17q25 locus is associated with white matter hyperintensity volume in ischemic stroke, but not with lacunar stroke status

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ABSTRACT

BACKGROUND: Recently, a novel locus at 17q25 was associated with white matter hyperintensities (WMH) on magnetic resonance imaging (MRI) in stroke-free individuals. We aimed to replicate the association with WMH volume (WMHV) in patients with ischemic stroke. If the association acts by promoting a small vessel arteriopathy it might be expected to also associate with lacunar stroke.

METHODS: We quantified WMH on MRI in the stroke-free hemisphere of 2588 ischemic stroke cases. Association between WMHV and six single nucleotide polymorphisms (SNPs) at chromosome 17q25 was assessed by linear regression. These SNPs were also investigated for association with lacunar stroke, in 1854 cases and 51939 stroke-free controls from METASTROKE. Meta-analyses with previous reports and a genetic risk score approach were applied to identify other novel WMHV risk variants and uncover shared genetic contributions to WMHV in community-participants without stroke and ischemic stroke.

RESULTS: SNPs at 17q25 were associated with WMHV in ischemic stroke, the most significant being rs9894383 (p=0.0006). In contrast there was no association between any SNP and lacunar stroke. A genetic risk score analysis revealed further genetic components to WMHV shared between community-participants without stroke and ischemic stroke.

CONCLUSION: This study provides support for an association between the 17q25 locus and WMH. In contrast, it is not associated with lacunar stroke suggesting that the association does not act by promoting small vessel arteriopathy or the same arteriopathy responsible for lacunar infarction.

INTRODUCTION

A recent report by the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium identified a novel genetic locus at chromosome 17q25 associated with white matter hyperintensities (WMH) on MRI in stroke-free community individuals.[1,2] WMH are common in stroke-free adults and increase with age. Prospective studies show WMH predict increased risks of cognitive decline, stroke and death.[3]

One might expect risk factors for WMH in community populations to also confer increased risk of WMH in stroke patients. However, the underlying pathology of WMH is heterogeneous; small punctate lesions are associated with mixed etiologies, while more confluent areas correspond primarily to small vessel disease (SVD).[4] In stroke patients, WMH are more frequent and extensive compared with healthy age-matched individuals and are usually associated with SVD.[4]

We hypothesized that risk factors for WMH in community populations without stroke also confer increased risk of WMH in stroke patients. Therefore, we assessed whether the 17q25 locus is associated with WMH as a quantitative trait in ischemic stroke. If the 17q25 locus acted to increase WMH through promotion of the small vessel arteriopathy, one might expect it to also increase risk of lacunar stroke, another SVD phenotype. To test this hypothesis, its association with lacunar stroke status was also examined in a cases-control analysis.

METHODS & MATERIALS

A. Association of 17q25 locus with WMHV

Subjects

Stroke cases were recruited from one community- and 8 hospital-based cohorts of ischemic strokes with genome-wide association study (GWAS) data available as well as MRI scans for WMH volume (WMHV) measurement. Details of populations are in Table 1. Inclusion criteria were: age>18 years, self-reported European ancestry, and a diagnosis of ischemic stroke of any subtype. Exclusion criteria were CADASIL, vasculitis, demyelinating and mitochondrial disorders.

Clinical characteristics

Hypertension was defined as antihypertensive prescription prior to stroke or systolic blood pressure >140 or diastolic >90mmHg more than one week post-stroke. Hypercholesterolemia was defined as lipid-lowering treatment prior to stroke, and/or elevated serum cholesterol (>5.2mmol/l) on stroke admission. Ever-smoker was defined as current and ex-smokers. Diabetes was defined as a previous diagnosis of diabetes mellitus. Ischemic stroke cases were subtyped according to the TOAST criteria[5] or similar (Supplementary Table S1).

MRI analysis

MRI was acquired as part of routine clinical practice in stroke evaluation using different scanners at individual centers. Fluid attenuated inversion recovery (FLAIR) sequences were primarily used for WMH analysis; however, in their absence, T2 sequences were used (Supplementary Table S2).

WMHV was measured in the hemisphere contralateral to the acute infarction to avoid confounding by T2 hyperintense signal due to acute stroke. All supratentorial white matter and deep gray matter lesions were included in WMHV with the exception of WMH corresponding to lacunar infarcts. MRI scans with excessive movement artefact, incomplete brain coverage, or bihemispheric infarcts (other than lacunar) were excluded. To account for normal inter-individual variability in head size, an estimate of total intracranial volume (TICV) was derived, using site-specific volumetric methodology.

Scans were analyzed anonymously, blinded to genetic information. MRIs from the Massachusetts General Hospital (MGH), Ischemic Stroke Genetics Study (ISGS), and Australian Stroke Genetics Collaborative (ASGC) studies were analyzed in Boston. FLAIR sequences were analyzed using a MRIcro (www.mricro.com) semi-automated method as previously described.[6] Using operator-mediated quality assurances, overlapping regions of interest (ROIs) corresponding to WMH produced the final maps for WMHV calculation.[6] To adjust for head size, intracranial area (ICA) was used as a validated marker of TICV.[7] The ICA constituted the average of two mid-sagittal slices traced using anatomical landmarks on T1 sequences.[7] SWISS scans were analyzed in the same way at the University of Virginia by the Boston-trained rater.

The Wellcome Trust Case Control Consortium-2 (WTCCC2) and Milan cohorts were analyzed in London using DISPunc semi-automated lesion drawing software[8]. A 'seed' at the lesion border was manually marked, following which the program automatically outlined the lesion based on the signal intensity gradient. Each ROI was visually inspected and manually corrected as required. To estimate TICV, T2 and, in its absence, FLAIR sequences were analyzed using an

automated segmentation program, SIENAX[9], and summing the cerebrospinal fluid, gray and white matter volumes.

WMH quantification agreement across the two main reading centers was performed for 50 randomly selected scans; agreement was very good (intraclass correlation coefficient 0.95, CI 0.91-0.97 n=50).

Genotyping

WTCCC2-UK and German cases were genotyped on the Illumina Human660W-Quad. Milan cases were genotyped using Illumina Human610-Quad or Human660W-Quad. ASGC samples were genotyped using the Human610-Quad. Both ISGS and SWISS samples were genotyped using the Illumina650K-Quad. MGH were genotyped on the Affymetrix 6.0, Illumina Human610-Quad or Illumina OmniExpress beadchips.

For all cohorts, individuals were removed if their inferred sex was discordant with the recorded sex or if >5% missing genotype data. Autosomal single nucleotide polymorphisms (SNPs) were excluded for minor allele frequency <1%, >5% missing data, or Hardy-Weinberg equilibrium $p<1x10^{-6}$. Each center performed checks for relatedness and population stratification. After quality-control procedures there was an effective sample size of 2588.

Imputation was performed in all centers using IMPUTE2.[10] All centers were imputed to HapMap3 and 1000 Genomes Project Phase pilot (June 2010) with the exception of MGH Omni, which was imputed to 1000 Genomes Integrated Release (June 2011). Post imputation, poorly

imputed SNPs (r^2<0.3) were removed resulting in 2706548 SNPs common to all centers.

Genome-wide Analyses and Meta-Analyses

To account for differences in MRI image acquisitions and population characteristics, we used a joint-analysis strategy; each cohort was analyzed independently and then meta-analyzed. Stroke cases with T2 images were analyzed separately from the FLAIR images. MGH samples were also sub-grouped based on genotyping platform (Supplementary Table S2).

Single hemisphere WMHV was doubled to obtain whole brain values and adjusted for normal inter-individual variation in head size by multiplying by the ratio of mean TICV to individual TICV. Values were natural log-transformed to a normal distribution. Within each group, rank-transformed residuals were derived from a linear regression model predicting WMHV with age, sex, and the first two ancestry principle components as covariates in GenABEL.[11] Thus the phenotype was adjusted for age because WMHV is highly age-dependent.[3,4] Principle components, derived using EIGENSTRAT (http://genepath.med.harvard.edu/~reich/Software.htm), were included to correct for potential population stratification. GWA analysis was undertaken in PLINK (http://pngu.mgh.harvard.edu/~purcell/plink/) using pseudo-dosages, a fractional count of 0 to 1 alleles for each genotype weighted by imputation probability, within a linear regression (additive) model. A meta-analysis of all the groups was performed under a fixed-effects inverse-variance model within METAL.[12] SNPs showing heterogeneity ($p<0.05$) were removed.

Two further analyses were performed, in which WMHV was additionally adjusted for

hypertension alone or combined cardiovascular risk factors (hypertension, hypercholesterolemia, ever-smoker and diabetes). Individuals with missing cardiovascular risk factors were removed from the latter analysis resulting in an effective sample size of 1932.

The CHARGE consortium reported 62 SNPs significant at $p<1x10^{-5}$ in their discovery meta-analysis; 61 of these were typed or imputed in at least nine out of thirteen groups. A Z-score meta-analysis was performed of the association of these SNPs in CHARGE and the WMH cohorts. P-values were weighted by sample size consistent with the CHARGE report.

SNPs at locus 17q25

Six SNPs in high linkage disequilibrium at locus 17q25 were examined (Figure 1). All SNPs were typed or imputed to high quality ($r^2 \geq 0.9$) in all cohorts with the exception of MGH-Affymetrix in which rs936393 was typed and only rs11869977 was well imputed (Supplementary Table S3).

Allelic dosage for the most significant SNP was extracted and used in a conditional analysis of SNPs within a 100kb window using ProbABEL[13], followed by meta-analysis within METAL. Also a meta-analysis was performed of the association results of these SNPs in discovery CHARGE report[1], Rotterdam replication report[2] and our WMH cohorts.

Genetic Risk Score

A genetic risk score (GRS) is a composite metric derived from a number of informative genetic variants associated with a phenotype of interest. We used SNPs highly associated with WMHV

($p<1x10^{-5}$) in stroke-free populations[1] to construct scores for ischemic strokes and test association with WMHV. SNPs were divided into 13 unique loci in relative linkage equilibrium ($r^2<0.2$) as determined by SNP Annotation and Proxy Search(www.broadinstitute.org/mpg/snap/). One SNP per locus was chosen based on the largest effect size across WMH cohorts, and the lowest p-value in the original CHARGE report. SNPs had to be typed or well imputed ($r^2>0.8$) in all WMH cohorts. Twelve SNPs were ultimately included in the GRS as rs10012573 was not adequately imputed in three centers and there were no other associated SNPs at this locus (Supplementary Table S3). Individuals with missing genotype(s) at these SNPs were excluded resulting in an effective sample size of 2564. The GRS was calculated by summing the risk allele doses and was not weighted because overall effect sizes are not available from the z-score meta-analysis employed in the CHARGE report.

Statistical Analysis

Statistical analyses were performed in R(http://R-project.org) and $p<0.05$ was considered significant. Within each group log-transformed WMHV normalized for TICV was adjusted for sex and age by obtaining standardized residuals from a linear regression model. The GRS both including, and excluding, the 17q25 locus was assessed as a predictor of adjusted WMHV by linear regression. The analyses were repeated with WMH additionally adjusted for hypertension, hypercholesterolemia, smoking and diabetes. Individuals with missing cardiovascular risk factors were removed from these analyses. The analyses were performed per center and meta-analyzed using inverse-variance method.

B. Association of 17q25 locus with lacunar stroke

Association between the 17q25 SNPs and lacunar stroke status was tested within METASTROKE, a consortium of ischemic stroke case-control GWA-studies.[14] 1854 lacunar strokes and 51939 controls free of symptomatic stroke from twelve cohorts were included (Table 2). Details of genotyping, quality-control, and imputation are available in the online supplement. All cases had brain imaging (CT and/or MRI) except in Atherosclerosis Risk in Communities Study (ARIC, 98% of cases), Australian Stroke Genetics Collaboration (ASGC, 97.4%), Cardiovascular Health Studies (CHS, 94.5%) and Heart and Vascular Health study (HVH, 96.5%).

Lacunar strokes were designated based on compatible clinical and neuroimaging findings using the TOAST criteria or similar.[5] TOAST requires normal neuroimaging or relevant brainstem/subcortical hemispheric lesions (<1.5cm), whereas HVH/CHS required normal CT with a typical lacunar syndrome or subcortical infarction (≤2cm). ARIC defined strokes as lacunar if the infarct was in a typical location (basal ganglia, brainstem, thalamus, internal capsule, or cerebral white matter) and of unstated size or ≤2cm. All cohorts excluded cases with recognized sources of emboli or large vessel atherosclerosis. Samples were not entirely independent of the WMH cohorts, with 16% of cases included in WMHV analysis.

For Genetics of Early Onset Stroke Study, rs3744028 and rs11869977 were not imputed. For Genes Affecting Stroke Risk and Outcome Study only rs936393 and rs1055129 were typed and rs3744017 was poorly imputed (r^2=0.64) and therefore not included in this SNP's meta-analysis. All six SNPs were typed or adequately imputed (r^2≥0.8) in the remaining cohorts (Supplementary Table S5). Association analyses were performed in each center by logistic

regression assuming an additive model. Results were adjusted by genomic control factor, followed by meta-analysis under an inverse-variance weighted model.

RESULTS

A. Association of 17q25 locus with WMHV

All SNPs at the 17q25 locus were significantly associated with WMHV as a quantitative trait in a direction and magnitude of effect consistent with previous reports. Most associations remained significant after Bonferroni adjustment for multiple comparisons ($p<0.008$) except for rs1055129 ($p=0.015$) (Table 3). The correction may be over-conservative given these SNPs are highly correlated ($r^2>0.85$), with the exception of rs1055129, which is in moderate correlated with the others ($r^2\approx0.5$)(Figure 1). The most significant SNP was rs9894383 (B=0.13 SE=0.04 p=0.0006) the risk allele was associated with a 13% (CI 6-22%) increase in the geometric mean of WMHV adjusted for age and sex (Figure 2). There was no evidence of between-study heterogeneity. The results remained significant when adjusting for hypertension alone or cardiovascular risk factors (Supplementary Table S6).

Conditioning on rs9894383 did not reveal any other significant variants within a 100kb window. Meta-analysis of the six 17q25 SNPs with the original CHARGE and Rotterdam replication reports revealed rs9894383 as most significantly associated with WMHV ($p=1.0 \times 10^{-11}$). Meta-analysis of moderately significant SNPs ($p<1\times10^{-5}$) reported by CHARGE only revealed a further genome-wide significant SNP at locus 17q25 (Supplementary Table S7).

The GRS was a significant predictor of WMHV adjusted for age and sex (p=0.001,B=0.031,CI 0.012-0.050) and after additional adjustment for hypertension (p=0.002,B=0.030,CI 0.011-0.049), and combined cardiovascular risk factors (p=0.003,B=0.031, CI 0.011-0.051). A GRS without the 17q25 locus was also significantly associated with WMHV (p=0.023,B=0.022,CI 0.003-0.042) and after adjustment for hypertension (p=0.020,B=0.023,CI 0.004-0.042) and cardiovascular risk factors (p=0.025,B=0.026,CI 0.003-0.048). Figure 3 shows an approximate linear relationship between mean WMHV residuals and quintiles of the GRS with and without 17q25 locus. Supplementary Table S8 gives the mean GRS for each quintile.

B. Association of 17q25 locus with lacunar stroke

There was no association between the six SNPs and lacunar stroke status in the Metastroke case-control analysis (Table 3). The 17q25 SNPs were not associated with cardioembolic or large artery stroke (data not shown).

DISCUSSION

This study provides further support for an association between the 17q25 locus and WMH, and replication in ischemic stroke supports the hypothesis of shared genetic contribution to WMHV in community participants without stroke and ischemic stroke cases. The most significant SNP here was rs9894383 and meta-analysis with two previous reports[1,2] revealed a combined p-value of 1.0×10^{-11}. Most SNPs within the 17q25 region were highly correlated and conditional analysis did not reveal more than one independent signal.

In contrast the 17q25 locus was not associated with another manifestation of SVD, lacunar

stroke. This may suggest that any causal variant linked to this locus does not act by directly promoting small vessel arteriopathy or the type of arteriopathy primarily underlying lacunar infarction. The study was powered to detect an association with an odds ratio between 1.1-1.15. Importantly, however, controls did not have brain imaging to exclude silent brain infarction, which is found in some 20% of healthy elderly[15] and this could have limited study power.

We performed a meta-analysis with the top SNPs reported by CHARGE; however, no novel loci reached genome-wide significance. To determine whether there were additional genetic variants shared by WMH occurring in community-participants without stroke and ischemic stroke, we calculated GRS excluding the 17q25 locus. This was a significant predictor of WMHV consistent with additional shared genetic variants.

There are several genes in the 17q25 region; however, cis-expression quantitative trait loci, primarily of HapMap lymphoblastoid cells, implicate *TRIM47*. TRIM47 could modulate brain responses to ischemic injury as its RING domain confers protein ubiquitination, which promotes proteolysis and cellular homestasis.[16] Imbalance in ubiquitin-proteasome pathways is integral to cerebral ischemic injury mechanisms[17] and also evident in WMH expression profiles.[18]

This study used volumetric MRI techniques, which have been demonstrated to be reliable and accurate[7-9] with good agreement across reading centers. A limitation is the variability in MR-imaging protocols, resulting from the use of clinical imaging in these GWAS databases. However, studies applying volumetric techniques have shown high reproducibility across

acquisition protocols[8] and scanner models.[19] To minimize effects of MRI heterogeneity, centers were analyzed separately and WMHV z-scores were derived prior to association testing. We measured whole brain rather than regional WMHV and therefore could not investigate genetic differences between periventricular and subcortical WMH, which are suggested to have differing pathological, risk factor and functional associations.[4] Another limitation is that genotyping was performed on multiple platforms. However, top SNPs were imputed to a high quality with consistent allele frequencies.

In conclusion, our data provide further support for an association between the 17q25 locus and WMHV in patients with ischemic stroke. Future studies are warranted to explore these genetic associations in order to understand biological underpinnings of this complex cerebrovascular phenotype. The lack of association with lacunar stroke may suggest that the 17q25 locus does not act via promoting small vessel arteriopathy.

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Figure 1. Linkage Disequilibrium Map of Six SNPs. Numbers indicate r-squared expressed as percentiles. Most SNPs are highly correlated (r^2 >0.85) and rs1055129 is moderately correlated ($r^2 \approx 0.5$).

Figure 2. Forest Plot showing inverse-variance weighted meta-analysis of the association of rs9894383 with adjusted WMH volume.

Figure 3. Mean adjusted WMHV residuals and 95% confidence intervals for quintiles of genetic risk scores. Analyses are shown both including (A), and excluding (B), the 17q25 locus.

Table 1. Clinical characteristics of ischemic stroke cohorts included in WMH analysis.

	Number	Mean(SD) age (years)	Male(%)	Hypertension (%)	Hypercholester-olaemia (%)	Ever Smoker (%)	Diabetes (%)
St George's*	381	70.6(13.5)	241(63.2)	300(78.7)	286(75.1)	263/380(69.2)	78(20.5)
Oxford*	170	67.0(13.7)	99(58.2)	114(67.1)	83(48.8)	91(53.5)	22(12.9)
Edinburgh*	72	68.6(13.9)	38(52.7)	34(47.2)	Unknown	51/68(75.0)	5(6.9)
Munich*	756	66.6(12.3)	467(61.7)	525(69.4)	346(45.7)	273(36.1)	166(21.9)
Milan	153	57.5(14.3)	92(60.1)	87(56.8)	94(61.4)	64(41.8)	21(13.7)
ISGS	209	67.9(13.8)	129(61.7)	127(60.7)	49/128(38.2)	51(24.4)	8/26(30.7)
ASGC	104	64.8(13.3)	59(56.7)	80(76.9)	52(50.0)	27/100(27.0)	18(17.3)
MGH	975	65.7(14.2)	606(62.2)	618(63.4)	408(41.8)	606(62.2)	199(20.4)
SWISS	115	66.3(11.4)	56(48.7)	85(73.9)	Unknown	Unknown	Unknown
Total	2935	66.4(14.3)	1734(60.9)	1911(65.1)	1318/2667(49.4)	1426/2811(50.7)	535/2628(20.4)

* Part of the Wellcome Trust Case Control Consortium 2 (WTCCC-2), ISGS=Ischemic Stroke Genetics Study, ASGC=Australian Stroke Genetics Collaborative, MGH=Massachusetts General Hospital, SWISS=Siblings with Ischemic Stroke Study.

Table 2. Demographics for lacunar stroke cases and stroke-free controls and effective sample size in METASTROKE

		Cases			Controls		
Continent	Study	n	Mean Age(SD)	% Males	n	Mean Age(SD)	% Male
Europe	WTCCC2-UK	474	75.4(12.5)	52.3%	5175	~52*	49.5%
	WTCCC2-Munich	106	65.1(12.9)	72.6%	797	62.7(10.9)	51.4%
	BRAINS	97	73.9(15.4)	52.5 %	407	≥65†	35.8%
	deCode	240	68.8(10.2)	56.5%	26970	57.3(21.4)	38.0%
	Milan	25	56.2(17.3)	56.7%	407	50.8(8.1)	87.7%
North	GEOS	54	44.3(4.1)	72.2%	498	39.5(6.7)	56.6%
America	GASROS	38	65.7(14.2)	60.3%	1202	47.5(8.5)	59.1%
	HVH	173	67.6(9.3)	32.9 %	1290	66.6(9.1)	47.7%
	ARIC	63	55.3(6.2)	58.7%	8803	54.1(5.7)	46.5%
	CHS	73	74.3(6.1)	34.2%	2817	85.8(5.6)	45.0%
	ISGS/SWISS	201	64.6(13.6)	60.3%	2329	64.8(12.6)	48.0%
Australia	ASGC	310	77.5(13.1)	57.4%	1244	70.2(12.1)	50.2%
Total		1854	70.2(14.0)	54.2%	51939	N/A	43.2%

CHS=Cardiovascular Health Study, ARIC=Atherosclerosis Risk in Communities, BRAIN=Bio-repository of DNA in stroke, GEOS=Genetics of Early Onset Stroke Study, GASROS=Genes Affecting Stroke Risk and Outcome Study, HVH=Heart and Vascular Health, ISGS= Ischemic Stroke Genetics Study.*Approximate age at genotyping of the 2738 controls from the 1958 Birth Cohort. Age was not available for the remaining controls.†All controls were aged 65 years or older at the time of genotyping. No further information was available

Table 3. Association statistics of SNPs at locus 17q25 with WMH volume and lacunar stroke

		WMH volume		Lacunar Stroke	
SNP	Risk Allele	Effect Size (SE)	P-value	Effect Size (SE)	P-value
rs3744028	C	0.12(0.04)	0.0030	-0.005(0.048)	0.92
rs9894383	G	0.13(0.04)	0.00064	0.005(0.046)	0.91
rs11869977	G	0.12(0.04)	0.00069	-0.001(0.047)	0.98
rs936393	G	0.11(0.04)	0.0012	0.014(0.045)	0.77
rs3744017	A	0.12(0.04)	0.0032	-0.001(0.046)	0.98
rs1055129	G	0.08(0.03)	0.015	0.031(0.039)	0.43