ORIGINAL RESEARCH

Identification of *PTCSC3* as a Novel Locus for Large-Vessel Ischemic Stroke: A Genome-Wide Association Study

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***Background*—**Ischemic stroke is a major cause of death and disability in the world. A major ischemic stroke subtype, large-vessel ischemic stroke (large artery atherosclerosis; LAA), has been shown to have some genetic components in individuals of European ancestry. However, it is not clear whether the genetic predisposition to LAA stroke varies among ethnicities. We sought to identify genetic factors that contribute to LAA stroke in 2 independent samples of Han Chinese individuals.

***Methods and Results*—**Novel genetic variants that predispose individuals to LAA stroke were identified using a genome-wide association study (GWAS) of 444 individuals with LAA stroke and 1727 controls in a Han Chinese population residing in Taiwan. The study was replicated in an independent Han Chinese population comprising an additional 319 cases and 1802 controls. We identified 5 single-nucleotide polymorphisms, including rs2415317 ($P=3.10\times10^{-8}$), rs934075 ($P=4.00\times10^{-9}$), rs944289 ($P=3.57\times10^{-8}$), rs2787417 ($P=1.76\times10^{-8}$), and rs1952706 ($P=2.92\times10^{-8}$), at one novel locus on chromosome 14q13.3 within *PTCSC3* (encoding papillary thyroid carcinoma susceptibility candidate 3) that were associated with LAA stroke at genome-wide significance ($P<5\times10^{-8}$).

***Conclusions*—**Our data provide strong support for future studies on the role of PTCSC3 in the pathogenesis of LAA stroke and the association between LAA stroke development and thyroid function. In addition, these findings provide insights into the genetic basis of LAA stroke and identify a novel pathway that might be applicable for future therapeutic intervention. **(*J Am Heart Assoc*. 2016;5:e003003 doi: 10.1161/JAHA.115.003003)**

Key Words: atherosclerosis • genome-wide association study • non-coding RNA • polymorphism • stroke

Stroke is a major cause of acquired disability and the second-leading cause of death in the world.[1,2] The primary causes of stroke are unclear[3]; however, family studies indicate that genetic factors may be involved.[4,5] A previous study demonstrated that 70% to 85% of strokes are ischemic, with the percentage varying by ethnicity.[6] Based on a nationwide surveillance program in Taiwan, including clinical data from 30 599 stroke admissions,[7] ischemic stroke is most frequent (74%), followed by intracerebral hemorrhage (16.1%), transient ischemic attack (6.7%), subarachnoid hemorrhage (2.8%), and cerebral venous thrombosis (0.2%).

Ischemic stroke includes 3 major subtypes according to the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) criteria: large-artery atherosclerosis (LAA); cardioembolism; and small-vessel occlusion. These subtypes may have different etiologies, resulting in disease subtype-specific processes.

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The LAA subtype, for example, has been shown to be more strongly correlated with family history than other subtypes.[8] Recent genome-wide association studies (GWASs) of LAA stroke subtypes conducted in Australian populations have reported 1 susceptibility loci in 6p21.1.[9] In addition, the International Stroke Genetics Consortium and the Wellcome Trust Case Control Consortium 2 have also identified a replicated genetic association with ischemic stroke by conducting a GWAS examining the major specific stroke subtypes.[10] Notably, based on 844 LAA stroke cases in a discovery population from 4 centers in Europe, a genetic variant at chromosome 7p21.1 (ie, *HDAC9*, which encodes the histone deacetylase [HDAC] 9 protein) is specifically associated with the LAA stroke subtype.[10] This association has been replicated in an independent Caucasian group.[11] However, although existing genetic data in Caucasian have demonstrated that unique genetic variants may predispose patients to a specific subtype of ischemic stroke,[9,10] it remains unclear whether other genetic variants are associated with LAA stroke. Furthermore, it is also important to identify genetic factors by conducting a GWAS in other ethnics. Therefore, in this study, we aimed to clarify the contributions of complex genetic effects to the pathogenesis of LAA stroke by identification of novel susceptibility loci and to validate the associations between previously reported loci in different ethnic groups.

Methods

Study Design and Patients

This study was approved by the Institutional Review Board and the Ethics Committee of the Institutional Review Board of Chang Gung Memorial Hospital and Academia Sinica, Taiwan. Written informed consent was obtained from the patients or their family members, in accord with institutional requirements and the principles of the Declaration of Helsinki. Individuals with LAA stroke (n=763; including the 444 patients with LAA stroke in the GWAS and the 319 patients with LAA stroke in the replication study) were consecutively recruited at different geographic medical centers, including Chang Gung Memorial Hospital Taipei Branch, Chang Gung Memorial Hospital Linkou Medical Center, Chang Gung Memorial Hospital Chiayi Branch, and Chang Gung Memorial Hospital Kaohsiung Medical Center (from the northern to southern regions of Taiwan). Patient enrollment and data management were performed in collaboration with the Translational Resource Center for Genomic Medicine of Taiwan. All of the cases were diagnosed according to TOAST criteria.[12] In addition, the cases received carotid ultrasound and transcranial color-coded Doppler to screen for large vessel disease. In those cases with suspected large vessel disease, digital subtraction, magnetic resonance, or computed tomographic angiography was performed for further confirmation. The clinical and imaging data were centralized and the classification of intracranial and extracranial LAA was identified and confirmed by Dr Tsong-Hai Lee. In the present study, we have included both intracranial atherosclerosis and extracranial atherosclerosis. Because identification of effects specific to more-refined phenotypes is critical for genetic studies of stroke,[9,10] we focused our GWAS on patients with carotid artery stenosis attributed to atherosclerotic mechanisms.[13] The controls used for our discovery and replication studies were independent groups for comparison in the GWAS study. The 1727 controls in the GWAS were randomly selected from the Taiwan Han Chinese Cell and Genome Bank in Taiwan, as reported previously.[14] The single-nucleotide polymorphism (SNP) genotyping results of another independent group of 1802 controls in the replication study were randomly selected from the publically available summary frequency of the Taiwan Biobank Website (https://taiwanview.twbiobank.org.tw/taiwanview/twbchipinfo.do).

Genotyping and Quality Control

Genomic DNA was extracted from blood using a Puregene DNA Isolation Kit (Gentra Systems, Inc., Minneapolis, MN). Each individual was genotyped using the Axiom Genome-Wide CHB (with 642 832 SNPs), which is the most comprehensive commercially available genome-wide coverage of the Han Chinese population, according to the manufacturer's protocols, at the National Center for Genome Medicine, Academia Sinica. All of the sample call rates were greater than 99%, and the mean individual sample call rate was 99.7±0.18%. First-degree relatives (parent-offspring and full sibling pairs) of patients with LAA stroke disease and controls were identified by kinship analysis and were excluded from further analysis. Genotyping quality control for each SNP was further evaluated by determining the total call rate (successful call rate) and minor allele frequency (MAF) in cases and controls. SNPs were excluded from further analysis if only 1 allele was successfully genotyped in cases and controls to avoid experimental errors. The total call rate was less than 0.95, or the total MAF was less than 0.05 and the total call rate was less than 0.99. In addition, SNPs that departed significantly from Hardy–Weinberg equilibrium were excluded ($P<1\times10^{-4}$).

Statistical Analysis

We estimated the variance inflation factor for genomic control. Genome-wide association analysis was carried out to compare allele and genotype frequencies between cases and controls, using the Cochran–Armitage trend test

implemented in PLINK 1.07. Heterogeneity tests (I^2 and P values of the Q statistics) between GWASs and replication groups were performed using the described methods.[15]

Validation and Replication

The top 58 SNPs ($P<5\times10^{-5}$) from the genome-wide association analysis of the 444 patients with LAA stroke and 1727 controls were further validated in 444 patients with LAA stroke, using either matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MassARRAY; Sequenom, Inc., San Diego, CA) or direct sequencing. Forty-one SNP genotypes with a successful rate of over 99% and over 99% concordance between 2 platforms were then genotyped in an additional 319 patients with LAA stroke for replication.

Results

We performed a case-control GWAS to identify loci associated with increased risk of LAA stroke in the Han Chinese population by using an Affymetrix Axiom CHB array containing 642 832 SNP probes (Affymetrix, Inc., San Diego, CA). We initially enrolled 444 patients with LAA stroke and 1727 controls in a Han Chinese population residing in Taiwan. After kinship analysis and strict quality control filtering, we analyzed 570 275 SNPs (representing 89% of array SNPs) in the discovery stage. Multidimensional scaling analysis (Figure 1A and 1B) and results of permutation tests for identity-by-state revealed no evidence for strong population stratification between LAA and control groups. Quantile-quantile (Q-Q) plots were used to examine P-value distributions (Figure 1C), and the lambda value is 1.07. In total, we found 58 SNPs associated with LAA ($P<5\times10^{-5}$). Forty-one SNPs were validated using either Sequenom MassARRAY or direct sequencing (Figure 2 and Table 1) and subsequently replicated in an independent cohort of 319 patients with LAA and 1802 controls (Table 2). In a combined analysis of the discovery and replication cohorts, the P values of 8 of the identified SNPs were lower than 10^{-6} (Table 3), and 5 exceeded the threshold for genome-wide significance in the joint analysis ($P<5\times10^{-8}$; Table 3). We observed no strong evidence of heterogeneity between samples from the discovery and the replication study for these 5 SNPs ($I^2=0$).

Two of the SNPs that reached genome-wide significance in the joint analysis were rs2415317 ($P=3.10\times10^{-8}$; odds ratio [OR]=1.37 [95% CI, 1.226–1.536]; Table 3) and rs934075 ($P=4.00\times10^{-9}$; OR=1.39 [95% CI, 1.247–1.558]; Table 3), which were located in the intron of the *PTCSC3* gene (encoding papillary thyroid carcinoma susceptibility candidate 3). Three additional SNPs reached genome-wide significance in the joint analysis: rs944289 ($P=3.57\times10^{-8}$; OR=1.37 [95% CI, 1.224–1.533]; Table 3); rs2787417 ($P=1.76\times10^{-8}$; OR=1.38 [95% CI, 1.232–1.538]; Table 3); and rs1952706 ($P=2.92\times10^{-8}$; OR=1.37 [95% CI, 1.226–1.534]; Table 3). These SNPs were located at upstream of the *PTCSC3* gene (Figure 3A). Two SNPs (rs2415317 and rs944289) were found to be in strong linkage disequilibrium (LD; $D'=0.971$ and $r^2=0.919$; Figures 3A and 4) and mapped to a 39.6-kb LD block (position 36 609 678–36 649 246) at 14q13.3; this block comprises the promoter, exon, and intron of *PTCSC3*.

Discussion

In this study, we sought to identify novel genetic variations that predisposed individuals to LAA stroke in a Han Chinese population residing in Taiwan. From 2 independent groups (Table 4), we found 5 new SNPs within the *PTCSC3* gene for LAA stroke that reached genome-wide statistical significance. These findings provide insights into the genetic basis of LAA stroke and identify a novel pathway that may be applicable for future therapeutic interventions.

In the discovery stage, we imputed 134 SNPs with P value $<1\times10^{-3}$. Notably, the rs934075 and rs944258 SNPs with high P value ($<1\times10^{-5}$) were directly genotyped in our discovery sample, whereas rs934075 could be imputed with an equally high statistical value ($<1\times10^{-5}$). In addition, the SNP rs944289, located in chromosome 14q13.3, is associated with an increased risk of papillary thyroid cancer (PTC) based on a GWAS including patients with thyroid cancer in Iceland, Spain, and the United States.[16] This SNP is located in the region upstream of a long intergenic noncoding RNA (lincRNA), named *PTCSC3*, and functional studies have indicated that this lincRNA is involved in PTC susceptibility.[17] *PTCSC3* is highly expressed in the thyroid and weakly expressed in the kidney,[17] and the downregulation of *PTCSC3* in thyroid tumors of PTC patients is significantly associated with the risk allele (T) in rs944289.[17] In addition, the risk allele (T) for PTC in the SNP rs944289 has also been shown to be associated with a low concentration of thyroid-stimulating hormone in the general population,[16] and a recent report also indicated that hyperthyroidism may be associated with an increased risk for ischemia stroke in young adults.[18] It raises a likelihood that *PTCSC3* may affect thyroid function, and the disruption of thyroid function may, in turn, be associated with a worse cardiovascular risk factor profile, potentially leading to progression of atherosclerosis.[19] Therefore, further studies are warranted to investigate the mechanism through which *PTCSC3* is involved in the susceptibility for LAA.

In a previous GWAS conducted in Caucasians, the SNP rs11984041, which had the strongest significance (ie, the lowest P values) for LAA stroke, was shown to be located in

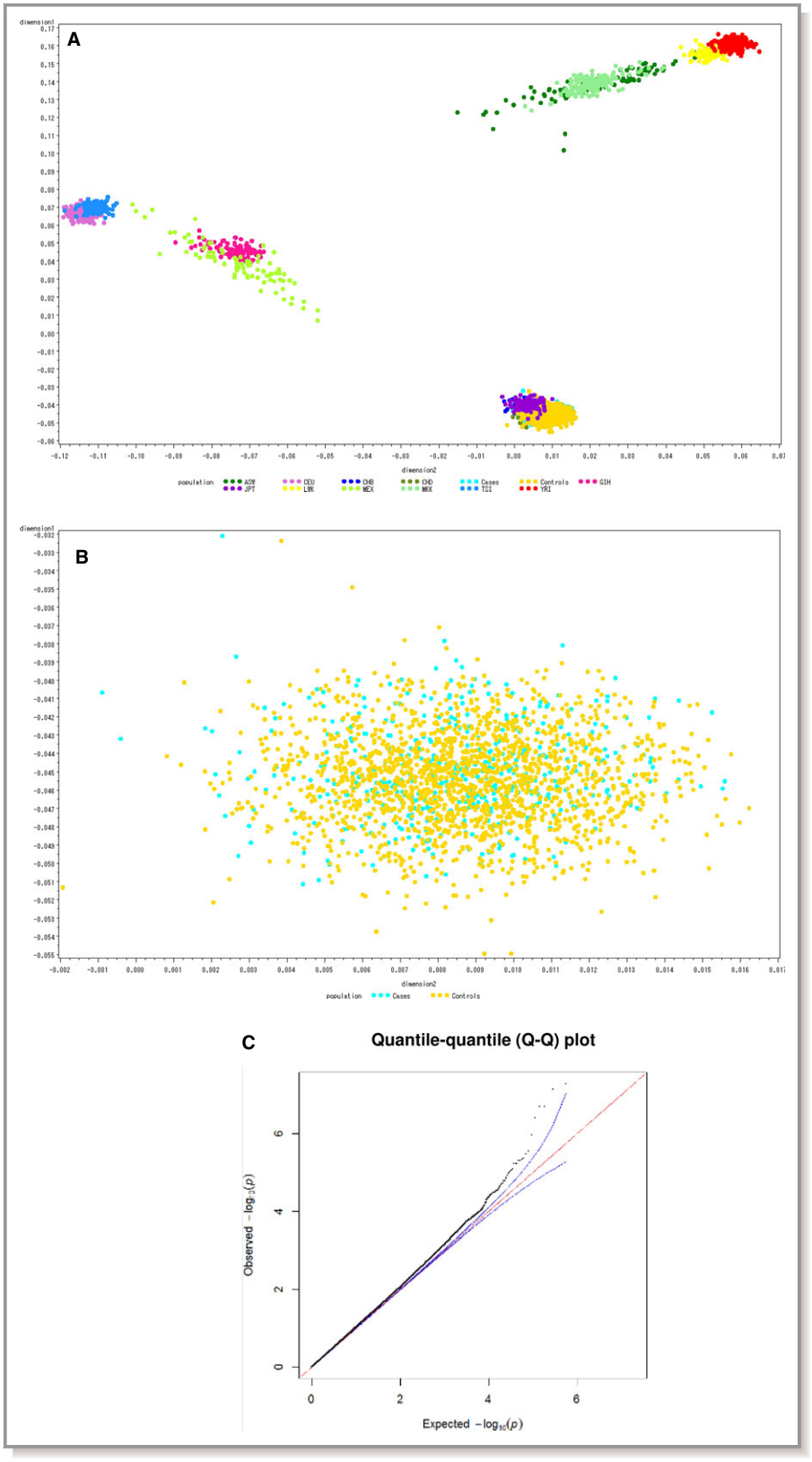

Figure 1. Multidimensional scaling analysis. A, Results of the multidimensional scaling analysis of the GWAS samples with HapMap populations. B, Results of the multidimensional scaling analysis of the GWAS samples with the GWAS samples only. C, Q-Q plot of the *P* values in Cochran–Armitage trend test. The lambda value is 1.07. GWAS indicates genome-wide association study.

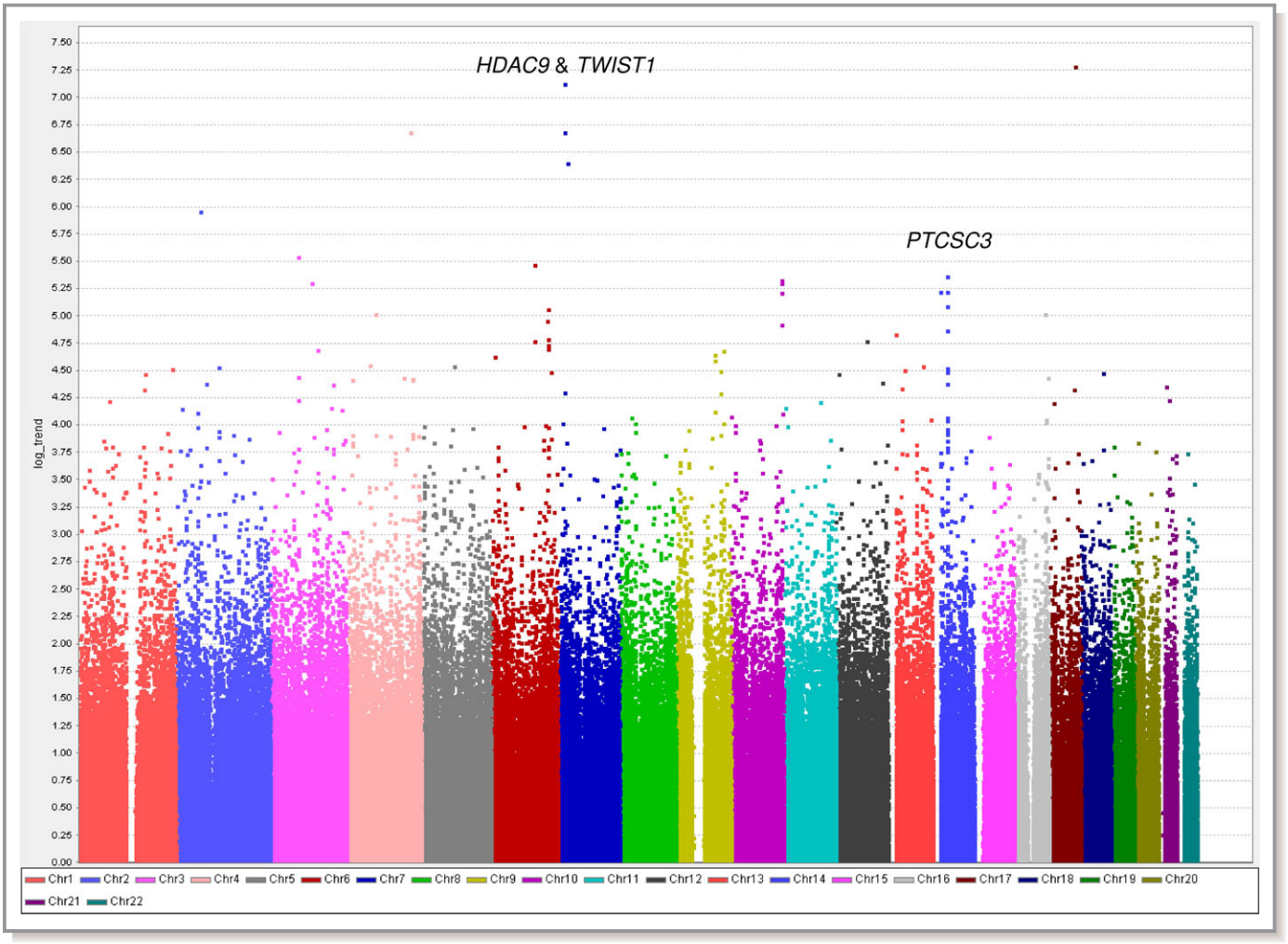

Figure 2. Results of genome-wide association analysis ($-\log_{10} P$) shown in chromosomal order for 570 275 SNPs tested for association in initial samples from 444 patients with LAA stroke and 1727 controls. The *x* axis represents each of the SNPs used in the primary scan. The *y* axis represents the $-\log_{10} P$ value of the trend test. Signals in *HDAC9*, *TWIST1*, and *PTCSC3* loci are indicated. LAA, large artery atherosclerosis; SNPs, single-nucleotide polymorphisms.

the final intron of the *HDAC9* gene.[10] Because this SNP (rs11984041) is not polymorphic in the Han Chinese population, an additional study investigated other potential SNPs associated with *HDAC9* in Han Chinese individuals.[20] This previous report showed that there are 2 SNPs, located in the different intron of *HDAC9* (ie, not in LD with rs11984041), which may be associated with LAA in Han Chinese individuals.[20] However, the *P* values of these SNPs (rs2389995 and rs2240419) did not reach the first criterion ($P<5\times10^{-5}$) in the discovery stage of our GWAS. In the present study, 2 novel suggestive SNPs (rs2074633 and rs28688791) represent a different, more significant locus in the *HDAC9* gene (ie, in LD with rs11984041) in Han Chinese individuals. The SNPs (rs28688791 and rs2074633) at chromosome 7p21 were associated with LAA in the discovery phase ($P<5\times10^{-5}$; Figure 2), and their *P* values in the joint analysis were close to reaching GWAS significance (rs28688791, $P=3.66\times10^{-7}$, OR=1.38 [95% CI, 1.195–1.496]; rs2074633, $P=3.20\times10^{-7}$; OR=1.34 [95% CI, 1.198–1.501]; Table 3). The SNP rs2074633 was located in the 3′-untranslated region (UTR) exon of *HDAC9*, and the SNP rs28688791 was located downstream of the *HDAC9* locus and in the 3′ UTR of *TWIST1* (encoding twist family bHLH transcription factor 1; Figure 3B) locus. Interestingly, a difference in the locations of the SNPs (rs28688791 and rs2074633) identified in Han Chinese and the SNP (rs11984041) identified in Caucasians implies multiple regulation pathways in expression of *HDAC9* gene in LAA stroke.

Study Limitations

This was a case-control GWAS conducted in a Han Chinese population residing in Taiwan for LAA stroke, a subphenotype of ischemia stroke differentiated based on TOAST

Table 1. Validated SNPs (n=41) With *P* Trend $<5\times10^{-5}$ in the Discovery Stage

Chr	SNP	Gene	Allele 1	Allele 2	Risk Allele	RAF in Control	RAF in Case	Discovery Trend *P*	Risk Allele OR (95% CI)
1	rs12120382	*LOC339535*	C	T	C	0.0894	0.1372	3.04E-05	1.620 (1.294–2.029)
1	rs1332824	*ELTD1*	A	C	A	0.4896	0.5643	6.00E-05	1.351 (1.164–1.567)
2	rs7601853	*ACOXL*	G	A	A	0.5064	0.5856	2.96E-05	1.377 (1.186–1.599)
2	rs79565251	*B3GNT2*	T	C	C	0.8041	0.8741	1.10E-06	1.692 (1.362–2.101)
3	rs11915881	*PDZRN3*	C	T	C	0.0721	0.1210	2.90E-06	1.772 (1.393–2.254)
3	rs73198741	*CBLB*	T	A	T	0.0772	0.1268	4.95E-06	1.736 (1.365–2.209)
3	rs9840967	*ADCY5*	T	C	C	0.5582	0.6380	2.03E-05	1.395 (1.197–1.625)
4	rs17600762	*FSTL5*	G	A	A	0.6551	0.7488	2.07E-07	1.569 (1.318–1.868)
4	rs3775488	*CXCL5*	C	T	T	0.7608	0.8291	9.61E-06	1.526 (1.257–1.851)
4	rs4273531	*LOC255130*	T	G	T	0.0932	0.1408	2.80E-05	1.593 (1.277–1.988)
5	rs12654219	*EDIL3*	C	T	C	0.2747	0.3473	2.86E-05	1.405 (1.200–1.644)
6	rs1111808	*UTRN*	G	A	G	0.2115	0.2788	1.62E-05	1.441 (1.218–1.704)
6	rs1999565	*LOC100506207*	G	T	G	0.1362	0.1919	2.36E-05	1.506 (1.241–1.828)
6	rs2297847	*UTRN*	A	G	A	0.2129	0.2827	8.66E-06	1.457 (1.233–1.722)
6	rs6933749	*LAMA4*	G	T	G	0.1436	0.2020	1.69E-05	1.509 (1.249–1.825)
6	rs6940518	*LAMA4*	A	G	A	0.1587	0.2251	3.35E-06	1.540 (1.283–1.848)
6	rs75523405	*UTRN*	C	A	C	0.1513	0.2106	1.98E-05	1.496 (1.242–1.803)
6	rs79375726	*UTRN*	T	C	T	0.1010	0.1532	1.09E-05	1.609 (1.300–1.992)
6	rs9403615	*UTRN*	G	A	G	0.1511	0.2106	1.86E-05	1.498 (1.244–1.805)
7	rs2074633	*HDAC9*	C	T	C	0.3400	0.4336	2.06E-07	1.486 (1.278–1.727)
7	rs28688791	*HDAC9 or TWIST1*	C	T	C	0.3555	0.4537	7.47E-08	1.506 (1.297–1.748)
7	rs56075816	*C7orf31*	T	A	A	0.6163	0.7120	4.01E-07	1.539 (1.305–1.816)
9	rs10046806	*LOC340508*	C	T	T	0.5806	0.6584	2.25E-05	1.392 (1.193–1.625)
9	rs10759468	*LOC340508*	C	T	T	0.5722	0.6493	2.55E-05	1.384 (1.187–1.615)
9	rs7040056	*TLR4*	A	T	T	0.6868	0.7604	2.06E-05	1.448 (1.219–1.719)
10	rs10765149	*FOXI2*	G	A	A	0.5452	0.6312	4.67E-06	1.428 (1.226–1.662)
10	rs11018272	*FOXI2*	T	C	C	0.5070	0.5923	6.08E-06	1.413 (1.217–1.641)
10	rs4237483	*FOXI2*	C	T	T	0.5061	0.5926	4.94E-06	1.419 (1.222–1.649)
10	rs7086441	*FOXI2*	G	C	C	0.5090	0.5916	1.21E-05	1.398 (1.203–1.623)
12	rs78567761	*SYT1*	G	A	G	0.0629	0.1056	1.69E-05	1.761 (1.359–2.280)
13	rs2812748	*ANKRD26P3*	T	G	T	0.0687	0.1129	1.47E-05	1.726 (1.348–2.211)
13	rs7984555	*MIR622*	A	T	T	0.7683	0.8341	2.89E-05	1.517 (1.243–1.851)
13	rs9525556	*VWA8*	A	C	C	0.5588	0.6324	3.13E-05	1.358 (1.166–1.581)
14	rs12891630	*OR4K15*	G	A	A	0.6323	0.7144	6.03E-06	1.455 (1.238–1.710)
14	rs1952706	*PTCSC3*	C	T	C	0.3683	0.4446	3.01E-05	1.373 (1.182–1.594)
14	rs2415317	*PTCSC3*	A	G	G	0.5223	0.6038	1.37E-05	1.394 (1.199–1.620)
14	rs2787417	*PTCSC3*	C	T	T	0.4612	0.5394	3.26E-05	1.368 (1.180–1.587)
14	rs934075	*PTCSC3*	G	A	G	0.4620	0.5473	6.02E-06	1.408 (1.214–1.633)
14	rs944289	*PTCSC3*	T	C	C	0.5162	0.6002	8.10E-06	1.407 (1.211–1.635)
16	rs7199119	*WWOX*	T	A	T	0.0467	0.0853	9.49E-06	1.906 (1.426–2.548)
17	rs17670925	*LOC440461*	G	C	G	0.3513	0.4508	5.18E-08	1.516 (1.300–1.767)

Chr indicates chromosome; gene, genes containing the SNP or the closest gene up to 50 kb upstream or downstream of the SNP; LAA, large artery atherosclerosis; OR, odds ratio for risk allele; RAF in case, risk allele frequency in LAA cases; RAF in control, risk allele frequency in controls; Risk allele, allele with higher frequency in cases compared to controls; SNP, single-nucleotide polymorphism.

Table 2. Validated SNPs (n=41) in the Replication Stage

Chr	SNP	Gene	Allele 1	Allele 2	Risk Allele	RAF in Control	RAF in Case	Replication Trend *P*	Risk Allele OR (95% CI)
1	rs12120382	*LOC339535*	C	T	T	0.8974	0.8996	8.83E-01	1.024 (0.748–1.402)
1	rs1332824	*ELTD1*	A	C	A	0.5191	0.5936	1.09E-03	1.353 (1.130–1.620)
2	rs7601853	*ACOXL*	G	A	A	0.5250	0.5429	4.56E-01	1.074 (0.889–1.298)
2	rs79565251	*B3GNT2*	T	C	T	0.1486	0.1572	5.67E-01	1.069 (0.847–1.349)
3	rs11915881	*PDZRN3*	C	T	T	0.9196	0.9204	9.52E-01	1.011 (0.713–1.432)
3	rs73198741	*CBLB*	T	A	T	0.0840	0.0930	5.02E-01	1.118 (0.805–1.553)
3	rs9840967	*ADCY5*	T	C	C	0.5639	0.5694	8.18E-01	1.023 (0.845–1.237)
4	rs17600762	*FSTL5*	G	A	A	0.6446	0.6469	9.18E-01	1.010 (0.829–1.231)
4	rs3775488	*CXCL5*	C	T	T	0.7819	0.7920	6.16E-01	1.062 (0.839–1.344)
4	rs4273531	*LOC255130*	T	G	G	0.8854	0.9020	2.63E-01	1.192 (0.870–1.633)
5	rs12654219	*EDIL3*	C	T	C	0.2900	0.3082	4.05E-01	1.091 (0.889–1.338)
6	rs1111808	*UTRN*	G	A	G	0.2224	0.2286	7.60E-01	1.036 (0.827–1.298)
6	rs1999565	*LOC100506207*	G	T	G	0.1547	0.1893	5.22E-02	1.276 (0.999–1.629)
6	rs2297847	*UTRN*	A	G	A	0.2220	0.2286	7.45E-01	1.039 (0.829–1.301)
6	rs6933749	*LAMA4*	G	T	G	0.1427	0.1481	7.49E-01	1.045 (0.800–1.365)
6	rs6940518	*LAMA4*	A	G	G	0.8314	0.8463	4.09E-01	1.117 (0.860–1.450)
6	rs75523405	*UTRN*	C	A	A	0.8258	0.8286	8.87E-01	1.020 (0.794–1.310)
6	rs79375726	*UTRN*	T	C	C	0.8940	0.8996	7.06E-01	1.062 (0.776–1.454)
6	rs9403615	*UTRN*	G	A	G	0.1612	0.1714	5.64E-01	1.077 (0.837–1.384)
7	rs2074633	*HDAC9*	C	T	C	0.3619	0.4025	5.05E-02	1.188 (1.000–1.411)
7	rs28688791	*HDAC9 or TWIST1*	C	T	C	0.3796	0.4151	8.93E-02	1.160 (0.977–1.377)
7	rs56075816	*C7orf31*	T	A	T	0.3657	0.3896	3.05E-01	1.107 (0.910–1.347)
9	rs10046806	*LOC340508*	C	T	C	0.3903	0.4294	9.60E-02	1.176 (0.975–1.418)
9	rs10759468	*LOC340508*	C	T	T	NA	0.6295	NA	NA
9	rs7040056	*TLR4*	A	T	T	0.6909	0.7286	9.52E-02	1.201 (0.972–1.483)
10	rs10765149	*FOXI2*	G	A	G	0.4320	0.4531	3.76E-01	1.089 (0.901–1.317)
10	rs11018272	*FOXI2*	T	C	T	0.4720	0.4855	5.74E-01	1.056 (0.873–1.277)
10	rs4237483	*FOXI2*	C	T	C	0.4714	0.4877	4.98E-01	1.068 (0.884–1.290)
10	rs7086441	*FOXI2*	G	C	G	0.4689	0.4816	5.97E-01	1.052 (0.871–1.272)
12	rs78567761	*SYT1*	G	A	G	0.0716	0.0928	9.67E-02	1.328 (0.949–1.857)
13	rs2812748	*ANKRD26P3*	T	G	T	0.0931	0.0975	7.54E-01	1.052 (0.763–1.451)
13	rs7984555	*MIR622*	A	T	T	0.7820	0.7971	4.32E-01	1.096 (0.867–1.385)
13	rs9525556	*VWA8*	A	C	A	0.4425	0.4571	5.48E-01	1.061 (0.878–1.282)
14	rs12891630	*OR4K15*	G	A	G	0.3405	0.3464	7.67E-01	1.027 (0.860–1.226)
14	rs1952706	*PTCSC3*	C	T	C	0.3813	0.4608	1.60E-04	1.387 (1.170–1.643)
14	rs2415317	*PTCSC3*	A	G	G	0.5336	0.6109	1.36E-03	1.372 (1.129–1.668)
14	rs2787417	*PTCSC3*	C	T	T	0.4569	0.5377	1.80E-04	1.382 (1.167–1.637)
14	rs934075	*PTCSC3*	G	A	G	0.4667	0.5586	1.46E-04	1.446 (1.193–1.752)
14	rs944289	*PTCSC3*	T	C	C	0.5294	0.6046	1.80E-03	1.359 (1.119–1.651)
16	rs7199119	*WWOX*	T	A	T	NA	0.0464	NA	NA
17	rs17670925	*LOC440461*	G	C	G	0.6332	0.6364	8.76E-01	1.014 (0.851–1.208)

Chr indicates chromosome; gene, genes containing the SNP or the closest gene up to 50 kb upstream or downstream of the SNP; LAA, large artery atherosclerosis; OR, odds ratio for risk allele; RAF in case, risk allele frequency in LAA cases; RAF in control, risk allele frequency in controls; Risk allele, allele with higher frequency in cases compared to controls; SNP, single-nucleotide polymorphism.

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Table 3. SNPs With *P* Values $<1\times10^{-6}$ in the Joint Analysis

Chr.	SNP	Position	Gene	Allele Format	Risk Allele	Stage	Control/Case	RAF Controls	RAF Cases	Trend *P*	OR	95% CI
1	rs1332824	79504372	*ELTD1*	AC	A	GWAS	1727/444	0.4896	0.5643	6.00E-05	1.35	1.164 to 1.567
				AC	A	Replication	1802/319	0.5191	0.5936	1.09E-03	1.35	1.130 to 1.620
				AC	A	Combined	3529/763	0.5047	0.5758	8.02E-07	1.33	1.188 to 1.493
7	rs2074633	19035920	*HDAC9*	CT	C	GWAS	1727/444	0.3400	0.4336	2.06E-07	1.49	1.278 to 1.727
				CT	C	Replication	1802/319	0.3619	0.4025	5.05E-02	1.19	1.000 to 1.411
				CT	C	Combined	3529/763	0.3512	0.4206	3.20E-07	1.34	1.198 to 1.501
7	rs28688791	19039605	*HDAC9 or TWIST1*	CT	C	GWAS	1727/444	0.3555	0.4537	7.47E-08	1.51	1.297 to 1.748
				CT	C	Replication	1802/319	0.3796	0.4151	8.93E-02	1.16	0.977 to 1.377
				CT	C	Combined	3529/763	0.3678	0.4376	3.66E-07	1.38	1.195 to 1.496
14	rs2415317	36140472	*PTCSC3*	AG	G	GWAS	1727/444	0.5223	0.6038	1.37E-05	1.39	1.199 to 1.620
				AG	G	Replication	1802/319	0.5336	0.6109	1.36E-03	1.37	1.129 to 1.668
				AG	G	Combined	3529/763	0.5281	0.6056	3.10E-08	1.37	1.226 to 1.536
14	rs934075	36169016	*PTCSC3*	AG	G	GWAS	1727/444	0.4620	0.5473	6.02E-06	1.41	1.214 to 1.633
				AG	G	Replication	1802/319	0.4667	0.5586	1.46E-04	1.45	1.193 to 1.752
				AG	G	Combined	3529/763	0.4644	0.5472	4.00E-09	1.39	1.247 to 1.558
14	rs944289	36180040	*PTCSC3*	CT	C	GWAS	1727/444	0.5162	0.6002	8.10E-06	1.41	1.211 to 1.635
				CT	C	Replication	1802/319	0.5294	0.6046	1.80E-03	1.36	1.119 to 1.651
				CT	C	Combined	3529/763	0.5230	0.6003	3.57E-08	1.37	1.224 to 1.533
14	rs2787417	36651803	*PTCSC3*	CT	T	GWAS	1727/444	0.4612	0.5394	3.26E-05	1.37	1.180 to 1.587
				CT	T	Replication	1802/319	0.4569	0.5377	1.80E-04	1.38	1.167 to 1.637
				CT	T	Combined	3529/763	0.4590	0.5387	1.76E-08	1.38	1.232 to 1.538
14	rs1952706	36651803	*PTCSC3*	CT	C	GWAS	1727/444	0.3683	0.4446	3.01E-05	1.37	1.182 to 1.594
				CT	C	Replication	1802/319	0.3813	0.4608	1.60E-04	1.39	1.170 to 1.643
				CT	C	Combined	3529/763	0.3750	0.4514	2.92E-08	1.37	1.226 to 1.534

Stage 1 (genome scan) included 444 cases and 1727 controls. Stage 2 (replication stage) included 319 cases and 1802 controls. SNPs with $P<1\times10^{-5}$ in the LAA GWAS collection and with $P<0.05$ in the LAA replication collection and the results of the joint analysis. GWAS indicates genome-wide association study; LAA, large artery atherosclerosis; SNPs, single-nucleotide polymorphisms.

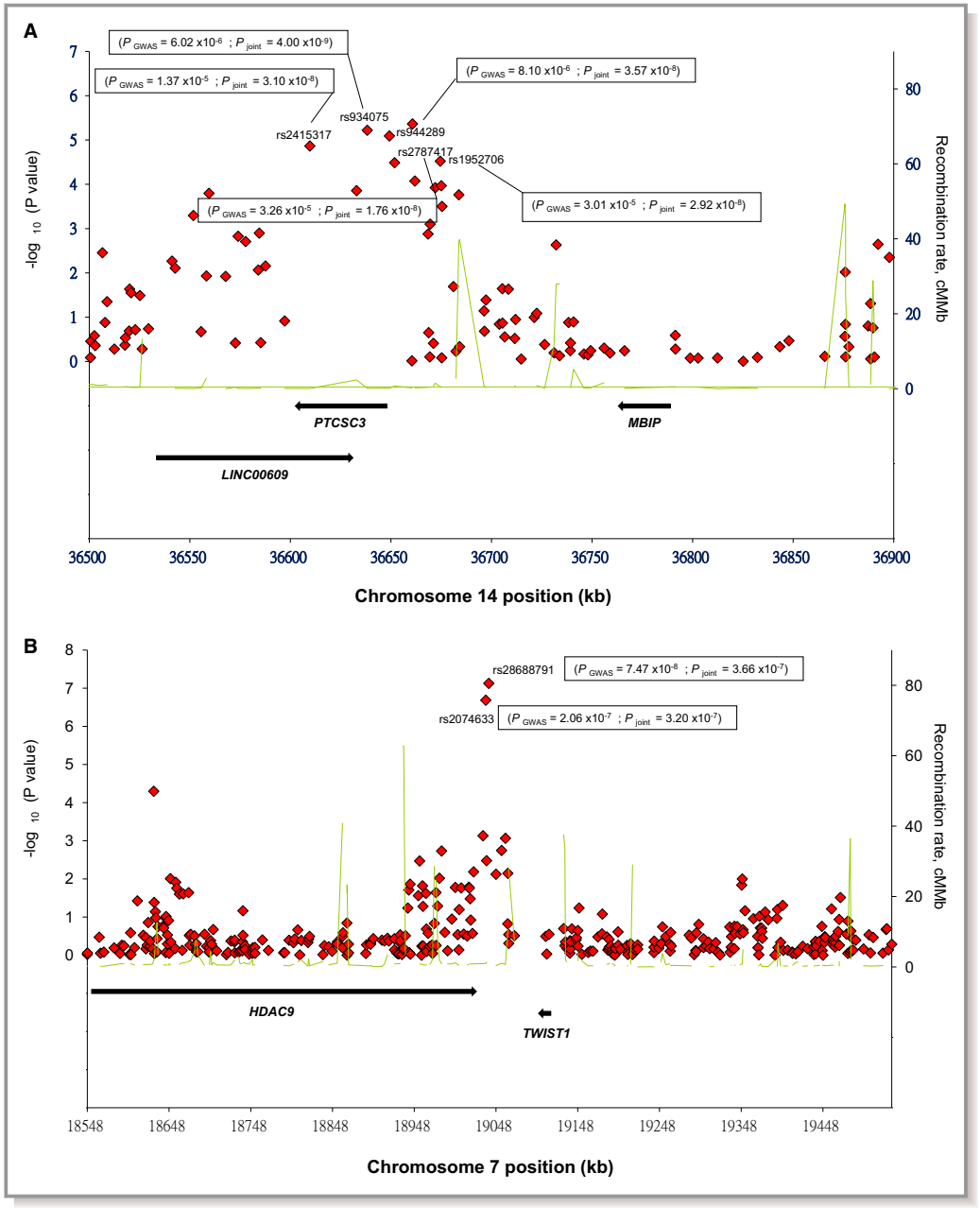
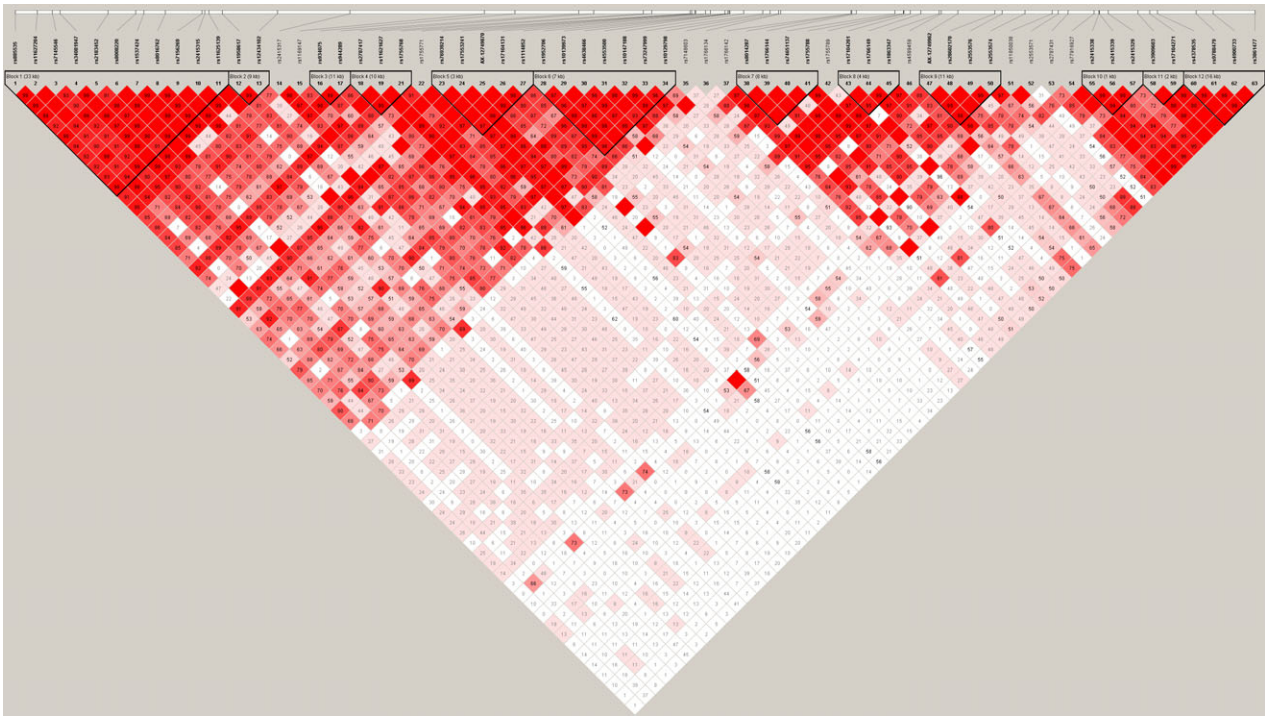


Figure 3. Association plots for *PTCSC3*, *MBIP*, *TWIST1*, and *HDAC9* loci. Regional association plot for *PTCSC3* and *MBIP* loci on chromosome 14 (A) or *TWIST1* and *HDAC9* loci on chromosome 7 (B), with gene annotations superimposed. Each SNP is plotted with respect to its chromosomal location (*x* axis) and its $-\log_{10}$ *P* values (left *y* axis) for the trend test from the primary GWAS scan and joint analysis at that region of the chromosome. The results from the discovery analysis and joint analysis for key SNPs are indicated using their rs numbers. GWAS indicates genome-wide association study; SNPs, single-nucleotide polymorphisms.

criteria. Although the findings identified a novel locus, PTCSC3, associated with LAA stroke and provide insight into the pathogenic mechanism for LAA stroke, the interpretation of the results of this study require caution owing to the limited number of ethnic groups considered. Future investigations of LAA stroke in other populations from Asia will be critical to clarify whether these newly identified genetic variants for LAA stroke are shared in other populations. Although the SNPs with genome-wide significance could be identified based on the combined analysis, there was still an incomplete validation of these SNPs in an independent cohort, which may be one of the study limitation in the present study. Therefore, further validation studies are needed to warrant our findings.

A



B

PTCSC3 SNP1	PTCSC3 SNP2	D'	r^2
rs2415317	rs934075	0.975	0.749
rs2415317	rs944289	0.971	0.919
rs2415317	rs2787417	0.834	0.540
rs2415317	rs1952706	0.796	0.339
rs934075	rs944289	0.997	0.802
rs934075	rs2787417	0.875	0.752
rs934075	rs1952706	0.825	0.461
rs944289	rs2787417	0.862	0.592
rs944289	rs1952706	0.809	0.359
rs2787417	rs1952706	0.954	0.627

Figure 4. LD structure and logistic regression analyses in *PTCSC3* region. A, shows −log10 (*P* values) of SNPs for the best test from the primary scan as a function of genomic positions for *PTCSC3* region (B) LD patterns, D' value, and r^2 among the disease-associated SNPs in PTCSC3 region. LD indicates linkage disequilibrium; SNPs, single-nucleotide polymorphisms.

Table 4. Baseline Demographic Summary of Patients (n=763)

	Discovery (n=444)	Replication (n=319)
Age, y, median (IQR)	68.0 (58.0–75.0)	67.0 (59.0–75.0)
Sex (male), %	82	77
Hypertension, %	79	76
Diabetes mellitus, %	38	32
Alcohol, %	20	36
Family history of stroke, %	38	25
HDL-C, median (IQR)	39.5 (33.0–47.0)	40.0 (34.0–48.0)
LDL-C, median (IQR)	111.0 (92.0–140.0)	113.0 (90.5–135.0)
VLDL, median (IQR)	26.0 (17.0–36.0)	25.0 (20.0–33.0)
Triacylglycerol, median (IQR)	136.5 (100.0–181.3)	133.0 (96.0–184.0)
Cholesterol, median (IQR)	181.5 (156.0–213.0)	182.0 (156.0–211.0)
Uric acid, median (IQR)	6.1 (5.1–7.1)	5.9 (4.9–7.1)

HDL-C indicates high-density lipoprotein; IQR, interquartile range; LDL-C, low-density lipoprotein; VLDL, very-low-density lipoprotein.

Conclusions

In the present study, we identified novel associations between LAA stroke and polymorphisms in the *PTCSC3* gene based on 5 SNPs with GWAS-significant *P* values. Because the *PTCSC3* gene also contains a risk locus correlated with PTC, our data provide strong support for future studies of the association between LAA and PTC. In addition, compared to previous SNP studies in Han Chinese individuals, here, we observed 2 SNPs with more-significant *P* values located in the *HDAC9* locus, which was in close proximity to the most significant SNP identified by GWAS in Caucasian individuals. These results support the notion that genetic predisposition to LAA may vary by ethnicity. In conclusion, our study revealed that the *PTCSC3* signaling pathway may be involved in the pathogenesis of LAA stroke and that *PTCSC3* may potentially serve as a therapeutic target for stroke prevention.

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Disclosures

None.

References

1. Roger VL, Go AS, Lloyd-Jones DM, Adams RJ, Berry JD, Brown TM, Carnethon MR, Dai S, de Simone G, Ford ES, Fox CS, Fullerton HJ, Gillespie C, Greenlund KJ, Hailpern SM, Heit JA, Ho PM, Howard VJ, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Makuc DM, Marcus GM, Marelli A, Matchar DB, McDermott MM, Meigs JB, Moy CS, Mozaffarian D, Mussolino ME, Nichol G, Paynter NP, Rosamond WD, Sorlie PD, Stafford RS, Turan TN, Turner MB, Wong ND, Wylie-Rosett J; American Heart Association Statistics C, Stroke Statistics S. Heart disease and stroke statistics—2011 update: a report from the American Heart Association. *Circulation*. 2011;123:e18–e209.
2. Feigin VL, Forouzanfar MH, Krishnamurthi R, Mensah GA, Connor M, Bennett DA, Moran AE, Sacco RL, Anderson L, Truelsen T, O'Donnell M, Venketasubramanian N, Barker-Collo S, Lawes CM, Wang W, Shinohara Y, Witt E, Ezzati M, Naghavi M, Murray C; Global Burden of Diseases I, Risk Factors S, the GBDSEG. Global and regional burden of stroke during 1990–2010: findings from the Global Burden of Disease Study 2010. *Lancet*. 2014;383:245–254.
3. Sacco RL, Ellenberg JH, Mohr JP, Tatemichi TK, Hier DB, Price TR, Wolf PA. Infarcts of undetermined cause: the NINCDS Stroke Data Bank. *Ann Neurol*. 1989;25:382–390.
4. Flossmann E, Schulz UG, Rothwell PM. Systematic review of methods and results of studies of the genetic epidemiology of ischemic stroke. *Stroke*. 2004;35:212–227.
5. Dichgans M. Genetics of ischaemic stroke. *Lancet Neurol*. 2007;6:149–161.
6. Khan NA, Quan H, Hill MD, Pilote L, McAlister FA, Palepu A, Shah BR, Zhou L, Zhen H, Kapral MK. Risk factors, quality of care and prognosis in South Asian, East Asian and White patients with stroke. *BMC Neurol*. 2013;13:74.
7. Hsieh FI, Lien LM, Chen ST, Bai CH, Sun MC, Tseng HP, Chen YW, Chen CH, Jeng JS, Tsai SY, Lin HJ, Liu CH, Lo YK, Chen HJ, Chiu HC, Lai ML, Lin RT, Sun MH, Yip BS, Chiou HY, Hsu CY, Registry TS. Get With the Guidelines-Stroke performance indicators: surveillance of stroke care in the Taiwan Stroke Registry Get With the Guidelines-Stroke in Taiwan. *Circulation*. 2010;122:1116–1123.
8. Jerrard-Dunne P, Cloud G, Hassan A, Markus HS. Evaluating the genetic component of ischemic stroke subtypes: a family history study. *Stroke*. 2003;34:1364–1369.
9. Holliday EG, Maguire JM, Evans TJ, Koblar SA, Jannes J, Sturm JW, Hankey GJ, Baker R, Golledge J, Parsons MW, Malik R, McEvoy M, Biros E, Lewis MD, Lincz LF, Peel R, Oldmeadow C, Smith W, Moscato P, Barlera S, Bevan S, Bis JC, Boerwinkle E, Boncoraglio GB, Brott TG, Brown RD Jr, Cheng YC, Cole JW, Cotlarciuc I, Devan WJ, Fornage M, Furie KL, Gretarsdottir S, Gschwendtner A, Ikram MA, Longstreth WT Jr, Meschia JF, Mitchell BD, Mosley TH, Nalls MA, Parati EA, Psaty BM, Sharma P, Stefansson K, Thorleifsson G, Thorsteinsdottir U, Traylor M, Verhaaren BF, Wiggins KL, Worrall BB, Sudlow C, Rothwell PM, Farrall M, Dichgans M, Rosand J, Markus HS, Scott RJ, Levi C, Attia J; Australian Stroke Genetics C, International Stroke Genetics C, Wellcome Trust Case Control C. Common variants at 6p21.1 are associated with large artery atherosclerotic stroke. *Nat Genet*. 2012;44:1147–1151.
10. International Stroke Genetics C, Wellcome Trust Case Control C, Bellenguez C, Bevan S, Gschwendtner A, Spencer CC, Burgess AI, Pirinen M, Jackson CA, Traylor M, Strange A, Su Z, Band G, Syme PD, Malik R, Pera J, Norrving B, Lemmens R, Freeman C, Schanz R, James T, Poole D, Murphy L, Segal H, Cortellini L, Cheng YC, Woo D, Nalls MA, Muller-Myhsok B, Meisinger C, Seedorf U, Ross-Adams H, Boonen S, Wloch-Kopec D, Valant V, Slark J, Furie K, Delavaran H, Langford C, Deloukas P, Edkins S, Hunt S, Gray E, Dronov S, Peltonen L, Gretarsdottir S, Thorleifsson G, Thorsteinsdottir U, Stefansson K, Boncoraglio GB, Parati EA, Attia J, Holliday E, Levi C, Franzosi MG, Goel A, Helgadottir A, Blackwell JM, Bramon E, Brown MA, Casas JP, Corvin A, Duncanson A, Jankowski J, Mathew CG, Palmer CN, Plomin R, Rautanen A, Sawcer SJ, Trembath RC, Viswanathan AC, Wood NW, Worrall BB, Kittner SJ, Mitchell BD, Kissela B, Meschia JF, Thijs V, Lindgren A, Macleod MJ, Slowik A, Walters M, Rosand J, Sharma P, Farrall M, Sudlow CL, Rothwell PM, Dichgans

M, Donnelly P, Markus HS. Genome-wide association study identifies a variant in HDAC9 associated with large vessel ischemic stroke. *Nat Genet*. 2012;44:328–333.

11. Traylor M, Farrall M, Holliday EG, Sudlow C, Hopewell JC, Cheng YC, Fornage M, Ikram MA, Malik R, Bevan S, Thorsteinsdottir U, Nalls MA, Longstreth W, Wiggins KL, Yadav S, Parati EA, Destefano AL, Worrall BB, Kittner SJ, Khan MS, Reiner AP, Helgadottir A, Achterberg S, Fernandez-Cadenas I, Abboud S, Schmidt R, Walters M, Chen WM, Ringelstein EB, O'Donnell M, Ho WK, Pera J, Lemmens R, Norrving B, Higgins P, Benn M, Sale M, Kuhlenbaumer G, Doney AS, Vicente AM, Delavaran H, Algra A, Davies G, Oliveira SA, Palmer CN, Deary I, Schmidt H, Pandolfo M, Montaner J, Carty C, de Bakker PI, Kostulas K, Ferro JM, van Zuydam NR, Valdimarsson E, Nordestgaard BG, Lindgren A, Thijs V, Slowik A, Saleheen D, Pare G, Berger K, Thorleifsson G; Australian Stroke Genetics Collaborative WTCCC, Hofman A, Mosley TH, Mitchell BD, Furie K, Clarke R, Levi C, Seshadri S, Gschwendtner A, Boncoraglio GB, Sharma P, Bis JC, Gretarsdottir S, Psaty BM, Rothwell PM, Rosand J, Meschia JF, Stefansson K, Dichgans M, Markus HS; International Stroke Genetics C. Genetic risk factors for ischaemic stroke and its subtypes (the METASTROKE collaboration): a meta-analysis of genome-wide association studies. *Lancet Neurol*. 2012;11:951–962.
12. Adams HP Jr, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, Marsh EE III. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in acute stroke treatment. *Stroke*. 1993;24:35–41.
13. Lee TH, Hsu WC, Chen CJ, Chen ST. Etiologic study of young ischemic stroke in Taiwan. *Stroke*. 2002;33:1950–1955.
14. Pan WH, Fann CS, Wu JY, Hung YT, Ho MS, Tai TH, Chen YJ, Liao CJ, Yang ML, Cheng AT, Chen YT. Han Chinese cell and genome bank in Taiwan: purpose, design and ethical considerations. *Hum Hered*. 2006;61:27–30.
15. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med*. 2002;21:1539–1558.
16. Gudmundsson J, Sulem P, Gudbjartsson DF, Jonasson JG, Sigurdsson A, Bergthorsson JT, He H, Blondal T, Geller F, Jakobsdottir M, Magnusdottir DN, Matthiasdottir S, Stacey SN, Skarphedinsson OB, Helgadottir H, Li W, Nagy R, Aguillo E, Faure E, Prats E, Saez B, Martinez M, Eyjolfsson GI, Bjornsdottir US, Holm H, Kristjansson K, Frigge ML, Kristvinsson H, Gulcher JR, Jonsson T, Rafnar T, Hjartarsson H, Mayordomo JI, de la Chapelle A, Hrafnkelsson J, Thorsteinsdottir U, Kong A, Stefansson K. Common variants on 9q22.33 and 14q13.3 predispose to thyroid cancer in European populations. *Nat Genet*. 2009;41:460–464.
17. Jendrzejewski J, He H, Radomska HS, Li W, Tomsic J, Liyanarachchi S, Davuluri RV, Nagy R, de la Chapelle A. The polymorphism rs944289 predisposes to papillary thyroid carcinoma through a large intergenic noncoding RNA gene of tumor suppressor type. *Proc Natl Acad Sci USA*. 2012;109:8646–8651.
18. Sheu JJ, Kang JH, Lin HC, Lin HC. Hyperthyroidism and risk of ischemic stroke in young adults: a 5-year follow-up study. *Stroke*. 2010;41:961–966.
19. Squizzato A, Gerdes VE, Brandjes DP, Buller HR, Stam J. Thyroid diseases and cerebrovascular disease. *Stroke*. 2005;36:2302–2310.
20. Han Y, Sun W, Wang L, Tao S, Tian L, Hao Y, Zhang W, Wu S, Li S, Lv H, Zheng SL, Sun J, Xu J. HDAC9 gene is associated with stroke risk in a Chinese population. *Exp Biol Med (Maywood)*. 2013;238:842–847.