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Genetic Variation in White Matter Hyperintensity Volume in the Framingham Study

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Background and Purpose—In a previous study of normal elderly male twins, the heritability of quantitative white matter hyperintensity (WMH) volume has been estimated to be high (0.73). We investigated heritability of WMH in a family-based sample of the Framingham Heart Study for sex differences and the impact of age.

Methods—Brain magnetic resonance scans were performed on 2012 individuals in the cohort and offspring of the Framingham study. This report was limited to 1330 stroke-free and dementia-free members (mean age 61.0 years) of the Framingham offspring. Individuals with a history of multiple sclerosis, stroke, dementia, or other neurological condition including traumatic brain injury were excluded from this analysis. WMH volume and total cranial volume (TCV) were quantified using a previously published algorithm. Because of extreme skewing, measures of WMH were log-transformed before analysis. Variance components methods were used to estimate heritability of WMH after adjusting for sex, age, age², and TCV.

Results—In the full dataset, WMH heritability was 0.55 ($P < 0.0001$). Heritability among women was 0.78 ($P < 0.0001$) whereas heritability among men was 0.52 ($P < 0.0003$). Heritability varied as average age increased, with a peak of 0.68 ($P < 0.0001$) in individuals aged 55 or older.

Conclusion—Using a family-based study design comprising generally healthy individuals, this study found high heritability of WMH overall and similar heritability for both men and women. In addition, the heritability of WMH remained high among individuals in whom the prevalence of cerebrovascular brain injury was generally low, suggesting that WMH is also likely to be an excellent genetic marker of brain aging. (*Stroke*. 2004;35:1609-1613.)

Key Words: hereditary disease ■ population genetics ■ MRI scans

Abnormalities of cerebral white matter, often seen as increased signal intensities on magnetic resonance imaging (MRI), have multiple causes but commonly occur with increasing age and accompanying cerebrovascular risk factors.¹⁻⁷ Although the pathophysiology of white matter hyperintensity (WMH) remains unclear, the consistent association between cerebrovascular risk factors and neuropathological evidence of concurrent cerebrovascular disease suggests an ischemic pathogenesis, especially among older individuals.⁸⁻¹⁴ In this regard, WMH may be considered part of the spectrum of cerebral vascular disease. Although cerebrovascular risk factors and various forms of cerebrovascular disease are thought to be under at least some genetic influence, the heritability of WMH has received only limited attention. In the only published study of WMH heritability, Carmelli et al¹⁵ reported high heritability (0.73) for WMH in a group of older World War II male veteran twins. The heritable nature of WMH was further supported by a follow-up family history study of the same population.¹⁶

These results suggest a surprisingly strong genetic influence on the extent of WMH but were limited by study of an older sample of men who also had a substantial burden of cerebrovascular disease.¹ In the current study, we examined the heritability of WMH in a large population-based sample of sibships who are a younger and generally healthy subset of the Framingham Heart Study. We sought to extend previous results by examining heritability in WMH among women and changes in heritability from middle to late life. High heritability of WMH in this generally younger and healthier population suggests that WMH signals are important phenotypic markers for brain aging as well as cerebrovascular brain injury.

Subjects and Methods

Study Population

The general design and demographics of the Framingham Heart Study (FHS) have been previously described.¹⁷ In brief, FHS is a population-based sample of 5209 men and women living in Framing-

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ham, Massachusetts. There was no ascertainment of any disease, ie, the entire community was recruited. Studies of the original Framingham cohort began in 1948. Subsequent research on 5241 children of the original cohort (The Framingham Offspring Study¹⁸) began in 1971. For this analysis, a subset of 1881 individuals from both cohorts received brain MRI. Individuals with stroke, multiple sclerosis, head trauma, dementia, or other clinically apparent neurological illness that might influence the extent of WMH volumes unrelated to the aging process were excluded. However, to preserve a population-based sample, individuals with cerebrovascular risk factors or clinically silent cerebrovascular disease were retained for this analysis. Subjects were excluded if they had medical contraindications for MRI.

To assure genetically meaningful information, the data were organized into discreet family structures. Once family structure organization was completed, there were 1330 individuals (704 women and 626 men) who were in genetically useful relationships, including 4 parent–offspring pairs, 608 sibling pairs, 43 avuncular pairs, 5 half-sibling pairs, 312 first cousin pairs, 26 first-cousin once-removed pairs, 4 second-cousin pairs, and 2 double first-cousin pairs. All participants gave informed consent and the Boston University Institutional Review Board approved all protocols.

MR Acquisition Parameters

Subjects were imaged on a Siemens Magnetom 1-tesla field strength magnetic resonance machine using a double spin-echo coronal imaging sequence of 4-millimeter contiguous slices from nasion to occiput with a repetition time (TR) of 2420 ms, echo time (TE) of TE1 20/TE2 90 ms, echo train length 8 ms, field of view (FOV) 22 centimeters, and an acquisition matrix of 182×256 interpolated to 256×256 with 1 excitation.

Image Analysis

After acquisition of the MR scans, the digital information was transferred to a central location for processing and analysis under supervision of 1 of the authors (C.D.), who was blinded to subject clinical and personal identification information. Quantitative analysis of the MR scans was performed with a custom-written computer program operating on a Unix Solaris platform. Image evaluation was based on a semiautomatic segmentation analysis that involves operator-guided removal of nonbrain elements, as previously described.^{19,20} In brief, nonbrain elements were manually removed from the image by operator-guided tracing of the dura matter within the cranial vault including the middle cranial fossa, but above the posterior fossa and cerebellum. The resulting measure of the cranial vault was defined as the total cranial volume (TCV) and served as an estimate of head size to correct for individual variation as well as recognized gender bases differences in brain volumes. Quantification of WMH volumes required a 2-step process that began with image segmentation to define brain matter from cerebral spinal fluid (CSF). For segmentation of brain parenchyma from CSF, a difference image was created by the subtraction of the second echo image from the first echo image. Image intensity nonuniformities were then removed from the difference image, and the resulting corrected image was modeled as a mixture of 2 gaussian probability functions. The segmentation threshold was determined at the minimum probability between the modeled CSF and brain matter intensity distributions.²⁰ For segmentation of WMH from brain matter, the first and second echo images were summed, and after removal of CSF and correction of image intensity nonuniformities, a repeat gaussian normal distribution was fitted to the summed image data after removal of 2 pixels along the image edge where partial volume effects of CSF occur. A

segmentation threshold for WMH was a priori determined as 3.5 standard deviations (SDs) in pixel intensity above the mean of the fitted distribution of brain parenchyma. Intrarater and interrater reliabilities of this method have been published.²⁰ Repeat measurement of intrarater and interrater reliabilities of WMH volumes from this data set were consistently >0.90. Intrarater and interrater measures of TCV consistently differed by <1%. All volumes were calculated as the sum of the pixels within the identified region of interest multiplied by pixel volume in milliliters.

Statistical Analyses: MRI Variables

Examination of the WMH distribution revealed strong rightward skewness (skewness=6.66); therefore, a natural logarithm transformation was performed to obtain a more normal distribution (skewness after transform=−0.07). All genetic analyses were based on the transformed WMH data. In addition, because WMH volumes are strongly age-related and may be correlated with head size, we used sex, age, age², and TCV as covariates for our analytical models.

Genetic Analysis

We assume that phenotypic variance can be decomposed into additive genetic, nonadditive genetic, and environmental sources of variation. The ratio of the additive genetic variance to the total phenotypic variance is called the narrow sense heritability and is referred to as heritability here. The theory of variance decomposition for human pedigrees has been developed by Amos²¹ and Almasy and Blangero.²² The components of variance were estimated by maximum likelihood as implemented in the SOLAR computer package,²² which is also capable of including variation caused by specific covariates. We used a multistep procedure to test the covariates and heritability for significance. First, we maximized the likelihood of a polygenic model that estimated genetic and all covariate effects. Significance of covariates was assessed by comparing the maximum likelihood of the full model to the maximum likelihood of the subset model in which the covariate was removed using a likelihood ratio test. Finally, the significance of the heritability estimate was assessed by comparing the polygenic model with the significant covariates to a “sporadic” model that had the genetic component removed. To assess heritability caused by differences in sex and age, we performed this procedure on the full data set and repeated the analysis with men only, women only, and on 4 age-specific subgroups comprised of individuals at least 40, 50, 60, and 70 years old, respectively.

Results

Subject demographics and average MRI variables are summarized in Table 1. The average age of the study population was 60.99±9.61 years (range 34 to 88) and the average WMH volume was 0.90±1.49 mL, with a natural log-transformed mean (*ln*WMH) of −0.64±1.01. Although there were no significant differences in WMH or *ln*WMH by sex ($P=0.41$ and 0.35 , respectively), average head size (TCV) was significantly smaller in women as compared with men ($P<0.0001$). *ln*WMH was also weakly correlated with TCV for the group as a whole, as well as for men and women (group $r=0.06$, $P=0.03$; male $r=0.05$, $P=0.22$; female $r=0.06$, $P=0.11$); therefore, we included TCV in all heritability estimates. In addition, *ln*WMH volumes were signifi-

TABLE 1. Descriptive Statistics for Brain Morphology Phenotypes

Group	N	Age	WMH	<i>ln</i> WMH	TCV
Men	704	60.74±9.51	0.94±1.68	−0.61±1.00	1343.43±107.43
Women	626	61.20±9.69	0.87±1.30	−0.66±1.01	1181.32±100.80
Total	1330	60.99±9.61	0.90±1.49	−0.64±1.01	1257.62±131.74

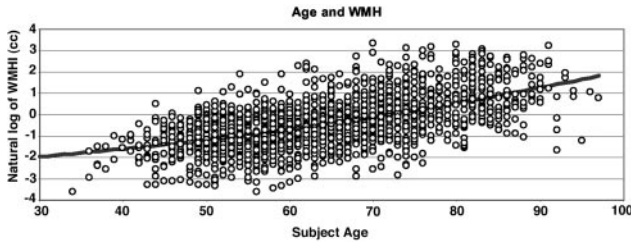


Figure 1. *ln*WMH versus age for the entire sample (n=1330).

cantly associated with age for men ($r=0.48, P<0.0001$) and women ($r=0.50, P<0.0001$). No sex-by-age interaction was found. Multiple regression analysis examining age-related differences in *ln*WMH that included TCV, age, and age² variables showed a significant relation between *ln*WMH and TCV and age², but not age. Figure 1 illustrates the relationship between *ln*WMH and age.

Table 2 summarizes estimates of heritability for the full data set as well as sex-specific and age-specific subsets. In the full data set, heritability was highly significant overall and significant for all age groups, except the oldest. Note that heritability increases as younger individuals are removed from the computation up to age 60, after which the heritability estimates decrease. Covariates were included in the model if they had even a marginal ($P<0.10$) effect. For the total data set, sex was highly significant as a covariate, both overall ($P=0.006$) and in each age group ($P<0.007$), except the oldest ($P=0.25$). Age² was significant in the 40 and older 50 and older age groups ($P<0.008$), but not significant ($P>0.50$) in the 60 and older and 70 and older age groups, and only marginally significant ($P=0.053$) in the full data set. TCV was significant ($P<0.002$) overall and in each age group. The proportion of variation accounted for by the covariates was relatively high in the groups containing younger individuals,

but decreased to negligible amounts in the older groups. Women had generally higher heritability than the full data set, reaching a peak of 0.906 when individuals older than age 40 were included in the analysis. The other major difference between women and the full data set was in the age² covariate; it was not significant in all women, and only in the 50 and older age group did it achieve even marginal significance ($P=0.08$). TCV was significant across all ages. The proportion of variation caused by covariates was low except when age² was included in the model.

Men had slightly lower heritabilities than the full data set, peaking at 0.664 in the oldest age group. In contrast to women, age² in men was significant ($P<0.05$) in the younger age groups. TCV was significant in all but the oldest group. Similar to women, the proportion of variation caused by covariates was relatively high when age² was included in the model but low otherwise.

Graphic display of age-related and gender-related differences in WMH heritability can be seen in Figure 2.

In this study, TCV was used primarily as a covariate to correct for the effect of head size. TCV, however, has also been previously shown to be heritable. We therefore calculated heritability of TCV. The heritability of TCV in the full data set was 0.938 ($P<0.0001$) and remained >0.91 in all age-specific subsets.

Discussion

Our results confirm and extend the initial findings of Carmelli et al.¹⁵ In a general population-based sample of men and women spanning a broad age range, we found that WMH volumes are highly heritable. Consistent with Carmelli et al,¹⁵ we also found that the genetic component accounted for the largest proportion of variation in WMH volumes, even in models that included environmental effects. Moreover, we

TABLE 2. Heritability and Significance of Covariates for White Matter Hyperintensity

	N	Heritability (P)	Covariates*			Proportion of Total Variance Caused by Covariates
All individuals	1330	0.553 (<0.0001)	Sex	Age ²	TCV	0.272
40 and older	1318	0.591 (<0.0001)	Sex	Age ²	TCV	0.263
50 and older	1157	0.654 (<0.0001)	Sex	Age ²	TCV	0.233
60 and older	688	0.666 (<0.0001)	Sex		TCV	0.024
70 and older	277	0.289 (0.1024)			TCV	0.032
Women	704	0.883 (<0.0001)			TCV	0.004
40 and older	698	0.906 (<0.0001)			TCV	0.005
50 and older	617	0.701 (<0.0001)		Age ²	TCV	0.239
60 and older	363	0.641 (0.0223)			TCV	0.031
70 and older	151	0.000 (0.5000)			TCV	0.059
Men	626	0.501 (<0.0005)		Age ²	TCV	0.251
40 and older	620	0.521 (0.0003)		Age ²	TCV	0.247
50 and older	540	0.598 (0.0004)		Age ²	TCV	0.220
60 and older	325	0.619 (0.0037)			TCV	0.019
70 and older	126	0.664 (0.0310)				0.000

*Significant at the 0.10 level and therefore included in the model.

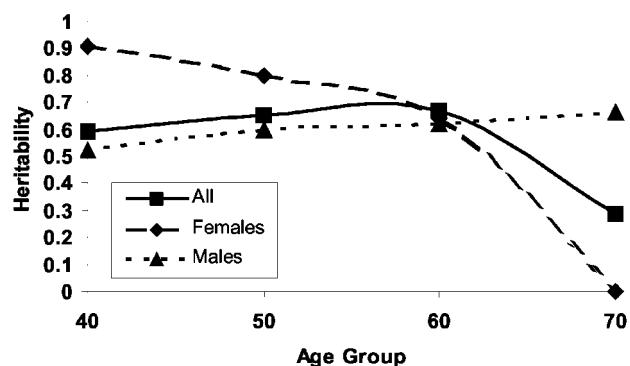


Figure 2. Heritability of \ln WMH by age and sex.

also extended the findings of Carmelli et al¹⁵ to show that heritability estimates were also high in women and remained high even at a relatively young age for both sexes. These findings raise questions that may reflect both differential biological effects and attributes of the design and analysis of this study.

Numerous studies suggest differences in brain aging for men and women (see Murphy et al²³ for review), including differences in WMH.^{5,24} In these semiquantitative studies, WMH measures were significantly greater in woman than in men. It is tempting to speculate that these sex-specific differences in WMH may reflect the impact of menopause on cerebrovascular risk factors among older women that are associated with WMH.²⁵ Given the high heritability of WMH shown by the results of this study and the results of Carmelli et al, it would not be surprising to see sex-related differences in heritability consistent with sex-specific differences in cross-sectional population studies. Although intriguing, these observations need confirmation and methodological limitations to the current study need to be examined. For example, WMH volumes at younger ages were quite small on average, particularly for women. In addition, the variance of these measures was equally low, suggesting a limited range of possible volumes and the potential for unstable estimates. This might explain why the heritability estimates changed most for the women of this study. Alternatively, the relation between WMH and age could differ among relatively younger versus older individuals. Clinically symptomatic cerebrovascular disease is relatively uncommon among individuals younger than 50 years of age; this is particularly true for women. Age- and sex-specific differences in heritability estimates, therefore, may also reflect age- and sex-specific differences in the cause of WMH.

Contrary to the age- and sex-specific differences in WMH heritability, we found consistently high heritability estimates for TCV, similar to the Carmelli et al¹⁵ study. Moreover, TCV heritability estimates did not differ by age. The observed constant heritability of TCV across age conforms to previous expectations that head size is strongly genetically determined; of course, once adulthood is reached, head size does not change.

The limitations of this study are those inherent in the assumptions of the genetic model and a large population spanning a broad age range. First, the estimate is only of

additive genetic variation. Although this may be robust, including a dominance component may be more realistic. Second, if there are unmeasured environmental effects that are family-specific, then heritability may be overestimated. Third, the calculation gives no insight into the number of genes or their relative effect; a high heritability estimate is not specific to a single gene with a large effect but may indicate a number of genes that together exert a strong effect. Moreover, as we have suggested, there may be multiple strong genetic influences on WMH (eg, aging and cerebrovascular disease). Understanding the effect of age, however, is problematic for the study of WMH. Aging and other genetic effects (eg, shared cerebrovascular diseases) may overlap. If this is true, then using age in the model could actually lead to underestimation of the genetic component(s). To investigate this, age was removed from the model and heritability was recomputed. The heritability increased to 0.775 in the full data set, similar to the heritability observed by Carmelli et al in the NHLBI twins data in which aging effects were insignificant because of the nature of the sample.

The overall heritability of WMH from this study and the study of Carmelli et al¹⁵ suggest a large genetic component to the individual expression of WMH volume. This evidence raises interesting questions regarding the potential cause of WMH. As we noted, WMH volumes are strongly affected by both age and the presence of cerebrovascular disease. High heritability estimates for WMH, therefore, might indicate pleiotropy with complex aging traits, complex cerebrovascular risk traits, or both. Importantly, linkage studies might suggest chromosomal regions or candidate genes that would clarify factors involved in genetic regulation of WMH. Further work on the heritability of cerebrovascular disease within this cohort, however, may also clarify possible genetic influences. For example, previous studies of cerebrovascular disease have focused on stroke incidence and prevalence with only limited results. Ongoing work with the Framingham Heart Study participants includes MRI detection of silent cerebral infarctions. Evidence for shared heritability between WMH and silent cerebral infarctions would support vascular-related genetic influences. Conversely, absence of such a relationship would favor complex aging traits, of which cerebrovascular disease may only be 1 part.

In conclusion, we found high heritability estimates of WMH volumes for men and women. Moreover, this high heritability is seen by middle age when symptomatic cerebrovascular disease is uncommon. These findings confirm those of Carmelli et al¹⁵ and support the hypothesis that the formation of WMH is under considerable genetic influence. Whether these influences result from genetic differences in the aging process or cerebrovascular disease is unknown. WMH, therefore, should serve as excellent phenotypes for linkage studies to determine the genetic influence of brain aging and cerebrovascular disease.

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