Replication Study of Chr17q25 With Cerebral White Matter Lesion Volume

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Background and Purpose—Recently, the first genomewide association study on cerebral white matter lesion burden identified chr17q25 to be significantly associated with white matter lesions. We report on the first independent replication study of this genetic association.

Methods—In a population-based cohort study, we investigated the association between the 6 genomewide significant single nucleotide polymorphisms at that locus and cerebral white matter lesion volume on MRI, measured quantitatively, adjusted for age, sex, and intracranial volume. Adjustments for ApoE4 carriership and cardiovascular risk factors were evaluated separately. Finally, we performed a meta-analysis of all published data for the single most significant single nucleotide polymorphism, rs3744028.

Results—The risk alleles of all the 6 single nucleotide polymorphisms were significantly associated with white matter lesion volume with $P < 1.1 \times 10^{-3}$ for rs3744028, adjusted for age, sex, and intracranial volume. Additional adjustments only had minor influence on these associations. A meta-analysis with all published data for rs3744028 resulted in a probability value of $5.3 \times 10^{-19}$.

Conclusions—This study further establishes chr17q25 as a novel genetic locus for WML volume. (Stroke. 2011;42:3297-3299.)

Key Words: epidemiology ■ genetic association ■ risk ■ WML

Cerebral white matter lesion (WML) burden is associated with an increased risk of stroke and dementia.¹ Unraveling the genetics of WMLs can identify potential targets for prevention and therapy. Recently, 6 single nucleotide polymorphisms mapping to 1 locus on chromosome 17q25 showed genomewide significance ($P < 5 \times 10^{-8}$) in the first genomewide association study on WML burden.² Although this association was replicated in 2 replication samples in the discovery report, further independent replication is needed to accumulate more evidence and thus strengthen the association. Here, we report on the first replication of the association of chr17q25 with WML burden after the discovery report.

Methods

Study Population

This study is based on Rotterdam Study III, the second expansion of the Rotterdam Study.³ No overlap exists between Rotterdam Study III and the samples from Rotterdam Study I and II, which were reported on in the original report.² In total, Rotterdam Study III comprised 3932 persons, of whom 2082 random persons were white and had good-quality genotype data. Of these, 1724 had MRI data available with good-quality brain segmentations. After excluding persons with cortical infarcts (n=45) and persons with dementia (n=2), 1677 persons were included in the analysis. Persons with cortical infarcts were excluded, because WMLs concomitant to cortical infarcts were considered to reflect a different pathology than the WMLs under study.

Genotyping

Genotyping and imputation were performed as part of a large project and are described in the Supplemental Methods (http://stroke.ahajournals.org). For this report, we extracted data on rs3744028, rs9894383, rs11869977, rs936393, rs3744017, and rs1055129. The quality of imputation of those single nucleotide polymorphisms was $>0.97$. APOE genotyping was performed on coded samples as described elsewhere.⁴

MRI Measurements

MRI was performed on a 1.5-Tesla scanner (GE Healthcare) with an 8-channel head coil and included T1-weighted, proton density-weighted and fluid-attenuated inversion recovery sequences.⁵ The
Table 1. Population Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n=1677</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>56.1 (5.3)</td>
</tr>
<tr>
<td>Female sex</td>
<td>55%</td>
</tr>
<tr>
<td>ApoE4 carriership</td>
<td>31%</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27.5 (4.4)</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>82.3 (10.5)</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>131.9 (18.1)</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.6 (1.1)</td>
</tr>
<tr>
<td>Hypertension*</td>
<td>46%</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>7%</td>
</tr>
<tr>
<td>Current smoker</td>
<td>22%</td>
</tr>
<tr>
<td>White matter lesion volume, mL</td>
<td>3.0 (3.9)</td>
</tr>
</tbody>
</table>

Values are means (SD) or percentages.

Statistical Analyses

An additive genetic model was assumed and significance of association was estimated using a 1-degree of freedom trend test relating genotype dosage, 0 to 2 copies of the minor allele, to the phenotype, that is, the log-transformed WML volume. Adjustments were for age, sex, and intracranial volume in Model I (model as in discovery study) and additionally for ApoE4-carriership and cardiovascular risk factors (body mass index, diastolic and systolic blood pressure, hypertension, total cholesterol, diabetes mellitus, current smoking) in Model II. Furthermore, we repeated our analyses using WML volume as percentage of intracranial volume to directly correct for head size (Model III). We used a threshold of $P=0.05$ for statistical significance because our aim was to replicate the previous findings. To evaluate the possible influence of familial relationships with individuals from Rotterdam Study I and II, we performed a subanalysis after excluding persons with first-degree relatives (n=36) in Rotterdam Study I or II. Finally, we performed a meta-analysis with results from the discovery study for rs3744028 by using a Z-score pooling method.

Table 2. Association of Chr17q25 With White Matter Lesion Volume

<table>
<thead>
<tr>
<th>Single Nucleotide Polymorphism</th>
<th>Chr: Position</th>
<th>Imputed</th>
<th>$R^2$</th>
<th>MA</th>
<th>MAF</th>
<th>Model I</th>
<th>Model II</th>
<th>Model III</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs3744028</td>
<td>17:714000267</td>
<td>Yes</td>
<td>0.17</td>
<td>1.1*10^{-3}</td>
<td>0.10 (0.04–0.16)</td>
<td>3.9*10^{-3}</td>
<td>0.09 (0.03–0.15)</td>
<td>5.8*10^{-4}</td>
</tr>
<tr>
<td>rs9894383</td>
<td>17:71377252</td>
<td>Yes</td>
<td>0.90</td>
<td>0.18</td>
<td>1.9*10^{-3}</td>
<td>0.10 (0.04–0.16)</td>
<td>2.7*10^{-3}</td>
<td>0.09 (0.03–0.16)</td>
</tr>
<tr>
<td>rs11869977</td>
<td>17:71368977</td>
<td>Yes</td>
<td>0.86</td>
<td>0.18</td>
<td>2.0*10^{-3}</td>
<td>0.10 (0.04–0.16)</td>
<td>2.8*10^{-3}</td>
<td>0.09 (0.03–0.16)</td>
</tr>
<tr>
<td>rs936393</td>
<td>17:71359208</td>
<td>Yes</td>
<td>0.86</td>
<td>0.23</td>
<td>2.3*10^{-3}</td>
<td>0.09 (0.03–0.15)</td>
<td>3.0*10^{-3}</td>
<td>0.09 (0.03–0.16)</td>
</tr>
<tr>
<td>rs3744017</td>
<td>17:71383062</td>
<td>No</td>
<td>0.86</td>
<td>0.17</td>
<td>5.1*10^{-4}</td>
<td>0.11 (0.05–0.17)</td>
<td>1.5*10^{-3}</td>
<td>0.10 (0.04–0.16)</td>
</tr>
<tr>
<td>rs1055129</td>
<td>17:71384543</td>
<td>Yes</td>
<td>0.49</td>
<td>0.28</td>
<td>5.7*10^{-5}</td>
<td>0.10 (0.05–0.16)</td>
<td>2.0*10^{-4}</td>
<td>0.10 (0.05–0.15)</td>
</tr>
</tbody>
</table>

Model I, adjusted for age, sex, and intracranial volume; Model II, same as in I, additionally adjusted for apoE4 carriership and cardiovascular risk factors; Model III, white matter lesion volume expressed as percentage of intracranial volume adjusted for age and sex.

Values are differences (95% CI) in white matter lesion volume per additional risk allele.

*Defined as systolic blood pressure $\geq 140$ mm Hg or diastolic blood pressure $\geq 90$ mm Hg or receiving antihypertensive treatment.

Discussion

In this study, we successfully replicated the association between a locus on chromosome 17q25 and WML volume. To our knowledge, this is the first replication study after the discovery report. The meta-analysis of the current and previous studies yielded compelling evidence that this locus is associated with WML volume in whites. The strength of our study is the use of the same methodology, WML quantification, and analyses similar to the discovery study. In addition, our study population is relatively young compared with the populations of the discovery study, which contributes to the generalizability of the results. A limitation of the study is that although no overlap existed with the Rotterdam Study I and II cohorts reported on previously, some individuals in the current study were related to persons from these cohorts. Although excluding these related persons did not influence

Results

Population characteristics are represented in Table 1. Table 2 shows the associations of the risk alleles of rs3744028 and the other 5 single nucleotide polymorphisms with WML volume. In Model I, presence of the risk allele of rs3744028 was associated with WML volume (difference in log-transformed mL: 0.10; 95% CI, 0.04–0.16; $P=1.1\times10^{-3}$). This corresponds to 9.3% of the mean WML volume. The other 5 single nucleotide polymorphisms were also significantly associated. Probability values survived Bonferroni correction. Additional adjustments (Model II) had minor influence on these associations. Expressing WML volume as a percentage of intracranial volume (Model III) slightly strengthened the associations. Finally, excluding persons with first-degree relatives in Rotterdam Study I or Rotterdam Study II did not change the results (rs3744028: 0.11; 95% CI, 0.04–0.17; $P=8.9\times10^{-4}$).

Results for other single nucleotide polymorphisms reaching $P<10^{-4}$ in the original report are listed in Supplemental Table I.

In the discovery report, rs3744028 was associated with WML volume with a $P$ value of $4.0\times10^{-15}$. After meta-analyzing the current data with the reported data, the $P$ value became even more significant and reached $5.3\times10^{-17}$.
the associations, any small residual effect cannot be ruled out entirely.

Together with the replication samples described in the original report, this is the third sample replicating the original findings. In a small sample of blacks in the original report, findings were not replicated. Studies investigating this locus in larger samples of blacks, and in other ethnicities such as Hispanics and Chinese, are therefore needed. This will further increase generalizability and contribute to pinpointing of the causative polymorphism. Alternatively, deep sequencing and expression studies can identify and unravel possible causative mechanisms. Furthermore, research is required to elucidate the potential role of this locus in diseases associated with WML volume such as stroke and dementia.

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Disclosures

None.

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