Multiethnic Genome-Wide Association Study of Cerebral White Matter Hyperintensities on MRI

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Background—The burden of cerebral white matter hyperintensities (WMH) is associated with an increased risk of stroke, dementia, and death. WMH are highly heritable, but their genetic underpinnings are incompletely characterized. To identify novel genetic variants influencing WMH burden, we conducted a meta-analysis of multiethnic genome-wide association studies.

Methods and Results—We included 21 079 middle-aged to elderly individuals from 29 population-based cohorts, who were free of dementia and stroke and were of European (n=17 936), African (n=1943), Hispanic (n=795), and Asian (n=405) descent. WMH burden was quantified on MRI either by a validated automated segmentation method or a validated visual grading scale. Genotype data in each study were imputed to the 1000 Genomes reference. Within each ethnic group, we

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investigated the relationship between each single-nucleotide polymorphism and WMH burden using a linear regression model adjusted for age, sex, intracranial volume, and principal components of ancestry. A meta-analysis was conducted for each ethnicity separately and for the combined sample. In the European descent samples, we confirmed a previously known locus on chr17q25 (P=2.7×10^{-19}) and identified novel loci on chr10q24 (P=1.6×10^{-9}) and chr2p21 (P=4.4×10^{-8}). In the multiethnic meta-analysis, we identified 2 additional loci, on chr1q22 (P=2.0×10^{-8}) and chr2p16 (P=1.5×10^{-8}). The novel loci contained genes that have been implicated in Alzheimer disease (chr2p21 and chr10q24), intracerebral hemorrhage (chr1q22), neuroinflammatory diseases (chr2p21), and glioma (chr10q24 and chr2p16).

Conclusions—We identified 4 novel genetic loci that implicate inflammatory and glial proliferative pathways in the development of WMH in addition to previously proposed ischemic mechanisms. **(*Circ Cardiovasc Genet*. 2015;8:398-409. DOI: 10.1161/CIRCGENETICS.114.000858.)**

Key Words: cerebral small vessel diseases ■ cerebrovascular disorders ■ genome-wide association study ■ hypertension ■ leukoencephalopathies ■ polymorphisms, single nucleotide

Cerebral white matter hyperintensities (WMH) are common in the aging population and are associated with an increased risk of stroke, vascular cognitive impairment, dementia, and death.[1] The prevalence and severity of WMH increase with advancing age and the presence of vascular risk factors, notably hypertension.[2] The pathophysiology of WMH is poorly understood but likely reflects ischemic or degenerative damage to the small vessels of the brain, leading to chronic cerebral hypoperfusion and myelin rarefaction.[3] Perivascular inflammation is a prominent pathological feature in WMH,[4] and WMH burden has been associated with circulating biomarkers of inflammation, including high-sensitivity C-reactive protein, interleukin-6, lipoprotein-associated phospholipase A2, and myeloperoxidase.[5,6]

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Twin and family studies suggest that the heritability of WMH is 55% to 80%.[7–9] Yet, few genetic variants have been identified and they explain only a small proportion of the phenotypic variance,[10] suggesting that additional variants remain to be discovered. The first meta-analysis of genome-wide association studies (GWAS) on WMH burden identified a new locus on Chr17q25,[11] which has been replicated in several studies.[12–14] It comprised 9361 individuals of European descent and used genome-wide genotype data imputed to the HapMap2 reference panel.[11] In recent years, the 1000 Genomes reference panel has become available for genotype imputation, enabling the study of millions of single-nucleotide polymorphisms (SNPs), including low-frequency variants. Furthermore, additional studies with brain MRI data have obtained genome-wide genotype data, including studies in populations of African, Hispanic, and Asian descent. Here, we conducted a meta-GWAS of WMH burden based on 1000 Genomes imputation data in 21 079 individuals from 4 ethnic groups. To gain pathophysiological insights, we also investigated the joint effect on WMH burden of genetic loci for high blood pressure levels, a strong predictor of WMH burden, and for Alzheimer disease and stroke, which, both, have comorbid loads of WMH.

Subject and Methods

Study participants were from 29 population-based cohorts. All participating studies worked cooperatively to address issues related to phenotype harmonization and covariate selection and to develop analytic plans for within-study GWAS analyses and for meta-analyses of results. Each study received institutional review board approval of its consent procedures, examination and surveillance, DNA collection and use, and data access and distribution. All participants in this study gave written informed consent for study participation, MRI scanning, and use of DNA. Details of cohort recruitment, risk factor assessment, phenotyping, and genotyping are described in the Data Supplement. Briefly, participants were excluded if they lacked information on MRI or genotypes or if they had clinical dementia or stroke. If data on clinical stroke were missing in a given cohort, the presence of MRI infarcts extending into the cortical grey matter was used as an exclusion criterion.

MRI Scans

In each study, MRI scans were performed and interpreted in a standardized fashion, without reference to demographic or clinical information. The field strength of the scanners used ranged from 0.5 to 3.0 Tesla. T1- and T2-weighted scans in the axial plane were obtained for all participants. These were complemented by either scans obtained with fluid attenuation inversion recovery or proton density sequences to allow better separation of WMH and cerebrospinal fluid. A validated automated segmentation method (23 cohorts) or a validated visual grading scale (6 cohorts) was used to quantify WMH burden. Details of the applied WMH quantification method per cohort can be found in the Data Supplement.

Comparability between the volumetric and visual scales has been evaluated previously and was shown to be similar across cohorts.[11] Details about the extensive phenotype harmonization procedures performed before GWAS have been previously reported.[11]

Genotyping and Imputation

As described in the Data Supplement, the participating studies used different genotyping platforms and performed extensive quality control analyses. Briefly, participant-specific quality controls filters were applied based on missing call rate, cryptic relatedness, sex mismatch, principal component analysis, and number of Mendelian errors per individual (for studies with family data). SNP-specific quality controls included filters for call rate, minor allele frequency Hardy–Weinberg equilibrium, differential missingness by outcome or genotype, and imputation quality. After quality control analysis, genotype data in each study were used to impute to the 1000 Genomes reference panel (version available in the Data Supplement). Because not all versions of 1000 Genomes that were used included copy number variations, we only analyzed SNPs, which totaled 14 227 402.

Statistical Analyses and Meta-Analysis

Statistical analyses were performed similar to the previous meta-GWAS on WMH burden.[8] Analyses were performed separately by

ethnic group. Briefly, within each study, we evaluated the relationship between each SNP and WMH burden using a linear regression model, assuming an additive genetic effect.[11] WMH burden was expressed as ln(WMH burden+1) to reduce the skewness of its distribution. We adjusted for age, sex, intracranial volume and, if indicated, principal components of ancestry. No adjustment for intracranial volume was performed in studies that used a visual grading scale because these scales take head size into account. Atherosclerosis Risk In Communities Study (ARIS) and Cardiovascular Health Study (CHS) also adjusted for study site, and Framingham Heart Study (FHS) adjusted for familial structure (Data Supplement).

We performed a weighted Z score–based fixed-effect meta-analysis implemented in the METAL software.[15] We chose this methodology for several reasons: first, the measures of WMH were not expressed on the same scale in the various studies, thus a random-effect meta-analysis was not possible. Second, the focus of our meta-analyses was to identify new loci for WMH, thus we sought to maximize power of our study. Fixed-effect models have been shown to be more powerful than random-effect models even in the presence of between-study heterogeneity.[16] Third, Senn stated that "the choice of fixed effects or random effects meta-analysis should not be made on the basis of perceived heterogeneity but on the basis of purpose."[17] Our purpose was to identify new associations rather than accurately estimating effect size of well-validated variants, which would need to account for possible between-population heterogeneity. For each SNP, the *z*-statistic, derived from the *P* value and direction of effect, was weighted by the effective sample size, which is the product of the sample size and the ratio of the empirically observed dosage variance to the expected binomial dosage variance for imputed SNPs. A combined estimate was obtained by summing the weighted *z*-statistics and dividing by the summed weights. Before meta-analysis, SNPs were filtered out within each cohort if they had a poor imputation quality (r^2>0.3), a minor allele frequency <0.005, and an effective sample size <50. The genomic control parameter was calculated and used to remove any residual population stratification within cohort and in the combined meta-analyses. We performed meta-analyses for each ethnicity separately and also combined results in a multiethnic meta-analysis, correcting for genomic inflation.

To gain a better understanding of each genome-wide significant locus ($P<5\times10^{-8}$), we performed a step-wise analysis to examine whether additional variants at the identified loci were independently associated with WMH burden, after adjusting for the effects of the most significant SNP. Each study repeated the primary analyses adjusting for the top-SNP at each of the significant loci (European ancestry sample only), and the results were then meta-analyzed as described above.

To study whether identified SNPs may cause damage of protein function, we used the prediction tools PolyPhen-2[18] and SIFT.[19] To examine whether identified SNPs had an impact on gene regulation, we used a heuristic scoring system implemented in RegulomeDB.[20]

In secondary analyses, we studied the joint effect of loci for WMH-related traits. We extracted SNPs from the meta-analysis that have been reported to be associated with blood pressure,[21] Alzheimer disease,[22] and stroke[23–25] and meta-analyzed their effects using a weighted *Z* score method.[26] Additional details are provided in the in the Data Supplement.

Results

Demographic and clinical characteristics of the participating cohorts are shown in the Data Supplement (Table I in the Data Supplement). In total, we included 17 936 individuals of European descent, 1943 African descent, 795 individuals of Hispanic descent, and 405 individuals of Asian descent (204 Chinese and 201 Malays). There was no evidence of test statistic inflation in the individual cohort analyses or the ethnic-specific and overall meta-analyses (Table I in the Data Supplement).

Table 1 shows the genome-wide significant loci ($P<5\times10^{-8}$) in the meta-analyses of the overall sample and of each ethnic group. Manhattan-plots are displayed in the Figure II in the Data Supplement. In the European descent samples, we identified 3 regions with genome-wide significant SNPs: on chr17q25 (top-SNP: rs7214628, $P=2.7\times10^{-19}$); on chr10q24 (top-SNP: rs72848980, $P=1.6\times10^{-9}$); and on chr2p21 (top-SNP: rs11679640; $P=4.4\times10^{-8}$; Table 1). In the samples of African, Hispanic, and Asian descent, no variant reached genome-wide significance likely because of limited power. In the multiethnic analyses, we identified 2 additional regions, on chr1q22 (top-SNP: rs2984613, $P=2.0\times10^{-8}$) and chr2p16 (top-SNP: rs78857879, $P=1.5\times10^{-8}$; Table 1). Directions of effect for these SNPs in each of the cohorts are shown in Table II in the Data Supplement and information on suggestive loci ($P<1.0\times10^{-5}$) in Table III in the Data Supplement.

The chr17q25 locus contained 147 genome-wide significant SNPs in the meta-analysis of the European descent samples (Figure). The top-SNP from chr17q25 (rs7214628) lies close (2.9 kb) to *TRIM65* and is in high linkage disequilibrium (LD) with rs3744028, reported in our previous GWAS (r^2=0.99).[11]

Table 1. Genome-Wide Significant Loci for White Matter Hyperintensities Burden

				P Value									
Locus	SNP	Chr:Position (hg19)	Nearest Gene	Total (n=21 079)	EUR (n=17 936)	AFR (n=1943)	HIS (n=795)	ASN (n=405)	RA	RAF EUR	RAF AFR	RAF HIS	RAF ASN
17q25.1	rs7214628	17:73882148	TRIM65	+5.1E–19	+2.7E–19	+0.12	+0.11	–0.32	G	0.19	0.40	0.28	0.13
10q24.33	rs72848980	10:105319409	NEURL (intron)	+2.6E–09	+6.3E–09	+0.09	+0.41	–0.31	G	0.80	0.96	0.93	0.97
	rs7894407	10:105176179	PDCD11 (intron)	+2.6E–08	+1.6E–09	–0.36	+4.4E–02	–0.46	T	0.65	0.80	0.69	0.61
	rs12357919	10:105438112	SH3PXD2A (intron)	+1.5E–08	+1.9E–08	+0.36	+0.31	+1.00	T	0.81	0.95	0.92	0.96
	rs7909791	10:105613178	SH3PXD2A (intron)	+2.9E–09	+1.7E–08	+0.33	+0.29	+0.09	A	0.34	0.35	0.32	0.16
2p16.1	rs78857879	2:56135099	EFEMP1 (intron)	+1.5E–08	+2.9E–07	+2.2E–02	+0.18	–0.67	A	0.10	0.02	0.05	0.04
1q22	rs2984613	1:156197380	PMF1–BGLAP (intron)	+2.0E–08	+1.4E–05	+6.5E–05	+1.5E–02	–0.80	C	0.65	0.72	0.69	0.68
2p21	rs11679640	2:43141485	HAAO	+2.1E–06	+4.4E–08	–0.37	–0.79	–0 0.74	C	0.80	0.84	0.85	0.98

Loci with corresponding *P* value are given for the association with white matter hyperintensities burden. The sign indicates the direction of the effect of the risk allele. Multiple single-nucleotide polymorphisms at the same locus indicate independent associations. AFR indicates African descent; ASN, Asian descent; Chr, chromosome; EUR, European descent; HIS, Hispanic descent; RA, risk allele; RAF, risk allele frequency; and SNP, single-nucleotide polymorphism.

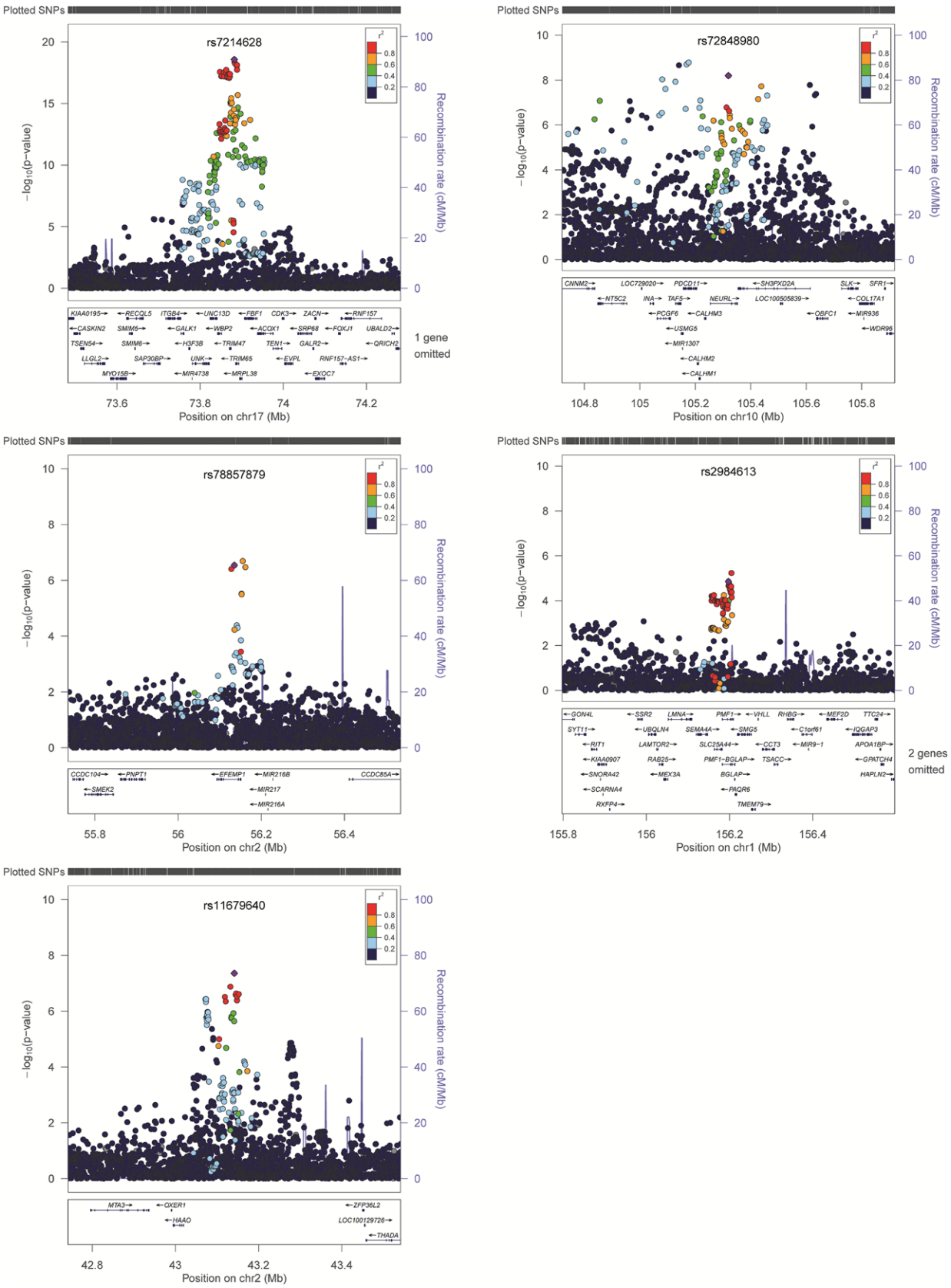

Figure. Regional plots of the genome-wide significant loci in individuals of European descent. Loci on chr17q25.1, chr10q24.33, chr2p16.1, chr1q22, and chr2p21 are shown. Each circle indicates a single-nucleotide polymorphism (SNPs) with a color scale corresponding to the r^2 value for that SNP and the top SNP from 1000 Genomes. Purple diamonds indicate the SNPs with the strongest association in the overall meta-analysis. Estimated recombination from 1000 Genomes are indicated blue lines. The bottom panels show the relative position of genes within each locus.

Analyses of association adjusting for the effect of rs7214628 were performed to determine whether secondary signals were present across the region. None of the 147 SNPs remained genome-wide significant after accounting for the effect of rs7214628 (Figure III in the Data Supplement). Ten were nominally significant ($P<0.05$), including 4 intronic variants and 1 missense variant in *ACOX1*, 3 intronic variants and 1 variant near *FBF1*, and 1 intronic variant in *MRPL38*, but would not survive a multiple testing significance threshold. Functional annotation of the genome-wide significant SNPs in the chr17q25 region identified 7 missense variants, 4 eQTLs influencing gene expression of *TRIM47*, 10 SNPs with a likely functional impact on gene regulation (RegulomeDB score ≤3), and 6 synonymous or intronic SNPs with high levels of evolutionary conservation. Association of these SNPs with WMH burden in each ethnic group is shown in Table 2. The direction of association was generally consistent in Europeans, Hispanics, and blacks but was opposite in Asians. This pattern was also observed across the larger set of 147 genome-wide significant SNPs, suggesting possible heterogeneity of effects in Asian populations. Among the putatively functional SNPs, those with the strongest LD with rs7214628 in Europeans were the *TRIM47* eQTL rs3744017 and the putatively regulatory SNP rs3744020, located in an intron of *TRIM47*. Interestingly, these 2 SNPs had also the lowest *P* value in blacks (rs3744017, $P=0.08$; rs3744020, $P=0.09$). We observed a nominally significant association ($P<0.05$) for the regulatory SNP rs1551619 in Hispanics. This SNP was in moderately high LD with rs7214628 in both Europeans and Hispanics ($r^2=0.74$).

The chr10q24 locus contained 12 genome-wide significant SNPs in the meta-analysis of the European descent samples and 7 SNPs in the overall meta-analysis. These mapped to a 555-kb region from base pair position 105080575 to 105635537 (Figure). The top-SNP from chr10q24 (rs7894407) lies within an intron of *PDCD11*. Analyses accounting for the effects of rs7894407 revealed that the SNPs in *SH3PXD2A (*rs12357919, $P=2.7\times10^{-3}$; rs4630220, $P=2.7\times10^{-3}$; rs7909791, $P=3.9\times10^{-7}$), and *NEURL* (rs72848980, $P=1.9\times10^{-3}$) were independently associated with WMH burden (Figure III in the Data Supplement). Ivn the multiethnic meta-analysis, rs72848980 (*NEURL*) had the lowest *P* value within the chr10q24 locus. These 4 SNPs were in low LD with rs7894407 (r^2 between 0 and 0.33), and in moderate to low LD with each other (Table IV in the Data Supplement). Functional annotation of the genome-wide significant SNPs identified a missense variant in TAF5 (rs10883859, Ser/Ala). The exonic variant in CALHM1 (rs4918016) was synonymous. Annotation of the genome-wide significant SNPs for predicted function on gene regulation identified 2 SNPs (RegulomeDB score ≤3): rs12357919 located in an intron of *SH3PXD2A*; and rs729211 located in the 3′untranslated region of CALMH1, and identified as an eQTL influencing gene expression of USMG5. rs11191772 was a highly conserved intronic SNP in *SH3PXD2A*. Association of these SNPs with WMH burden in each ethnic group is shown in Table 3. rs729211, rs4918016, and rs11191772, identified in the multiethnic meta-analyses, show trends toward nominal significance in blacks and Hispanics.

The chr2p16 locus contained 1 genome-wide significant SNP (rs78857879) in the multiethnic meta-analysis. This SNP maps to an intron of *EFEMP1* (Figure) and was predicted to have a putatively functional impact on gene regulation (RegulomeDB score=3a). This SNP was nominally significant in the groups of European and African descent ($P=2.9\times10^{-7}$ and 2.2×10^{-2}, respectively; Table 1).

The chr1q22 locus contained 1 genome-wide significant SNP (rs2984613) in the multiethnic meta-analysis. Although the association of rs2984613 with WMH burden was only nominally significant in individuals of European, African, and Hispanic descent ($P=2.4\times10^{-5}$, 1.4×10^{-5}, and 1.5×10^{-2}, respectively), it reached genome-wide significance in the multiethnic meta-analysis combining all ethnic groups. This SNP is located in an intron of PMF1/PMF1-BGLAP (Figure).

The chr2p21 locus contained 1 genome-wide significant SNP (rs11679640) near HAAO (122 kb) and THADA (316 kb) in individuals of European descent only (Table 1; Figure). The association of rs11679640 with WMH burden was no longer genome-wide significant in the overall meta-analysis and showed opposite direction of effect in the other ethnic groups (Table 1), suggesting possible heterogeneity by ethnicity. Although a genome-wide significant SNP for multiple sclerosis (rs6718520)[22] is also nearby (184 kb), this SNP was not in LD with rs11679640 and was not significantly associated with WMH burden in our study.

To gain additional pathophysiological insights, we investigated whether genetic loci identified for WMH-related traits are related to WMH burden.

We showed that genetic loci for blood pressure were significantly related to a higher WMH burden (*P* for systolic blood pressure <0.0001; *P* for diastolic blood pressure=0.007). We did not find a significant association between WMH and loci for Alzheimer disease ($P=0.12$) or stroke ($P=0.46$, in opposite direction).

Discussion

We performed a meta-analysis of genome-wide association studies in samples of European, African, Hispanic, and Asian descent. We identified 4 novel loci on chr10q24, chr2p21, chr1q22, and chr2p16 and confirmed a previously identified locus on chr17q25. Three of the 4 novel loci (chr10q24, chr1q22, and 2p16.1) were associated with WMH burden in >1 ethnic group. In addition, we showed that genetic loci influencing systolic blood pressure and diastolic blood pressure are associated with WMH burden.

Strengths of our study include the large and diverse sample, the population-based setting, and the comprehensive set of common genetic variants examined. However, several limitations must be acknowledged. First, the use of different WMH quantification methods has constrained our analytic methodology to the meta-analysis of *P* values, which is less powerful and prevented us to determine an estimate of effect size for the associated SNPs. Second, we had a limited sample size of blacks, Hispanics, and Asians. This limited sample size has reduced our ability to identify new variants in these populations and to replicate findings from the larger European sample. However, the inclusion of these groups in a multiethnic meta-analysis permitted the identification of 2 additional loci, albeit likely because of increased sample size. Finally,

Table 2. Association of Putatively Functional SNPs at the 17q25.1 Locus by Ethnic Group

Chr	Position (hg19)	SNP	Putative Function and Location	RA	LD With rs7214628 (EUR)	*P* Value EUR	*P* Value AFR	*P* Value HIS	*P* Value ASN	RAF EUR	RAF AFR	RAF HIS	RAF ASN
17	73827205	rs1135688	Missense (K867E, *UNC13D*)	C	0.32	+1.7E−08	+0.60	+0.22	−0.12	0.31	0.81	0.50	0.37
17	73839366	rs3744009	Regulatory (RDB=3a) (intronic, *UNC13D*)	T	0.29	+1.8E−10	−0.89	+0.22	−0.23	0.26	0.44	0.31	0.15
17	73841285	rs2410859	Regulatory (RDB=2b) (5′-UTR *UNC13D*)	C	0.44	+1.9E−11	+0.48	+0.16	−0.20	0.32	0.82	0.51	0.38
17	73841702	rs9900122	Regulatory (RDB=2b) (3′-UTR, WBP2)	C	0.44	+1.5E−11	−0.98	+0.19	−0.21	0.32	0.76	0.48	0.38
17	73844748	rs2290771	Regulatory (RDB=2b) (intronic, *WBP2*)	G	0.46	+8.1E−11	+0.39	+0.17	−0.17	0.32	0.82	0.50	0.16
17	73847613	rs936393	Regulatory (*TRIM47* eQTL; RDB=1f) (intronic, *WBP2*)	G	0.86	+2.7E−18	+0.74	+0.96	−0.46	0.19	0.26	0.21	0.14
17	73851113	rs55868394	Regulatory (RDB=2b) (intronic, *WBP2*)	A	0.63	+1.5E−13	−0.87	+0.22	−0.34	0.13	0.03	0.08	0.09
17	73852008	rs936394	Regulatory (RDB=2b) (5′-UTR, *WBP2*)	A	0.89	+6.0E−18	+0.62	+0.65	−0.41	0.19	0.26	0.21	0.14
17	73865657	rs9894383	Regulatory (*TRIM47* eQTL; RDB=2b) (4.6kb 3′ of *TRIM47*)	G	0.91	+7.6E−18	+0.18	+0.34	−0.30	0.19	0.59	0.35	0.14
17	73871467	rs3744017	Regulatory (*TRIM47* eQTL; RDB=1f) (intronic, *TRIM47*)	A	0.93	+6.7E−18	+0.09	+0.16	−0.27	0.19	0.29	0.23	0.13
17	73871773	rs3744020	Regulatory (RDB=2a) (intronic, *TRIM47*)	A	0.93	+4.1E−18	+0.10	+0.16	−0.29	0.19	0.29	0.22	0.13
17	73873394	rs9908862	Regulatory (RDB=2b), Conserved (intronic, *TRIM47*)	G	0.73	+7.9E−16	+0.21	+0.13	−0.31	0.14	0.50	0.28	0.12
17	73874071	rs4600514	Missense (R187W, *TRIM47*)	A	0.74	+6.3E−16	+0.20	+0.11	−0.30	0.14	0.32	0.21	0.12
17	73874138	rs4072479	Conserved, synonymous (A164A, *TRIM47*), regulatory (RDB=2b)	C	0.72	+5.6E−15	+0.43	+0.21	−0.30	0.14	0.44	0.26	0.12
17	73885805	rs1551619	Regulatory (RDB=2b) (3′-UTR, *TRIM65*)	T	0.74	+2.2E−14	+0.24	+4.4E−02	−0.34	0.23	0.33	0.27	0.13
17	73886888	rs3760128	Missense (L509P, *TRIM65*)	G	0.46	+6.9E−12	+0.65	+0.12	−0.06	0.33	0.82	0.51	0.20
17	73888427	rs7222757	Missense (V222G, *TRIM65*)	C	0.56	+1.3E−14	−0.95	+0.34	−0.07	0.28	0.71	0.45	0.20
17	73922941	rs2305913	Missense (R151G, *FBF1*)	C	0.41	+4.7E−11	+0.92	+0.13	−2.1E−02	0.34	0.76	0.50	0.19
17	73926121	rs1135889	Missense (G65V, *FBF1*)	A	0.29	+9.5E−11	−0.79	+0.16	−4.9E−03	0.23	0.19	0.18	0.13
17	73949540	rs1135640	Missense (I312M, *ACOX1*)	G	0.41	+3.3E−10	+0.88	−0.17	−7.0E−03	0.35	0.67	0.49	0.19

AFR indicates African descent; ASN, Asian descent; Chr, chromosome; EUR, European descent; HIS, Hispanic descent; LD, linkage disequilibrium; RA, risk allele; RAF, risk allele frequency; and SNP, single-nucleotide polymorphism.

we used different versions of the 1000 Genomes reference panel for genotype imputation and did not study copy number variations.

We confirmed the association of the locus on chr17q25 in individuals of European descent. The genome-wide significant SNPs in this locus include all previously reported SNPs.[11] However, the most significantly associated SNP in this analysis (rs7214628) was not previously identified. It lies 2.9 kb away from *TRIM65* and in strong LD with rs3744028 from the original report. Analyses accounting

Table 3. Association of Top-SNPs and Putatively Functional SNPs at the 10q24 Locus by Ethnic Group

Chr	Position (hg19)	SNP	Putative Function and Location	RA	LD with rs7894407 (EUR)	*P* Value EUR	*P* Value AFR	*P* Value HIS	*P* Value ASN	RAF EUR	RAF AFR	RAF HIS	RAF ASN
10	105128134	rs10883859	Missense (S130A, *TAF5*)	T	0.64	+1.2E–08	–0.13	+0.09	–0.27	0.67	0.75	0.67	0.57
10	105214932	rs729211	Regulatory (*USMG5* eQTL, RDB=1f) (3′-UTR, *CALHM1*)	T	0.65	+1.7E–07	+0.21	+0.08	–0.69	0.67	0.63	0.62	0.61
10	105218254	rs4918016	Conserved, synonymous (P85P, *CALHM1*)	C	0.66	+8.1E–08	+0.38	+0.06	–0.71	0.67	0.80	0.70	0.61
10	105438112	rs12357919	Regulatory (RDB=2b) (intronic, *SH3PXD2A*)	T	0.11	+1.9E–08	+0.36	+0.31	0.99	0.81	0.95	0.92	0.96
10	105459834	rs11191772	Conserved (intronic, *SH3PXD2A*)	T	0.04	+1.0E–06	+0.07	+0.22	0.17	0.60	0.66	0.61	0.43

AFR indicates African descent; ASN, Asian descent; Chr, chromosome; EUR, European descent; HIS, Hispanic descent; LD, linkage disequilibrium; RA, risk allele; RAF, risk allele frequency; and SNP, single-nucleotide polymorphism.

for the effects of rs7214628 showed a strong attenuation of effects for all genome-wide significant SNPs, suggesting little evidence for multiple independent association signals in this region. Several genome-wide significant SNPs in the chr17q25 locus are missense variants in the *UNC13D*, *TRIM47*, *TRIM65*, *FBF1*, and *ACOX1* genes. In addition, several SNPs were predicted to have a functional impact on gene regulation, including 2 eQTLs of the *TRIM47* gene. The direction of associations of SNPs at this locus was generally consistent among populations of European, Hispanic, and African descent but not Asians. Power to detect genetic effects in ethnic groups other than Europeans was limited. However, SNPs potentially affecting regulation of *TRIM47* and *TRIM65* showed the strongest associations in this region in Hispanics and blacks, whereas SNPs encoding missense mutations in *FBF1*, *ACOX1*, and *TRIM65* were nominally associated in Asians.

The novel locus on chr10q24 contained genome-wide significant SNPs in introns of *PDCD11*, *NEURL*, and *SH3PXD2A*, *TAF5*, and *CALHM1*, of which *PDCD11*, *NEURL*, and *SH3PXD2A* were shown to be independent from each other. *PDCD11* encodes the programmed cell death 11 and is involved in T-cell–induced apoptosis.[27] It is expressed in glial cells,[28] which make up a large proportion of the white matter. *NEURL* encodes the neuralized homolog (Drosophila), an E3 ubiquitin ligase, which has been implicated in malignant brain tumors.[29,30] *NEURL* reportedly causes apoptosis and downregulates NOTCH target genes in medulloblastoma.[29] *NEURL* maps to a region that is frequently deleted in astrocytoma.[30] The SNP in *NEURL* was also nominally associated in Hispanics in the same direction (P=0.04). The SNP in *PDCD11* only showed significant associations in individuals of European descent. *SH3PXD2A*, which codes for *SH3 and PX domain-containing protein 2A*, has also been implicated in gliomas.[31] In addition, it has been reported to be involved in amyloid-β neurotoxicity[32] and implicated in Alzheimer disease.[33] *TAF5* contained a missense variant, although without predicted damage on protein function. *TAF5* codes for *transcription initiation factor TFIID subunit 5*, which is involved in the initiation of transcription by RNA polymerase II. *CALHM1* codes for *calcium homeostasis modulator 1*, which influences calcium homeostasis and increases cerebral amyloid-β (Aβ) peptide production. Interestingly, a missense variant of *CALHM1* (rs2986017) has been associated with late-onset Alzheimer disease and Creutzfeldt–Jakob disease,[34,35] but this SNP was only nominally associated with WMH burden (in the same direction) in our study (P=2.5×10^{-2} in Europeans and P=3.5×10^{-2} in the total group). The genome-wide significant SNP rs729211, located in the 3′untranslated region of *CALHM1,* had a predicted functional impact on *USMG5* gene expression. *USMG5* encodes a small subunit of the mitochondrial ATP synthase, which is phylogenetically conserved and is thought to have a role in cellular energy metabolism.

The novel locus on chr2p21 that reached genome-wide significance in Europeans but not the overall group was located near *HAAO*. *HAAO* codes for *3-hydroxyanthranilate 3,4-dioxygenase*, which catalyzes the synthesis of quinolinic acid (QUIN) from 3-hydroxyanthranilic acid. QUIN is an excitotoxin whose toxicity is mediated by its ability to activate glutamate N-methyl-D-aspartate receptors. QUIN has been implicated in neuroinflammatory diseases and may participate in the pathogenesis of Parkinson disease, Alzheimer disease, and Huntington disease.[36–39] Within the brain, QUIN is produced and released by infiltrating macrophages and activated microglia, which are prominent during neuroinflammation.[36]

The novel genome-wide significant SNP on chr1q22 is located in an intron of the read-through *PMF1-BGLAP* sequence, which encodes a variant isoform of the *polyamine-modulated factor 1* (PMF1). PMF is a member of a kinetochore-associated multiprotein complex, involved in chromosomal alignment and segregation during mitosis.[40] Moreover, it is a cofactor for the regulation of expression of the rate-limiting enzyme in the catabolic pathway of polyamine metabolism.[41] Polyamines are important regulators of cell growth and cell death, and epigenetic modification of *PMF1* has been implicated in cancer.[42] The SNP identified in our analysis (rs2984613) was also identified in a GWAS of nonlobar intracerebral hemorrhage.[43] In one study involving two of the cohorts included in this work, WML burden was associated with an increased risk of

intracerebral hemorrhage.[44] Both intracerebral hemorrhage and WMH share common risk factors, such as hypertension, and may share common underlying pathological mechanisms involving microangiopathy. Our finding supports such a hypothesis.

The locus on chr2p16 contained its top-hit in the intron of *EFEMP1*, which codes for *EGF containing fibulin-like extracellular matrix protein 1. EFEMP1* is uniquely upregulated in malignant gliomas (different grades) and promotes tumor cell motility and invasion.[45] It encodes a novel soluble activator of Notch signaling that antagonizes DLL3, an autocrine inhibitor or Notch, and promotes tumor cell survival and invasion in a Notch-dependent manner.[46] *EFEMP1* was originally cloned from senescent fibroblasts derived from a patient with Werner syndrome a disease of premature aging with diffuse structural abnormalities in the brain white matter.[47,48]

Intriguingly, 3 of the 5 regions significantly associated with WMH burden and 1 suggestive locus contained variants in genes implicated in malignant brain tumors of the white matter that involve glial cells (*TRIM47*, *NEURL, SH3PXD2A, EFEMP1*, and *NBEAL1*). Although these tumors can appear as WMH on MRI,[49] given the population-based setting of the participating studies, the exclusion criteria used in WMH quantification, as well as the very low incidence of gliomas (<5 per 100 000 persons per year),[50] the presence of unrecognized glioma cases is very unlikely to explain these associations. However, our findings suggest that WMH in aging and glioma may share common pathophysiological mechanisms, perhaps involving glial cell activation, apoptosis, or both. The role of microglia in white matter injury has been demonstrated in several animal models. For example, activated microglia have a critical role in the formation of the excitotoxic white matter lesion in a mouse model of periventricular leukomalacia.[51] In the rat 2-vessel occlusion model, microglial activation was shown to be an early marker of subsequent white matter injury[52] and may contribute to induce apoptosis of oligodendrocytes in the white matter of these animals.[53]

In addition to the identification of novel WMH loci, we showed that loci for blood pressure were also associated with WMH burden. This further establishes the role of blood pressure in WMH. We were not able to identify effects of loci for Alzheimer disease and stroke on WMH. Pathological processes other than those affecting WMH may be stronger determinants of Alzheimer disease and therefore variants identified to date may capture mostly other mechanisms leading to Alzheimer disease. Similarly, stroke is heterogeneous and the stroke risk variants tested here are not those reflecting small-vessel disease stroke subtypes. Shared mechanisms between WMH and stroke are expected mostly for these subtypes.

In summary, in a meta-analysis of genome-wide association studies in individuals of European, African, Hispanic, and Asian descent, we identified 4 novel loci and confirmed a previous locus. Furthermore, we also report significant associations of blood pressure loci with WMH burden. Although additional fine mapping at each of the identified loci will be needed to uncover the causal genes and variants, a unifying hypothesis emerging from this work suggests a central role of neuroinflammation, possibly involving pathological mechanisms related to microglial activation and common to gliomas. Additional work will be needed to establish the importance of these findings in understanding the cause and pathophysiology of WMH and bring us closer to reducing WMH burden and its associated clinical manifestations.

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CLINICAL PERSPECTIVE

White matter hyperintensities (WMH) are commonly identified on MRI, and their burden increases with age. These MRI findings cannot be considered benign accompaniments of aging because their burden in the elderly is associated with impairments in cognition, mobility, and mood and with an increased risk of subsequent stroke, dementia, and death. Small-vessel angiopathy is presumed to play a major causal role given associations with vascular risk factors, especially hypertension, but the precise pathophysiologic pathways responsible for accumulation of WMH with aging remain obscure. Genetics likely is key, and the heritability of WMH is higher than other MRI findings. The hope is that understanding the genetic underpinnings of WMH may lead to a better understanding of these pathways and thus novel means to prevent the clinical consequences of WMH. Genome-wide association studies are one of the initial steps in coming to that understanding. Here, we describe a multiethnic genome-wide association study including 21 079 middle-aged to elderly participants from 29 population-based cohorts, who were free of dementia and stroke. The findings not only support a vascular cause, further highlighting the relationship between blood pressure and WMH burden, but also suggest a central role of neuroinflammation in WMH, possibly involving pathological mechanisms related to microglial activation and common to gliomas. More work is needed to learn whether these findings will lead to the discovery of interventions, beyond those directed at vascular risk factors, to prevent the development of WMH and their clinical consequences.