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Stroke

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Common Genetic Variation Indicates Separate Causes for Periventricular and Deep White Matter Hyperintensities

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BACKGROUND AND PURPOSE: Periventricular white matter hyperintensities (WMH; PVWMH) and deep WMH (DWMH) are regional classifications of WMH and reflect proposed differences in cause. In the first study, to date, we undertook genome-wide association analyses of DWMH and PVWMH to show that these phenotypes have different genetic underpinnings.

METHODS: Participants were aged 45 years and older, free of stroke and dementia. We conducted genome-wide association analyses of PVWMH and DWMH in 26,654 participants from CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology), ENIGMA (Enhancing Neuro-Imaging Genetics Through Meta-Analysis), and the UKB (UK Biobank). Regional correlations were investigated using the genome-wide association analyses -pairwise method. Cross-trait genetic correlations between PVWMH, DWMH, stroke, and dementia were estimated using LDSC.

RESULTS: In the discovery and replication analysis, for PVWMH only, we found associations on chromosomes 2 (*NBEAL*), 10q23.1 (*TSPAN14/FAM231A*), and 10q24.33 (*SH3PXD2A*). In the much larger combined meta-analysis of all cohorts, we identified ten significant regions for PVWMH: chromosomes 2 (3 regions), 6, 7, 10 (2 regions), 13, 16, and 17q23.1. New loci of interest include 7q36.1 (*NOS3*) and 16q24.2. In both the discovery/replication and combined analysis, we found genome-

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wide significant associations for the 17q25.1 locus for both DWMH and PVWMH. Using gene-based association analysis, 19 genes across all regions were identified for PVWMH only, including the new genes: *CALCRL* (2q32.1), *KLHL24* (3q27.1), *VCAN* (5q27.1), and *POLR2F* (22q13.1). Thirteen genes in the 17q25.1 locus were significant for both phenotypes. More extensive genetic correlations were observed for PVWMH with small vessel ischemic stroke. There were no associations with dementia for either phenotype.

CONCLUSIONS: Our study confirms these phenotypes have distinct and also shared genetic architectures. Genetic analyses indicated PVWMH was more associated with ischemic stroke whilst DWMH loci were implicated in vascular, astrocyte, and neuronal function. Our study confirms these phenotypes are distinct neuroimaging classifications and identifies new candidate genes associated with PVWMH only.

Key Words: brain ■ genome-wide association study ■ neuroimaging ■ risk factors ■ white matter

Radiological white matter hyperintensities (WMH) of presumed ischemic origin are the most prevalent sign of cerebral small vessel disease (SVD) and represent 40% of all SVD disease burden.[1] They are detected as incidental lesions on T2-weighted magnetic resonance imaging.[1] WMH are associated with increased risk for ischemic and hemorrhagic stroke, cognitive decline, and motor gait disorders.[2–6] Two regional classifications, based on their anatomic relationship to the lateral ventricles in the brain, are periventricular WMH (PVWMH) and deep WMH (DWMH).[5,7–9] PVWMH have been associated with declines in cognitive performance and increased systolic and arterial pressure, whereas DWMH are linked to body mass index, mood disorders, gait impairment, and arterial hypertension.[10–12] This categorization reflects proposed differences in underlying pathophysiology.[5,7,8] DWMH lesions occur in the subcortex, areas primarily supplied by long microvessels, with lower estimated blood pressures, possibly subject to damage secondary to hypertension and possibly with consequent hypoperfusion.[1,8,13,14] PVWMH are related to alterations in short penetrating microvessels ending in close approximation to larger arterial blood vessels with different vascular architecture such as 2 leptomeningeal layers and enlarged perivascular spaces.[1,15] They are hypothesized to be affected more directly by hypertension and risk factors associated with stroke.[1,8,13,14]

These subclassifications may also reflect differences in associated underlying genetic factors.[16] Twin and family studies report that both PVWMH and DWMH have high heritability and genetic correlations.[16,17] Recently, genome-wide association analyses (GWAS) for total WMH volume identified a major genetic risk locus on chromosome 17q25.1[18–21] and several other loci (eg, 10q24, 2p21, 2q33, 6q25.1).[19,21,22] However, the genetic determinants of regional WMH burden, specifically DWMH and PVWMH, remain elusive.

We combined all available participants aged 45 and above with both DWMH and PVWMH measurements from the CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology) and the ENIGMA (Enhancing Neuro-Imaging Genetics Through Meta-Analysis) consortia, and the UKB (UK Biobank). This is the only GWAS to date examining WMH subclassifications. We hypothesized that separating the two WMH subclassifications would mitigate phenotype heterogeneity, allowing us to identify additional risk loci and show that DWMH and PVWMH have different genetic underpinnings and pathophysiology.

METHODS

Summary data for this meta-analysis will be available through the database of Genotypes and Phenotypes Cohorts for Heart and Aging Research in Genomic Epidemiology Summary Results site, which can be downloaded via authorized access.

Study Cohorts

Study participants (total N=26 654) were drawn from cohorts in the CHARGE and ENIGMA consortia and the UKB. Detailed Methods are in the Data Supplement. All cohorts followed standardized procedures for participant inclusion, genotype calling, phenotype harmonization, covariate selection, and study-level analysis. Participants were included if they had phenotype, genotype, and covariate data available and were aged 45 years and over without stroke, dementia, or any neurological abnormality at the time of magnetic resonance imaging scanning. All participants provided written informed consent, and each study received ethical approval to undertake this work.

Phenotype and Covariates

The magnetic resonance imaging and WMH extraction methods for each study are detailed in the Data Supplement. In brief, PVWMH and DWMH volumetric data were extracted using automated methods for all studies except HUNT, LBC, and AGES, which used visual rating scales (Table I in the Data Supplement). Hypertension was defined as systolic blood pressure ≥140 mm Hg and diastolic blood pressure ≥90 mm Hg or on current antihypertensive treatment.

Statistical Analysis

Each study fitted linear regression models to test the association of DWMH and PVWMH (continuous measures) with individual single nucleotide polymorphisms (SNPs). Additive genetic effects were assumed, and the models were adjusted for age (years), sex, and intracranial volume (where applicable). In addition, principal

components for population stratification and other covariates, such as familial structure, were included if necessary. Models were also fitted with hypertension as an additional covariate.

Fixed-effects, inverse variance–weighted meta-analysis was carried out in METAL,[23] with correction for genomic control. Two meta-analyses were carried out: all cohorts excluding UKB (discovery, phase I) and all cohorts (phase II). Post meta-analysis QC was also performed (see in the Data Supplement).

Genetic Correlations With Stroke and Dementia

Cross-trait genetic correlation between the 2 subclassifications of WMH, stroke, and dementia was estimated using LDSC[24] on the GWAS summary statistics from phase II, MEGASTROKE (European ancestry only).[25] Linkage disequilibrium scores were based on the HapMap3 European reference panel. Regional level correlation was investigated using the GWAS-PW and HESS methods.[26,27]

RESULTS

Detailed study descriptions are provided in Tables I through III in the Data Supplement. The discovery cohort was comprised of ≈18 234 older adults (≥45 years, 16 studies) and was primarily white, with 736 blacks and 658 Hispanics. The predominantly white UKB was used as the replication cohort (n=8428).

In the discovery analysis (phase I), genome-wide significant associations ($P<5\times10^{-8}$) were observed in the 17q25.1 region for both phenotypes (Tables IV and V in the Data Supplement). Only the PVWMH analysis found additional genome-wide significant associations on chromosomes 2 and 10 (2 regions). Two of these regions had previously been described for total WMH burden (chromosomes 2, *NBEAL*,[19,21] 10q24.33, *SH3PXD2A*[21]), whereas 10q23.1 had not been described. Adjusting for hypertension made little difference to our findings (Tables VI through VII in the Data Supplement). Replication of the majority of genome-wide significant results for both phenotypes was observed after adjustment for multiple testing (DWMH $P<3.6\times10^{-4}$, PVWMH $P<2.76\times10^{-4}$, Tables VIII and IX in the Data Supplement).

Given the relatively large size of the replication cohort, a combined meta-analysis (phase II) was undertaken using all samples (N≈26 654). Removing either the small subsample of nonwhites or the cohorts with visual ratings did not substantially change the findings (beta value $r^2>0.93$). The phase II GWAS meta-analyses identified 236 for DWMH and 513 genome-wide significant SNPs for PVWMH (Figure 1A, Table 1, Tables X and XI in the Data Supplement, respectively). Figure 1B shows the zoom plot of the single locus identified for DWMH on chr17q25.1. The associations of the identified genome-wide and suggestive associations for each phenotype for the alternate trait are also provided in Tables X and XI in the Data Supplement. The only SNPs genome-wide significant for both phenotypes (n=209) were located on 17q25.1 (Figure 2A).

Ten chromosomal regions containing 290 genome-wide significant SNPs for PVWMH only were identified on chromosomes 2 (3 regions), 6, 7, 10 (2 regions), 13, 16, and 17q23.1 (Results, Table XI, and Figures I and II in the Data Supplement). Four loci had not been previously reported for associations with total WMH at the genome-wide significant level: (1) 7q36.1 (7.2 kb) containing 2 exonic SNPs in the *NOS3* gene; (2) 10q23.1 (50.5 kb) containing 4 intronic SNPs in *TSPAN14* & *FAM231A;* (3) 16q24.2 (1.2 kb) containing 2 intergenic SNPs; (4) 17q21.31 (27.2 kb) containing 8 SNPs, most of which are intronic and in the *NMT1* gene. Many of these are expression quantitative trait loci or participate in long-range chromatin interactions (Figure 2B). Further descriptions of the PVWMH findings are found in the Results in the Data Supplement.

As expected, the association of the 17q25.1 locus with both phenotypes was confirmed. The size of this region, including genome-wide significant SNPs only, was similar for both DWMH (236 SNPs, BP 73757836-74025656, Figure 1B) and PVWMH (223 SNPs, BP 73757836-74024711, Figure IA in the Data Supplement). The top results in this locus were rs3744020 for DWMH ($P=7.06\times10^{-35}$, *TRIM47* intronic SNP) and rs35392904 for PVWMH ($P=3.989\times10^{-28}$, *TRIM65* intronic SNP), which are in high linkage disequilibrium ($R^2=0.902$; Table 1). Many of these SNPs are expression quantitative trait loci or have long-range chromatin interactions (Figure 2B and 2C). For further details, see the Results in the Data Supplement.

Using gene-based tests, 13 genes in the 17q25.1 locus reached genome-wide significance ($P<2.66\times10^{-6}$) with both phenotypes (Table 2, Figure 2D, Tables XII and XIII in the Data Supplement). For PVWMH, an additional 19 genes were identified, covering the majority of regions/loci found in the SNP-based analysis (Figure 2D, Table 2, Table XIII in the Data Supplement). Four genes were located in previously unidentified regions: *CALCRL* (2q32.1), *KLHL24* (3q27.1), *VCAN* (5q27.1), and *POLR2F* (22q13.1).

Heritability analyses revealed low to moderate heritability for both traits (see Results in the Data Supplement). A high genetic correlation between DWMH and PVWMH was observed ($r_g=0.927$, $P=1.1\times10^{-65}$), indicating a shared genetic architecture. Figure 3 shows the genetic correlations with DWMH, PVWMH, stroke, and Alzheimer disease. Positive genetic correlations with both phenotypes were found for all stroke, ischemic stroke, and SVD. Intracerebral hemorrhage (all types) was correlated with DWMH only. No significant correlations were found with Alzheimer disease (Table XIV in the Data Supplement).

Using GWAS-PW,[26] we observed several regions with high probability (>90%) for harboring a shared genetic variant between PVWMH and DWMH (Table XV in the Data Supplement). These regions encompass several genome-wide significant loci that were identified

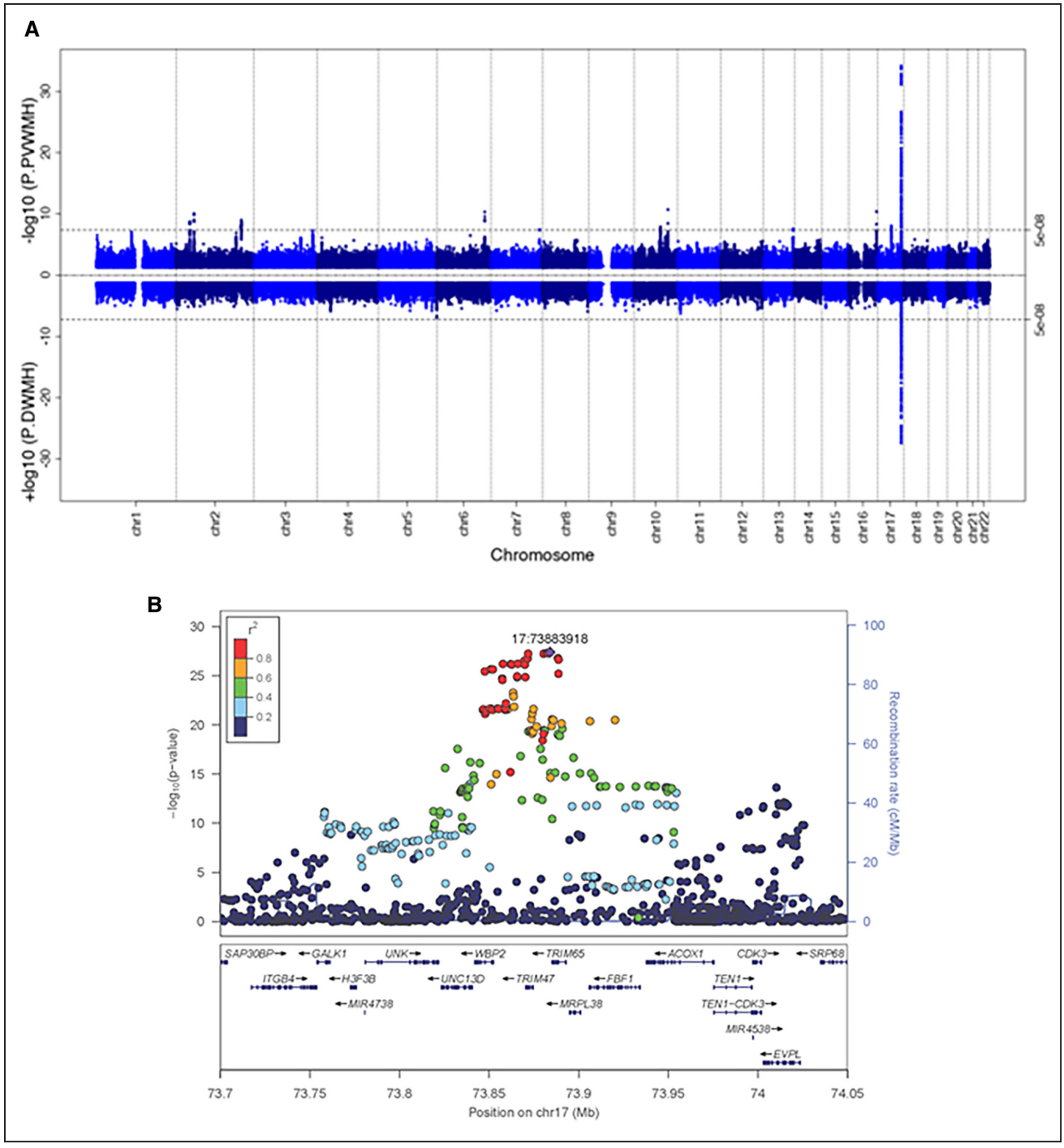

Figure 1. Phase II genome-wide association analyses meta-analysis.
A, Miami plot for periventricular white matter hyperintensities (PVWMH; **upper**) and deep white matter hyperintensities (DWMH; **lower**). Dashed line shows genome-wide significance threshold ($P<5\times10^{-8}$). **B**, Chromosome 17 regional plot of genome-wide significant SNPs for DWMH. Colors of the SNPs indicate the level of linkage disequilibrium with the top SNP (purple), rs35392904.

for PVWMH (2p16.1 [*EFEMP1*], 2q33.2 [*CARF* and *NBEAL*], 6q25.1 [*PLEKHG1*[22]], 16q24.2 [*C16orf95*], and 17q25.1 [*TRIM47*, *TRIM65*]). Additionally, by using HESS,[27] regional level correlation estimates were derived for those regions identified by the Bayesian approach (GWAS-PW).

Finally, we investigated local regions of a shared genetic variant between the WMH subtypes and stroke (Table XV in the Data Supplement). A region on chromosome 7 (encompassing the PVWMH *NOS3* exonic SNP) exhibited shared genetic influence of all stroke with both phenotypes. Other regions of shared influence with all

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Table 1. Top Genome-Wide Significant SNP Results From Each Genomic Locus Identified From the Phase II GWAS Meta-Analysis for Deep and PV WMH

WMH	rsID	CHR	POS	Nearest Gene	Function/ Position	A1	A2	Freq (A1)	Beta (SE)	N	Direction	*P* Value
PV	rs3744020	17q25.1	73871773	*TRIM47*	Intronic	A	G	0.1897	0.0899 (0.0073)	26 438	+++−+++?+++ ++++++++++	7.06×10^{-35}
Deep	rs35392904	17q25.1	73883918	*TRIM65*	Intronic	T	C	0.7981	−0.0765 (0.0070)	26 642	————+———— ———+———	3.99×10^{-28}
PV	rs3758575	10q24.33	105454881	*SH3PXD2A*	Intronic	A	G	0.4904	0.0388 (0.0058)	26 654	+++−++−++++ ++++++++++	2.00×10^{-11}
PV*	rs12928520	16q24.2	87237568	*C16orf95*	Intergenic	T	C	0.4252	0.0431 (0.0065)	26 327	+++++−?−+−− +++++++−++	4.22×10^{-11}
PV	rs275350	6q25.1	151016058	*PLEKHG1*	Intronic	C	G	0.4202	0.0374 (0.0057)	26 654	+−+−+++++− ++++++−−−++	4.86×10^{-11}
PV	rs7596872	2p16.1	56128091	*EFEMP1*	Intronic	A	C	0.0975	0.0642 (0.0099)	25 730	−++++++−+− −+++???++++	8.66×10^{-11}
PV	rs72934583	2q33.2	204009057	*NBEAL1*	Intronic	T	G	0.8740	0.0529 (0.0087)	25 730	−+++++++++− +++???+−++	1.03×10^{-9}
PV	rs57242328	2p21	43073247	*AC098824.6*	Intergenic	A	G	0.3317	−0.0368 (0.0061)	25 730	———+———+ ———???−++−	1.85×10^{-9}
PV*	rs7213273	17q21.31	43155914	*NMT1*	Intronic	A	G	0.6668	0.0341 (0.0059)	26 111	+++++−??++− ++−+++++++	8.89×10^{-9}
PV*	rs1993484	10q23.1	82222698	*TSPAN14*	Intronic	T	C	0.2388	0.0378 (0.0067)	26 654	++++−+−−++− ++−+++++++	1.36×10^{-8}
PV	rs11838776	13q34	111040681	*COL4A2*	Intronic	A	G	0.2793	0.0350	26 654	−+++++−++−+ ++++++−+−+	2.82×10^{-8}
PV*	rs1799983	7q36.1	150696111	*NOS3*	Exonic	T	G	0.3201	0.0373	26 654	++++++−−++− ++−++———+	3.68×10^{-8}

Effect allele is A1. A1 indicates allele 1; A2, allele 2; Chr, chromosome; GWAS, genome-wide association analyses; POS, base pair position; PV, periventricular; and WMH, white matter hyperintensities.

*These loci have not been previously associated with total WMH.

stroke were observed for PVWMH only. For the subtypes of stroke, significant regions were identified for DWMH and PVWMH, but none were found for both phenotypes except the chromosome 7 region for ischemic stroke (also identified for all stroke). Similar to the GW level correlation, a positive regional level genetic correlation was observed between the WMH subtypes and stroke (all stroke, all-ischemic, cardio-embolic and small vessel), by using HESS.[27]

DISCUSSION

In our meta-analyses using all available individuals (N=26 654, phase II), PVWMH had significant independent associations with loci containing genes implicated in large and SVD, as well as ischemic and deep hemorrhagic stroke suggesting a unique genetic and pathophysiological underpinning. Although our phase II GWAS were only slightly larger than the previous biggest GWAS on total WMH burden with 21 079 participants,[21] our detection rate of significant SNPs was substantially higher.[18,19,21] This improved detection may be the result of reduced heterogeneity by separately analyzing the DWMH and PVWMH phenotypes.

We identified 11 independent loci for PVWMH and one locus for DWMH. Significant genes associated with WMH for the first time in PVWMH include *CALCRL*, *VCAN, TSPAN,* and *NOS3*. Most genes and loci previously reported as significant in total WMH.[28–32] were now found to be associated with PVWMH alone, including *PLEKHG1*,[22] *SH3PXD2A,*[25,28,33] and *COL4A2.*[33]. Similarly, genes viewed as potential candidates[18,19,21] in prior studies we now find to be significantly associated only with PVWMH, including *DYDC2* and *NEURL1,* as well as *NMT1*, *GALK1*, *H3F3B*, *UNK*, *UNC13D*, *EVPL*, *ICAL1*, *WDR12/CARF*, *NBEAL1*, and *EFEMP1*.

Many of these genes associated with PVWMH affect vascular function or vascular diseases, such as ischemic stroke or coronary artery disease. The *NOS3* gene is associated with coronary artery disease, migraine, vascular dysfunction, SVD, and ischemic stroke.[22,29,30,34] *PLEKHG1* is associated with dementia and ischemic stroke,[35] and *SH3PXD2A* has been previously associated with total WMH and ischemic stroke.[19,25]

The most notable associated vascular gene is *COL4A2* that encodes for a subunit of type IV collagen, which has been associated with SVD, ischemic stroke, intracranial hemorrhage, and coronary artery disease.[31,35–38] It is a proposed therapeutic target for the prevention of intracranial hemorrhage.[32,39] The association of this vascular gene with PVWMH and deep intracerebral hemorrhage is suggestive of

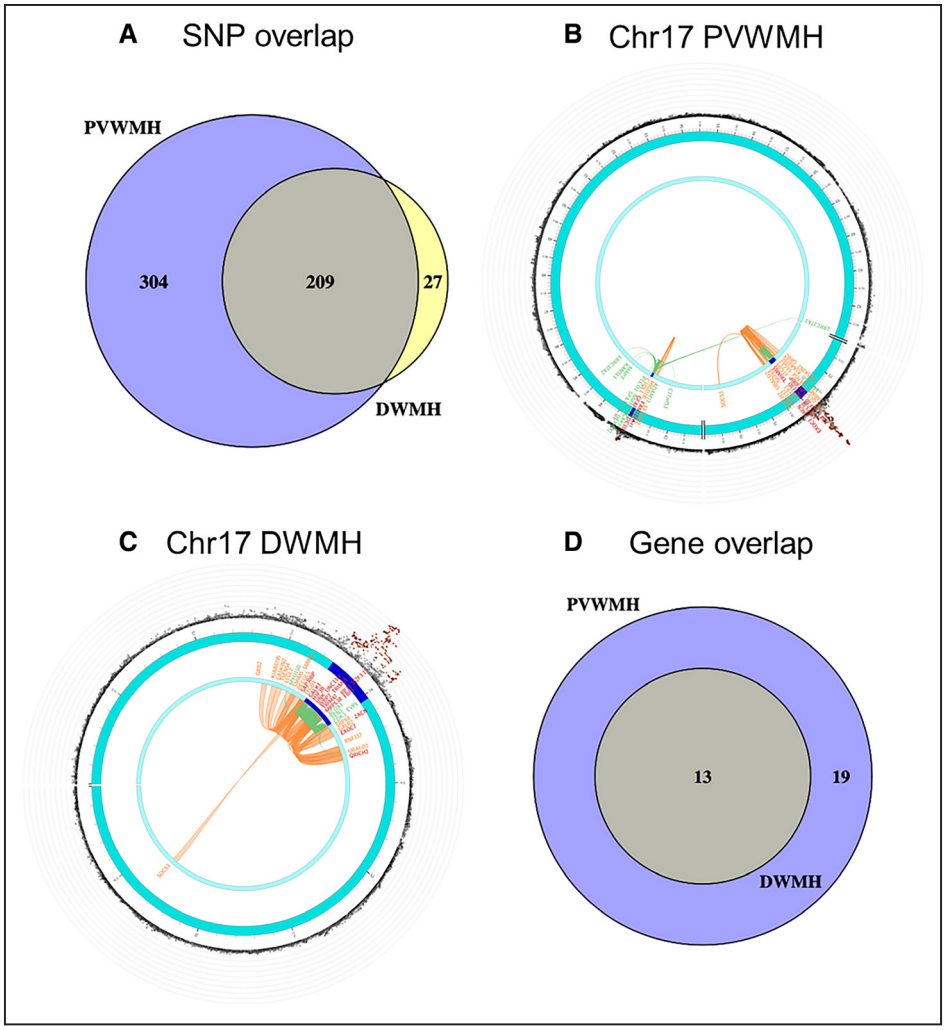

Figure 2. Overlap between significant SNPs and genes and Circos plots of the chromosome 17 region for deep (DWMH) and periventricular (PVWMH) white matter hyperintensities.
A, Overlap between genome-wide significant SNPs (P<5×10–8) for DWMH and PVWMH. **B** and **C**, Circos plots for chromosome 17 for both phenotypes, showing two identified regions for PVWMH (**B**) but only one for DWMH (**C**). Outer ring shows SNPs <0.05 with the most significant SNPs located towards the outermost ring. SNPs in high linkage disequilibrium (LD) with the independent significant SNPs in each locus are colored in red (r^2>0.8)-blue (r^2>0.2); no LD (gray). Genomic risk loci are colored in dark blue (second layer). Genes are mapped by chromatin interaction (orange), expression quantitative trait loci (green), or both (red). **D**, Overlap between significant genes identified by MAGMA for both phenotypes.

underlying regional gene effects of the *COL4A2* gene on the microvasculature affecting the risk of vascular injury in the periventricular region. These include potential weakening of the structural integrity of the regional microvasculature by altered collagen type 4 structural integrity, dysregulated gene expression of *COL4A1* and *COL4A2*, and toxic cytosolic accumulations of COL4A2 within microvascular structural cells.[40] When comparing PVWMH and DWMH anatomy, these mechanisms may enhance the direct mechanical effects of hypertension, or the other stroke risk factors, on the unique microvascular structure of the PVWMH region that also has predicted higher ambient blood pressure.[1,6,13]

We also discovered a new set of putative PVWMH genes. These include: *TSPAN14*, which encodes one of the tetraspanins which organize a network of interactions referred to as the tetraspanin web, *ADAM10*, a metalloprotease that cleaves the precursor of cell surface proteins,[41] *KLHL24* encodes a ubiquitin ligase substrate receptor,[42] *VCAN* encodes a large chondroitin sulfate proteoglycan that is found in the extracellular matrix. In a recent meta-analysis, *VCAN* was associated with white matter microstructural integrity.[43] These candidate genes for PVWMH may influence the immediate tissues surrounding microvessels and may contribute to SVD-associated biological changes.

Table 2. Thirty-Two Significant Genes Were Identified for PVWMH Using Gene-Based Tests ($P<2.66\times10^{6}$)

Gene	CHR	START	STOP	N SNPs	N	*P* PVWMH	*P* DWMH
WBP2	17	73841780	73852588	28	24 682	3.19×10^{-26}	1.16×10^{-21}*
TRIM65	17	73876416	73893084	52	24 555	7.73×10^{-24}	9.12×10^{-19}*
TRIM47	17	73870242	73874656	13	24 185	1.70×10^{-23}	9.04×10^{-19}*
RP11-552F3.12	17	73894726	73926210	53	24 351	2.15×10^{-20}	1.76×10^{-15}*
FBF1†	17	73905655	73937221	55	24 338	3.98×10^{-17}	1.23×10^{-13}*
GALK1†	17	73747675	73761792	36	24 307	6.34×10^{-16}	3.23×10^{-14}*
MRPL38	17	73894724	73905899	21	24 481	7.62×10^{-15}	1.18×10^{-13}*
UNC13D	17	73823306	73840798	73	23 788	3.10×10^{-14}	1.22×10^{-13}*
UNK	17	73780681	73821886	120	22 768	3.28×10^{-13}	4.85×10^{-10}*
H3F3B	17	73772515	73781974	23	24 009	4.43×10^{-12}	1.41×10^{-10}*
SH3PXD2A	10	105348285	105615301	788	24 847	8.43×10^{-12}	0.21731
ACOX1	17	73937588	73975515	151	24 198	7.72×10^{-11}	1.1×10^{-9}*
EVPL	17	74000583	74023533	67	24 582	1.26×10^{-10}	2.82×10^{-14}*
PLEKHG1	6	150920999	151164799	1022	24 922	1.59×10^{-10}	0.011765
WDR12†	2	203739505	203879521	322	23 753	2.53×10^{-10}	0.00104
ICA1L†	2	203640690	203736708	224	23 843	8.44×10^{-10}	0.001301
CARF†	2	203776937	203851786	157	24 076	2.41×10^{-9}	0.001763
NMT1†	17	43128978	43186384	221	24 766	7.18×10^{-8}	0.00034
CDK3†	17	73996987	74002080	12	24 433	8.54×10^{-8}	1.82×10^{-8}*
OBFC1†	10	105642300	105677963	99	25 461	1.41×10^{-7}	0.054127
NOS3†	7	150688083	150711676	58	24 608	1.73×10^{-7}	0.000371
DCAKD†	17	43100708	43138473	111	25 229	2.60×10^{-7}	0.000363
DYDC2†	10	82104501	82127829	91	25 050	2.88×10^{-7}	0.003460
NBEAL1	2	203879602	204091101	367	23 413	3.83×10^{-7}	0.040539
NEURL1	10	105253736	105352309	296	25 038	4.84×10^{-7}	0.098303
MAT1A†	10	82031576	82049440	66	25 295	4.90×10^{-7}	0.002421
TSPAN14†	10	82213922	82292879	213	24 731	6.73×10^{-7}	0.006605
CALCRL†	2	188207856	188313187	278	24 309	7.87×10^{-7}	0.000574
KLHL24†	3	183353356	183402265	207	24 356	1.29×10^{-6}	0.002571
POLR2F†	22	38348614	38437922	105	23 525	1.94×10^{-6}	0.252540
VCAN†	5	82767284	82878122	316	24 248	2.52×10^{-6}	0.065044
COL4A2	13	110958159	111165374	1140	24 876	2.61×10^{-6}	0.365300

Chr indicates chromosome; DWMH, deep white matter hyperintensities; and PVWMH, periventricular white matter hyperintensities.
*Thirteen of these genes (chr17) were also significant for DWMH.
†Those loci bolded have not been previously associated with total WMH.

The only significant locus observed for DWMH was the previously reported total WMH 17q25.1 locus,[18,19,21,22] which was also found for PVWMH. This locus contained the SNPs with the largest effect sizes for both phenotypes. The top genome-wide significant hits for DWMH and PVWMH (17q25.1) were either identical with the SNP recently reported by Traylor et al[22] for total WMH (PVWMH rs3744020) or in high linkage disequilibrium (R^2>0.9) with the previously identified top ranked SNPs in the same locus (rs3744028, Fornage et al,[18] rs7214628, Verhaaren et al[21]). Our identified SNPs were only in moderate linkage disequilibrium (R^2≤0.396) with the top SNP (rs3760128) identified in a recent exome association analysis.[19] All of these SNPs fall within or between the previously reported *TRIM47* and *TRIM65* genes.[18,21,22,35] This gene-rich locus contains genes that influence glial cell proliferation and have been hypothesized to influence gliosis, which is a histological and magnetic resonance imaging marker of microvascular injury.[1] It includes previously identified total WMH genes, such as *TRIM47/TRIM65* (glial proliferation, astrocytoma's),[18,21]

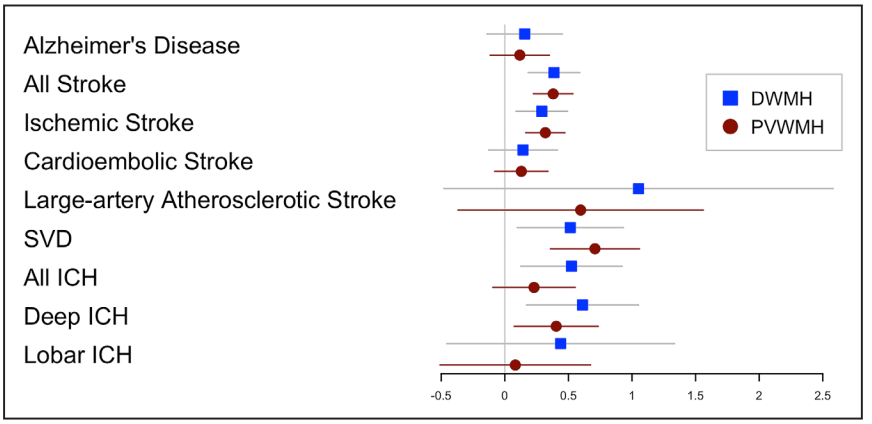

Figure 3. Genetic correlations (rg) between deep white matter hyperintensities (DWMH), periventricular white matter hyperintensities (PVWMH), Alzheimer disease (AD), and stroke phenotypes.
Horizontal bars represent SE, and the size of the square corresponds precision. ICH indicates intracranial hemorrhage; and SVD, small vessel disease stroke.

ACOX1 (cell replication, hepatic cancer)[18,19,21] and *MRPL38* (protein synthesis).[19] Genes associated with neuronal injury or neurodegenerative disorders are also found in the 17q25.1 locus, including *CDK3* (neuronal cell death in stroke),[44] *H3F3B* (schizophrenia pathogenesis) and *GALK1* (galactosemia).[45] Interestingly, 2 genome-wide significant intronic *UNC13D* SNPs identified in this study and reported previously for total WMH burden,[21] rs9894244 and rs7216615, have been reported as expression quantitative trait loci for *GALK1* and *H3F3B*, respectively.[46] The PVWMH specific loci also contained genes that potentially influence astrocytic function and gliosis, several previously reported for total WMH. These include *NBEAL1*,[19,21] *WDR12*,[19] *NEURL1*,[18,19,21] *CARF*,[47] and *EFEMP1*.[37] Newly identified PVWMH genes potentially affecting astrocytic functioning include *NMT1*,[48] *ICA1L*,[49] *POLR2F*, *OBFC1*, and *DYDC2*.

Shortcomings of this study include the potential variability due to the different WMH extraction algorithms used, with a minority of samples using visual ratings. However, this is a common problem encountered in this type of study.[18,19,21] Although our results suggest improved power and reduction in potential bias through the discrimination of PVWMH from DWMH, the Euclidean methodology used by the majority of studies undoubtedly missed PVWMH lesions outside this boundary. The majority of the participants in this study were white, and hence these results may not apply to other ethnicities. Sex differences have been previously reported but were not examined in the current study.[50] For the phase II meta-analysis, we did not have an independent replication cohort. Older adults were included in this study, and the majority of participants had both DWMH and PVWMH and not one or the other. However, selection of individuals with only one subtype of these lesions present may be more appropriate to identify differences but would only be possible in younger cohorts. Future studies should aim to address these shortcomings, including continuing to improve and harmonize WMH measurement methods but also using consistent DWMH and PVWMH measurement methods across studies.

CONCLUSIONS

Our study confirms PVWMH and DWMH have distinct and shared genetic architecture. Genetic analyses indicated PVWMH was more associated with ischemic stroke and vascular function (*PLEKHG1*, *SH3PXD2*, *COL4A2, CALCRL*, *VCAN, NOS3*), whereas DWMH loci were implicated in vascular, astrocyte and neuronal function (*TRIM47/TRIM 65*, *ACOX1*, *MRPL38*, *H3F3B*, *GALK*, *UNC13D*, *GALK1*). New genes for PVWMH, potentially affecting the extravascular connective tissue, were also identified (*TSPAN14, ADAM10*, *KLHL24, VCAN).* Our study confirms that PVWMH and DWMH are distinct neuroimaging classifications and identifies new candidate genes associated with PVWMH only.

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