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Siblings with Ischemic Stroke Study (SWISS): Results of a Genome-wide Scan for Stroke Loci

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Abstract

Background and Purpose—Ischemic stroke has a strong familial component to risk. The Siblings with Ischemic Stroke Study (SWISS) is a genome-wide family-based analysis that included use of imputed genotypes. SWISS was conducted to examine associations between SNPs and risk of stroke and stroke subtypes within pairs.

Methods—SWISS enrolled 312 probands with ischemic stroke across 70 US and Canadian centers. Affected siblings were ascertained by centers and confirmed by central record review; unaffected siblings were ascertained by telephone contact. Ischemic stroke was subtyped using TOAST criteria. Genotyping was performed using an Illumina 610 quad array (probands) and an Illumina linkage V array (affected siblings). SNPs were imputed using 1000 Genomes Project data and MACH software. Family-based association analyses were conducted using the sibling-transmission disequilibrium test.

Results—For all pairs, the correlation of age at stroke within pairs of affected siblings was r = 0.83 (95%CI, 0.78 to 0.86; $P < 2.2\times10^{-16}$). The correlation did not differ substantially by subtype. The concordance of stroke subtypes among affected pairs was 33.8% (kappa = 0.13; $P = 5.06\times10^{-4}$) and did not differ by age at stroke in the proband. Although no SNP achieved genome-wide significance for risk of ischemic stroke, there was clustering of the most associated SNPs on chromosomes 3p (*NOS1*) and 6p.

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*See Appendix for a list of participating centers and investigators.

Conflicts of Interest/Disclosure
The funding source had no involvement in study design beyond peer review of the grant. The funding source had no involvement in collection, analysis, or interpretation of data; in writing of the report; or in the decision to submit the article for publication.

Conclusions—Stroke subtype and age at stroke in affected sibling pairs exhibit significant clustering. No individual SNP reached genome-wide significance. However, two promising candidate loci were identified, including one that contains *NOS1*, though these risk loci warrant further examination in larger sample collections.

Ischemic stroke has long been recognized to cluster in families. This clustering has been attributed to both genetic factors and common environmental exposures. Gene mutations have been identified that lead to rare ischemic stroke syndromes like CADASIL and MELAS. However, search for genetic loci associated with ischemic stroke risk has yielded meager results thus far despite several candidate gene [1] and large-scale genome-wide association studies. [2] [3] The Siblings with Ischemic Stroke Study (SWISS) provides a different and promising approach to discover novel risk factors for ischemic stroke through the study of unrelated families of affected and unaffected siblings. Here we present the results of the family-based genome-wide scan of SWISS after achieving the original recruitment goal.

Methods

Study Subjects

Probands were recruited at 70 US medical centers and Canadian medical centers. Probands were adult (>18 years old) men and women presenting to a participating center with a study neurologist-confirmed ischemic stroke. Stroke was defined as rapidly developing signs of a focal or global disturbance of cerebral function, with symptoms lasting at least 24 hours or leading to death with no apparent cause other than vascular origin (WHO definition). [4] Stroke was defined as ischemic if CT or MR imaging of the brain was performed within 7 days of onset of stroke symptoms and identified the symptomatic cerebral infarct or failed to identify an alternative cause of symptoms. Probands were required to have reported at least one living full sibling with a history of stroke. No probands were enrolled with iatrogenic vasospastic, or vasculitic stroke or if the stroke occurred in the setting of a mechanical heart valve or in the setting of untreated or actively treated bacterial endocarditis. Probands were also excluded if they were known to have CADASIL, Fabry disease, homocysteinuria, MELAS, or sickle cell anemia. Study neurologists at each center assigned to the qualifying ischemic stroke of each proband a TOAST subtype diagnosis.[5]

Stroke-affected siblings of the proband (concordant siblings) were recruited using proband-initiated contact.[6] Telephone interviews were performed to obtain demographic and clinical information and to gain permission for obtaining medical records pertaining to treatment for stroke. Medical records were compiled and adjudicated by a central committee (JFM; TGB), to verify the diagnosis of ischemic stroke and to assign a TOAST subtype diagnosis. Assignment of TOAST subtype diagnoses to SWISS concordant siblings has moderate inter-rater reliability.[7] Unaffected siblings were ascertained by telephone contact and interview.

Genotyping Considerations

The establishment of lymphoblastoid cell lines, quality control of genomic DNA, acquisition of genetic data, and genotyping quality control metrics were performed using standard procedures. Please see http://stroke.ahajournals.org for these details.

Consensus SNPs that passed QC in both phases (genome-wide association and family-based phases) were merged for all available sibships (2239 SNPs were imputed in the probands). Using all 5612 SNPs in the merged dataset, reported relationships were verified using pi_hat estimates. Sibships were confirmed if pairwise pi_hats values were between 0.35 and 0.65; samples were removed from a sibship if estimated pi_hat was not in this range. This dataset

of the combined genotyping phases represents the final dataset for all subsequently described analyses. The flow of patients in the study is shown in Figure 1.

Genetic Data Analysis

All family-based analyses were conducted using PLINK 1.07 software. [8] The dFam utility within PLINK implements a siblings-based transmission-disequilibrium test and was used to conduct these analyses. The dFam option is a powerful test for sibling-only datasets, incorporating data across sibships as well as using data from estimated parental genotypes to calculate expected allele frequencies for comparison to observed allele frequencies. The association test is based on the Cochran-Mantel-Haenszel test. Bonferroni correction for the number of tested SNPs corresponds to a minimum p-value for genome-wide significance of $P < 8.91\times10^{-6}$.

Additional Statistical Analyses

Frequencies of stroke risk factors (hypertension, hyperlipidemia and diabetes) between affected and unaffected participants were compared using chi-squared tests. The correlation between affected sibling age at stroke was estimated using the Pearson test of correlation. These analyses were conducted across all TOAST subtypes as well as following stratification by concordant and discordant subtypes among affected sibling pairs. Linear regression was used to determine the confidence intervals and linear fit of the age association as show in Figure 2 Kappa statistics were calculated to quantify concordance of phenotypes of interest within sibling pairs for all ages and stroke subtypes as well as models stratified by age (< 65 year proband as defining age strata) and stroke subtype. All analyses that did not include genetic data were conducted using scripts written in R (R Development Core Team (2008)). [9]

Results

A total of 312 affected sibling pairs (312 probands) were enrolled at 70 centers across the US and Canada. After quality control filtering, the final study population consisted of 223 probands, 248 stroke-affected siblings, and 84 stroke-unaffected siblings (total sample size, 555). Ischemic stroke-affected individuals had expected high rates of conventional atherosclerotic risk factors (Table 1). Stroke-affected individuals (probands and affected-siblings) were significantly more likely to have hypertension ($P < 0.0001$), hyperlipidemia ($P= 0.002$) and diabetes ($P = 0.008$) than stroke-unaffected individuals. Stroke-affected siblings were somewhat older than the probands. This difference of 2 years ($P = 0.057$) is expected as an older sibling of the proband would be more likely to have a stroke than a younger sibling.

Sibling age at time of stroke strongly correlated with proband age at time of stroke, despite the sibling being older. As shown in Figure 2 for all the sibling pairs, the correlation coefficient was r = 0.83 (95%CI, 0.78 to 0.86; $P < 2.2\times10^{-16}$). For affected sibling pairs who have the same stroke subtype, the correlation coefficient was not different from all pairs, r = 0.83 (95%CI, 0.75 to 0.89; $P < 2.2\times10^{-16}$). This was the same for sibling pairs in which the affected siblings had different stroke subtypes, r = 0.83 (95%CI, 0.77 to 0.87; $P < 2.2\times10^{-16}$). Over 50% of the variance in age at stroke onset in siblings could be predicted by the age of proband at time of stroke. As shown in Table 2, there was significant concordance with affected siblings for TOAST subtype (kappa = 0.13; $P = 5.06\times10^{-4}$); this relationship remained significant for sibling pairs where the proband was less than 65 years old at time of stroke and for sibling pairs in which the proband was 65 years or older.

Results of the genome-wide analyses are shown in Figure 3. Although no SNP association with ischemic stroke achieved genome-wide level of significance, the ten most associated SNPs exhibited clustering in several genomic regions. These ten most significantly associated SNPs, their locations, frequencies and effect estimates are shown in Table 3. The ten SNPs represent 8 genomic loci, with minor allele frequencies ranging from 0.38–0.48 (common alleles). The effects for each are small, with odds ratios ranging from 0.96 to 1.04. The location of the most significantly associated SNPs (as well as others within 2.5 Mb) is shown in Figure 3 (indicated by blue shading). There are clusters of associated SNPs on chromosomes 3p and 6p. The SNPs on chromosome 3p lie in a strong candidate gene, *NOS1*.

Discussion

Our genome-wide scan for risk factors for ischemic stroke was performed in the largest collection of affected sibling pairs to date and showed potential loci of interest including a locus on *NOS1*. We are not aware of other genetic studies showing *NOS1* variants to be associated with human ischemic stroke risk. However, knock-out mouse models show that *NOS1* gene deficiency causes worsening neointimal formation and constrictive vascular remodeling. [10] Genetic variants theoretically could impart elevated ischemic stroke risk by a vascular mechanism, by a thrombotic mechanism or even by a parenchymal mechanism where brain tissue is sensitized to focal ischemia. In the case of *NOS1*, it seems that variants would be more likely to impart risk by a vascular mechanism than by a parenchymal mechanism, since *NOS1* knockout mice have smaller infarcts than control wild-type mice in an MCA occlusion model. [11] It is important not to speculate beyond the strength of our observations, as no locus achieved genome-wide statistical significance.

Genome-wide studies have had mixed results in ischemic stroke. When SWISS was initiated, the human genome had only been sequenced in draft form. [12] SWISS was predicated on the hypothesis that ischemic stroke obeyed the common disease, common variant hypothesis, which states that the genetic influences on many common disease are attributable to a limited number of allelic variants present in > 1–5% of the population. [13] It has since become less clear that the hypothesis holds for ischemic stroke. No single locus has been identified in two genome-wide association studies at a genome-wide level of significance. [14] Our study supports the idea that no single locus substantially contributes to ischemic stroke risk from the perspective of common variants contributing to disease risk, although future sequencing-based studies of rare variants may meet with substantially more success.

SWISS was designed to treat all types of ischemic stroke as a single phenotype. The phenotypic heterogeneity of ischemic stroke has long been appreciated, but categorizing subtypes of ischemic stroke historically has been done with little consistency in genetic research.[15] Despite this methodological limitation, genetic risk factors have been identified that appear to be specific for certain ischemic stroke subtypes. For example, the chromosome 9p21 locus appears to impart risk for so-called large vessel atherosclerotic stroke. [16, 17] The atrial fibrillation locus 4q25 appears to impart risk for cardioembolic stroke. [18, 19] Collaborating with investigators from Sweden, we previously assessed whether ischemic stroke subtypes clustered among affected sibships, showing low aggregation rates. [20] We continue to see low aggregation rates, but the relationship is significant. In addition to having larger numbers, the current analysis is restricted to those affected sibling pairs confirmed to be full siblings through genomic analysis. It is not known whether more complex systems of classifying stroke also show a tendency toward aggregation within families.[21]

Age at onset of stroke may be a quantitative phenotype more tractable to genomic analysis. In an interim analysis, we had observed a significant association of proband age at stroke onset and sibling age at stroke onset. [22] As with the subtype aggregation reanalysis, the current analysis involves a larger sample size and is restricted to those affected sibling pairs confirmed to be full siblings through genomic analysis. As a phenotype, age at stroke onset has the limitation that it does not necessarily reflect the burden of ischemic disease at any given moment in the lifespan of a patient. Some cerebral infarcts are asymptomatic [23] while other cerebral infarcts may be symptomatic but undiagnosed. [24]

In summary, we have described here an affected relative-based genetic analysis of ischemic stroke. This work provides preliminary evidence for the involvement of several loci in risk for this disease, and these loci certainly warrant follow-up. This work also suggests that any individual risk variants involved in ischemic stroke are likely to have a low population-attributable risk. Attributable risk could be low if the risk conferred is relatively low; it could also be low if there is extensive allelic and/or genetic heterogeneity in stroke, with no single locus being a common, high risk conferring locus. Clearly we can hope that the future application of now- and next-generation technologies in large and extremely well characterized cohorts will enable identifying genetic risks for ischemic stroke.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study utilized the high-performance computational capabilities of the Biowulf Linux cluster at the National Institutes of Health, Bethesda, MD. (http://biowulf.nih.gov).

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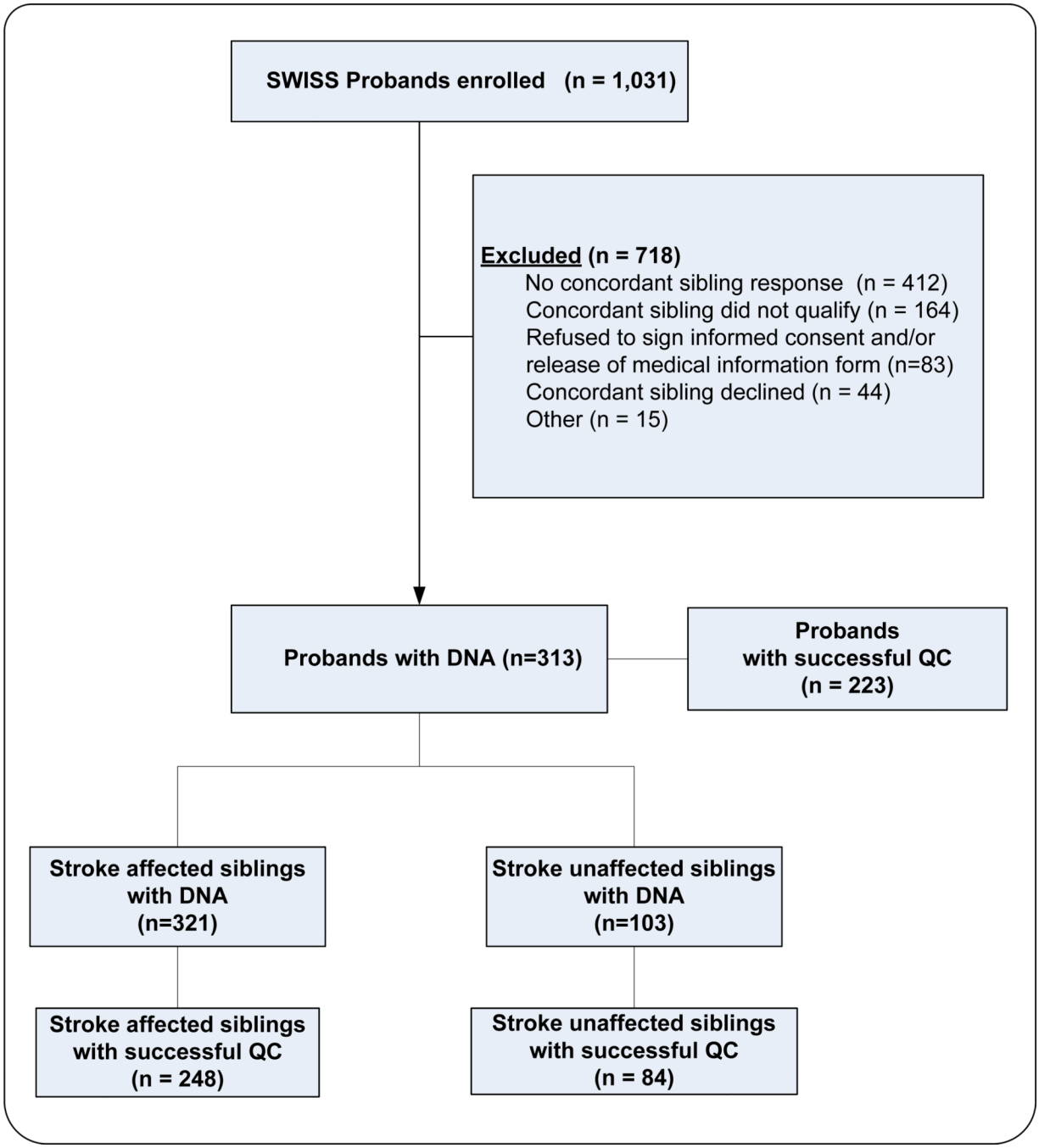

Figure 1.
Flow of participants in the study.

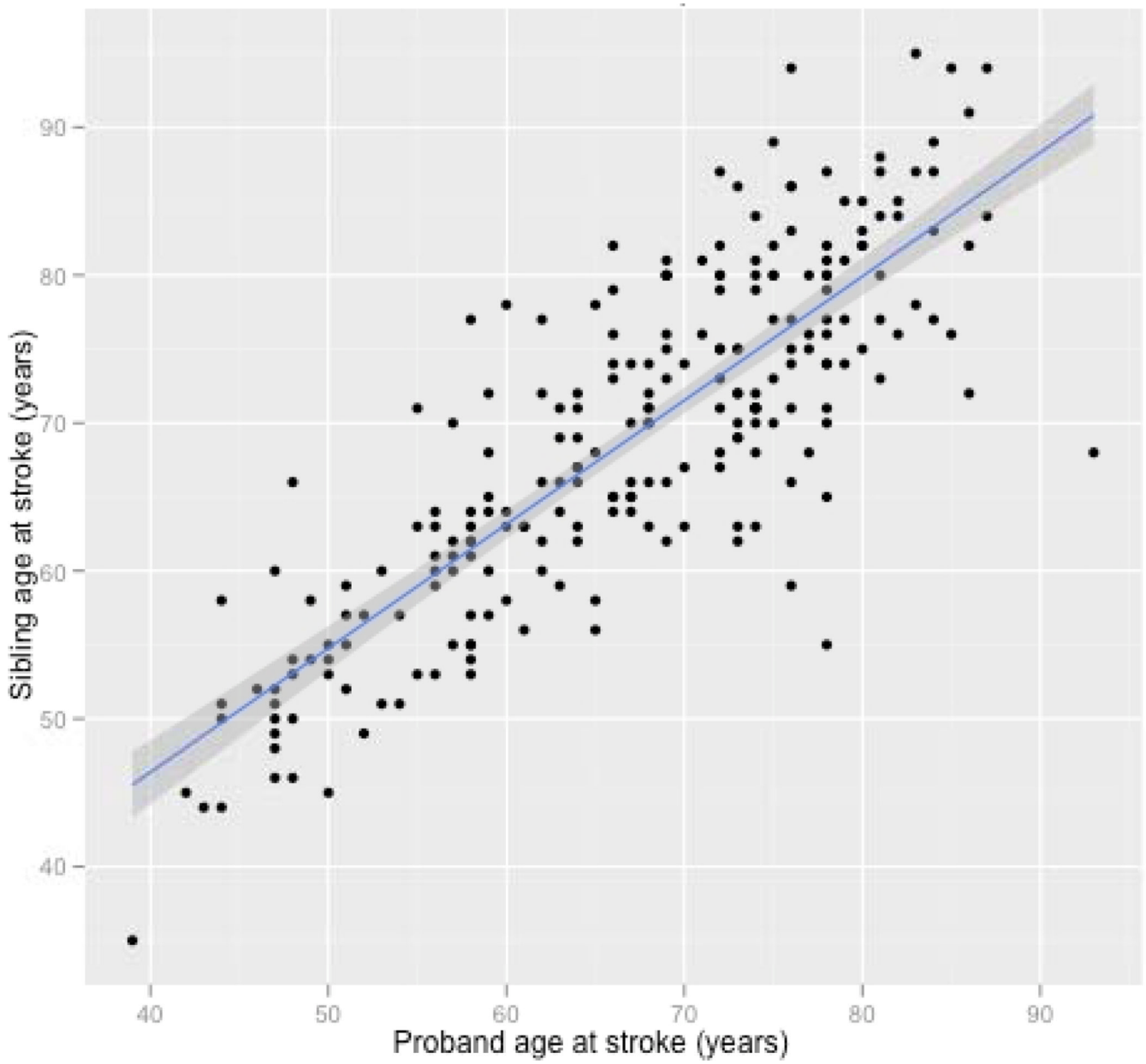

Figure 2.
Correlation between proband and sibling age at stroke. Correlation coefficient = 0.83. P-value < 0.0001. Pairs are points, Blue line = linear model, grey shading = 95% CI.

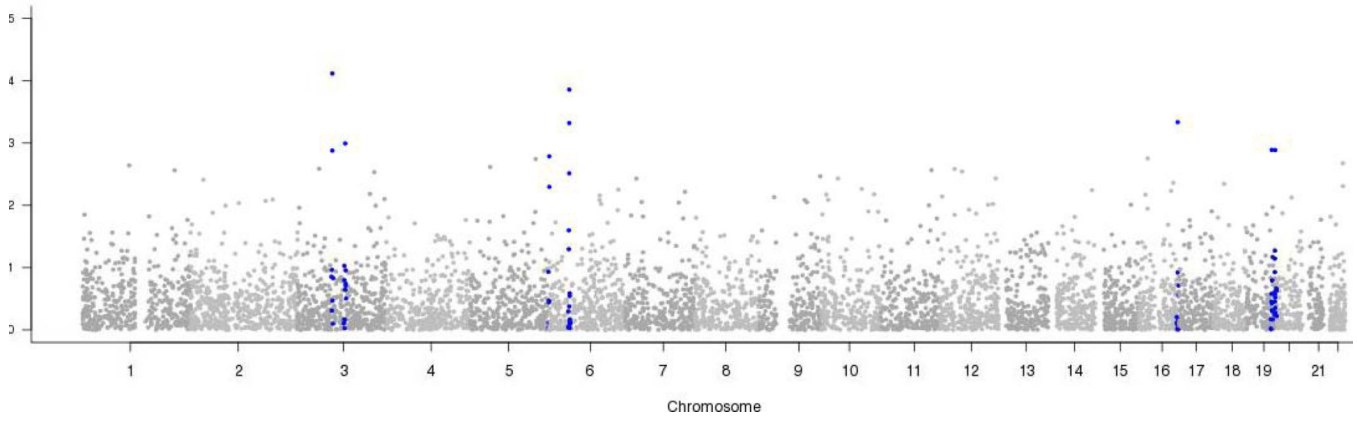

Figure 3.
Summary of transmission-disequilibrium test results. All SNPs within 2.5 megabases of SNPs shown in table 1 are highlighted in blue.

Table 1

Characteristics of the Study Population after passing genomic quality controls.

	Probands	Affected Siblings	Unaffected Siblings	Total
N total (%)	**223 (40)**	**248 (45)**	**84 (15)**	**555 (100)**
Age (mean, SD)	66.99, 11.39	69.01, 11.55	66.00, 11.12	67.74, 11.46
% Female	48.43	45.97	58.33	48.83
TOAST Criteria **N (%)**				
Cardioembolic	25 **(11)**	23 **(9)**	n.a.	48/471 **(10)**
Large Vessel	58 **(26)**	47 **(19)**	n.a.	105/471 **(22)**
Small Vessel	66 **(30)**	76 **(31)**	n.a.	142/471 **(30)**
Other	13 **(6)**	10 **(4)**	n.a.	23/471 **(5)**
Undetermined	61 **(27)**	92 **(37)**	n.a.	153/471 **(33)**
Hypertension **N (%)**				
Yes	155 **(70)**	175 **(71)**	38 **(45)**	368 **(66)**
No	68 **(30)**	72 **(29)**	46 **(55)**	186 **(34)**
Unknown	0	1 **(<1)**	0	1 **(<1)**
Atrial Fibrillation **N (%)**				
Yes	24 **(11)**	57 **(23)**	12 **(14)**	93 **(17)**
No	197 **(88)**	188 **(76)**	72 **(86)**	457 **(82)**
Unknown	2 **(1)**	3 **(1)**	0	5 **(1)**
Hyperlipidemia **N (%)**				
Yes	140 **(63)**	162 **(65)**	39 **(46)**	341 **(61)**
No	83 **(37)**	84 **(34)**	45 **(54)**	212 **(38)**
Unknown	0	2 **(1)**	0	2 **(1)**
Diabetes **N (%)**				
Yes	50 **(22)**	61 **(25)**	9 **(11)**	120 **(22)**
No	173 **(78)**	185 **(75)**	75 **(89)**	433 **(78)**
Unknown	0	2 **(<1)**	0	2 **(<1)**
Smoking **N (%)**				
Current	44 **(20)**	45 **(18)**	14 **(17)**	103 **(19)**
Never	104 **(47)**	91 **(37)**	37 **(44)**	232 **(42)**
Former	74 **(33)**	109 **(44)**	33 **(39)**	216 **(39)**
Unknown	1 **(<1)**	3 **(1)**	0	4 **(<1)**

* n.a.=Not Applicable

Table 2

Concordance for ischemic stroke subtype within affected sibling pairs.

	All ages			Proband < 65 years			Proband ≥ 65 years		
Proband stroke type	**Total pairs**	**% Expected Concordant**	**% Observed Concordant**	**Total pairs**	**% Expected Concordant**	**% Observed Concordant**	**Total pairs**	**% Expected Concordant**	**% Observed Concordant**
All Strokes	233	--	34.8	92	--	33.7	141	--	35.5
Cardioembolic	29	1.5	24.1	13	2.0	30.8	16	1.3	18.8
Large Vessel	58	6.2	31.0	26	8.0	38.5	32	5.2	25.0
Small Vessel	68	8.5	36.8	22	5.7	45.4	46	10.6	32.6
Other	13	0.3	7.7	7	0.6	14.3	6	0.2	0
Unknown	65	7.8	46.2	24	6.8	25.0	41	8.5	58.5
Kappa Statistic	--	--	0.13	--	--	0.14	--	--	0.12
Kappa SE	--	--	0.04	--	--	0.06	--	--	0.05
Kappa Z	--	--	3.48	--	--	2.46	--	--	2.45
Kappa P-value	--	--	5.06×10^{-4}	--	--	1.38×10^{-2}	--	--	1.44×10^{-2}

Note: Kappa statistics calculated for all pairs by age strata

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Table 3

Top 10 most significant SNPs representing 8 genomic loci.

SNP	CHR	MB	cM	Minor Allele	MAF	Odds Ratio for Minor Allele	Chi-Squared Statistic	P-value
rs1383407	3	78.991	107.18	C	0.4488	0.961	15.65	7.63×10^{-5}
rs328049	3	79.068	107.21	A	0.4766	0.968	10.31	1.32×10^{-3}
rs986692	3	107.766	116.1	T	0.412**0**	1.04**0**	10.8**0**	1.01×10^{-3}
rs1053110	5	180.421	205.94	T	0.4704	1.034	9.92	1.64×10^{-3}
rs1293457	6	44.866	68.46	T	0.4372	1.036	12.2**0**	4.79×10^{-4}
rs3778507	6	45.005	68.69	A	0.4075	1.043	14.53	1.38×10^{-4}
rs179209	16	19.215	41.4	A	0.4749	0.967	9.773	1.77×10^{-3}
rs750740	16	87.335	129.03	T	0.4695	1.037	12.26	4.63×10^{-4}
rs897783	19	56.723	88.79	A	0.3779	1.036	10.36	1.29×10^{-3}
rs976192	20	1.444	5.81	C	0.3797	1.041	10.35	1.30×10^{-3}

SNP denotes single nucleotide polymorphism; CHR, chromosome; MB, megabase; cM, centiMorgan; MAF, minor allele frequency.