Ischemic Stroke and Six Genetic Variants in *CRP*, *EPHX2*, *FGA*, and *NOTCH3* Genes: A Meta-Analysis

Yeimy González-Giraldo, BSc,* George E. Barreto, MSc, PhD,*†‡ Cristiano Fava, MD, PhD,§‖ and Diego A. Forero, MD, PhD¶#

Background: Ischemic stroke (IS) is a leading cause of death and disability worldwide. As genetic heritability for IS is estimated at about 35%-40%, the identification of genetic variants associated with IS risk is of great importance. The main objective of this study was to carry out a meta-analysis for polymorphisms in *CRP*, *EPHX2*, *FGA*, and *NOTCH3* genes and the risk for IS. *Methods:* Literature search for 6 candidate polymorphisms and IS was conducted using HuGE Navigator, PubMed, and Google Scholar databases. Meta-Analyst program was used to calculate pooled odds ratios (ORs) with a random effects model. *Results:* Twenty-five published studies for 6 candidate polymorphisms were included: *CRP*-rs1800947 (5 studies), *CRP*-rs1205 (3 studies), *EPHX2*-rs751141 (5 studies), *FGA*-rs6050 (6 studies), *NOTCH3*-rs3815188 (3 studies), and *NOTCH3*-rs1043994 (3 studies), for a total number of 7,825 IS cases and 56,532 control subjects. We did not find significant pooled ORs (P values > .05) for any of the genetic variants evaluated in this work. *Conclusions:* Our meta-analysis results did not show significant associations between these 6 polymorphisms in 4 candidate genes and IS, despite the functional role of some of these single nucleotide polymorphisms (e.g., rs6050 in *FGA* gene). Future studies are needed to identify additional main genetic risk factors for IS in different populations. **Key Words:** Candidate gene—genetic factors—ischemic stroke—meta-analysis—polymorphism—risk factor.

From the *Departamento de Nutrición y Bioquímica, Facultad de Ciencias, Pontificia Universidad Javeriana, Bogotá, Colombia; †Universidad Científica del Sur, Lima, Peru; ‡Instituto de Ciencias Biomédicas, Universidad Autónoma de Chile, Santiago, Chile; §Department of Medicine, University of Verona, Verona, Italy; ‖Department of Clinical Sciences, University of Lund, Malmö, Sweden; ¶Laboratory of NeuroPsychiatric Genetics, Biomedical Sciences Research Group, School of Medicine, Universidad Antonio Nariño, Bogotá, Colombia; and #Laboratory of NeuroPsychiatric Genetics, School of Medicine, Universidad Antonio Nariño, Bogotá, Colombia.

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Address correspondence to Diego A. Forero, MD, PhD, Laboratory of NeuroPsychiatric Genetics, School of Medicine, Universidad Antonio Nariño, Bogotá, Colombia. E-mail: diego.forero@uan.edu.co.

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Introduction

Ischemic stroke (IS) is a multifactorial disease and a leading cause of death and disability worldwide,[1] as it generates large negative effects on health and economy, such as long-term disability and high economic costs.[2] Increased risk for IS is attributable to environmental and genetic factors, which can be prevented by lifestyle changes in most cases. A genetic predisposition for an ischemic event might predispose to IS during the adulthood. In this context, there has been a great interest in the study of genetic factors for IS,[1,3] given the fact that heritability for IS is estimated at about 35%-40%.[4]

Alterations in coagulation and inflammation mechanisms are involved in the development of IS,[5] in which some of these dysfunctions might be produced by inherited changes in molecules that participate in those processes.[6-8] Single nucleotide polymorphisms (SNPs) in genes that encode proteins involved in coagulation and inflammation mechanisms have been proposed as

promising candidates for IS.[1] Previous meta-analyses for commonly studied candidate genes have identified significant associations of IS with variants in *F5*, *ACE*, *APOE*, *MTHFR*, and *FGB* genes (Table S1).[9-14]

In addition, an increasing number of recent studies have focused on other candidate genes (such as *CRP*, *EPHX2*, *FGA*, and *NOTCH3*), with some conflicting results,[15-19] suggesting that additional meta-analyses studies are needed to address possible associations of these genes with the pathogenesis of IS.[2] The main objective of this study was to carry out a meta-analysis of published studies for 6 polymorphisms in *CRP*, *EPHX2*, *FGA*, and *NOTCH3* genes and the risk for IS.

Materials and Methods

Literature Search

This meta-analysis was conducted following the recommendations of the PRISMA statement.[20] We used HuGE Navigator database[21] for an initial search of genetic association studies for stroke; PubMed and Google Scholar databases were used to complete and refine the search of studies that analyzed the association between the following genetic variants and IS: *CRP* (rs1800947, rs1205), *EPHX2* (rs751141), *FGA* (rs6050), and *NOTCH3* (rs3815188, rs1043994) (Table 1). We combined the name and symbol of the genes: *CRP* (C-reactive protein); *EPHX2* (epoxide hydrolase 2); *FGA* (fibrinogen alpha chain); and *NOTCH3* with the terms "stroke" and "ischemic stroke" for the literature search.

Selection Criteria

We included original articles that were published in English in peer-reviewed journals, describing case–control and cohort studies that evaluated the association between the selected candidate genes and IS. Studies that evaluated IS recurrence, pharmacogenetics, or comparison between subtypes were excluded.

Data Extraction

Information of each study was extracted by 2 independent investigators, including the following data: first author, publication year, country, ethnicity, sample size, percent of male subjects, mean ages, genotyping methods, Hardy–Weinberg equilibrium in controls, and allele and genotype frequencies.[22,23] The authors of primary articles were contacted by email if genotype frequencies were not available in the text of the respective publications.

Statistical Analysis

The Meta-Analyst program[24] was used for meta-analytical procedures, as previously described.[25] Odds ratios (ORs) and 95 percent confidence intervals were calculated for each study, and pooled ORs for each gene were calculated using a random effects model, following previous recommendations for meta-analyses of genetics studies.[26,27] Heterogeneity between studies was evaluated with the I^2 test. Three genetic models were tested (dominant, recessive, and allelic models), and forest plots were generated to show the pooled ORs for each model analyzed, as previously described.[28]

Results

An initial screening identified 48 eligible studies for the 6 candidate polymorphisms and, after applying inclusion and exclusion criteria, 25 studies were included in this meta-analysis (Fig S1): *CRP*-rs1800947 (5 studies)[15,16,29-31]; *CRP*-rs1205 (3 studies)[16,29,32]; *EPHX2*-rs751141 (5 studies)[17,33-36]; *FGA*-rs6050 (6 studies)[7,18,37-40]; *NOTCH3*-rs3815188 (3 studies); and *NOTCH3*-rs1043994 (3 studies),[19,41,42] for a total number of 7,825 IS cases and 56,532 control subjects. General information about the studies included in the analysis is shown in Table 2 and allele and genotype frequencies are shown in Table S2.

Three genetic models (dominant, recessive, and allelic) were tested for the 4 SNPs in *CRP*, *FGA*, and *EPHX2* genes. For the 2 candidate SNPs in *NOTCH3* gene, only the allelic model was tested (genotype frequencies were not available). Pooled ORs, using a random effects model, did not show significant associations for the 6 polymorphisms in the 4 candidate genes (P values > .05) and the risk for IS (Fig 1 and Table S3). Finally, a subgroup analysis was carried out for *EPHX2* and *CRP* genes (case–control and

Table 1. *Details about selected candidate polymorphisms*

Gene	dbSNP ID	Alias	Gene region	Genomic position	Protein effect	MAF CEU
C-reactive protein	rs1800947	1059 G/C	Exon 2	159.683.188	Leu184Leu	.067
C-reactive protein	rs1205	1846 G/A	3′UTR	159.681.983	—	.341
Epoxide hydrolase 2	rs751141	—	Exon 8	27.373.865	Arg287Gln	.098
Fibrinogen alpha chain	rs6050	—	Exon 5	155.507.590	Thr312Ala	.237
Notch 3	rs3815188	381 C/T	Exon 3	15.303.225	Thr101Thr	.158
Notch 3	rs1043994	684 G/A	Exon 4	15.302.844	Ala202Ala	.157

Abbreviations: CEU, Utah residents (CEPH) with Northern and Western European ancestry; MAF, minor allele frequency.