White matter lesion progression: A genome-wide search for genetic influences

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Abstract

Background and Purpose—White matter lesion (WML) progression on magnetic resonance imaging (MRI) is related to cognitive decline and stroke, but its determinants besides baseline WML burden are largely unknown. Here, we estimated heritability of WML progression, and sought common genetic variants associated with WML progression in elderly participants from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium.

Methods—Heritability of WML progression was calculated in the Framingham Heart Study. The genome-wide association study included 7773 elderly participants from 10 cohorts. To assess the relative contribution of genetic factors to progression of WML, we compared in seven cohorts risk models including demographics, vascular risk factors plus single nucleotide polymorphisms (SNPs) that have been shown to be associated cross-sectionally with WML in the current and previous association studies.

Results—A total of 1085 subjects showed WML progression. The heritability estimate for WML progression was low at 6.5%, and no SNPs achieved genome-wide significance (p-value < 5×10^{-8}). Four loci were suggestive (p-value < 1×10^{-5}) of an association with WML progression: 10q24.32 (rs10883817, p=1.46×10^{-6}); 12q13.13 (rs4761974, p=8.71×10^{-7}); 20p12.1 (rs6135309, p=3.69×10^{-6}); and 4p15.31 (rs7664442, p=2.26×10^{-6}). Variants that have been previously related to WML explained only 0.8% to 11.7% more of the variance in WML progression than age, vascular risk factors and baseline WML burden.

Conclusions—Common genetic factors contribute little to the progression of age-related WML in middle-aged and older adults. Future research on determinants of WML progression should focus more on environmental, life-style or host-related biological factors.

Keywords

magnetic resonance imaging; aging; cerebral small vessel disease

Introduction

The etiology of white matter lesions (WML) on magnetic resonance imaging (MRI) is still incompletely understood. WML burden was shown to be highly heritable.[1-3] Age and hypertension are the main known risk factors for WML but explain only a small proportion of lesion presence and burden.[4] Prior to the era of genome-wide association studies (GWAS) candidate gene studies investigated variants in 19 genes and found associations between WML extent and polymorphisms in the apolipoprotein E, the methylentetrahydrofolate reductase, the angiotensin-converting enzyme and the angiotensinogen genes.[5, 6] Moreover, genetic variants in the NOTCH3 gene may not only play a role in CADASIL, a monogenic cerebral small vessel disease, but are also likely to be involved in the etiology of age-related WML burden.[7] In 2011, the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium[8] performed the first genome-wide association study on WML burden in the general population.[9] The CHARGE investigators identified 6 SNPs mapping to a locus on chromosome 17q25 to be related to WML burden. The findings of the discovery meta-analyses had been confirmed in an independent sample of 1607 AGES-Reykjavik participants and in 1417 and 1677 elderly white participants from the Three-City-Dijon Study[9] and the Rotterdam Study III[10] as well as in a study of WML burden in persons with a clinical ischemic stroke.[11] The association was also confirmed in an Asian population including 1190 Japanese persons with a mean age of 66 years.[12] The region on chromosome 17 is approximately 100 kb long and harbors several genes with diverse functions such as the 2 tripartite motif-containing genes (*TRIM65* and *TRIM47*) the WW domain binding protein 2 gene (*WBP2*), the mitochondrial ribosomal protein L38 gene (*MRPL38*), the Fas-binding factor 1 gene (*FBF1*), the acyl-coenzyme A oxidase 1 gene (ACOX1) and the C-Elegans homolog (*UNC13D*) gene. Although genetic factors may play an important role in the occurrence of WML in middle-aged to older adults, whether these genes or others influence the further progression of WML is unknown. Using data on WML progression from all the cohorts currently available within the CHARGE consortium, we examined the heritability of WML progression and performed a meta-analysis of GWAS data in 7773 individuals of European descent from 10 cohorts to identify common single nucleotide polymorphisms (SNPs) that influence the risk for WML progression. We also assessed the relative contribution of genetic factors in predicting WML progression beyond information that can be obtained from baseline clinical and MRI data alone.

Materials and Methods

Study Population

Study participants were from 10 prospective cohort studies collaborating in the CHARGE Consortium[8]: the Aging Gene-Environment Susceptibility Reykjavik Study (AGES-Reykjavik)[13], the Atherosclerosis Risk in Communities (ARIC) study[14], the Austrian Stroke Prevention Study (ASPS)[15, 16], the Cardiovascular Health Study (CHS)[17], the Framingham

Heart Study (FHS)[18, 19], the Prospective Study of Pravastatin in the Elderly at Risk (PROSPER)[20, 21], the Rotterdam Study II (RS II) and the Rotterdam Study III (RS III)[22], the Tasmanian Study of Cognition and Gait (TASCOG)[23] and the 3C-Dijon study.[24] All participating studies agreed on phenotype harmonization, covariate selection, pre-specified analytic plans for within-study analyses, and meta-analysis of results. Each study secured approval from Institutional Review Boards, and all participants provided written informed consent for study participation, MRI scanning, and use of DNA for genetic research.

Participants were eligible for the current study if they had genotyping, serial MRI, and lacked a history of transient ischemic attacks, strokes, dementia, or any combination of these conditions. All the individuals in the present analysis were whites of European descent. The number of participants and their characteristics in each cohort are shown in Supplemental Table I in the online-only Data Supplement.

WML Progression Assessment

In each study, eligible participants were invited to undergo serial MRI scans, which were performed and interpreted in a standardized fashion without knowledge of demographic, clinical or genetic information (Section II in the online-only Data Supplement). Except for ARIC and CHS whose readers used a 10-point scale, readers in the other cohorts measured the volume of WML on each MRI scan. WML progression was defined as absent or present (Section III in the online-only Data Supplement). In brief, WML progression was considered to be present if visual rating increased by at least 1 grade between baseline and follow-up in ARIC and CHS, or if WML volume increased by at least one standard deviation of the study-specific mean of volume change in AGES-Reykjavik, ASPS, FHS, PROSPER, RS II, RS III, TASCOG and 3C-Dijon. WML regression was rare in all studies and was not considered separately in these analyses.

Genotyping

The consortium was formed after individual studies had finalized their GWAS platforms, which differed across studies. All studies used their genotype data to impute to the 2.5 million non-monomorphic, autosomal, single nucleotide polymorphisms (SNPs) described in HapMap's European population panel. Extensive quality control analyses were performed in each cohort. Details on the genotyping, quality control and imputation efforts are described in Supplemental Tables II - IV in the online-only Data Supplement.

Heritability of WML Progression

Heritability of WML progression was calculated based on family structure in FHS, which is the only family study among the participating cohorts. Calculations were based on annual WML change and two models were assessed. The first model adjusted for age and sex, while the second model additionally adjusted for WML volume at baseline. The ratio of the genetic variance to the phenotypic variance in FHS was determined using variance component models in SOLAR.[25]

Genome-wide association analyses (GWAS)

For the GWAS each study fitted an additive genetic model with a 1-degree-of-freedom trend test relating genotype dosage, 0 to 2 copies of the minor allele, to presence or absence of WML progression. We used logistic regression models to calculate regression estimates with corresponding standard errors. Initial analyses were adjusted for age, sex, interval between scans as well as principal components of populatsion structure if appropriate. Subsequent analyses included additional adjustment for WML volume at baseline. In addition, ARIC and CHS also adjusted for study site, and FHS, for familial structure. We then conducted a meta-analysis of logistic regression estimates and standard errors using a fixed effects inverse-variance weighting approach with genomic control correction as implemented in METAL.[26]

There was no evidence of inflated test statistics in the individual cohort analyses (Figures I and II in the online-only Data Supplement).

The genome-wide significance threshold was set a priori at 5×10^{-8}. Associations were considered highly suggestive for SNPs with $5\times10^{-8}<p<1\times10^{-5}$.

To evaluate if the lengths of the observational period influenced the effect size of highly suggestive SNPs and WML progression we performed GWAS meta-analyses separately for cohorts with short mean follow-up and those with long mean follow-up time. Short-term studies were AGES-Reykjavik, PROSPER and TASCOG. Long-term cohorts were ARIC, ASPS, CHS and FHS (for follow-up intervals between scans see Supplemental Table I in the online-only Data Supplement). Identical analyses were also done for younger (ARIC, ASPS, FHS, RSII and RSIII) and older (AGES-Reykjavik, CHS, PROSPER, TASCOG and 3C-Dijon) cohorts (for mean age of cohorts see Supplemental Table I in the online-only Data Supplement). The meta-analysis regression coefficients β of the SNPs were then compared between the investigational subsets using a z-test.

Annotation

Key SNPs were functionally annotated with SNPnexus[27] and ANNOVAR.[28] The SNAP[29] web application of the Broad Institute was used to determine linkage-disequilibrium (LD). Regional association plots were generated with LocusZoom.[30] Furthermore we used the NCBI Genotype-Tissue Expression eQTL Browser[31] to check if a given SNP was associated with a quantitative gene expression trait.

Performance of WML progression risk models

To estimate the relative importance of genetic factors for progression of WML we compared the explained variance between three models in ASPS, CHS, FHS, PROSPER, RS II, RS III, and 3C-Dijon. Model 1 served as the reference and included age, sex, hypertension, diabetes, current smoking and the time interval between scans; model 2 included model 1 variables and those SNPs with the lowest p-value at each highly suggestive locus according to the present GWAS meta-analysis with adjustment for WML burden at baseline. We also included a missense SNP identified by annotation analyses. Model 3 included all model 2 variables plus genetic polymorphisms which were shown to be associated with WML in

previous publications.[5, 7, 9, 32-43] We included only those SNPs for which an association with WML was reported by at least 2 studies. In case that any of these SNPs was not present in our database, a proxy SNP ($r^2 > 0.8$) was selected to be included.[9] Based on this search strategy we selected ACE[5, 32-34] (rs4343), MTHFR[5, 35, 36] (rs1801133), AGT[5, 37-39] (rs2478539), TRIM47[9] (rs1055129), TRIM65[9] (rs3744028), NOTCH3[7] (rs10404382) and APOE4[5, 40-43] genotypes to be included in model 3. APOEε4 status was included as a binary variable (E4+ included the e3/e4 and e4/e4 genotypes; E4- included e2/e2, e2/e3 and e2/e4 genotypes). The three models were also calculated with additional adjustment for WML volume at baseline. Models 2 and 3 were adjusted for principal components of population structure if appropriate. The goodness of fit for each model was assessed by Nagelkerke's R^2.[44, 45]

Results

The analyses included 7773 participants from 10 cohort studies (Supplemental Table I in the online-only Data Supplement). A total of 4342 (56%) of study participants were women; 4030 (52%), hypertensive; and 690 (8.9%), diabetic. The mean systolic blood pressure ranged between 121.9mmHg and 157.8mmHg and the mean diastolic blood pressure between 69.6mmHg and 86.5mmHg. The mean time interval between the baseline and follow-up MRI was shortest in AGES-Reykjavik with 29.1 months and longest in ARIC with 123.6 months. Overall, WML progression was observed in 1085 (14.0%) study participants with the study-specific mean annual volume increase ranging between 0.2 cm^3 and 1.4 cm^3. The association between mean WML volume at baseline and mean annual volume increase in our cohorts was moderate (Pearson correlation coefficient r=0.59), but did not reach statistical significance (p-value=0.095). The relationship for each cohort is displayed in Supplemental Figure III in the online-only Data Supplement.

Heritability of WML progression

After adjustment for age and sex the heritability estimate for WML progression observed in 1368 FHS individuals was 6.5%. Additional adjustment for WML burden at baseline resulted in a heritability estimate of 4.7%.

Genome-wide associations of WML progression

Figure 1 illustrates the meta-analysis results of genome-wide association analyses on WML progression without and with adjustment for WML burden at baseline. In both meta-analyses no SNPs achieved genome wide significance (p-value $< 5\times10^{-8}$). Meta-analysis without adjustment for WML burden at baseline revealed 45 SNPs in 7 loci on 7 chromosomes with a highly suggestive association with WML progression at p-values $< 1\times10^{-5}$ (Supplemental Table V in the online-only Data Supplement). After additional adjustment for WML burden at baseline, 8 highly suggestive associations were identified (Supplemental Table VI in the online-only Data Supplement). These SNPs are located in 6 loci on 6 chromosomes. There is an overlap of four suggestive loci (1q41, 5q12.1, 12q13.13 and 13q34) between the two meta-analysis results. In the meta-analysis without adjustment for WML burden at baseline 35 of 45 suggestive SNPs in total were located at 10q24.32. The SNP rs10883817 had the lowest p-value (1.46×10^{-6}) at this locus, with an odds ratio

(OR) of 1.27, a mean minor allele frequency of 0.41, and a mean imputation quality score of 0.98. The suggestive variants at locus 10q24.32 were in LD (r^2 between 0.336 and 1) and reside either in introns of genes AS3MT, CNNM2, NT5C2, in the intergenic regions between these genes, or in exons of CNNM2 and NT5C2 (Figure 2 A).

At the second locus of interest, 12q13.13, we identified 3 highly suggestive variants in the analysis without adjustment for WML burden at baseline and only 1 with adjustment. In the meta-analysis without adjustment for baseline WML burden rs4761974 had the lowest p-value (8.71×10^{-7}), with an odds ratio of 0.5. After additional adjustment for baseline WML volume the p-value of the association changed to 2.94×10^{-6} (OR=0.5). The SNP had a mean minor allele frequency of 0.057 and a mean imputation quality score of 0.98. Suggestive SNPs at this locus were in high LD (r^2 between 0.786 and 1) and are located either in introns or in close proximity to the gene SLC4A8 (Figure 2 B).

At the third locus 20p12.1 in the analysis without adjustment for WML burden at baseline, the top SNP was rs6135309 with a p-value of 3.69×10^{-6}, an odds ratio of 0.78, a mean minor allele frequency of 0.29, and a mean imputation quality score of 0.97. Both suggestive variants at this locus were in LD ($r^2=0.642$) and reside in introns of MACROD2 (Figure 2 C). The fourth locus suggestive of an association with WML progression was located on chromosome 4p15.31 in the analysis with adjustment for WML burden at baseline. The top SNP at this locus was rs7664442 (p-value=2.26×10^{-6}; OR=0.65), which had a mean minor allele frequency of 0.079 and a mean imputation quality score of 0.96. These three SNPs at 4p15.31 were in perfect LD ($r^2=1$) and are located in the intergenic region near GBA3 (Figure 2 D).

Functional annotation of all highly suggestive SNPs and SNPs in linkage-disequilibrium with them was performed. The two suggestive exonic variants at locus 10q24.32, rs2275271 (p-value=2.26×10^{-6}, OR=0.79) and rs3740387 (p-value=5.10×10^{-6}, OR=1.25), were synonymous. Two other suggestive SNPs at this locus have been reported in previous genome-wide association studies investigating schizophrenia[46-48] (rs7897654; p-value=5.26×10^{-6}, OR=0.79 and rs7914558; p-value=2.14×10^{-6}, OR=1.26). No association between suggestive SNPs and quantitative gene expression traits could be determined. A comparison of the effect size of suggestive SNPs on WML progression between studies with short versus long term follow-up failed to demonstrate significantly increased effect sizes in studies with long-term follow-up. There were also no significant differences in the effect sizes of suggestive SNPs on WML progression between younger and older cohorts (data not shown).

Performance of WML progression risk models

As can be seen from Table 1, model 1, which included demographics and risk factors, explained between 2.3% and 14.9% of the variance in WML progression in the various cohorts. Amongst studies that assessed WML progression by volume change, 17.2% to 43.3% of the WML progression variance was explained if baseline WML was included (model 1). In CHS, which used a visual rating scale for assessment of WML progression, only 4.5% of the variance could be explained. Highly suggestive SNPs from the current GWAS and all genetic variants previously described as being related to WML accounted for

additional 1.1% to 8.6% (model 2) and 0.8% to 11.7% (model 3) of the variance of WML progression beyond age, sex, vascular risk factors and baseline WML volume.

Discussion

In this first genome-wide association study on WML progression, our data indicate that genetic factors contribute only little to WML progression in the general elderly population. This conclusion relies on three major findings. First, the family-based heritability estimate for WML progression was only 6.5% versus a heritability of 55% for baseline WML burden in the same sample.[2] Second, our genome-wide analysis yielded no associations at a genome-wide significance level. Third, risk prediction models including highly suggestive SNPs according to the present GWAS meta-analysis plus all genetic polymorphisms that have been shown to be associated with WML burden in previous literature explained only between 0.8% and 11.7% more of the variance of WML progression than that explained by baseline clinical and MRI data. These results for WML progression oppose previous cross-sectional studies in which the contribution of genetic factors for WML burden was substantial.[5, 7, 9, 32-43] This finding is puzzling because cross-sectionally assessed WML burden and WML progression are both quantitative measures with presumably the same biological basis. In this context it is of note, however that in our study initial burden of WML predicted annual WML volume increase moderately, but this relationship was not significant. Moreover, for both, brain volume and cognitive functions, similar results of limited genetic variance in longitudinal measures have been shown.[49-51] In line with our findings on white matter lesions these investigations also found that measures of brain atrophy and cognitive performance assessed cross-sectionally are highly heritably but predominantly environmental factors account for the rate of change of these cerebral phenotypes over time.

We identified four suggestive loci for WML progression on chromosomes 10q24.32, 12q13.13, 20p12.1 and 4p15.31. None of the SNPs in these loci reached genome-wide significance; however, some of the neighboring genes within these loci have been related to neuropsychiatric or vascular diseases.

Locus 10q24.32 includes AS3MT (arsenic (+3 oxidation state) methyltransferase), CNNM2 (Cyclin M2) and NT5C2 (5′-nucleotidase, cytosolic II). These genes have previously been identified to be associated with schizophrenia[46-48, 52, 53] and blood pressure[54-57] in GWAS and replication studies. CNNM2 and NT5C2 were associated with coronary artery disease,[58] and CNNM2 was additionally associated with intracranial aneurysm.[59] Moreover, a suggestive variant in an intron of AS3MT, rs10748835 (p-value=2.79×10^{-6}, OR=1.25), is known to modify cognitive function in persons with low-level arsenic exposure.[60]

The locus at 12q13.13 includes SLC4A8, a sodium bicarbonate co-transporter of the designated solute carrier family 4. It transports sodium and bicarbonate ions across the cell membrane.[61, 62] The gene is highly expressed in all major regions of the brain and is involved in pH regulation in human neurons.[63]

MACROD2 (MACRO domain containing 2) at locus 20p12.1 is a protein-coding gene that has been found to be associated with MRI-defined brain infarcts in a previous GWAS.[64] This is particularly intriguing as recent studies have suggested new lacunes are most likely to develop in areas of white matter progression.[65] MACROD2 has also been associated with autistic traits.[66]

Although we included a large number of participants from ten cohort studies with longitudinal assessment of WML change, we cannot exclude the possibility that we missed significant associations of WML progression to SNPs with small effect sizes or low risk allele frequencies. Moreover, three other factors need to be considered when interpreting our results. First, we used a binary phenotype based on visual rating or cut-off values of volumetric change to define WML progression. This conservative definition decreased the likelihood of false positive ratings but, among those with WML at baseline, might have led to an underestimation of WML progression. Second, the average time period between scans among contributing cohorts was 54 months, and despite the fact that single studies had followed their participants for more than 10 years, it is conceivable that this period was too short to reveal the full impact of genetic factors on WML progression. Our findings that the effect sizes of suggestive GWAS SNPs on WML progression were not significantly larger in cohorts with long-term follow-up versus cohorts with short-term follow-up and in older versus younger cohorts do not support this assumption. Third, we by design concentrated on elderly people. Genetic factors may play a larger role for white matter progression in younger populations.

Our study findings have important implications for future research on age-related white matter changes. They suggest that, although the contribution of genetic factors seems to be large during the initiating phase of white matter damage, the propagating phase of WML appears to be mainly influenced by non-genetic determinants. With the exception of high blood pressure these non-genetic risk factors for WML progression remain largely unknown.[67, 68] Based on current data, we need to intensify the search for potentially modifiable environmental and lifestyle factors that influence the progression of age-related white matter changes and the associated morbidity. Moreover, while this study focused on the influence of genetics on age-related WML progression, the effects of heritability on injury-induced WML progression are an interesting direction for future studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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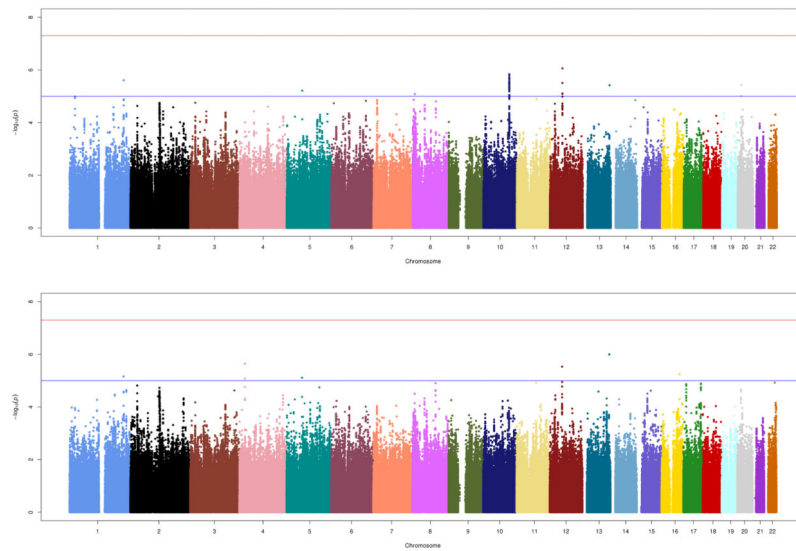
Figure 1. Genome-wide Manhattan plot for progression of WML
The Manhattan plot for the GWAS without adjustment for WML burden at baseline is shown in the upper panel and the Manhattan plot for the GWAS adjusted for WML burden at baseline is shown in the lower panel. The plot shows the −log10(p-values) for all SNPs in the analysis against their genomic position. Within each chromosome, shown on the *x*-axis, the results are plotted left to right from the p-terminal end. The red line represents the threshold for genome wide significance (5×10^{-8}), and the blue line represents the threshold for highly suggestive SNPs (1×10^{-5}).
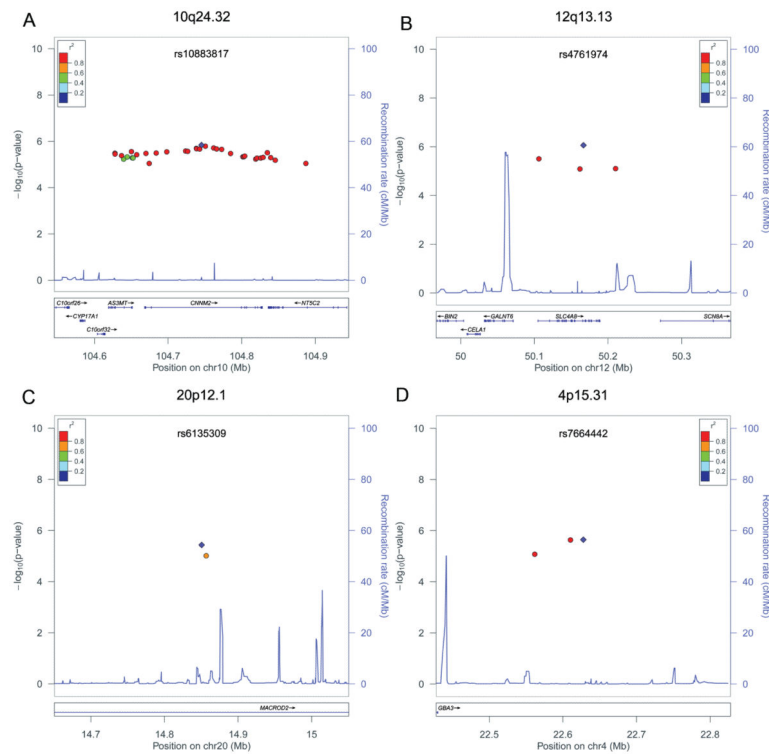
Figure 2. Regional association plots for WML progression SNPs in loci 10q24.32, 12q13.13, 20p12.1, 4p15.31

The plots for locus 10q24.32 (A) and 12q13.13 (B) are shown in the upper panel, and the plots for locus 20p12.1 (C) and 4p15.31 (D) are shown in the lower panel. For loci 10q24.32, 12q13.13, and 20p12.1 associations were determined by WML progression GWAS meta-analysis in the model without adjustment for WML burden at baseline. For locus 4p15.31 the associations were determined by WML progression GWAS meta-analysis in the model with adjustment for WML burden at baseline. Plots are centered on the most significant SNP at a given locus along with the meta-analysis results for SNPs in a region surrounding it (± 200kb). All SNPs are plotted with their meta-analysis p-values against their genomic position, with the most significant SNP in the region indicated as a diamond and other SNPs shaded according to their pairwise correlation (r2) with the signal SNP. The blue line represents the estimated recombination rates. Gene annotations are shown as dark blue line. The boxes at the end of the genes show the 5′ and 3′ UTRs, while boxes within the gene represent exons. Arrows indicate the direction of transcription.

Table 1

Performance of WML progression risk models.

		Model 1	Model 2	Model 3	Model 2 – Model 1	Model 3 – Model 1
ASPS	Without WML burden adjustment	0.146	0.226	0.275		
	With WML burden adjustment	0.433	0.519	0.550	0.086	0.117
CHS	Without WML burden adjustment	0.023	0.051	0.068		
	With WML burden adjustment	0.045	0.072	0.089	0.027	0.044
FHS	Without WML burden adjustment	0.113	0.130	0.143		
	With WML burden adjustment	0.172	0.183	0.194	0.011	0.022
PROSPER	Without WML burden adjustment	0.057	0.095	0.143		
	With WML burden adjustment	0.320	0.392	0.410	0.072	0.090
RS II	Without WML burden adjustment	0.149	0.164	0.199		
	With WML burden adjustment	0.391	0.408	0.453	0.017	0.062
RS III	Without WML burden adjustment	0.119	0.146	0.158		
	With WML burden adjustment	0.397	0.419	0.405	0.022	0.008
3C-Dijon	Without WML burden adjustment	0.054	0.106	0.118		
	With WML burden adjustment	0.203	0.247	0.259	0.044	0.056

Data are Nagelkerke's R^2.

Model 1: WML progression ~ age + sex + time + hypertension + diabetes + current smoking [*] without or with adjustment for WML burden at baseline

Model 2: WML progression ~ age + sex + time + hypertension + diabetes + current smoking [*] + GWAS SNPs without or with adjustment for WML burden at baseline

Model 3: WML progression ~ age + sex + time + hypertension + diabetes + current smoking [*] + GWAS SNPs + previously reported SNPs + APOE4 without or with adjustment for WML burden at baseline

[*] Frequency of current smoking ranged between 5.9% in 3C-Dijon and 24.5% in RS III.

ASPS: Austrian Stroke Prevention Study, CHS: Cardiovascular Health Study, FHS: Framingham Heart Study, PROSPER: Prospective Study of Pravastatin in the Elderly at Risk, RS: Rotterdam Study, RS II: Rotterdam Study II, RS III: Rotterdam Study III, 3C-Dijon: Three-City Dijon study