Gene Variation of the Transient Receptor Potential Cation Channel, Subfamily M, Member 7 (TRPM7), and Risk of Incident Ischemic Stroke

Prospective, Nested, Case-Control Study

José R. Romero, Paul M. Ridker, Robert Y.L. Zee

Background and Purpose—Transient receptor potential cation channel, subfamily M, member 7 (TRPM7), has been implicated in ischemic brain damage, a major source of morbidity and mortality in westernized society. We hypothesized that TRPM7 gene variation might play a role in the risk of ischemic stroke.

Methods—From a group of DNA samples collected at baseline in a prospective cohort of 14,916 initially healthy American men, we assessed 16 TRPM7 tag–single-nucleotide polymorphisms (SNPs) (dbSNP: rs11854949, rs4775899, rs11635825, rs12905120, rs16973487, rs7173321, rs7163283, rs17520378, rs17520350, rs4775892, rs7174839, rs17645523, rs3109894, rs616256, rs11070795, and rs313158) from 245 white men who subsequently had an incident ischemic stroke and from 245 age- and smoking habit–matched white men who remained free of reported vascular disease during follow-up (controls).

Results—All SNPs examined were in Hardy-Weinberg equilibrium. Overall allele, genotype, and haplotype distributions were similar between cases and controls. Marker-by-marker conditional logistic-regression analysis, adjusted for potential risk factors, showed no evidence for an association between any of the SNPs tested and ischemic stroke. Further investigation with an Entropy Blocker–defined, haplotype-based approach showed similar null findings. Prespecified analysis limited to participants without baseline diabetes and hypertension (ie, low-risk group) again showed similar null findings.

Conclusions—The present prospective investigation provides no evidence of a role for the TRPM7 gene in the risk of incident ischemic stroke. (Stroke. 2009;40:2965-2968.)

Key Words: TRPM7 tag–single-nucleotide polymorphisms ischemic stroke risk factors

Disordered regulation of magnesium and calcium levels has been reported in the ischemic brain.1–4 A recent report of the Atherosclerosis Risk In Communities Study provided evidence that serum magnesium levels were inversely associated with the incidence of ischemic stroke in a cohort of 14,221 men and women.5 These findings are consistent with reports showing that a low intake of magnesium may increase the risk for ischemic stroke.6 Furthermore, there is evidence of an inverse association between either serum magnesium levels or intake and risk factors for stroke, such as hypertension and diabetes.7–9 However, the mechanisms for the role of magnesium homeostasis in the pathophysiology of ischemic stroke are unclear.

Magnesium homeostasis is in part mediated by plasma membrane channels and transporters. Transient receptor potential cation channel, subfamily M, member 7 (also known as transient receptor potential melastatin 7; TRPM7), is a ubiquitously expressed member of the TRP family of ion channels. It is permeable to magnesium, calcium, and divalent trace-metal ions and has been suggested to play a role in the homeostasis of these ions.10 There is an expanding literature suggesting an important role for TRPM7 in the pathophysiology of stroke.11–13 Furthermore, TRPM7 is expressed in brain tissue as well as in vascular smooth muscle cells,14 and it has been implicated in anoxia-induced neuronal cell death.15 Thus, the association of magnesium homeostasis with the incidence of ischemic stroke could be modified by genetic factors associated with the TRPM7 gene.

We therefore hypothesized that TRPM7 (MIM: 605692; 15q21) gene variations might contribute to the pathogenesis of ischemic stroke. To test this hypothesis, we evaluated the potential association of 16 TRPM7-tagging single-nucleotide polymorphisms (SNPs) with the risk of incident ischemic stroke in participants drawn from the Physicians’ Health Study (PHS) cohort.

Subjects and Methods

Study Design

We used a prospective, nested, case-control design within the PHS, a completed, randomized, controlled trial of aspirin and β-carotene.
initiated in 1982 among 22,071 male, predominantly white (>94%), US physicians 40 to 84 years old at study entry. Before randomization, 14,916 participants provided an EDTA-anticoagulated blood sample that was stored for further analyses. All participants were free of prior myocardial infarction, stroke, transient ischemic attack, and cancer at study entry. A history of cardiovascular risk factors, such as hypertension, diabetes, or hyperlipidemia, was defined by self-report at entry into the study. For all reported incident vascular events that occurred after study enrollment, relevant hospital records, death certificates, and autopsy reports were requested and reviewed by an outcomes committee according to standardized diagnostic criteria.

Stroke was defined by the presence of a new, focal, neurologic deficit, with symptoms and signs persisting for >24 hours, and was ascertained from a blinded review of medical records, autopsy results, and the judgment of a board-certified neurologist, based on clinical reports and computed tomographic or magnetic resonance imaging scanning.

For each case, a control matched by age, smoking history, and length of follow-up was chosen; 259 ischemic stroke case–control pairs were identified for the present investigation, all white men. Ischemic stroke was classified as 33% embolic, 29% thrombotic, and 38% nondifferentiable embolic–thrombotic among the ischemic stroke cases in the present study. The study was approved by the Brigham and Women’s Hospital institutional review board for human subjects research.

**SNP Selection and Genotype Determination**

We selected a set of tagging SNPs that capture common variation and linkage disequilibrium structure across the TRPM7 gene with use of the Tagger program implemented in Haplovie v4.1 software. The data source for tagging SNP selection was from CEPH Utah residents of European ancestry. Selection of tagging SNPs was based on a pairwise correlation coefficient (r2) of 0.80 or greater–between tagging SNPs and untyped SNPs–and a minor allele frequency of 5% or greater. A total of 16 SNPs were identified (5’ to 3’ orientation): dbSNP rs11854949, rs4775899, rs11635825, rs13005120, rs14973487, rs7173321, rs7163283, rs17520378, rs17520350, rs4775892, rs1748393, rs17645523, rs3108984, rs616256, rs11070795, and rs313158. Genotype was determined by Sequenom matrix-assisted laser desorption and ionization–time-of-flight mass spectrometry according to standard protocol.

To confirm genotype assignment, scoring was performed by 2 independent observers. Discordant results (<1% of all scoring) were resolved by a joint reading and, if necessary, a repeat genotyping. In 14 subjects, we encountered difficulties in obtaining unambiguous amplifications; these subjects along with their matched counterparts were excluded from the analysis (N = 245 case-control pairs for subsequent analysis). Five percent of randomly selected samples were re-genotyped for quality control (100% concordance rate). Results were scored blinded as to case-control status.

**Statistical Analysis**

Allele and genotype frequencies among cases and controls were compared with values predicted by Hardy-Weinberg equilibrium with the exact test. Odds ratios of ischemic stroke associated with each genotype were calculated separately by logistic-regression analysis conditioned on age, smoking status, and length of follow-up since randomization and further adjusted for randomized treatment assignment, body mass index, history of hypertension (≥140/90 mm Hg or on antihypertensive medication), and the presence or absence of diabetes. We performed a conditional logistic-regression analysis after assuming an additive, dominant, or recessive model. Haplotype estimation and inference were determined by an expectation-maximization algorithm. Haplotype blocks were defined by Entropy Blocker (EB). As described previously, unlike most methods for discovering haplotype blocks, EB does not aim to discover haplotype tagging single-base variations (also known as SNPs) but rather aims to differentiate between regions populated by weakly correlated single-base variations and regions populated by at least several single-base variations in strong linkage disequilibrium.

Haplotype distributions (as defined by EB) between cases and controls were examined by the exact test. In addition, the relation between haplotypes and incident ischemic stroke was examined by haplotype-based conditional logistic-regression analysis, after adjusting for the same potential confounders or risk factors. Furthermore, prespecified analysis limited to participants without baseline diabetes or hypertension (ie, low-risk group), was performed. All analyses were carried out with the SAS v9.1 package (SAS Institute Inc). For each odds ratio, we calculated 95% CIs. A 2-tailed probability value of 0.05 was considered statistically significant.

**Results**

Baseline characteristics of the study population are shown in Table 1. Baseline, cases had a higher prevalence of traditional cardiovascular risk factors at baseline than did controls. The observed genotype distributions were in Hardy-Weinberg equilibrium in controls (all P>0.20) and cases (all P>0.20). According to standard marker-by-marker χ2 analysis, the genotype distribution was similar between cases and controls for all SNPs tested (supplemental Table I; available online at http://stroke.ahajournals.org). Results from the conditional logistic-regression analysis showed no evidence for an association with risk of ischemic stroke after assuming an additive (Table 2), dominant (data not shown), or recessive (data not shown) model. Supplemental Table II shows the pairwise linkage disequilibrium among the 16 SNPs evaluated. Four haplotype blocks were defined by EB (supplemental Table III) and were used for subsequent analyses. Overall, the EB haplotype frequencies were similar between cases and controls (supplemental table III). Results from the haplotype-based conditional logistic-regression analysis showed no evidence for an association with risk of incident ischemic stroke (data not shown). Again, virtually identical null findings were observed in our prespecified “low-risk” group (data not shown).

**Table 1. Baseline Characteristics of Study Participants**

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 245)</th>
<th>Cases (n = 245)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>61.9 ± 0.5</td>
<td>62.3 ± 0.5</td>
<td>MV</td>
</tr>
<tr>
<td>Smoking status, %</td>
<td></td>
<td></td>
<td>MV</td>
</tr>
<tr>
<td>Never</td>
<td>39.6</td>
<td>39.6</td>
<td></td>
</tr>
<tr>
<td>Past</td>
<td>42.0</td>
<td>42.0</td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>18.4</td>
<td>18.4</td>
<td></td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.8 ± 0.2</td>
<td>25.3 ± 0.2</td>
<td>0.06</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>129.4 ± 0.8</td>
<td>134.9 ± 0.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diastolic</td>
<td>79.7 ± 0.5</td>
<td>82.5 ± 0.5</td>
<td>0.0002</td>
</tr>
<tr>
<td>Hyperlipidemia (&gt;240 mg/dL), %</td>
<td>15.8</td>
<td>19.6</td>
<td>0.26</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>30.7</td>
<td>51.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>2.4</td>
<td>11.9</td>
<td>0.0001</td>
</tr>
<tr>
<td>Aspirin use, %</td>
<td>48.2</td>
<td>49.8</td>
<td>0.72</td>
</tr>
<tr>
<td>Family history of premature coronary artery disease (&lt;60 y), %</td>
<td>7.8</td>
<td>8.3</td>
<td>0.87</td>
</tr>
</tbody>
</table>

MV indicates matching variable. Values are mean ± SE unless otherwise stated. Continuous and categorical variables were tested by paired t test and McNemar’s test, respectively.
ischemic stroke are available, a direct cross-reference com-

pathophysiology of stroke. TRPM7, a believed to play an important role in the pathogenesis of vascular disorders, including ischemic stroke.1–4 TRPM7, a able to divalent cations such as magnesium, calcium, and potential channel that is expressed in brain tissue, is perme-

ation of the 

anoxia-induced cell death via the production of reactive trace elements. In neurons, TRPM7 can detect changes in the body mass index, history of hypertension, and the presence or absence of diabetes.17 In our study, we had the ability to detect, on the basis of the present sample size and assuming 80% power and an α of 0.05, a risk ratio >1.65 if the minor allele frequency was 0.50 and of >2.50 if the minor allele frequency was 0.05, after assuming a univariable-additive model.

**Discussion**

To the best of our knowledge, the present prospective, nested, case-control investigation is the first to examine the potential involvement of TRPM7 gene variations in the risk of incident ischemic stroke, and we found no evidence for any association of the TRPM7 gene polymorphisms/haplotypes tested. Similar null findings were observed in analyses limited to participants with neither baseline diabetes nor baseline hypertension (low-risk group).

Stroke is a major cause of morbidity and mortality around the world, and magnesium and calcium homeostasis is believed to play an important role in the pathogenesis of vascular disorders, including ischemic stroke.1–4 TRPM7, a potential channel that is expressed in brain tissue, is permeable to divalent cations such as magnesium, calcium, and trace elements. In neurons, TRPM7 can detect changes in the levels of divalent cations and has been shown to mediate anoxia-induced cell death via the production of reactive oxygen and nitrogen species. Thus, these previous reports suggest functional involvement of the TRPM7 gene in the pathophysiology of stroke. Because no epidemiologic data on TRPM7 and the risk of ischemic stroke are available, a direct cross-reference comparison with the present null findings cannot be made. Nonetheless, the present investigation suggests that TRPM7 does not play a role in the underlying pathophysiology of ischemic stroke.

The prospective nature of the PHS cohort, and the use of a closed, prospective cohort in which the determination of case status was based solely on the subsequent development of disease rather than on any arbitrary selection criteria designed by the investigators, greatly reduces the possibility that our findings are due to bias and/or confounding. Nonetheless, our sample population consisted of white men only, so the data cannot be generalized to other ethnic groups, women, or populations with different socioeconomic backgrounds. On the basis of the power of the present investigation, we cannot rule out a modest risk of ischemic stroke associated with the polymorphisms/haplotypes tested. The gene ontology and relevant pathway(s) relating to TRPM7 were explored with the use of public databases, including the NCBI PubMed, the UCSC Genome Bioinformatics Browser, and the Gene Ontology Database. Because of the limited information available, further studies for examining the potential pathway(s) that TRPM7 influences and the gene locus(1) whose variants might interact with TRPM7 are warranted. Furthermore, the phylogenetic history of TRPM7 intragenic variation and how this might influence subsequent haplotype-based analysis should be examined in future studies. Because we had limited information on ischemic stroke subtype classification and the small sample sizes for our available subtypes (namely, embolic, thrombotic, or nondifferentiable), the potential association of these TRPM7 polymorphisms/haplotypes with subtypes of ischemic stroke could not be examined in the present context.

In conclusion, these prospective data from a large cohort of apparently healthy white US men provide no evidence of an association between the TRPM7 SNPs tested and risk of incident ischemic stroke. If corroborated in other prospective studies, our data further suggest that TRPM7 gene variation is not informative for risk assessment of ischemic stroke.

**Disclosures**

None.

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**References**


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