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Gene Variation of the Transient Receptor Potential Cation Channel, Subfamily M, Member 7 (*TRPM7*) and Risk of Incident Ischemic Stroke: A Prospective, Nested Case-Control Study

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Abstract

Background—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 the *TRPM7* gene variation may 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-SNPs (dbSNP: rs11854949, rs4775899, rs11635825, rs12905120, rs16973487, rs7173321, rs7163283, rs17520378, rs17520350, rs4775892, rs7174839, rs17645523, rs3109894, rs616256, rs11070795, and rs313158) from 245 Caucasian men who subsequently suffered an incident ischemic stroke, and from 245 age- and smoking-matched Caucasian 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 of any of the SNPs tested with ischemic stroke. Further investigation using an Entropy Blocker-defined haplotype-based approach showed similar null findings. Pre-specified analysis limited to participants without baseline diabetes and hypertension (i.e. low-risk group) again showed similar null findings.

Conclusions—The present prospective investigation provides no evidence for a role of TRPM7 gene in the risk of incident ischemic stroke.

Keywords

TRPM7; tag-SNP; ischemic stroke; risk factors

Introduction

Disordered regulation of magnesium and calcium levels have been reported in ischemic brain [1-4]. A recent report of the Atherosclerosis Risk in Communities Study provides evidence that serum magnesium levels are 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 low intake of magnesium may increase risk for ischemic stroke [6]. Furthermore, there is evidence of an inverse association of either serum magnesium levels or intake on risk factors

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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 (a.k.a. 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 is 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 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 variation may 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 risk of incident ischemic stroke in participants drawn from the Physicians Health Study (PHS) cohort.

Methods

Study Design

We employed a prospective, nested case-control design within PHS, a completed randomized controlled trial of aspirin and beta-carotene initiated in 1982 among 22,071 male, predominantly white (>94%), US physicians, 40 to 84 years old at study entry [16]. Before randomization, 14916 participants provided an EDTA-anticoagulated blood sample that was stored for further analyses. All participants were free of prior MI, stroke, transient ischemic attacks, and cancer at study entry. 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 occurring after study enrollment, relevant hospital records, death certificates, and autopsy reports were requested and reviewed by an outcomes committee using standardized diagnostic criteria.

Stroke was defined by the presence of a new focal neurological deficit, with symptoms and signs persisting for >24 hours, and was ascertained from blinded review of medical records, autopsy results, and the judgment of a board-certified neurologist, on the basis of clinical reports, computed tomographic or magnetic resonance image 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 Caucasian men. Ischemic stroke was classified as 33% embolic, 29% thrombotic, and 38% non-differentiable 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 (LD) structure across the *TRPM7* gene using the Tagger program implemented in Haploview v4.1 software. The data source for tagging SNP selection was from the CEPH Utah residents with European ancestry. Selection of tagging SNPs was based on a pairwise correlation coefficient (r-square) of 0.80 or greater --between tagging SNPs and untyped SNPs-- and a minor allele frequency (MAF) of 5% or greater. A total of 16 SNPs were identified [5′ to 3′ orientation: dbSNP rs11854949, rs4775899, rs11635825, rs12905120, rs16973487,

rs7173321, rs7163283, rs17520378, rs17520350, rs4775892, rs7174839, rs17645523, rs3109894, rs616256, rs11070795, and rs313158].

Genotype was determined using the Sequenom matrix-assisted laser desorption and ionization-time of flight (MALDI-TOF) mass spectrometry according to standard protocol.

To confirm genotype assignment, scoring was carried out 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 samples along with the matched counterparts were excluded from the analysis (N=245 case-control pairs for subsequent analysis). Five percent 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 using the exact test. Odds ratios (ORs) 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, bodymass index, history of hypertension (≥140/90 mmHg or on antihypertensive medication), and presence/absence of diabetes. We performed conditional logistic regression analysis assuming an additive, dominant, or recessive model. Haplotype estimation and inference were determined by expectation-maximization algorithm. Haplotype blocks were defined with Entropy Blocker [EB] [17]. As described previously, unlike most methods for discovering haplotype blocks, EB does not aim to discover haplotype tagging single-base variations [also known as single-nucleotide polymorphisms (SNPs)], but rather to differentiate between regions populated by weakly correlated single-base variations and regions populated by at least several single-base variations in strong LD. Haplotype distributions (as defined by EB) between cases and controls were examined by exact test. In addition, the relationship between haplotypes and incident ischemic stroke was examined by haplotype-based conditional logistic regression analysis, adjusting for the same potential confounders/risk factors. Furthermore, pre-specified analysis, limited to participants without baseline diabetes nor hypertension (i.e. low-risk group), was performed. All analyses were carried out using SAS v9.1 package (SAS Institute Inc). For each OR, we calculated 95% confidence intervals (CIs). A 2-tailed *p*-value of 0.05 was considered a statistically significant result.

Results

Baseline characteristics of the study population are shown in Table 1. As expected, the case patients had a higher prevalence of traditional cardiovascular risk factors at baseline than the 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 χ^2-analysis, the genotype distribution was similar between cases and controls for all SNPs tested (Supplementary Data Table 1). Results from the conditional logistic regression analysis showed no evidence for an association with risk of ischemic stroke assuming an additive (Table 2), dominant (data no shown), or recessive model (data not shown). Supplementary Data Table 2 shows the pairwise LD amongst the 16 SNPs evaluated. Four haplotype-blocks were defined by EB (Supplemental Data Table 3), and used for subsequent analyses. Overall, the EB haplotype frequencies were similar between cases and controls (Supplemental Data Table 3). Results from the haplotype-based conditional logistic regression analysis showed no evidence for an associated with risk of incident ischemic stroke (data not shown). Again, virtually identical null findings were observed in our pre-specified ‘low-risk’ group (data not shown).

In our study, we had the ability to detect, based on the present sample size, assuming 80% power, at an alpha of 0.05, a risk ratio of >1.65 if the MAF is 0.50, and of >2.50 if the MAF is 0.05 assuming an 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 variation 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 without baseline diabetes nor hypertension (low-risk group).

Stroke is a major cause of morbidity and mortality around the world, and magnesium and calcium homeostasis are believed to play important roles 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 [3], and has been shown to mediate anoxia-induced cell death via the production of reactive oxygen and nitrogen species [15]. Thus, these previous reports suggest a functional involvement of the *TRPM7* gene in the pathophysiology of stroke.

As no epidemiological data of *TRPM7* on the risk of ischemic stroke are available, a direct cross-reference comparison cannot be made on the present null findings. Nonetheless, the present investigation suggests that *TRPM7* do 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 consists of Caucasian males only, so the data cannot be generalized to other ethnic groups, women, or to population with different socio-economic background. Based on 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* was explored via public databases including the NCBI PubMed, the UCSC Genome Bioinformatics-Browser and the Gene Ontology Database. Because of limited information available, further studies examining the potential pathway(s) that TRPM7 influences, and gene locus(i) whose variants may 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. As we have limited information on ischemic stroke subtype classification, and small sample size for our available subtypes (namely embolic, thrombotic, or non-differentiable), 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 Caucasian US men provide no evidence of an association between *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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Baseline characteristics of study participants.

	Controls (N=245)	**Cases (N=245)**	***p***
Age (years)	61.9±0.5	62.3±0.5	m.v.
Smoking Status (%)			m.v.
Never	39.6	39.6	
Past	42.0	42.0	
Current	18.4	18.4	
Body Mass Index (kg/m^2)	24.8±0.2	25.3±0.2	*0.06*
Blood Pressure (mmHg)			
Systolic	129.4±0.8	134.9±0.8	*<0.0001*
Diastolic	79.7±0.5	82.5±0.5	*0.0002*
Hyperlipidemia >240 mg/dL (%)	15.8	19.6	*0.26*
Hypertension (%)	30.7	51.4	*<0.0001*
Diabetes (%)	2.4	11.9	*0.0001*
Aspirin use (%)	48.2	49.8	*0.72*
Family History of Premature CAD <60 years (%)	7.8	8.3	*0.87*

Mean±SE unless otherwise stated.

m.v.=matching variable, CAD=coronary artery disease.

Continuous and categorical variables were tested by paired t-test and McNemar's test, respectively.

Table 2

Conditional logistic regression analysis assuming an additive model.

Variant	Adjusted OR, 95%CI, *p*	Adjusted OR, 95%CI, *p* (Low risk group)*
rs11854949	1.209, 0.762-1.919, *0.421*	1.758, 0.669-4.617, *0.252*
rs4775899	1.100, 0.831-1.456, *0.506*	1.057, 0.604-1.851, *0.846*
rs11635825	1.007, 0.682-1.489, *0.970*	1.088, 0.555-2.135, *0.805*
rs12905120	1.209, 0.748-1.955, *0.438*	1.659, 0.617-4.457, *0.316*
rs16973487	1.289, 0.789-2.107, *0.311*	1.735, 0.648-4.651, *0.273*
rs7173321	1.185, 0.742-1.893, *0.478*	1.758, 0.669-4.617, *0.252*
rs7163283	1.098, 0.832-1.448, *0.509*	1.115, 0.633-1.964, *0.707*
rs17520378	0.903, 0.625-1.305, *0.587*	0.974, 0.478-1.983, *0.942*
rs17520350	0.885, 0.606-1.292, *0.526*	0.573, 0.275-1.194, *0.137*
rs4775892	0.915, 0.625-1.339, *0.648*	0.593, 0.279-1.260, *0.174*
rs7174839	0.906, 0.679-1.208, *0.502*	0.773, 0.441-1.355, *0.369*
rs17645523	0.991, 0.741-1.326, *0.951*	0.991, 0.579-1.695, *0.973*
rs3109894	0.982, 0.727-1.326, *0.904*	0.970, 0.563-1.671, *0.912*
rs616256	1.104, 0.828-1.472, *0.501*	1.056, 0.600-1.857, *0.851*
rs11070795	1.101, 0.826-1.468, *0.510*	1.100, 0.617-1.962, *0.746*
rs3131583	0.991, 0.680-1.443, *0.962*	1.443, 0.729-2.854, *0.292*

Adjusted=conditional on age, smoking status, time of follow-up and further controlled for randomized treatment group, BMI, history of hypertension, presence or absence of diabetes.

Additive=Minor-homozygotes versus heterozygotes versus major-homozygotes

* Only participants without baseline diabetes nor hypertension.