Genome-Wide Genotyping Demonstrates a Polygenic Risk Score Associated With White Matter Hyperintensity Volume in CADASIL

Christian Opherk, Mariya Gonik, Marco Duering, Rainer Malik, Eric Jouvent, Dominique Hervé, Poneh Adib-Samii, Steve Bevan, Luigi Pianese, Serena Silvestri, Maria Teresa Dotti, Nicola De Stefano, Michael Liem, Elles M.J. Boon, Francesca Pescini, Chahin Pachai, Luc Bracoud, Bertram Müller-Myhsok, Thomas Meitinger, Natalia Rost, Leonardo Pantoni, Saskia Lesnik Oberstein, Antonio Federico, Michele Ragno, Hugh S. Markus, Elisabeth Tournier-Lasserve, Jonathan Rosand, Hugues Chabriat and Martin Dichgans

Stroke. published online February 27, 2014;
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0039-2499. Online ISSN: 1524-4628

Original Contribution

Genome-Wide Genotyping Demonstrates a Polygenic Risk Score Associated With White Matter Hyperintensity Volume in CADASIL

Christian Opherk, MD*; Mariya Gonik, PhD*; Marco Duering, MD; Rainer Malik, PhD; Eric Jouvent, MD, PhD; Dominique Hervé, MD; Poneh Adib-Samii, MBBS; Steve Bevan, PhD; Luigi Pianese, MSc, PhD; Serena Silvestri, MSc; Maria Teresa Dotti, MD; Nicola De Stefano, MD; Michael Liem, MD; Elles M.J. Boon, PhD; Francesca Pescini, MD, PhD; Chahin Pachai, PhD; Luc Bracoud, MSc; Bertram Müller-Myhsok, MD; Thomas Meitinger, MD; Natalia Rost, MD, MPH; Leonardo Pantoni, MD, PhD; Saskia Lesnik Oberstein, MD, PhD; Antonio Federico, MD; Michele Ragno, MD; Hugh S. Markus, MD, FRCP; Elisabeth Tournier-Lasserve, MD; Jonathan Rosand, MD, MSc; Hugues Chabriat, MD, PhD; Martin Dichgans, MD

Background and Purpose—White matter hyperintensities (WMH) on MRI are a quantitative marker for sporadic cerebral small vessel disease and are highly heritable. To date, large-scale genetic studies have identified only a single locus influencing WMH burden. This might in part relate to biological heterogeneity of sporadic WMH. The current study searched for genetic modifiers of WMH volume in cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), a monogenic small vessel disease.

Methods—We performed a genome-wide association study to identify quantitative trait loci for WMH volume by combining data from 517 CADASIL patients collected through 7 centers across Europe. WMH volumes were centrally analyzed and quantified on fluid attenuated inversion recovery images. Genotyping was performed using the Affymetrix 6.0 platform. Individuals were assigned to 2 distinct genetic clusters (cluster 1 and cluster 2) based on their genetic background.

Results—Four hundred sixty-six patients entered the final genome-wide association study analysis. The phenotypic variance of WMH burden in CADASIL explained by all single nucleotide polymorphisms in cluster 1 was 0.85 (SE=0.21), suggesting a substantial genetic contribution. Using cluster 1 as derivation and cluster 2 as a validation sample, a polygenic score was significantly associated with WMH burden (P=0.001) after correction for age, sex, and vascular risk factors. No single nucleotide polymorphism reached genome-wide significance.

Conclusions—We found a polygenic score to be associated with WMH volume in CADASIL subjects. Our findings suggest that multiple variants with small effects influence WMH burden in CADASIL. The identification of these variants and the biological pathways involved will provide insights into the pathophysiology of white matter disease in CADASIL and possibly small vessel disease in general. **(*Stroke*. 2014;45:00-00.)**

Key Words: CADASIL ■ cerebral small vessel diseases ■ genetics ■ genome-wide association study ■ leukoaraiosis

Received December 9, 2013; accepted December 30, 2013.

From Institute for Stroke and Dementia Research (C.O., M.G., M. Duering, R.M., M. Dichgans), and Department of Neurology (C.O.), Klinikum der Universität München, Ludwig-Maximilians-University, Munich, Germany; Department of Neurology, DHU NeuroVasc, Hopital Lariboisiere, APHP, Paris, France (E.J., H.C.); Université Paris Diderot, Sorbonne Paris Cité, Génétique des Maladies Vasculaires, INSERM UMR-S740, Paris, France (D.H., E.T.-L.); Stroke and Dementia Research Centre, St George's University of London, London, United Kingdom (P.A.-S., S.B.); Neurology Division and Molecular Medicine Section, Mazzoni Hospital, Ascoli Piceno, Italy (L.P., S.S., M.R.); Department of Medicine, Surgery and Neurosciences, University of Siena, Italy (M.T.D., N.D.S., A.F.); Departments of Radiology (M.L.) and Clinical Genetics (E.M.J.B., S.L.O.), Leiden University Medical Center, Leiden, the Netherlands; Stroke Unit and Neurology, Azienda Ospedaliero Universitaria Careggi, Florence, Italy (F.P., L.P.); BioClinica Inc, Lyon, France (C.P., L.B.); Max-Planck Institute of Psychiatry, Munich, Germany (B.M.-M.); Institute of Human Genetics, Helmholtz Center, Munich, Germany (T.M.); Center for Human Genetic Research and Department of Neurology, Massachusetts General Hospital, Boston, MA (N.R., J.R.); Department of Clinical Neurosciences, University of Cambridge, Cambridge, United Kingdom (H.S.M.); Program in Medical and Population Genetics, Broad Institute, Cambridge, MA (J.R.); and Munich Cluster for Systems Neurology (SyNergy), Munich, Germany (M. Dichgans).

*Drs Opherk and Gonik contributed equally.

The online-only Data Supplement is available with this article at http://stroke.ahajournals.org/lookup/suppl/doi:10.1161/STROKEAHA.113.004461/-/DC1.

Correspondence to Martin Dichgans, MD, Institute for Stroke and Dementia Research, Klinikum der Universität München, Ludwigs-Maximilians University, Marchioninistr. 15, 83177 Munich, Germany. E-mail martin.dichgans@med.uni-muenchen.de

Stroke **is available at http://stroke.ahajournals.org** **DOI: 10.1161/STROKEAHA.113.004461**

Radiographic white matter lesions, visible on T2-weighted and fluid attenuated inversion recovery (FLAIR) MRI as signal hyperintensities, are the most common manifestation of cerebrovascular disease in elderly persons.[1] In population-based surveys of individuals >50 years of age, well over two thirds have some degree of white matter hyperintensities (WMH), and their prevalence substantially increases with age.[2–4] Because radiographic WMH predict the risk of stroke, cognitive decline, late-life depression, and deterioration of gait,[1] preventing WMH progression holds the promise of markedly reducing age-related disability.

Twin, sibling, and family history studies have demonstrated a strong genetic contribution to WMH. Previous reports have estimated that between 50% and 70% of interindividual differences in WMH volumes result from differences in genetic background.[3,5,6] However, specific genetic variants or chromosomal regions underlying this variability are still largely unknown. Most genetic studies conducted previously offered limited insights on individual genes.[3,7,8] In contrast, a recent report using genome-wide approaches provided evidence for an association between a locus on chromosome 17q25 and WMH burden, which was subsequently replicated in an independent study.[9,10]

The identification of genetic determinants accounting for the high degree of heritability of WMH has been challenging, despite large sample sizes available for study. A presumed explanation has been heterogeneity within study samples and frequent coexistence of age-related pathologies and vascular risk factors. Cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), a hereditary small vessel disease (SVD) caused by mutations in *NOTCH3*, may offer a unique opportunity to identify genetic determinants of WMH[11] because of its relative homogeneity. WMH volumes in CADASIL show a striking variability that is not related to different mutations described at the *NOTCH3* locus or to demographic factors (Figure 1). Instead, previous data suggest a strong influence of genetic modifiers[12] on CADASIL-related WMH.

The aim of the current study, therefore, was to perform a genome-wide search for common genetic variants that may modify WMH volume in CADASIL with the hope of identifying the loci that may play a role in sporadic SVD as well. This was done by combining data from 517 CADASIL patients recruited through 7 centers across Europe.

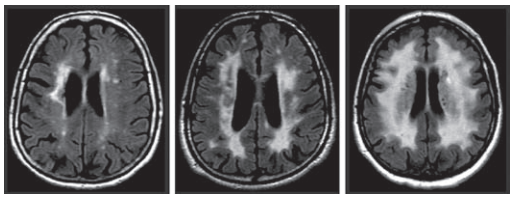

Figure 1. Variability of white matter hyperintensity (WMH) volume in cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy. Three patients carrying the same (R1006C) *NOTCH3* mutation show striking differences in WMH volumes. FLAIR indicates fluid attenuated inversion recovery; and nWMHV, normalized WMH volume.

Materials and Methods

Consortium Organization and Study Sample

The CADASIL GWAS (genome-wide association study) consortium is an academic collaboration aiming to identify genetic modifiers of radiographic and clinical features in CADASIL patients with the primary objective to identify quantitative trait loci for WMH volume. Patients were recruited through 7 sites across Europe (Florence, Leiden, London, Siena, Ascoli, Paris, and Munich; Figure 2). Inclusion criteria were: (1) a diagnosis of CADASIL documented by mutational analysis of the *NOTCH3* gene or skin biopsy; (2) electronic MRI data with at least FLAIR-weighted imaging; and (3) availability of demographic and clinical information including vascular risk factor profiles. The study protocol was approved by the ethics committee of the coordinating site. In addition, each site had institutional review board approval of its consent procedures, cohort examination, and surveillance. All participants of the study gave informed consent for study participation, MRI scanning, and use of DNA.

Hypertension was defined as antihypertensive treatment or systolic blood pressure >140 mm Hg or diastolic >90 mm Hg. Hypercholesterolemia was defined as lipid-lowering treatment or elevated serum cholesterol (>5.2 mmol/L). Smoking was determined by pack-years (packs of cigarettes per day×years). Diabetes mellitus was defined as any previous diagnosis of diabetes mellitus.

MRI Scans and WMH Quantification

At each site, standardized MRI scans were performed using scanners operating at field strengths between 0.5 and 3 Tesla. Scans were electronically transferred to BioClinica Inc (Lyon, France) for quality checks and processing. WMH volumes were measured on FLAIR MR scans using a semiautomated method and custom 2-dimensional and 3-dimensional editing tools from BioClinica as described previously.[13] All hyperintense subcortical lesions on FLAIR images (regardless of whether they occurred in white or subcortical grey matter) were labeled WMH. Segmentations were checked and corrected by trained raters (neurologists and neuroscientists with >5 years of experience with SVD-related lesions). Intracranial cavity was assessed from T2- or proton density–weighted images using an automated 3-dimensional image segmentation algorithm followed by manual correction. WMH volumes were divided by intracranial cavity volume to normalize for head size. All raters were blinded with regard to clinical and demographic data. Interrater reliability for normalized WMH volume was very high, with an intraclass correlation coefficient of 0.996.

Genotyping and Statistical Analysis

CADASIL cases were genotyped at the Helmholtz Center (Munich, Germany) and the Broad Institute (Cambridge, MA) using the Affymetrix 6.0 platform. Rigorous quality control analyses were performed. Samples with low call rate (<0.96), excess autosomal heterozygosity (false discovery rate >0.05), or close family relationships characterized by a high identity-by-state (>0.95) were excluded. In total, 487 samples passed quality controls. Identity-by-state–based principal component analysis was performed to identify clusters of samples that differ in genetic background. Two main clusters emerged (Figure in the online-only Data Supplement): the first cluster (n=339) consisted of samples mostly from London, Munich, and Leiden, whereas the second cluster (n=127) included samples from Ascoli, Florence, and Siena. The Paris sample was represented in both clusters. For both clusters, we used marker inclusion thresholds of minor allele frequency >0.01, single nucleotide polymorphism (SNP) call rate >0.9, and deviation from Hardy–Weinberg equilibrium P>0.0001. This yielded 650 006 autosomal SNPs in cluster 1 and 583 499 autosomal SNPs in cluster 2. Eighteen individuals were identified as outliers (Figure in the online-only Data Supplement) and were thus excluded. In total, 466 patients were available with high-quality genotyping data.

GWAS analyses were conducted with the GenABEL package for R[14] under an additive model and correction for age, sex, and

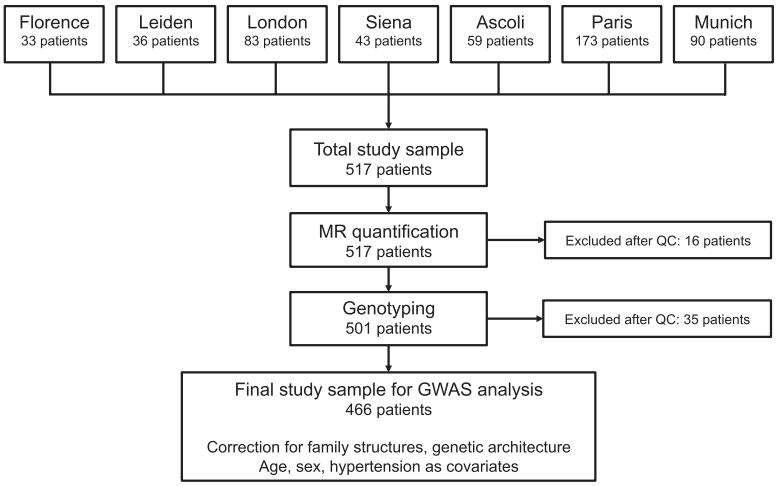

Figure 2. Study profile. Schematic representation of patient recruitment, MR quantification, and genotyping. GWAS indicates genome-wide association study; and QC, quality control.

hypertension. The mmscore function within GenABEL was used to implement a powerful family-based association test with control for stratification in admixed samples by providing the estimates of ancestry of each individual as covariates.[14] Results were corrected for possible genomic inflation. We used GWAMA[15] for meta-analysis of summary statistics generated from GWAS of quantitative traits. Genomic control of summary statistics was performed to correct for population structure within each study and potential variation between studies. Results from the analyses of both clusters were meta-analyzed using an inverse-variance fixed-effects approach to form the final result.

Heritability Calculation

Heritability was approximated by the phenotypic variance of WMH explained by autosomal SNPs using the GCTA software package.[16] In a strict sense, only the proportion of phenotypic variance (V_g/V_p) attributable to the variation in genotyped SNPs is measured, where V_g is the component of phenotypic variance attributable to the variation in genotyped SNPs, and V_p is the total observed phenotypic variance. To account for population substructure, heritability was calculated solely within the largest cluster 1. A linear mixed model was used to estimate the contribution of genotyped SNPs (and causal variants in linkage disequilibrium with genotyped SNPs) to the variance in phenotype. Age, sex, hypertension, and eigenvectors from 20 principal components of the population structure were used as covariates.

Polygenic Model

To estimate the association of WMH burden with the combined effects of multiple genetic variants of very small effect, we used a polygenic score analysis.[17] Polygenic scores were derived in PLINK[18] by using all SNPs passing nominal significance thresholds (P threshold=1e−4, 0.001, 0.01, 0.1, 0.5) in cluster 1 as a derivation cohort to calculate the weighted allele dosages in cluster 2 as a validation cohort. For this analysis, a pruned subset of approximately independent autosomal SNPs (maximum pairwise r^2=0.25 within sliding windows of 100 SNPs) was used. The associations between WMH volume and polygenic scores adjusted for age, sex, and hypertension as covariates were calculated by computing an ANOVA table for 2 linear model fits: a full model including the intercept, the polygenic score, and all covariates; and a reduced model that only included the intercept and covariates. The analysis was performed using R (http://www.R-project.org). Adjusted R^2 for the full and reduced models was used as a measure to describe the explained variance in WMH volume.

Results

A total of 466 patients entered the final GWAS analysis (Table 1). There was no evidence for genomic inflation within cluster 1 and cluster 2 (data not shown) or in the meta-analysis. Figure 3 shows the genome-wide plot of P values for individual SNPs against their genomic position adjusted for age, sex, and

Table 1. Clinical Characteristics of Individuals With CADASIL Included Into the Final Genome-Wide Association Analysis

	Florence (n=27)	Leiden (n=29)	London (n=69)	Siena (n=21)	Ascoli (n=57)	Paris (n=173)	Munich (n=90)	Total (n=466)
Demographics								
Age, y (median; IQR; range)	51 (29.2; 22–78)	53 (12; 28–67)	49 (15; 19–69)	48 (15; 31–74)	58.5 (18.75; 31–83)	51 (15.2; 24–74)	47 (13; 22–72)	**51 (16; 19–83)**
Male sex, n (%)	7 (25.9)	15 (51.7)	32 (46.3)	10 (47.6)	26 (46.4)	77 (44.2)	35 (38.8)	**202 (43.3)**
Vascular risk factors								
Hypertension, n (%)	9 (33.3)	2 (6.8)	12 (17.3)	4 (19)	17 (30.3)	34 (19.5)	21 (23.3)	**99 (21.2)**
Hypercholesterolemia, n (%)	7 (25.9)	3 (10.3)	48 (69.5)	6 (35.3)	28 (50)	76 (43.6)	24 (26.6)	**192 (41.2)**
Diabetes mellitus, n (%)	2 (7.4)	1 (3.4)	0 (0)	0 (0)	4 (7.1)	4 (2.2)	2 (2.2)	**13 (2.7)**
Smoking, pack-years (median; IQR; range)	12.6 (10; 8–72)	1 (1; 0–1)	0.5 (10; 0–64)	0 (0; 0–250)	0 (0; 0–720)	20 (21.75; 2–60)	17 (13.7; 2–75)	**1 (13.8; 0–720)**
nWMHV, % (median; IQR; range)	2.2 (5.85; 0–11.17)	5.85 (5.05; 0–12.85)	3.9 (4.65; 0–14.72)	4.8 (3.82; 0–14.82)	3.51 (4.26; 0.03–14.97)	5.7 (6.94; 0.07–21.93)	6.45 (8.01; 0.05–24.27)	**5.24 (6.4; 0–24.27)**

CADASIL indicates cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy; IQR, interquartile range; and nWMHV, normalized white matter hyperintensity volume.

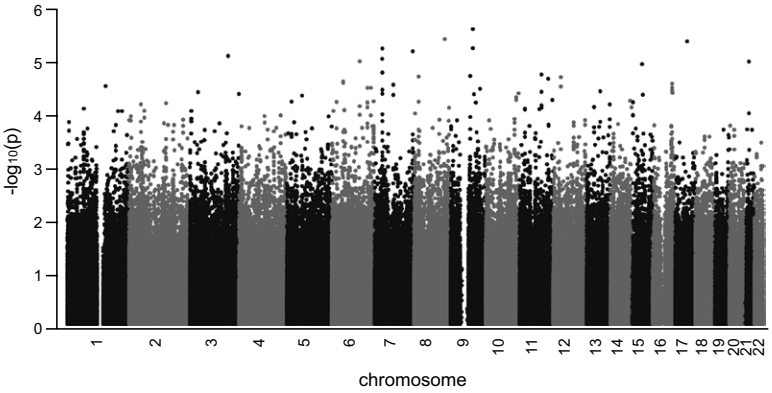

Figure 3. Genome-wide association results for white matter hyperintensity burden in cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy. Meta-analysis *P* values are plotted against their genomic position.

hypertension. GCTA analysis resulted in a heritability estimate of 0.85 (SE=0.21) explained by common SNPs in cluster 1.

No SNP reached the genome-wide significance level of $P<5\times10^{-8}$. Six SNPs showed association with $P<1\times10^{-5}$ (Table in the online-only Data Supplement). However, there was no association with variants at the previously reported locus for WMH burden on chr17q25 (Figure 3). rs7218738, the SNP with the lowest *P* value (*P*=0.27) in this region defined as ±50 kb around the lead SNP, is in low linkage disequilibrium (r^2=0.275) with rs3744028, the lead SNP in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) study.[9]

Because of the high heritability estimate, we investigated whether a polygenic score could be created for the association with WMH. Applying different thresholds of statistical significance, a polygenic score composed of all SNPs with a *P* value <0.5 in the derivation sample (cluster 1) was associated with WMH volume in the validation sample (cluster 2) after correction for age, sex, and hypertension (*P*=0.001; Table 2).

Discussion

Our results demonstrate a substantial role for genetic modifiers of WMH burden in patients with CADASIL. Because the genome-wide platform we used examines only common variants and could not evaluate mutations in *NOTCH3*, we can be confident that the sequence variants responsible for WMH burden are novel. Our finding that a polygenic risk score predicts WMH burden points to the presence of particular variants for further study, but cannot single out any one gene or gene region. The heritability estimate derived from genotype data was remarkably high and close to conventional estimates obtained in CADASIL,[12] normal elderly subjects,[5,6] and hypertensive sibships,[3] suggesting that continuing the search for individual sequence variants underlying WMH in both CADASIL and sporadic WMH is likely to prove fruitful.

Our findings suggest that multiple common variants with very small effect sizes, rather than single variants with moderate effect sizes, modify WMH burden in CADASIL. With 466 subjects in our analysis, we had 90% power to detect an association at a genome-wide level ($P<5\times10^{-8}$) for a variant explaining 8.67% of variance of WMH volume assuming additive effects. Depending on minor allele frequency, this would correspond to an increase of 2.1% to 4.7% in normalized WMH volume per allele. We, therefore, think that any strong signals related to common variants would have been picked up by our approach. However, it should be noted that our genotyping protocol did not cover rare variants, which may likewise contribute to quantitative traits.[19] Thus, it is possible that some of the heritability of WMH volume in CADASIL is due to rare variants. Of note, there was no indication for a disease-modifying influence of common or rare variants at the *NOTCH3* locus (Figure 3; data not shown). Future studies with full sequencing of the *NOTCH3* locus will further address this question.

The absence of signals of moderate to strong effect that reach genome-wide significance is broadly consistent with findings from a GWAS in 9361 stroke-free individuals from the general population, which found no variants to be significantly associated with WMH burden except for a single locus on chromosome 17q25,[9] which was recently replicated in stroke patients.[10] In the current study, we found no association signal with variants at 17q25. This might relate to differences in the protocols used for measuring WMH burden or to a differential impact of 17q25 region on WMH burden in normal elderly subjects and CADASIL. In fact, previous studies have demonstrated slight differences in the distribution of WMH in CADASIL and sporadic disease,[20] indicating that the mechanisms might partially differ. Further studies are needed to address this question.

Heritability, as estimated in our study, is unexpectedly high but largely consistent with previous measurements obtained without genome-wide genotyping.[12] Cryptic relatedness and lack of power might have led to some overestimation.

Table 2. Association of Polygenic Scores With White Matter Hyperintensity Volume

P Value Threshold (Derivation Sample)	Number of SNPs*	*P* Value (Validation Sample)	Adjusted R^2
1e–4	14	0.32	0.41
0.001	105	0.88	0.41
0.01	1035	0.28	0.41
0.1	10 574	0.001	0.46
0.5	52 125	0.001	0.46

*Linkage disequilibrium–pruned single nucleotide polymorphisms (SNPs); R^2 describes the variance explained by the full model (polygenic score plus covariates); R^2 for the reduced model (covariates only) was 0.41. Note that increasing *P* thresholds in the derivation sample leads to an increase in statistical significance and variance explained in the validation sample. After additional correction for 20 principal components, association of the polygenic score at *P*=0.5 in the derivation sample remained statistically significant (*P*=0.02).

Nevertheless, in conjunction with our findings on polygenic score, this adds to the notion that white matter integrity is strongly influenced by genetic variation. A recent study in individuals at high risk of mood disorders found a polygenic score for major depressive disorder to be significantly associated with reduced fractional anisotropy, an index of white matter integrity.[21] Diffusion tensor imaging parameters, such as fractional anisotropy, capture subtle alterations in the white and grey matter not visible on conventional scans. They may thus be considered complementary to the quantification of WMH volume. Future large-scale genetic studies will have to determine whether genetic variants impacting on WMH volumes and diffusion tensor imaging metrics overlap.

Specific strengths of this study include the unique sample of patients with pure SVD covering a broad range of WMH volumes, centralized image analysis with volumetric WMH measurements using validated protocols, and the inclusion of known risk factors for WMH into the models. The main limitations include small sample size and the fact that the data originated from different MR scanners. Also, we did not assess lacunar lesion volume, another quantitative marker of SVD. However, differentiating lacunes from other imaging findings, such as dilated Virchow–Robin spaces, may be difficult,[22] and a recent study in patients with acute ischemic stroke showed that the signals for WMH and lacunes do not necessarily overlap.[10]

In conclusion, we found a polygenic risk score to be associated with WMH volume in CADASIL. Our results suggest that multiple genetic variants with small effect sizes influence WMH burden. The identification of causative genetic variants is likely to shed light on the pathophysiology of WMH formation in CADASIL and possibly in sporadic SVD as well.

Acknowledgments

We would like to thank all study individuals and their relatives for their support to this study.

Sources of Funding

This study was supported by the Corona Foundation, the Vascular Dementia Research Foundation, the Deutsche Forschungsgemeinschaft (OP 212/1-1), the Dr Werner Jackstädt Foundation, Fondation Leducq (Transatlantic Network of Excellence on the Pathogenesis of Small Vessel Disease of the Brain), the American Heart Association–Bugher Foundation, the Massachusetts General Hospital Deane Institute for Integrative Research in Atrial Fibrillation and Stroke, and the National Institute of Neurological Disorders and Stroke (5P50NS051343; R01NS059727). Dr Markus is supported by a National Institute for Health Research Senior Investigator award. Drs Pianese and Pescini were supported by MIUR (Ministero dell'Istruzione, dell'Università e della Ricerca) programmi di ricerca cofinanziati-2006 (MIUR 2006-prot. 2006065719) and programmi di ricerca cofinanziati-2009 (MIUR 2009-prot. 20095JPSNA) and Regione Toscana, programma per la Ricerca Regionale in Materia di Salute, 2009 (Evaluation of *NOTCH3* mutations and correlation with clinical phenotypes; prot. reg. AOO GRT 190899/Q.20.70.20).

Disclosures

Dr Rosand has received significant research funding from the Deane Institute for Integrated Research on Atrial Fibrillation and Stroke at Massachusetts General Hospital and the National Institutes of Health. Dr Rost is supported by the National Institute of Neurological Disorders and Stroke (K23NS064052). Dr Pantoni is member of the editorial boards of *Cerebrovascular Diseases*, *Acta Neurologica Scandinavica*, and *International Journal of Alzheimer Disease*, and section editor (Vascular Cognitive Impairment) of *Stroke*.

References

1. Pantoni L. Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. *Lancet Neurol*. 2010;9:689–701.
2. Schmidt R, Enzinger C, Ropele S, Schmidt H, Fazekas F; Austrian Stroke Prevention Study. Progression of cerebral white matter lesions: 6-year results of the Austrian Stroke Prevention Study. *Lancet*. 2003;361:2046–2048.
3. Turner ST, Jack CR, Fornage M, Mosley TH, Boerwinkle E, de Andrade M. Heritability of leukoaraiosis in hypertensive sibships. *Hypertension*. 2004;43:483–487.
4. de Leeuw FE, de Groot JC, Achten E, Oudkerk M, Ramos LM, Heijboer R, et al. Prevalence of cerebral white matter lesions in elderly people: a population based magnetic resonance imaging study. The Rotterdam Scan Study. *J Neurol Neurosurg Psychiatry*. 2001;70:9–14.
5. Atwood LD, Wolf PA, Heard-Costa NL, Massaro JM, Beiser A, D'Agostino RB, et al. Genetic variation in white matter hyperintensity volume in the Framingham Study. *Stroke*. 2004;35:1609–1613.
6. Carmelli D, DeCarli C, Swan GE, Jack LM, Reed T, Wolf PA, et al. Evidence for genetic variance in white matter hyperintensity volume in normal elderly male twins. *Stroke*. 1998;29:1177–1181.
7. DeStefano AL, Atwood LD, Massaro JM, Heard-Costa N, Beiser A, Au R, et al. Genome-wide scan for white matter hyperintensity: the Framingham Heart Study. *Stroke*. 2006;37:77–81.
8. Paternoster L, Chen W, Sudlow CL. Genetic determinants of white matter hyperintensities on brain scans: a systematic assessment of 19 candidate gene polymorphisms in 46 studies in 19,000 subjects. *Stroke*. 2009;40:2020–2026.
9. Fornage M, Debette S, Bis JC, Schmidt H, Ikram MA, Dufouil C, et al. Genome-wide association studies of cerebral white matter lesion burden: the CHARGE consortium. *Ann Neurol*. 2011;69:928–939.
10. Adib-Samii P, Rost N, Traylor M, Devan W, Biffi A, Lanfranconi S, et al; Australian Stroke Genetics Collaborative; Wellcome Trust Case-Control Consortium-2 (WTCCC2); METASTROKE; International Stroke Genetics Consortium. 17q25 Locus is associated with white matter hyperintensity volume in ischemic stroke, but not with lacunar stroke status. *Stroke*. 2013;44:1609–1615.
11. Chabriat H, Joutel A, Dichgans M, Tournier-Lasserve E, Bousser MG. Cadasil. *Lancet Neurol*. 2009;8:643–653.
12. Opherk C, Peters N, Holtmannspötter M, Gschwendtner A, Müller-Myhsok B, Dichgans M. Heritability of MRI lesion volume in CADASIL: evidence for genetic modifiers. *Stroke*. 2006;37:2684–2689.
13. Duering M, Zieren N, Hervé D, Jouvent E, Reyes S, Peters N, et al. Strategic role of frontal white matter tracts in vascular cognitive impairment: a voxel-based lesion-symptom mapping study in CADASIL. *Brain*. 2011;134(pt 8):2366–2375.
14. Aulchenko YS, Ripke S, Isaacs A, van Duijn CM. GenABEL: an R library for genome-wide association analysis. *Bioinformatics*. 2007;23:1294–1296.
15. Mägi R, Morris AP. GWAMA: software for genome-wide association meta-analysis. *BMC Bioinformatics*. 2010;11:288.
16. Yang J, Lee SH, Goddard ME, Visscher PM. GCTA: a tool for genome-wide complex trait analysis. *Am J Hum Genet*. 2011;88:76–82.
17. Dudbridge F. Power and predictive accuracy of polygenic risk scores. *PLoS Genet*. 2013;9:e1003348.
18. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*. 2007;81:559–575.
19. Grarup N, Sulem P, Sandholt CH, Thorleifsson G, Ahluwalia TS, Steinthorsdottir V, et al. Genetic architecture of vitamin B12 and folate levels uncovered applying deeply sequenced large datasets. *PLoS Genet*. 2013;9:e1003530.
20. Auer DP, Pütz B, Gössl C, Elbel G, Gasser T, Dichgans M. Differential lesion patterns in CADASIL and sporadic subcortical arteriosclerotic encephalopathy: MR imaging study with statistical parametric group comparison. *Radiology*. 2001;218:443–451.
21. Whalley HC, Sprooten E, Hackett S, Hall L, Blackwood DH, Glahn DC, et al. Polygenic risk and white matter integrity in individuals at high risk of mood disorder. *Biol Psychiatry*. 2013;74:280–286.
22. Wardlaw JM, Smith EE, Biessels GJ, Cordonnier C, Fazekas F, Frayne R, et al; STandards for ReportIng Vascular changes on nEuroimaging (STRIVE v1). Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. *Lancet Neurol*. 2013;12:822–838.