Published in final edited form as:
Eur J Neurol. 2013 February ; 20(2): 300–308. doi:10.1111/j.1468-1331.2012.03846.x.

Genetic variants associated with myocardial infarction in the *PSMA6* gene and Chr9p21 are also associated with ischemic stroke

Michael G. Heckman, MS[1,*], **Alexandra I. Soto-Ortolaza, BS**[2], **Nancy N. Diehl, BS**[1], **Sruti Rayaprolu, BS**[2], **Thomas G. Brott, MD**[3], **Zbigniew K. Wszolek, MD**[3], **James F. Meschia, MD**[3], and **Owen A. Ross, PhD**[2]

[1]Biostatistics Unit, Mayo Clinic, Jacksonville, Florida, USA

[2]Department of Neuroscience, Mayo Clinic, Jacksonville, Florida, USA

[3]Department of Neurology, Mayo Clinic, Jacksonville, Florida, USA

Abstract

Background—Ischemic stroke shares common traditional risk factors with coronary artery disease (CAD) and myocardial infarction (MI). This study evaluated whether genetic risk factors for CAD and MI also affect susceptibility to ischemic stroke in Caucasians and African Americans.

Methods—Included in the study were a Caucasian series (713 ischemic stroke patients, 708 controls) and a small African American series (166 ischemic stroke patients, 117 controls). Twenty single nucleotide polymorphisms (SNPs) previously shown to be associated with CAD or MI were genotyped and assessed for association with ischemic stroke and ischemic stroke subtypes using odds ratios (ORs) from multivariable logistic regression models.

Results—In Caucasians, four SNPs on chromosome 9p21 were significantly associated with risk of cardioembolic stroke, the strongest of which was rs1333040 (OR=1.55, P=0.0007); similar but weaker trends were observed for small vessel stroke, with no associations observed regarding large vessel stroke. Chromosome 9p21 SNPs were also associated with risk of ischemic stroke in African Americans (rs1333040: OR=0.65, P=0.023; rs1333042, OR=0.55, P=0.070; rs2383207: OR=0.55, P=0.070). The *PSMA6* SNP rs1048990 on chromosome 14q13 was associated with overall ischemic stroke in both Caucasians (OR: 0.80, P=0.036) and African Americans (OR: 0.31, P=0.020).

Conclusions—Our results provide evidence that chromosome 9p21 variants are associated with cardioembolic ischemic stroke in Caucasians and with overall ischemic stroke in African Americans. The *PSMA6* variant rs1048990 also appears to affect susceptibility to ischemic stroke in both populations. These findings require validation, particularly the preliminary findings regarding African Americans given the small size of that series.

Keywords

genetics; single nucleotide polymorphism; ischemic stroke; coronary artery disease; myocardial infarction

[*]Corresponding author's contact information: Michael G. Heckman, MS, Biostatistics Unit, Mayo Clinic Jacksonville, 4500 San Pablo Road, Jacksonville, FL 32224, Tel: (904)-953-1049, Fax: (904)-953-0277, heckman.michael@mayo.edu.

Conflict of interest statement
The authors report no conflicts of interest.

Introduction

Stroke is a leading cause of death and disability in the United States and worldwide, with approximately 15 million people throughout the world suffering a stroke every year [1–2]. With a prevalence of at least 80% among stroke patients, ischemic stroke is the by far the most common type of stroke. Traditional risk factors for ischemic stroke are well understood, and these include, among others, older age, hypertension, atrial fibrillation, smoking, and diabetes mellitus. Reports involving twin and family studies have provided evidence that genetics also has an influence on risk of ischemic stroke [3–6], and in recent years there has been substantial research evaluating specific genetic risk factors [7–8]. Several notable discoveries have been made, with some of the most promising findings involving a replicated association with the chromosome 9p21 locus [9–12]. However, despite these positive reports, genetic risk factors for ischemic stroke remain poorly understood, and this is particularly true for individuals of African descent, for whom very little research has been undertaken despite the high risk of stroke in this population.

Ischemic stroke shares common traditional risk factors with both coronary artery disease (CAD) and myocardial infarction (MI), and similar to ischemic stroke, these two cardiovascular diseases have a well-established genetic component [13–14]. A number of promising risk variants for CAD and MI have emerged, with the most consistent of these being the locus on chromosome 9p21 [13–14]. Although a recent study by Cheng et al. examining eleven single nucleotide polymorphisms (SNPs) reported by the Myocardial Infarction Genetics Consortium (MIGC) did not find any association with ischemic stroke [15], the fact that the 9p21 locus has been shown to be associated with risk of ischemic stroke and CAD/MI, combined with the shared conventional risk factors between ischemic stroke, CAD, and MI, suggests that genes that have been implicated in CAD and MI should be further studied in relation to ischemic stroke. Herein, we evaluate twenty SNPs that have been shown to affect risk of CAD or MI for association with risk of ischemic stroke and ischemic stroke subtypes in Caucasians and African Americans.

Methods and Patients

Subjects

Three different ischemic stroke patient-control series' from the United States were utilized in these analyses (Table 1), yielding a total of 879 ischemic stroke patients and 825 controls. The first series, which we refer to as the familial Caucasian series, utilized a group of 264 unrelated Caucasian familial ischemic stroke patients collected through the Siblings with Ischemic Stroke Study (SWISS) and 374 controls who were seen at Mayo Clinic Florida. The second series was a patient-control series that was obtained through the Ischemic Stroke Genetics Study (ISGS), and consisted of 449 ischemic stroke patients and 334 controls. A smaller third series of African American ischemic stroke patients and controls also obtained through the ISGS was utilized, and involved 166 ischemic stroke patients and 117 controls. The ISGS and SWISS Caucasian ischemic stroke patients, but not the ISGS African American stroke patients, were a subset of those utilized by Cheng et al. in their meta-analysis, which among other series' involved a large group of ISGS/SWISS patients (N=991) [15].

Age and gender information was collected for all series', while information regarding atrial fibrillation, CAD, diabetes, hypertension, and current smoking was also collected for both of the ISGS patient-control series' and the familial stroke patients (Table 1). Stroke was defined according to the World Health Organization criteria as rapidly developing signs of a focal or global disturbance of cerebral function, with symptoms lasting 24 hours or longer or

leading to death, with no apparent cause other than vascular origin [16]. Details on the diagnosis of ischemic stroke and phenotypic characterization for the SWISS and ISGS studies have been previously described [17, 18]. Type of ischemic stroke was classified according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) system [19]. The ethical review board at each institution approved the study. All patients provided informed consent.

Genetic Analysis

We selected 20 SNPs that have been shown to be associated with either CAD or MI for analysis in this study [20–29]. All SNPs were selected based on findings from prior genome-wide association studies (GWAS's) with the exception of rs1048990 in *PSMA6*, which was selected due to several independent replications in different populations. Details of these SNPs and their reasons for inclusion are displayed in Table 2. DNA was extracted from whole blood samples using standard protocols. Variants were genotyped on a Sequenom MassArray iPLEX platform (San Diego, CA; primer sequences are available on request) and analyzed with Typer 4.0 software. Genotype call rates were ≥98% for each SNP for each series, and there was no departure from Hardy Weinberg equilibrium ($P>0.01$) in controls for any of the SNPs with the exception of rs6725887 in the ISGS African American series, where the minor allele frequency was 2.6% and one rare homozygote was observed.

Nine of the SNPs included in this study were also examined by Cheng et al. in their series of Caucasian and South Asian individuals (highlighted in Table 2) [15]. Therefore, our study provides unique data on all 20 of the selected SNPs in the ISGS African American series, and on 11 of the 20 selected SNPs in the familial Caucasian series and ISGS Caucasian series. Additionally, data was not presented separately for the ISGS and familial Caucasian series' by Cheng et al. [15], and series-specific associations with ischemic stroke and ischemic stroke subtypes were presented only for SNPs with the most significant associations. As a result, of the 9 SNPs included in both studies, the only SNPs whose associations are presented for the combined familial/ISGS Caucasian series both in our study and in the study by Cheng et al. were those involving rs11206510 (overall ischemic stroke), rs9818870 (cardioembolic stroke), and rs4977574 (large vessel stroke). Thus, the vast majority of the associations with ischemic stroke and ischemic stroke subtypes presented in our study were not described by Cheng et al.

Statistical analysis

Single SNP associations with ischemic stroke were evaluated using logistic regression models, separately for the familial proband Caucasian series, ISGS Caucasian series, combined Caucasian series, and ISGS African American series. Models for the familial Caucasian series and combined Caucasian series were adjusted for age and gender, with additional adjustment for series in the combined Caucasian series. Models for the ISGS Caucasian series and ISGS African American series were adjusted for additional variables available in those series' for both ischemic stroke patients and controls, specifically age, gender, CAD, diabetes, hypertension, current smoking, and atrial fibrillation (ISGS Caucasian series only). Atrial fibrillation was not adjusted for in the ISGS African American series because no control had atrial fibrillation. In the combined Caucasian series, familial Caucasian series, and ISGS Caucasian series, single SNP associations with specific ischemic stroke subtypes (cardioembolic stroke, large vessel stroke, small vessel stroke) were also evaluated; these associations were not evaluated in the ISGS African American series owing to its small sample size. Additive models (effect of each additional minor allele) were utilized in the primary analysis, while dominant models (presence vs. absence of the minor allele) were also considered in secondary analysis. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated.

Haplotype analysis was performed regarding association with ischemic stroke and ischemic stroke subtypes using score tests for association [30], adjusting for the same variables as in the previously described logistic regression analysis; p-values were obtained from the asymptotic distribution of the score statistic, and haplotypes with a frequency of less than 1% were not considered. P-values of 0.05 or less were considered as statistically significant in all analyses. No adjustment for multiple testing was made in these exploratory analyses, and as such our findings require validation. All statistical analyses were performed using the SAS software package (SAS Institute; Cary, North Carolina) and S-Plus (version 8.0.1; Insightful Corporation; Seattle, Washington).

Results

An evaluation of single SNP associations with ischemic stroke under an additive model is presented in Table 3, separately for each series. In the familial Caucasian series, the only SNP significantly associated with ischemic stroke was rs1048990 in *PSMA6* (OR: 0.72, P=0.038). For the ISGS Caucasian series, significant associations with ischemic stroke were identified for rs6922269 in *MTHFD1L* (OR: 1.28, P=0.050), rs2048327 in *SLC22A3* (OR: 1.32, P=0.026), and rs1333040 on chromosome 9p21 (OR: 1.27, P=0.040), and these associations were independent of traditional risk factors for stroke. When combining the familial and ISGS Caucasian series', the only SNP whose association with ischemic stroke remained significant was rs1048990 (OR: 0.80, P=0.036). In the small ISGS African American series, associations with ischemic stroke independent of traditional risk factors were noted for rs1333040 (OR: 0.65, P=0.023) and rs1048990 (OR: 0.31, P=0.020), with non-significant trends also observed for rs1333042 (OR: 0.55, P=0.070) and rs2383207 (OR: 0.55, P=0.070) on chromosome 9p21, and rs9982601 on chromosome 21q22 (OR: 0.65, P=0.055). Associations were consistent under a dominant model (online-only Table 1).

Associations of the twenty SNPs with cardioembolic, large vessel, and small vessel ischemic stroke are displayed in Table 4 for the combined Caucasian series. There was evidence of an association between all four chromosome 9p21 SNPs and cardioembolic stroke. This association was strongest for rs1333040 (OR: 1.55, P=0.0007), with other significant associations observed for rs4977574 (OR: 0.69, P=0.0035), rs1333042 (OR: 1.42, P=0.0065), and rs2383207 (OR: 1.39, P=0.010). The only SNP that was significantly associated with large vessel stroke was rs1048990 (OR: 0.65, P=0.023), while rs1333040 was the only SNP showing statistically significant evidence of an association with small vessel ischemic stroke (OR: 1.31, P=0.044). Of note, these associations were similar in magnitude between the two Caucasian series' (online-only Tables 2 and 3). Single SNP associations with each ischemic stroke subtype were consistent under a dominant model (data not shown). Allele and genotype counts and frequencies in ischemic stroke patients (including different subtypes) and controls are provided in online-only Tables 4, 5, 6 and 7 for the familial Caucasian series, ISGS Caucasian series, combined Caucasian series, and ISGS African American series, respectively.

A previously nominated four-SNP (rs2048327-rs3127599-rs7767084-rs10755578) haplotype spanning the *SLC22A3*, *LPAL2*, and *LPA* genes [23] was not associated with ischemic stroke in the familial Caucasian series (P=0.72), ISGS Caucasian series (P=0.20), combined Caucasian series (P=0.82) or ISGS African American series (P=0.54); these results are presented in detail in Table 5. This lack of association was also consistent for the different ischemic stroke subtypes in the familial, ISGS, and combined Caucasian series' (all P≥0.18, online-only Table 8).

Discussion

Due to the fact that ischemic stroke shares common risk factors with CAD and MI, genetic variants that have been shown to be associated with these two cardiovascular phenotypes are promising candidates for study in relation to risk of ischemic stroke. In this study, we identified significant associations of SNPs located on chromosome 9p21 and a SNP in the *PSMA6* gene with risk of ischemic stroke in Caucasians and African Americans. Though our findings regarding African Americans should be viewed as preliminary owing to the small size of that series, it is important to highlight the fact that associations identified in African Americans were independent of traditional risk factors for ischemic stroke, including CAD. It is also worth noting that literature involving studies of genetic risk factors for ischemic stroke in individuals of African descent is scarce. In Caucasians, while we were not able to adjust for traditional risk factors in the combined series due to the lack of this information in the familial series controls, associations regarding *PSMA6* rs1048990 and chromosome 9p21 SNPs were similar in magnitude in the ISGS Caucasian series where we were able to adjust for these factors.

The most consistently replicated genetic risk factor for ischemic stroke has involved SNPs in the chromosome 9p21 region [9–12]. Our study found that chromosome 9p21 SNPs were associated with risk of overall ischemic stroke in our small African American series, however associations in the combined Caucasian series were not apparent until we considered ischemic stroke subtypes, where association was strong for cardioembolic ischemic stroke and weaker but significant or borderline-significant (rs1333040, rs4977574) for small vessel ischemic stroke. Though the minor allele was associated with an increased risk of ischemic stroke in Caucasians while the effect was protective in African Americans where three of the four chromosome 9p21 SNPs were much less frequent, these apparently discordant results are most likely due to the different genetic backgrounds of these two populations [31]. Of note, Cheng et al. evaluated one chromosome 9p21 SNP, rs4977574, in regard to association with ischemic stroke and its subtypes and did not identify a significant association, which may be due to the population stratification in the large series [15].

We observed evidence of a protective association between rs1048990, a SNP in the *PSMA6* gene on chromosome 14q13, and overall ischemic stroke in both Caucasians and African Americans. In Caucasians, this protective effect was observed in the individual as well as combined series', where it was fairly consistent in magnitude across all three stroke subtypes, though strongest for large vessel stroke with an odds ratio of 0.65 (P=0.023). These results are similar to those shown by Freilinger et al. [32], where the minor allele of rs1048990 was associated with decreased risk of overall ischemic stroke (OR: 0.80, P=0.037) and large vessel stroke (OR: 0.70, P=0.042) in a German series. However, these associations were not observed in a replication sample from the United Kingdom in the same study [32]. Despite this lack of replication, given the consistency of our findings with those seen in the German series examined by Freilinger et al., a role of *PSMA6* in ischemic stroke appears promising.

Several caveats should be considered when interpreting our results. The sample size is relatively small, and therefore the possibility of Type II error is important to consider, especially in the small African American series, in examination of associations with stroke subtypes, and for SNPs with low minor allele frequencies. Related to this, the small sample size of the African American series did not allow for a reasonable examination of associations with ischemic stroke subtypes; additional study in larger African American series' is needed. Also, traditional risk factors for stroke were not available in the familial Caucasian series controls, which prevented the adjustment of these risk factors in the combined Caucasian series. Though we cannot conclude that the associations identified in

the larger combined Caucasian series are independent of other risk factors for ischemic stroke such as CAD, it is important to note that these associations were very similar in the ISGS Caucasian series where we were able to adjust for these factors. Additionally, we did not make any adjustment for multiple testing in these analyses despite performing a relatively large number of statistical tests, and it is therefore very important to highlight that our findings require validation in independent series'. This is particularly true for findings regarding African Americans given the small size of that series.

In conclusion, the results of this study provide evidence that variants on chromosome 9p21 are associated with cardioembolic ischemic stroke in Caucasians and with overall risk of ischemic stroke in African Americans. Furthermore, the *PSMA6* SNP rs1048990 was associated with decreased risk of ischemic stroke in both populations. Given previous findings suggesting that chromosome 9p21 variants are specifically associated with large vessel stroke, and also the lack of a consistently documented association of ischemic stroke with *PSMA6*, further study is needed to determine the role of these genetic variants on risk of ischemic stroke and its subtypes. Future large meta-analyses involving GWAS data on both cerebrovascular and cardiovascular disease are likely to provide a clearer understanding of shared genetic risk factors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

OAR is a recipient of a James and Ester King Foundation New Investigator Award from the Department of Health, Florida State. OAR is also funded by the American Heart Association (AHA) and the Myron and Jane Hanley Award in Stroke research. The Siblings with Ischemic Stroke Study was funded by a grant from the NINDS (R01 NS39987; JFM, PI) and by a Marriott Disease Risk and Regenerative Medicine Initiative Award in Individualized Medicine. The Ischemic Stroke Genetics Study (ISGS) was funded by a grant from the NINDS (R01 NS42733). ZKW is partially supported by the NIH/NINDS 1RC2NS070276, NS057567, P50 NS072187-01S2, Mayo Clinic Florida (MCF) Research Committee CR program (MCF #90052030), and Dystonia Medical Research Foundation. For further details on our research in cerebrovascular disease please visit our web-site; http://mayoresearch.mayo.edu/mayo/research/ross_lab/

References

1. Mackay, J.; Mensah, G. The Atlas of Heart Disease and Stroke. Geneva: World Health Organizaton; 2004.
2. Roger VL, Go AS, Lloyd-Jones DM, et al. Executive Summary: Heart Disease and Stroke Statistics--2012 Update: A Report From the American Heart Association. Circulation. 2012; 125:188–197. [PubMed: 22215894]
3. Bak S, Gaist D, Sindrup SH, Skytthe A, Christensen K. Genetic liability in stroke: a long-term follow-up study of Danish twins. Stroke. 2002; 33:769–774. [PubMed: 11872902]
4. Liao D, Myers R, Hunt S, et al. Family history of stroke and stroke risk. The Family Heart Study. Stroke. 1997; 28:1908–1912. [PubMed: 9341694]
5. Flossmann E, Schulz UG, Rothwell PM. Systematic review of methods and results of the genetic epidemiology of ischemic stroke. Stroke. 2004; 35:212–227. [PubMed: 14684773]
6. Seshadri S, Beiser A, Pikula A, et al. Parental occurrence of stroke and risk of stroke in their children: the Framingham study. Circulation. 2010; 121:1304–1312. [PubMed: 20212282]
7. Markus HS. Stroke genetics. Human Molecular Genetics. 2011; 20:R124–131. [PubMed: 21831884]
8. Meschia JF, Worrall BB, Rich SS. Genetic susceptibility to ischemic stroke. Nature Reviews Neurology. 2011; 7:369–378.

9. Matarin M, Brown WM, Singleton A, Hardy JA, Meschia JF. ISGS investigators. Whole genome analyses suggest ischemic stroke and heart disease share an association with polymorphisms on chromosome 9p21. Stroke. 2008; 39:1586–1589. [PubMed: 18340101]
10. Smith JG, Melander O, Lövkvist H, et al. Common genetic variants on chromosome 9p21 confers risk of ischemic stroke: a large-scale genetic association study. Circulation Cardiovascular Genetics. 2009; 2:159–164. [PubMed: 20031580]
11. Gschwendtner A, Bevan S, Cole JW, et al. Sequence variants on chromosome 9p21.3 confer risk for atherosclerotic stroke. Annals of Neurology. 2009; 65:531–539. [PubMed: 19475673]
12. Anderson CD, Biffi A, Rost NS, Cortenelli L, Furie KL, Rosand J. Chromosome 9p21 in ischemic stroke: population structure and meta-analysis. Stroke. 2010; 41:1123–1131. [PubMed: 20395606]
13. Musunuru K, Kathiresan S. Genetics of coronary heart disease. Annual Review of Genomics and Human Genetics. 2010; 11:91–108.
14. Schunkert H, Erdmann J, Samani NJ. Genetics of myocardial infarction: a progress report. European Heart Journal. 2010; 31:918–925. [PubMed: 20219748]
15. Cheng YC, Anderson CD, Bione S, et al. Are Myocardial Infarction-Associated Single-Nucleotide Polymorphisms Associated with Ischemic Stroke? Stroke. 2012; 43:980–986. [PubMed: 22363065]
16. WHO MONICA Project Principal Investigators. The World Health Organization MONICA Project (monitoring trends and determinants in cardiovascular disease): a major international collaboration. J Clin Epidemiol. 1988; 41:105–114. [PubMed: 3335877]
17. Meschia JF, Brown RD Jr, Brott TG, Chukwudelunzu FE, Hardy J, Rich SS. The Siblings With Ischemic Stroke Study (SWISS) protocol. BMC Med Genet. 2002; 3:1. [PubMed: 11882254]
18. Meschia JF, Brott TG, Brown RD Jr, et al. The Ischemic Stroke Genetics Study (ISGS) Protocol. BMC Neurol. 2003; 3:4. [PubMed: 12848902]
19. Adams HP Jr, Bendixen BH, Kappelle LJ, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. Stroke. 1993; 24:35–41. [PubMed: 7678184]
20. Kathiresan S, Voight BG, Purcell S, et al. Genome-wide association of early-onset myocardial infarction with single nucleotide polymorphisms and copy number variants. Nature Genetics. 2009; 41:334–341. [PubMed: 19198609]
21. Erdmann J, Grosshennig A, Braund PS, et al. New susceptibility locus for coronary artery disease on chromosome 3q22.3. Nature Genetics. 2009; 41:280–282. [PubMed: 19198612]
22. Samani NJ, Erdmann J, Hall AS, et al. Genomewide association analysis of coronary artery disease. New England Journal of Medicine. 2007; 357:443–453. [PubMed: 17634449]
23. Trégouët DA, König IR, Erdmann J, et al. Genome-wide haplotype association study identifies the SLC22A3-LPAL2-LPA gene cluster as a risk locus for coronary artery disease. Nature Genetics. 2009; 41:283–285. [PubMed: 19198611]
24. Helgadottir A, Thorleifsson G, Manolescu A, et al. A common variant on chromosome 9p21 affects the risk of myocardial infarction. Science. 2007; 316:1491–1493. [PubMed: 17478679]
25. McPherson R, Pertsemlidis A, Kavaslar N, et al. A common allele on chromosome 9 associated with coronary heart disease. Science. 2007; 316:1488–1491. [PubMed: 17478681]
26. Welcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. 2007; 447:661–678. [PubMed: 17554300]
27. Ozaki K, Sato H, Iida A, et al. A functional SNP in PSMA6 confers risk of myocardial infarction in the Japanese population. Nature Genetics. 2006; 38:921–925. [PubMed: 16845397]
28. Barbieri M, Marfella R, Rizzo MR, et al. The -8 UTR C/G polymorphism of PSMA6 gene is associated with susceptibility to myocardial infarction in type 2 diabetic patients. Atherosclerosis. 2008; 201:117–123. [PubMed: 18358479]
29. Bennett DA, Xu P, Clarke R, et al. The exon 1-8C/G SNP in the PSMA6 gene contributes only a small amount to the burden of myocardial infarction in 6946 cases and 2720 controls from a United Kingdom population. European Journal of Human Genetics. 2008; 16:480–486. [PubMed: 18231128]

30. Schaid DJ, Rowland CM, Tines DE, Jacobson RM, Poland GA. Score tests for association between traits and haplotypes when linkage phase is ambiguous. American Journal of Human Genetics. 2002; 70:425–434. [PubMed: 11791212]

31. Lin PI, Vance JM, Pericak-Vance MA, Martin ER. No gene is an island: the flip- flop phenomenon. American Journal of Human Genetics. 2007; 80:531–538. [PubMed: 17273975]

32. Freilinger T, Bevan S, Ripke S, et al. Genetic variation in the lymphotoxin-alpha pathway and the risk of ischemic stroke in European populations. Stroke. 2009; 40:970–972. [PubMed: 19182073]

Table 1

Patient characteristics for each series

	Familial Caucasian series		ISGS Caucasian series		ISGS African American series	
Variable	**Stroke patients (N=264)**	**Controls (N=374)**	**Stroke patients (N=449)**	**Controls (N=334)**	**Stroke patients (N=166)**	**Controls (N=117)**
Age	72 ± 12 (33 – 96)	72 ± 11 (33 – 93)	71 ± 15 (28 – 100)	67 ± 15 (27 – 97)	61 ± 13 (25 – 98)	59 ± 14 (27 – 95)
Gender (Male)	147 (56%)	205 (55%)	265 (59%)	169 (51%)	82 (49%)	48 (41%)
Age at stroke	67 ± 11 (27 – 93)	N/A	66 ± 15 (22 – 94)	N/A	56 ± 13 (20 – 93)	N/A
Atrial fibrillation	33 (13%)	N/A	33 (7%)	8 (2%)	7 (4%)	0 (0%)
Coronary artery disease	39 (15%)	N/A	122 (27%)	35 (10%)	18 (11%)	7 (6%)
Diabetes	61 (23%)	N/A	105 (23%)	35 (10%)	51 (31%)	26 (22%)
Hypertension	186 (70%)	N/A	291 (65%)	124 (37%)	133 (80%)	53 (46%)
Current smoking	46 (18%)	N/A	100 (22%)	26 (8%)	62 (37%)	19 (16%)
Type of stroke						
Cardioembolic	34 (13%)	N/A	123 (27%)	N/A	29 (17%)	N/A
Large vessel	67 (25%)	N/A	90 (20%)	N/A	28 (17%)	N/A
Small vessel	81 (31%)	N/A	60 (13%)	N/A	46 (28%)	N/A
Other	15 (6%)	N/A	25 (6%)	N/A	2 (1%)	N/A
Undetermined	67 (26%)	N/A	151 (34%)	N/A	61 (37%)	N/A

- The sample mean ± SD (minimum – maximum) is given for age and age at stroke. In the Familial Caucasian series, information in stroke patients was unavailable regarding atrial fibrillation (N=2), coronary artery disease (N=1), and current smoking (N=2), and information was unavailable in all controls regarding atrial fibrillation, coronary artery disease, diabetes, hypertension, and current smoking. In the ISGS Caucasian series, information was unavailable regarding atrial fibrillation (N=3), coronary artery disease (N=1), and hypertension (N=1). In the ISGS African American series, information was unavailable for atrial fibrillation (N=4), coronary artery disease. (N=1), and hypertension (N=2). ISGS=Ischemic Stroke Genetics Study.

Table 2

SNP information and reason for inclusion

SNP information				Reason for inclusion in current study	
SNP	**Chromosome**	**Position (bp)[a]**	**Gene or [b]Nearest gene**	**Associated Disease**	**Reference(s)**
rs646776[c]	1p13	109818530	*CELSR2-PSRC1-SORT1*[b]	MI	20
rs11206510[c]	1p32	55496039	*PCSK9*[b]	MI	20
rs6725887[c]	2q33	203745885	*WDR12*	MI	20
rs9818870 [c]	3q22	138122122	*MRAS*	CAD	21
rs12526453 [c]	6p24	12927544	*PHACTR1*	MI	20
rs6922269	6q25	151252985	*MTHFD1L*	CAD	22
rs2048327	6q25	160863532	*SLC22A3*	CAD	23
rs3127599	6q25	160907134	*LPAL2*	CAD	23
rs7767084	6q25	160962503	*LPA*	CAD	23
rs10755578	6q25	160969738	*LPA*	CAD	23
rs1333040	9p21	22083404	*CDKN2BAS*[b]	CAD, MI	22, 24, 25, 26
rs4977574 [c]	9p21	22098574	*CDKN2BAS*[b]	CAD, MI	22, 24, 25, 26
rs1333042	9p21	22103813	*CDKN2BAS*[b]	CAD, MI	22, 24, 25, 26
rs2383207	9p21	22115959	*CDKN2BAS*[b]	CAD, MI	22, 24, 25, 26
rs1746048 [c]	10q11	44775824	*CXCL12*[b]	MI	20
rs2259816	12q24	121435587	*HNF1A-C12orf43*[b]	CAD	21
rs1048990	14q13	35761675	*PSMA6*	MI	27, 28, 29
rs17228212	15q22	67458639	*SMAD3*	CAD	22
rs1122608 [c]	19p13	11163601	*LDLR*[b]	MI	20
rs9982601 [c]	21q22	35599128	*SLC5A3-MRPS6-KCNE2*[b]	MI	20

[a]Chromosomal positions based on the February 2009 (GRCH37/hg19) genome assembly.

[c]SNP examined in Cheng et al. [15].

CAD=coronary artery disease. MI=myocardial infarction.

Table 3

Single SNP associations with ischemic stroke under an additive model

		Familial Caucasian series (264 patients, 374 controls)			ISGS Caucasian series (449 patients, 334 controls)			Combined Caucasian series (713 patients, 708 controls)			ISGS African American series (166 patients, 117 controls)		
SNP	**MA[a]**	**MAF**	**OR (95% CI)**	**P-value**	**MAF**	**OR (95% CI)**	**P-value**	**MAF**	**OR (95% CI)**	**P-value**	**MAF**	**OR (95% CI)**	**P-value**
rs646776	C	19.8%	1.04 (0.79, 1.39)	0.77	23.2%	1.10 (0.85, 1.42)	0.49	21.7%	1.06 (0.88 – 1.27)	0.54	36.2%	0.79 (0.55, 1.15)	0.22
rs11206510	C	18.8%	1.01 (0.76, 1.34)	0.97	19.3%	0.93 (0.70, 1.23)	0.60	19.1%	0.99 (0.82 – 1.19)	0.89	12.2%	0.85 (0.48, 1.51)	0.58
rs6725887	C	12.8%	0.79 (0.56, 1.11)	0.17	11.8%	0.88 (0.62, 1.25)	0.48	12.2%	0.84 (0.67 – 1.06)	0.14	2.5%	0.94 (0.31, 2.87)	0.91
rs9818870	T	16.3%	0.84 (0.62, 1.13)	0.25	16.2%	0.87 (0.64, 1.17)	0.35	16.2%	0.84 (0.69 – 1.03)	0.090	10.1%	1.06 (0.56, 2.04)	0.85
rs12526453	G	35.2%	1.12 (0.87, 1.42)	0.38	33.0%	1.06 (0.84, 1.33)	0.65	34.0%	1.04 (0.89 – 1.23)	0.61	15.9%	1.21 (0.70, 2.08)	0.49
rs6922269	A	28.8%	0.81 (0.63, 1.03)	0.090	29.1%	1.28 (1.00, 1.65)	0.050	29.0%	1.01 (0.86 – 1.20)	0.87	53.4%	1.18 (0.81, 1.71)	0.40
rs2048327	C	34.8%	0.84 (0.66, 1.07)	0.15	35.2%	1.32 (1.03, 1.67)	0.026	35.0%	1.06 (0.91 – 1.25)	0.46	8.0%	0.79 (0.40, 1.55)	0.49
rs3127599	T	26.7%	1.02 (0.80, 1.32)	0.85	31.7%	1.17 (0.92, 1.48)	0.21	29.5%	1.08 (0.92 – 1.27)	0.36	28.3%	1.09 (0.71, 1.65)	0.70
rs7767084	C	17.0%	0.90 (0.67, 1.21)	0.49	15.5%	0.94 (0.69, 1.28)	0.69	16.2%	0.93 (0.75 – 1.14)	0.46	2.7%	0.68 (0.22, 2.12)	0.51
rs10755578	G	45.8%	0.88 (0.70, 1.10)	0.26	47.1%	1.21 (0.97, 1.52)	0.092	46.5%	1.02 (0.88 – 1.19)	0.80	33.0%	0.89 (0.59, 1.33)	0.56
rs1333040	C	41.0%	0.98 (0.78, 1.23)	0.88	38.0%	1.27 (1.01, 1.60)	0.040	39.3%	1.12 (0.96 – 1.30)	0.16	38.5%	0.65 (0.45, 0.94)	0.023
rs4977574	G	47.2%	0.95 (0.76, 1.18)	0.65	51.3%	0.90 (0.72, 1.12)	0.34	49.4%	0.93 (0.80 – 1.08)	0.32	20.5%	1.01 (0.64, 1.60)	0.98
rs1333042	A	50.8%	1.07 (0.86, 1.34)	0.52	46.9%	1.11 (0.89, 1.39)	0.34	48.7%	1.08 (0.93 – 1.26)	0.30	8.5%	0.55 (0.29, 1.05)	0.070
rs2383207	A	49.9%	1.07 (0.86, 1.33)	0.55	45.9%	1.09 (0.88, 1.36)	0.44	47.7%	1.07 (0.92 – 1.24)	0.38	8.5%	0.55 (0.29, 1.05)	0.070
rs1746048	T	14.4%	0.88 (0.64, 1.21)	0.42	12.4%	1.10 (0.77, 1.55)	0.61	13.3%	0.99 (0.79 – 1.24)	0.92	45.8%	1.17 (0.80, 1.70)	0.42
rs2259816	T	34.2%	0.88 (0.70, 1.11)	0.29	35.4%	1.14 (0.90, 1.44)	0.28	34.9%	1.00 (0.85 – 1.17)	0.99	13.1%	0.74 (0.43, 1.28)	0.28
rs1048990	G	16.9%	0.72 (0.52, 0.98)	0.038	15.6%	0.82 (0.60, 1.14)	0.24	16.2%	0.80 (0.64 – 0.99)	0.036	3.5%	0.31 (0.12, 0.83)	0.020
rs17228212	C	30.0%	1.24 (0.97, 1.60)	0.092	29.0%	0.80 (0.63, 1.02)	0.073	29.4%	1.02 (0.86 – 1.20)	0.83	13.6%	1.29 (0.74, 2.25)	0.38
rs1122608	T	25.5%	1.17 (0.92, 1.51)	0.20	26.2%	0.95 (0.74, 1.21)	0.66	25.9%	1.11 (0.94 – 1.31)	0.24	4.1%	0.91 (0.36, 2.32)	0.85
rs9982601	T	12.0%	0.82 (0.58, 1.17)	0.28	10.7%	1.13 (0.80, 1.61)	0.49	11.3%	0.97 (0.76 – 1.22)	0.77	25.8%	0.65 (0.42, 1.01)	0.055

[a]The minor allele was considered to be the least frequent allele across all series'.

ORs and p-values result from logistic regression models adjusted for age and gender (Familial Caucasian series), age, gender, atrial fibrillation, coronary artery disease, diabetes, hypertension, and current smoking (ISGS Caucasian series), age, gender, and series (combined Caucasian series), and age, gender, coronary artery disease, diabetes, hypertension, and current smoking (ISGS African American series). ORs correspond to an additional minor allele.

SNP=single nucleotide polymorphism. MA=minor allele. MAF=minor allele frequency. OR=odds ratio. CI=confidence interval. ISGS=Ischemic Stroke Genetics Study.

Table 4

Single SNP associations with ischemic stroke subtypes in the combined Caucasian series under an additive model

			Association with cardioembolic stroke (157 patients, 708 controls)		Association with large vessel stroke (157patients, 708 controls)		Association with small vessel stroke (141 patients, 708 controls)	
SNP	**MA[a]**	**MAF**	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**
rs646776	C	21.7%	0.96 (0.71 – 1.31)	0.80	1.07 (0.79 – 1.44)	0.68	0.94 (0.68 – 1.29)	0.69
rs11206510	C	19.1%	0.93 (0.68 – 1.29)	0.67	0.96 (0.70 – 1.31)	0.79	0.86 (0.61 – 1.21)	0.38
rs6725887	C	12.2%	0.79 (0.53 – 1.19)	0.26	0.76 (0.51 – 1.14)	0.18	0.81 (0.54 – 1.22)	0.31
rs9818870	T	16.2%	1.04 (0.74 – 1.45)	0.83	0.70 (0.49 – 1.01)	0.058	0.88 (0.62 – 1.25)	0.46
rs12526453	G	34.0%	0.82 (0.62 – 1.10)	0.18	1.04 (0.80 – 1.36)	0.78	1.07 (0.81 – 1.40)	0.64
rs6922269	A	29.0%	0.98 (0.74 – 1.31)	0.91	0.91 (0.69 – 1.21)	0.53	1.30 (0.98 – 1.73)	0.066
rs2048327	C	35.0%	1.20 (0.92 – 1.57)	0.19	1.07 (0.82 – 1.39)	0.61	0.99 (0.75 – 1.30)	0.93
rs3127599	T	29.5%	1.06 (0.80 – 1.39)	0.69	1.23 (0.94 – 1.61)	0.13	0.98 (0.73 – 1.30)	0.86
rs7767084	C	16.2%	0.98 (0.70 – 1.39)	0.93	0.76 (0.53 – 1.10)	0.14	1.02 (0.72 – 1.44)	0.90
rs10755578	G	46.5%	1.00 (0.77 – 1.29)	1.00	1.08 (0.84 – 1.38)	0.56	1.03 (0.80 – 1.34)	0.81
rs1333040	C	39.3%	1.55 (1.20 – 2.01)	0.0007	0.88 (0.68 – 1.14)	0.35	1.31 (1.01 – 1.69)	0.044
rs4977574	G	49.4%	0.69 (0.53 – 0.89)	0.0035	1.09 (0.85 – 1.39)	0.51	0.79 (0.61 – 1.03)	0.079
rs1333042	A	48.7%	1.42 (1.10 – 1.84)	0.0065	0.97 (0.76 – 1.24)	0.78	1.23 (0.95 – 1.58)	0.12
rs2383207	A	47.7%	1.39 (1.08 – 1.80)	0.010	0.95 (0.74 – 1.21)	0.68	1.22 (0.94 – 1.57)	0.13
rs1746048	T	13.3%	1.06 (0.72 – 1.56)	0.78	0.70 (0.47 – 1.06)	0.095	1.07 (0.73 – 1.55)	0.74
rs2259816	T	34.9%	1.14 (0.87 – 1.49)	0.34	1.00 (0.77 – 1.29)	0.99	1.08 (0.83 – 1.41)	0.55
rs1048990	G	16.2%	0.88 (0.61 – 1.26)	0.48	0.65 (0.44 – 0.94)	0.023	0.83 (0.57 – 1.20)	0.32
rs17228212	C	29.4%	1.10 (0.83 – 1.44)	0.52	0.97 (0.74 – 1.27)	0.80	1.15 (0.88 – 1.52)	0.31
rs1122608	T	25.9%	1.06 (0.79 – 1.41)	0.72	1.11 (0.84 – 1.46)	0.46	1.11 (0.83 – 1.48)	0.47
rs9982601	T	11.3%	1.06 (0.71 – 1.60)	0.77	1.09 (0.75 – 1.59)	0.67	1.17 (0.79 – 1.73)	0.43

[a]The minor allele was considered to be the least frequent allele across all series'.

ORs and p-values result from logistic regression models adjusted for age, gender, and series. ORs correspond to an additional minor allele. SNP=single nucleotide polymorphism.

MA=minor allele. MAF=minor allele frequency. OR=odds ratio. CI=confidence interval.

Table 5

Association of haplotypes of rs2048327-rs3127599-rs7767084-rs10755578 with overall ischemic stroke

	Haplotype frequency		
Haplotype (rs2048327-rs3127599-rs7767084-rs10755578)	**Ischemic stroke patients**	**Controls**	**P-value for association**
Familial Caucasian series (264 patients, 374 controls)			0.72
C-C-T-G	2.4%	3.8%	
C-C-C-G	15.3%	16.7%	
C-T-T-G	12.6%	13.5%	
C-C-T-C	2.2%	2.2%	
T-T-T-G	13.2%	11.7%	
T-C-T-C	52.3%	49.7%	
ISGS Caucasian series (449 patients, 334 controls)			0.20
T-C-T-C	46.9%	51.2%	
C-C-C-G	14.2%	14.3%	
T-T-T-G	12.6%	13.4%	
T-T-T-C	1.8%	2.0%	
C-C-T-C	1.7%	1.6%	
C-C-T-G	3.6%	2.2%	
C-T-T-G	18.1%	13.8%	
Combined Caucasian series (713 patients, 708 controls)			0.82
C-C-C-G	14.6%	15.6%	
T-C-T-C	48.9%	50.3%	
C-C-T-C	1.6%	1.5%	
C-C-T-G	12.8%	12.5%	
T-T-T-G	3.1%	3.0%	
T-T-T-C	1.9%	2.0%	
C-T-T-G	16.1%	13.7%	
ISGS African American series (166 patients, 117 controls)			0.54
T-C-T-G	2.9%	5.4%	
C-C-C-G	2.1%	3.0%	
C-T-T-G	3.0%	2.3%	
C-C-T-C	2.7%	2.1%	
T-C-T-C	62.8%	61.9%	
T-T-T-G	23.7%	23.7%	
T-T-T-C	2.5%	0.9%	

- P-values result from score tests of association. Haplotypes with a frequency of less than 1% were not considered. Adjustments were made for age and gender (familial Caucasian series), age, gender, atrial fibrillation, coronary artery disease, diabetes, hypertension, and current smoking (ISGS Caucasian series), age, gender, and series (combined Caucasian series), and age, gender, coronary artery disease, diabetes, hypertension, and current smoking (ISGS African American series).

ISGS=Ischemic Stroke Genetics Study.