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The Prothrombin G20210A Mutation is Associated with Young-Onset Stroke: The Genetics of Early Onset Stroke Study and Meta-Analysis

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Abstract

Background and Purpose—Although the prothrombin G20210A mutation has been implicated as a risk factor for venous thrombosis, its role in arterial ischemic stroke is unclear, particularly among young-adults. To address this issue, we examined the association between prothrombin G20210A and ischemic stroke in a Caucasian case-control population and additionally performed a meta-analysis

Methods—From the population-based Genetics of Early Onset Stroke (GEOS) study we identified 397 individuals of European ancestry aged 15-49 years with first-ever ischemic stroke and 426 matched-controls. Logistic regression was used to calculate odds ratios in the entire population and for subgroups stratified by gender, age, oral contraceptive use, migraine and smoking status. A meta-analysis of 17 case-control studies (n=2305 cases <55 years) was also performed with and without GEOS data.

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Results—Within GEOS, the association of the prothrombin G20210A mutation with ischemic stroke did not achieve statistical significance (OR=2.5,95%CI=0.9-6.5,p=0.07). However, among adults aged 15-42 (younger than median age), cases were significantly more likely than controls to have the mutation (OR=5.9,95%CI=1.2-28.1,p=0.03), whereas adults ages 42-49 were not (OR=1.4,95%CI=0.4-5.1,p=0.94). In our meta-analysis, the mutation was associated with significantly increased stroke risk in adults <=55 years (OR=1.4;95%CI=1.1-1.9;p=0.02) with significance increasing with addition of the GEOS results (OR=1.5;95%CI=1.1-2.0;p=0.005).

Conclusions—The prothrombin G20210A mutation is associated with ischemic stroke in young-adults and may have an even stronger association among those with earlier onset strokes. Our finding of a stronger association in the younger-young adult population requires replication.

Keywords

ischemic stroke; young; risk modifiers; risk factors; genetics

Introduction

Prothrombin (coagulation factor II, or FII) G20210A (rs1799963) is a single-nucleotide polymorphism (guanine to adenine; G→A) at position 20210 located at the 3' untranslated region of the non-coding region of the prothrombin gene on chromosome 11.[1] The minor A allele of this polymorphism is found in 2% of individuals of European-ancestry and is slightly less common than the minor A allele associated with Factor V Leiden (3.5%; rs6025).[2,3] The prothrombin G20210A mutation (A allele) is exceedingly rare in those of African- or Asian-ancestry.[2,3] Prothrombin is a precursor to thrombin, a key regulator of blood coagulation in the clotting cascade, and carriers of the prothrombin G20210A mutation have elevated blood plasma prothrombin levels.[2] The prothrombin G20210A mutation plays a role in hypercoagulability and has been associated with a two-to-fourfold higher risk for venous thrombosis.[3,4] Although this polymorphism has been well characterized for venous thrombosis, its role in arterial vascular disease still remains uncertain, particularly in young-adults with ischemic stroke. To address this issue, we examined the association between the prothrombin G20210A mutation and first-ever ischemic stroke in young Caucasian adults from the Genetics of Early Onset Stroke (GEOS) study. In addition, we also performed a meta-analysis of 17 previously published association studies of the prothrombin G20210A polymorphism and ischemic stroke in young-adults aged <=55

Methods

GEOS Study

The GEOS Study is a population-based case-control study designed to identify the genetic determinants of early-onset ischemic stroke and to characterize the interactions of identified stroke genetic variants with environmental risk factors such as smoking and oral contraceptive (OC) use. Cases aged 15-49 years with a first ischemic stroke were identified between 1992-2007 by discharge surveillance from one of 59 hospitals in the greater Baltimore/Washington, DC area and by direct referral from regional neurologists. Details of

the recruitment of cases and controls have been previously published.[5,6,7] In brief, cases and controls were recruited in 3 different time periods: Stroke Prevention in Young Women-1 (SPYW-1) conducted from 1992-1996, Stroke Prevention in Young Women-2 (SPYW-2) conducted from 2001-2003, and Stroke Prevention in Young Men (SPYM) conducted from 2003-2007. SPYW-1 included cases between 15-44 years of age who were recruited within one year of stroke and was designed with a 1:2 case-to-control ratio. SPYW-2 and SPYM included cases 15-49 years of age who were recruited within three years of stroke and was designed with a 1:1 case-to-control ratio. Control participants without a history of stroke were identified by random-digit dialing. Controls were balanced to cases by age and region of residence in each study and were additionally balanced for ethnicity in SPYW-2 and SPYM. Given the rarity of the prothrombin G20210A mutation in non-European ethnicities (only 2 of 392 non-Caucasians in our study had the mutation; both were cases), we limited our analyses to Caucasians, for whom there were 397 cases and 426 matched non-stroke controls.

The abstracted hospital records of cases were reviewed and adjudicated for ischemic stroke subtype by a pair of vascular neurologists according to previously published procedures[5,6,7] with disagreements resolved by a third vascular neurologist. Stroke subtypes were classified using the Trial of ORG 10172 in Acute Stroke[8] (TOAST) system. Ischemic strokes with the following characteristics were excluded from participation: stroke occurring as an immediate consequence of trauma, stroke within 48 hours after a hospital procedure, stroke within 60 days after the onset of a non-traumatic subarachnoid hemorrhage, and cerebral venous thrombosis. Additional exclusions for these genetic analyses were as follows: known single-gene or mitochondrial disorders recognized by a distinctive phenotype (e.g., cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy [CADASIL], mitochondrial encephalopathy with lactic acidosis and stroke-like episodes [MELAS], homocystinuria, Fabry disease, or sickle cell anemia); mechanical aortic or mitral valve at the time of index stroke; untreated or actively treated bacterial endocarditis at the time of the index stroke; neurosyphilis or other central nervous system infections; neurosarcoidosis; severe sepsis with hypotension at the time of the index stroke; cerebral vasculities by angiogram and clinical criteria; postradiation arteriopathy; left atrial myxoma; major congenital heart disease; and cocaine use in the 48 hours preceding their stroke.

Clinical and medical information, including age, ethnicity, and established stroke risk factors including history of hypertension, diabetes, myocardial infarction (MI), migraine with or without aura, current smoking status, and current OC use (both defined as use within 1 month before the event for cases and at a comparable reference time for controls), were collected during a standardized face-to-face interview and were included as covariates in our analysis. Blood chemistries were not measured among the controls, precluding case-control comparisons of hyperlipidemia.

The prothrombin G20210A polymorphism (rs1799963) was genotyped in all cases and controls at the Centers for Disease Control and Prevention as part of a custom 384-SNP GoldenGate assay according to the manufacturer's protocol (Illumina). Processed Universal-32 Beadchips were imaged on an Illumina BeadArray Reader, and GenomeStudio

software (version 2011.1) was used to assess sample and assay quality. The genotyping call rate for rs1799963 was 100%.

Statistical analysis was performed using SAS software (version 9.2; SAS institute, Cary NC). The distributions of prothrombin G20210A and other characteristics among cases and controls were compared using *t*-tests for continuous variables and Mantel-Haenzel Chi-square tests for categorical variables. The association between prothrombin G20210A and ischemic stroke was then examined within pre-defined subgroups using Chi-square tests (or Fisher exact tests in the event of small sample sizes). Comparisons were repeated using a logistic regression model adjusted for age and gender in a "basic" model and for the basic model plus hypertension, diabetes, history of MI, current OC use, current smoking status, and migraine with aura in a "full" model. Two-tailed *P* values of <.05 were considered statistically significant.

Meta-Analysis

Using the key words "Prothrombin G20210A mutation", "ischemic stroke", and "young-adults" we searched PubMed and Web of Science data bases for case-control studies of ischemic stroke in young-adults published before June 2012. Any identified articles were then hand-searched for references to identify additional relevant studies. Studies were included in the meta-analysis according to standard criteria[9] if: (1) neuroimaging was used to confirm clinical diagnoses of ischemic stroke; (2) controls were derived from the same population as cases; (3) prothrombin G20210A genotypes were available for all participants; (4) the numbers of cases and controls with and without prothrombin G20210A were provided in the article; and (5) the study included only cases with first stroke less than or equal to 55 years of age (most identified studies classified young stroke as ≤55 rather than less than or equal to ≤49 as consistent with GEOS), or the number of cases in this age group with and without prothrombin G20210A could be clearly obtained from the study. We also excluded case-series restricted to those with known patent foramen ovale (PFO).

Data analysis was performed using Comprehensive Meta-Analysis version 2.0 by Biostat (http://www.meta-analysis.com). The numbers of cases and controls stratified by prothrombin G20210A carrier status were extracted from each study, and odds ratios calculated. A pooled odds ratio was calculated using a variance-weighted approach under both fixed-effects and random-effects models. The fixed-effects model assumes the effects are the same across studies while the random-effect model allows for heterogeneity of effects. The calculation for the genetic effect of prothrombin variant assuming fixed-effects model was repeated with each of the studies individual removed from the analysis to confirm that no single study was principally responsible for the findings. Between-study heterogeneity was assessed using the Q-test, which is based on comparing the estimated study-specific treatment effects to the estimated overall treatment effect. We additionally computed the I^2 statistic, which describes the proportion of variation across studies attributable to heterogeneity rather than chance.[10]

Results

GEOS

Clinical characteristics of cases (n=397) and controls (n=426) are summarized in Table 1. Cases were older than controls and were more likely to report a history of hypertension, diabetes, myocardial infarction, to be current smokers, to have history of migraine with aura and, among women, to be oral contraceptive users. A total of 20 subjects were carriers of a prothrombin G20210A minor A allele, 14 cases (3.5%) and 6 controls (1.4%). This difference was statistically significant (p = 0.05). Controls were in Hardy-Weinberg equilibrium for the prothrombin G20210A mutation. There was 1 homozygote in the study population, a case, which was also a Factor V Leiden heterozygote. As an established hypercoaulable state, by TOAST[8] subtype criteria, this homozygote case was classified as a stroke of other determined etiology. Evaluating the distribution of 13 heterozygote cases with the prothrombin 20210A allele by TOAST[8] stroke subtype demonstrated 1 cardioembolic, 1 large-artery atherosclerotic, 4 small vessel, and 7 undetermined etiology (cryptogenic).

Analysis of prothrombin G20210A and ischemic stroke risk are presented in Table 2 stratified by demographic and established risk factors for stroke, including gender, current smoking status, migraine, and OC use. In the overall Caucasian population, cases had more than a 2-fold greater odds of having the prothrombin G20210A mutation than controls, although this difference did not achieve statistical significance (14/397 cases vs. 6/426 controls; OR=2.5;95%=CI 0.9-6.5;p=0.07). Stratification by gender, current OC use (among women), current smoking status, and migraine did not alter these results. However, in a post-hoc analysis, the association was more pronounced in younger-onset cases (i.e., < the median age of 42 years; OR=5.9;95%CI=1.2-28.1;p=0.03) than in older onset cases (i.e., ≥ age 42; OR=1.4;95%CI=0.4-5.1;p=0.94).

Meta-analyses without and with GEOS data

Seventeen studies matched our selection criteria and were included in the meta-analysis.[11-27] Table 3 shows the demographic characteristics of studies included in the meta-analysis. The results of the meta-analysis performed with and without GEOS data are shown in Figure 1. The studies were conducted in several European countries (prothrombin G20210A prevalence is highest in Southern European countries[2]), the United States, and Brazil, and the majority of participants were of Caucasian ancestry. In 13 of these studies, the reported odds ratio for prothrombin G20210A and ischemic stroke was greater or equal to 1, and in two of these studies the odds ratio was significantly greater than 1 (p<.05). Across prior studies, prothrombin G20210A was detected in 80 out of 1908 cases (4.2%) and in 175 out of 5551 controls (3.2%) yielding an OR of 1.4 (95%CI=1.1-1.9;p-value=0.02). After including GEOS data, prothrombin G20210A was present in 94 of 2305 cases (4.0%) and in 181 of 5977 controls (3.0%), yielding a pooled OR of 1.5 (95%CI=1.1-2.0;p-value=0.005), based on fixed effect. There was no significant heterogeneity between the studies (Q-value=13.2;I^2=0.0). Repeating the meta-analysis with each of the studies removed individually did not significantly alter the calculated odds ratio (data not shown). To assess publication bias, we created a funnel plot of all the studies used in the meta-analysis (Figure

2), which conformed to the expected shape of the curve and demonstrated overall left-right symmetry.[28]

Discussion

Our meta-analysis of 2305 young-onset stroke cases reveals a moderately strong association between the prothrombin 20210A minor allele and young-onset ischemic stroke. While the GEOS results were not significant, they were of a similar magnitude and direction, and adding them to the meta-analysis increased the significance of the association. Moreover, stratifying the GEOS data by age of stroke-onset revealed the effect of the prothrombin allele to be most pronounced in the youngest subjects.

When we analyzed our GEOS population as a whole, we failed to find an overall statistically significant association between the prothrombin G0210A polymorphism and ischemic stroke in those of European ancestry. We further hypothesized that prothrombin G20210A might be associated with ischemic stroke risk in specific subpopulations, such as those with cryptogenic stroke or those having one or more vascular risk factors such as hypertension, diabetes, history myocardial infarction, oral contraceptive use, migraine headache, and current smoking status. We did not find an association among cryptogenic stroke patients, or those with cardiovascular risk factors as stratified individually or when analyzed in aggregate (results not shown). This could be due to the fact that stratification of the GEOS study population yielded small sample sizes with limited power to detect such associations.

However, in the GEOS population, we demonstrated that the prothrombin G20210A mutation is a significant risk factor in the youngest population of young-adults with ischemic stroke (age less than the median age of 42 in our study). This suggests that the prothrombin minor A allele has a stronger association among patients with earlier onset strokes. In the youngest group of young-adults with a ‘light’ disease burden, the prothrombin minor A allele could be a significant risk factor for ischemic stroke whereas in older individuals, the progression of disease and other risk factors makes the presence of the prothrombin minor A allele less of a contributing stroke risk factor. In other words, in the absence of traditional risk factors (e.g. smoking, oral contraceptive use, heart disease), the heritability of stroke risk may be enhanced. We also emphasize that from a clinical standpoint it is generally not advocated to perform screening “hypercoagulability workups” in all stroke patients.[29, 30] Typically, patients to be screened for coagulation defects will have a prior history of one or more unexplained thromboembolic events. The yield for diagnosing a hypercoagulable state is typically greatest for young stroke patients or those with a family history of thrombosis and who have no other explanations for their stroke (i.e. cryptogenic stroke). [29, 30]

While the GEOS study is among the largest study to date to have examined the association between prothrombin G20210A and ischemic stroke in young-adults, our study has several limitations. First, the low frequency of prothrombin 20210A minor allele in our study population limits our power to detect an association with ischemic stroke, further limiting subsequent stratified analysis by stroke subtype and vascular risk factors. Furthermore, because of the low frequency of the mutation in non-Caucasians, our study does not provide

useful information about other ethnic subgroups. Second, because all but one of those with the prothrombin 20210A minor allele were heterozygotes, we do have statistical power to evaluate the effect of homozygosity. Third, the population-based design of the GEOS study with recruitment at more than 50 regional hospitals precluded consistent assessment of the presence of patent foramen ovale (PFO) and potential paradoxical embolism among cases. This is important because the prothrombin minor A allele can cause ischemic stroke through venous thrombosis and paradoxical venous-to-arterial embolus through a PFO, or potentially via the PFO in and of itself. In our meta-analysis, we specifically excluded studies that considered only stroke patients with the prothrombin 20210A minor allele and PFO, as risk has been shown to be consistently higher in this setting.[31] However it is important to note that among the studies included in our meta-analyses, the absence or presence of a PFO was not consistently reported. As such, paradoxical embolism may have played a significant role in the pathogenesis of cryptogenic stroke occurring in those studies. Lastly, because blood chemistries were not obtained for the GEOS controls, we were unable to evaluate potential relationships between dislipidemia and the prothrombin 20210A mutation.

All studies included in this meta-analysis were case-control association studies. The case-control approach is an efficient design for studying genetic risk factors for early-onset stroke because the outcome, stroke in young-adults, is rare with a potentially long latency period. Although case-control studies in general can be prone to selection bias, this is unlikely in our study because the exposure is genotype. However, unlike cohort studies, case-control studies may be subject to survival bias because cases characterized by high fatality rates are less likely to be included in the study sample. Lastly, to reduce genotyping error, GEOS cases and controls were plated together for genotyping.

In conclusion, we report that the prothrombin G20210A mutation is associated with ischemic stroke in young-adults and may have an even higher association among the youngest group of young-adults. Specific to the GEOS data, in adults with first ever ischemic stroke before the age of 42, the prothrombin G20210A mutation may be a contributing factor. In PFO cases where a venous source is not identified, positive prothrombin G20210A screening might increase the likelihood that the PFO was involved. Our results suggest the need for the studies included in the meta-analysis to stratify their data by age groups to determine if the association is truly stronger in the younger age ranges, as suggested by the GEOS data.

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a.

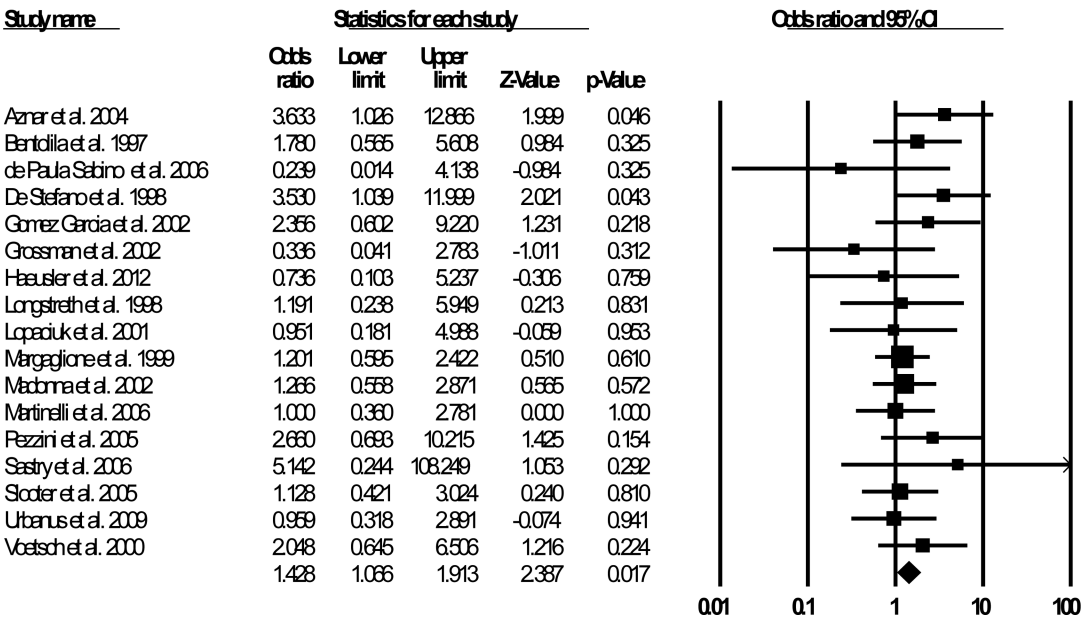

Figure 1a.
Meta-analysis results without GEOS data

b.

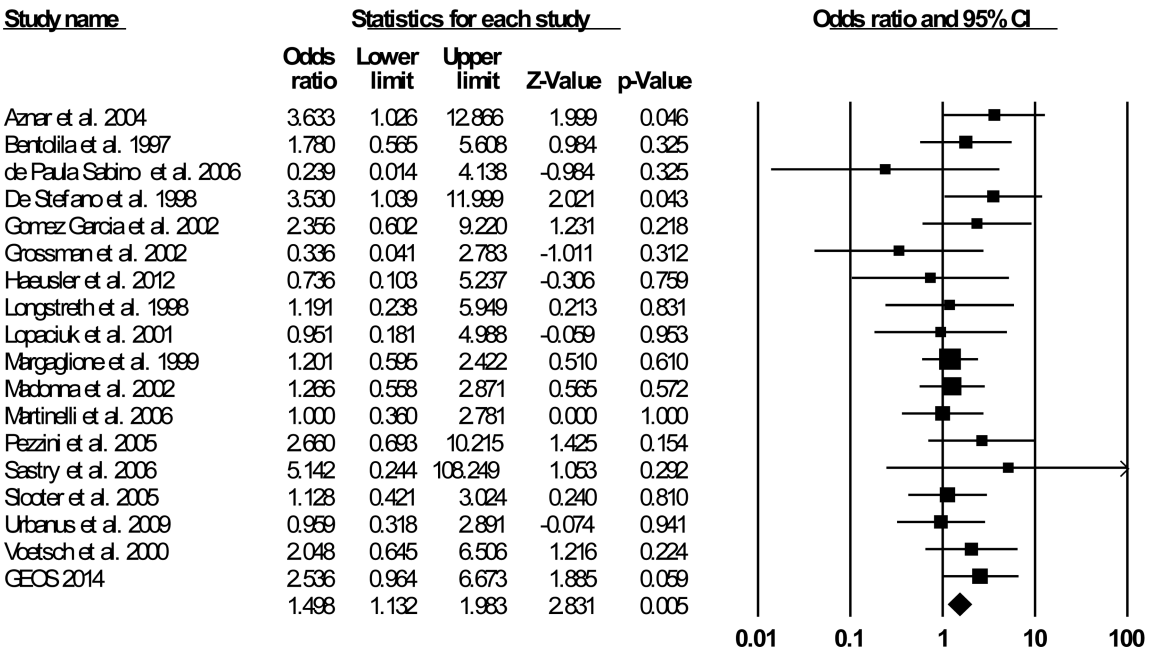

Figure 1b.
Meta-analysis with GEOS data

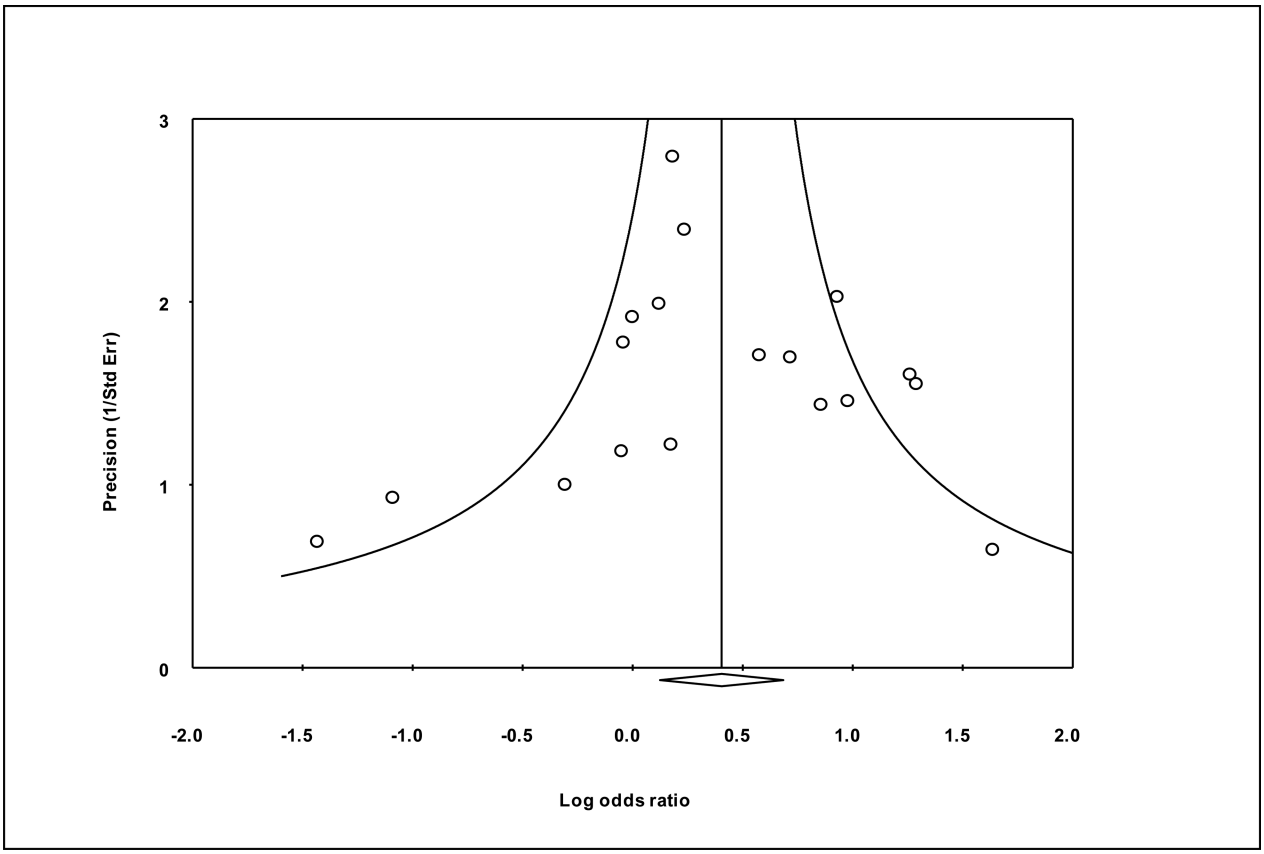

Figure 2.
Funnel plot of precision by log odds ratio

Table 1

Demographic and clinical characteristics of cases and controls of European ancestry in the Genetics of Early Onset Stroke (GEOS) Study.

	Cases (n=397)	Controls (n=426)	P value
Gender, male	62.7%	55.4%	0.03
Mean age, years	41.1	39.4	0.0004
Hypertension	31.7%	16.0%	<.0001
Diabetes Mellitus	11.4%	2.1%	<.0001
Previous MI	5.1%	0.7%	0.0002
Current Oral contraceptive use*	23.0%	10.5%	0.002
Current smoking	42.6%	24.2%	<.0001
History of migraines	33.2%	29.1%	0.21
Migraine with aura	26.8%	20.2%	0.03
Migraine without aura	6.4%	8.9%	0.17
Prothrombin G20210A mutation	**3.5%**	**1.4%**	**0.05**

*women only

Table 2

Odds ratios for Prothrombin G20210A and ischemic stroke in young-adults of European Ancestry as stratified by risk factors.

		No. FII/No. of Cases	No. FII/No. of Controls	OR (basic model)	95%CI	P value (* basic model)	P value (ψ full model)
Entire Population		14/397	6/426	2.5	0.9 - 6.5	0.07	0.07
Gender	Male	11/249	4/236	2.8	0.9 - 8.9	0.09	0.09
	Female	3/148	2/190	1.9	0.3 - 11.8	0.47	0.47
OC use	Yes	1/34	0/20	NE	NE	NE	NE
	No	2/114	2/170	1.7	0.2 - 12.0	0.62	0.45
Current Smoking	Yes	4/169	1/103	2.2	0.2 - 20.3	0.49	0.38
	No	10/228	5/323	3.0	1.0 - 8.8	0.05	0.12
Migraine with Auraξ	Yes	3/105	0/86	NE	NE	NE	NE
	No	10/287	6/340	2.0	0.7 - 5.5	0.20	0.24
Age	**<42**	**8/167**	**2/235**	**5.9**	**1.2 - 28.1**	**0.03**	**0.03**
	≥42	6/230	4/191	1.4	0.4 - 5.1	0.63	0.94

*Includes: age and gender.

ψIncludes: age, gender, hypertension, diabetes, previous myocardial infraction, current oral contraceptive use, current smoking, migraine with aura. NE = not estimable

ξFive case subjects did not have migraine information available.

Table 3

Demographic Characteristics of Studies Included in Meta-Analysis

Author(Year)	Country	N	Males/Females	Ethnicity	Mean Age (case/control)
Aznar(2004)	Spain	343	105/238	White	<50
Bentolila(1997)	France	259	139/120	White	40.6/34
de Paula Sabino(2006)	Brazil	328	72/256	White/black/mestizo	31/33.5
De Stefano(1998)	Italy	270	113/157	White	33.9/49
Gomez Garcia 2002)	Netherlands	136	61/75	White	36/37
Grossman(2002)	Germany	279	138/141	White	36/33
Haeusler(2012)	Germany	326	18/26	White	36/38.5 (median)
Longstreth(1998)	USA	496	0/496	White/black	36.6/37.7
Lopaciuk(2001)	Poland	338	207/131	White	38.1/33.2
Madonna(2002)	Italy	394	183/211	White	38.4/36
Margaglione(1999)	Italy	1238	545/693	White	39/35 (median)
Martinelli(2006)	Italy	398	0/398	White	34.7/34.9
Pezzini(2005)	Italy	321	169/152	White	35.0/34.8
Sastry(2006)	UK	202	*	White	33.2/33.2
Slooter(2005)	Netherlands	960	0/960	White	38.6/39.7
Urbanus(2009)	Netherlands	779	0/779	White	39/39
Voetsch(2000)	Brazil	378	153/217	White/black	33.3/34.1

*Data Not Available