, systolic BP, diastolic BP, BMI, homocysteine, heart attack, atrial fibrillation and stroke in that order is indicated as a string; 1=significant; 0=not-significant.

TABLE IV. Heritability estimates under the sex heterogeneity model

WMH region	Female ICC MZ (95 % CI)	Female ICC DZ (95 % CI)	Male ICC MZ (95 % CI)	Male ICC DZ (95 % CI)	Male-Female ICC DZ (95 % CI)	Female h^2 (95% CI)	Male h^2 (95% CI)	Test of Homogeneity
Total (whole brain)	0.78 (0.66,0.86)	0.41 (0.34,0.54)	0.72 (0.52,0.83)	0.64 (0.28,0.81)	0.22 (0.00,0.40)	0.74 (0.46,0.85)	0.15 (0.00,0.81)	0.646
Periventricular	0.78 (0.65,0.85)	0.44 (0.35,0.58)	0.67 (0.46,0.81)	0.66 (0.28,0.80)	0.23 (0.00,0.43)	0.67 (0.37,0.84)	0.03 (0.00,0.72)	0.282
Deep	0.78 (0.66,0.86)	0.39 (0.33,0.48)	0.75 (0.54,0.86)	0.40 (0.27,0.72)	0.26 (0.00,0.39)	0.78 (0.59,0.86)	0.69 (0.04,0.86)	0.985
Frontal	0.66 (0.49,0.78)	0.35 (0.25,0.51)	0.56 (0.30,0.73)	0.50 (0.16,0.72)	0.18 (0.00,0.37)	0.62 (0.22,0.77)	0.11 (0.00,0.72)	0.468
Temporal	0.74 (0.60,0.82)	0.41 (0.31,0.59)	0.41 (0.12,0.64)	0.27 (0.07,0.55)	0.22 (0.00,0.38)	0.65 (0.24,0.82)	0.28 (0.00,0.64)	0.178
Parietal	0.72 (0.56,0.82)	0.36 (0.28,0.47)	0.66 (0.41,0.81)	0.41 (0.21,0.71)	0.24 (0.00,0.40)	0.72 (0.47,0.82)	0.50 (0.00,0.81)	0.975
Occipital	0.83 (0.72,0.89)	0.41 (0.36,0.50)	0.62 (0.37,0.77)	0.31 (0.19,0.49)	0.21 (0.08,0.29)	0.83 (0.65,0.89)	0.62 (0.22,0.77)	0.243
Cerebellum	0.54 (0.37,0.68)	0.53 (0.27,0.67)	0.47 (0.17,0.68)	0.35 (0.13,0.63)	0.19 (0.00,0.35)	0.01 (0.00,0.56)	0.24 (0.00,0.64)	0.059
Brainstem	0.75 (0.63,0.83)	0.47 (0.32,0.67)	0.52 (0.23,0.72)	0.29 (0.13,0.61)	0.17 (0.01,0.30)	0.56 (0.13,0.83)	0.46 (0.00,0.71)	0.156

Intra-class correlations (ICC), heritability (H^2) and 95% confidence intervals for WMH in different ROIs obtained using heterogeneity ACE model (age, scanner and ICV adjusted). The p-value from the likelihood ratio test of homogeneity (common variances and co-variances versus separate parameters for male and female samples) is also presented.

TABLE V. Heritability of white matter hyperintensities (WMH) as a function of age and sex

ROI	Age	Parameter Label	Lower Limit	Estimate	Upper Limit
Total volume	65	Female_H2	0.46	0.76	0.88
	70	Female_H2	0.49	0.78	0.86
	73	Female_H2	0.08	0.78	0.87
	75	Female_H2	0.01	0.78	0.88
	80	Female_H2	0.00	0.76	0.91
	83	Female_H2	0.00	0.74	0.93
	85	Female_H2	0.00	0.72	0.94
	65	Male_H2	0.00	0.16	0.87
	70	Male_H2	0.00	0.16	0.81
	73	Male_H2	0.00	0.16	0.79
	75	Male_H2	0.00	0.16	0.79
	80	Male_H2	0.00	0.17	0.80
	83	Male_H2	0.00	0.16	0.82
	85	Male_H2	0.00	0.16	0.84
Peri	65	Female_H2	0.36	0.68	0.87
	70	Female_H2	0.34	0.67	0.85
	73	Female_H2	0.18	0.67	0.86
	75	Female_H2	0.07	0.67	0.87
	80	Female_H2	0.00	0.66	0.91
	83	Female_H2	0.00	0.65	0.93
	85	Female_H2	0.00	0.65	0.94
	65	Male_H2	0.00	0.03	0.76
	70	Male_H2	0.00	0.01	0.72
	73	Male_H2	0.00	0.00	0.70
	75	Male_H2	0.00	0.00	0.69
	80	Male_H2	0.00	0.01	0.47
	83	Male_H2	0.00	0.02	0.57
	85	Male_H2	0.00	0.04	0.64
Deep	65	Female_H2	0.62	0.82	0.90
	70	Female_H2	0.42	0.74	0.87
	73	Female_H2	0.24	0.66	0.86
	75	Female_H2	0.12	0.59	0.86
	80	Female_H2	0.00	0.42	0.88
	83	Female_H2	0.00	0.32	0.89
	85	Female_H2	0.00	0.26	0.90
	65	Male_H2	0.02	0.50	0.91
	70	Male_H2	0.02	0.53	0.85
	73	Male_H2	0.01	0.53	0.82

	75	Male_H2	0.01	0.53	0.81
	80	Male_H2	0.00	0.48	0.81
	83	Male_H2	0.00	0.41	0.82
	85	Male_H2	0.00	0.35	0.83
Frontal	65	Female_H2	0.00	0.58	0.81
	70	Female_H2	0.00	0.17	0.80
	73	Female_H2	0.00	0.01	0.81
	75	Female_H2	0.00	0.01	0.35
	80	Female_H2	0.00	0.23	0.74
	83	Female_H2	0.00	0.35	0.85
	85	Female_H2	0.00	0.41	0.89
	65	Male_H2	0.11	0.70	0.88
	70	Male_H2	0.00	0.38	0.75
	73	Male_H2	0.00	0.13	0.70
	75	Male_H2	0.00	0.03	0.69
	80	Male_H2	0.00	0.07	0.58
	83	Male_H2	0.00	0.19	0.70
	85	Male_H2	0.00	0.27	0.76
Temporal	65	Female_H2	0.17	0.60	0.80
	70	Female_H2	0.15	0.56	0.82
	73	Female_H2	0.08	0.52	0.86
	75	Female_H2	0.03	0.49	0.88
	80	Female_H2	0.00	0.41	0.94
	83	Female_H2	0.00	0.36	0.96
	85	Female_H2	0.00	0.33	0.97
	65	Male_H2	0.00	0.33	0.66
	70	Male_H2	0.00	0.33	0.63
	73	Male_H2	0.00	0.32	0.65
	75	Male_H2	0.00	0.31	0.68
	80	Male_H2	0.00	0.28	0.78
	83	Male_H2	0.00	0.25	0.83
	85	Male_H2	0.00	0.23	0.86
Parietal	65	Female_H2	0.48	0.71	0.83
	70	Female_H2	0.35	0.70	0.82
	73	Female_H2	0.21	0.66	0.84
	75	Female_H2	0.12	0.62	0.85
	80	Female_H2	0.00	0.50	0.90
	83	Female_H2	0.00	0.42	0.93
	85	Female_H2	0.00	0.36	0.95
	65	Male_H2	0.00	0.27	0.79
	70	Male_H2	0.00	0.29	0.76
	73	Male_H2	0.00	0.31	0.76

	75	Male_H2	0.00	0.32	0.77
	80	Male_H2	0.00	0.35	0.82
	83	Male_H2	0.00	0.36	0.86
	85	Male_H2	0.00	0.35	0.89
Occipital	65	Female_H2	0.70	0.87	0.94
	70	Female_H2	0.50	0.82	0.89
	73	Female_H2	0.29	0.79	0.88
	75	Female_H2	0.18	0.77	0.88
	80	Female_H2	0.03	0.71	0.88
	83	Female_H2	0.00	0.68	0.88
	85	Female_H2	0.00	0.66	0.89
	65	Male_H2	0.33	0.74	0.90
	70	Male_H2	0.29	0.67	0.81
	73	Male_H2	0.14	0.63	0.78
	75	Male_H2	0.06	0.61	0.77
	80	Male_H2	0.00	0.55	0.77
	83	Male_H2	0.00	0.52	0.78
	85	Male_H2	0.00	0.50	0.79
Cerebellum	65	Female_H2	0.00	0.02	0.57
	70	Female_H2	0.00	0.11	0.60
	73	Female_H2	0.00	0.17	0.66
	75	Female_H2	0.00	0.22	0.70
	80	Female_H2	0.00	0.32	0.79
	83	Female_H2	0.00	0.38	0.83
	85	Female_H2	0.00	0.41	0.86
	65	Male_H2	0.00	0.50	0.83
	70	Male_H2	0.00	0.28	0.68
	73	Male_H2	0.00	0.16	0.62
	75	Male_H2	0.00	0.09	0.59
	80	Male_H2	0.00	0.00	0.55
	83	Male_H2	0.00	0.01	0.63
	85	Male_H2	0.00	0.03	0.69
Brainstem	65	Female_H2	0.00	0.32	0.82
	70	Female_H2	0.12	0.54	0.80
	73	Female_H2	0.26	0.65	0.83
	75	Female_H2	0.22	0.71	0.86
	80	Female_H2	0.32	0.78	0.93
	83	Female_H2	0.22	0.78	0.96
	85	Female_H2	0.16	0.77	0.97
	65	Male_H2	0.00	0.02	0.69
	70	Male_H2	0.00	0.12	0.61
	73	Male_H2	0.00	0.23	0.62

75	Male_H2	0.00	0.31	0.66
80	Male_H2	0.00	0.45	0.80
83	Male_H2	0.00	0.48	0.87
85	Male_H2	0.00	0.48	0.90

Heritability estimates of WMH and their 95% confidence intervals for male (Male_H2) and female (Female_H2) at different ages under the age and sex moderated ACE model (Figure I).

Supplemental Video I. A 3D movie of voxel-wise heritability of white matter hyperintensities using the binary AE model has been provided.