

# Large-scale meta-analysis of genetic studies in ischemic stroke: Five genes involving 152,797 individuals

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**BACKGROUND:** Ischemic stroke descent has a genetic basis. Stroke represents a complex trait, which is assumed to be polygenic. On this topic, the role of a wide number of candidate genes has been investigated in stroke through association studies.

**MATERIALS AND METHODS:** We performed a literature-based systematic review of genetic association studies in stroke about several populations. Odds ratios (ORs) and 95% confidence intervals (CIs) were determined for each gene-disease association. Following a review of 300 manuscripts, five candidate gene variants were analyzed among 152,797 individuals (45,433 cases and 107,364 controls).

**RESULTS:** For these five candidate genes studied, the prothrombin OR is 1,57 (1,23-2,89), the factor V Leiden OR is 1,43 (0,67-6,24), the mean OR of angiotensin I converting enzyme (ACE) insertion/deletion (I/D) polymorphism is 1,11 (1,02-1,25), the summary OR for the C677T variant of 5,10-methylenetetrahydrofolate reductase (MTHFR) is 1,23 (0,61-1,47) and the pooled OR for the apolipoprotein E (APOE) gene is 0,95 (0,77-1,14).

**CONCLUSION:** These data suggest the genetic associations of some genes with ischemic stroke and it is necessary to compete with other genes. Our findings could represent an epidemiological base and a useful tool to address further molecular investigations and to realize more detailed meta-analyses.

**Key words:** Angiotensin I converting enzyme, apolipoprotein E, factor V Leiden, factor II prothrombin, meta-analysis, MTHFR, stroke

## Introduction

Stroke is believed to be a complex multifactorial and polygenic disease arising from a wide number of gene-gene and gene-environment interactions. Genetic factors could act by predisposing to conventional risk factors. Studies on stroke genetics present some methodological difficulties. Therefore, the major line of multifactorial stroke investigation is the candidate-gene approach, which consists of identifying molecular variants within a functional relevant gene and establishing its function in stroke risk by association case-control. Following some reports of positive association ischemic disease, a wide number of candidate genes have been investigated in stroke, even if, so far, only a few polymorphisms have been consistently associated with stroke occurrence. Most of these studies have been criticized for some bias related to small sample size, lack of classification by stroke phenotype or subtype, use of ethnically different populations, and unmatched controls (Flossmann *et al.*, 2004<sup>[1]</sup>; Dichgans and Markus *et al.*, 2005<sup>[2]</sup>). The differences in patient's characteristics together with the heterogeneity in study design, could explain much of the inconsistency between studies (Dichgans *et al.*, 2007<sup>[3]</sup>).

Although association studies are considered as a powerful instrument to identify risk factors, both methodological and appropriate studies and replication of results are necessary to demonstrate a causal relationship between a genetic marker and stroke. Therefore, the results of existing studies identified a list of possible candidate genes associated with stroke (Meschia *et al.*, 2005<sup>[4]</sup>). In our work, we want to provide

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a meta analysis reporting studies published on genetic of stroke, and we analyzed only studies in which genotype frequency was reported. In our literature revision, we focused on ischemic stroke, excluding association analyses on hemorrhagic stroke patients.

The aim of this work is to give a panel of possible genes associated with ischemic stroke risk that could be useful both for future epidemiological data and for deciding new research strategies for stroke genetics studies.

## Materials and Methods

Electronic databases (MEDLINE, EMBASE, PUBMED, SCOPUS...) were searched up until 2010 for all case-control studies evaluating any candidate gene and stroke. The medical subject headings terms and text words used for the search were *cerebrovascular disease*, *stroke*, *cerebral ischemia*, and *brain infarction* in combination with *polymorphism(s)*, *mutation*, *genetic*, *genotype*, or *genes*. All languages were searched initially, but only English language articles were selected. The references of all computer- identified publications were searched for any additional studies, and the MEDLINE option *related articles* was used for all relevant articles. In addition, a search to identify previous genetic meta-analyses in stroke was also performed. Studies were selected if neuroimaging (magnetic resonance imaging or computed tomography) had been used to confirm the diagnosis of ischemic stroke. Studies were excluded if the patients were children (aged 18 years), quantitative traits or intermediate phenotypes were being investigated, or genotype frequency was not reported. For duplicate publications, the smaller data set was discarded. First search generated 300 relevant articles, of which 60 met the inclusion criteria. Data for analysis were extracted and entered into separate databases by two of us (K.H. and A.T).

### Data synthesis:

Thirty two candidate gene case-control studies in which the presence or absence of stroke were analyzed. In total, 5 polymorphisms in 5 genes were identified. From the 5 polymorphisms analyzed in detail for a total of 152797 individuals (representing 45433 cases

and 107364 controls), the mean number of studies per candidate gene was 12. The ACE I/D polymorphism was evaluated in 12 studies (7709 cases and 17284 controls), and a summary OR of 1.11 (95% CI, 1.02-1.25;  $P = .002$ ), under a fixed-effects model, was observed for individuals homozygous for the *D* allele compared with heterozygous (*D/I*) and homozygous (*I/I*) individuals combined [Table 1]. The prothrombin G20210A mutation was evaluated in 12 studies, with a total of 5644 cases and 13459 controls. The summary OR under a fixed-effects model showed that carriers of the mutation were 1.57 times more likely to develop stroke (95% CI, 1.23- 2.89;  $P = .005$ ) [Table 2]. The Factor V Leiden G1691A mutation was evaluated in 12 studies, with a total of 7749 cases and 26101 controls. The summary OR under a fixed-effects model showed that carriers of the mutation were 1.43 times more likely to develop stroke (95% CI, 0.67- 6.24;  $P = .005$ ) [Table 3]. A total of 12 studies (13657 cases and 17090 controls) were identified that evaluated the polymorphism in the gene encoding methylenetetrahydrofolate reductase where cytosine is replaced by thymidine at base position 677 of the gene (*MTHFR*C677T). A summary OR, under the fixed-effects model, of 1.23 (95% CI, 0.61-1.47;  $P = .001$ ) was observed for individuals homozygous for the *T* allele compared with *C* allele carriers (*C/T* plus *C/C*) [Table 4]. The apolipoprotein E genotype has been by far the most

**Table 1: Results of published studies of the association between the ACE I/D polymorphism and ischemic stroke. Odds ratios for the outcome compared individuals homozygous for the *D* allele with those with the heterozygous (*D/I*) plus wild type (*I/I*). CI indicates confidence interval.**

ACE			
Publication	Cases	Controls	OR
Roshan Ariyaratnam <i>et al</i> 2007	1042	2530	1,90(1,23-2,93)
Roshan Ariyaratnam <i>et al</i> 2007 <sup>[5]</sup>	644	957	1,74(0,88-3,42)
Szolnoki <i>et al</i> <sup>[6]</sup>	689	652	1,29 (1,00-1,65)
Peterlin <i>et al</i> <sup>[7]</sup>	124	165	1,41(0,85-2,34)
Markus <i>et al</i> 1995 <sup>[8]</sup>	101	188	1,98 (1,11-3,51)
Kostulas <i>et al</i> 1999 <sup>[9]</sup>	100	93	0,99 (0,54-1,84)
Pfohl <i>et al</i> 1998 <sup>[10]</sup>	91	297	0,94 (0,57-1,55)
Zee <i>et al</i> 1999 <sup>[11]</sup>	338	338	1,10 (0,80-1,51)
Casas <i>et al</i> <sup>[12]</sup>	2990	11305	1,21 (1,08-1,35)
Zhang <i>et al</i> 2004 <sup>[13]</sup>	151	150	2,13 (1,10-4,13)
Szolnoki <i>et al</i> <sup>[6]</sup>	407	295	1,3 (0,7-2,1)
Ueda <i>et al</i> 1995 <sup>[14]</sup>	488	73	1,26 (0,84-1,88)
Cato <i>et al</i> 1996 <sup>[15]</sup>	406	137	0,94 (0,65-1,35)
Kario <i>et al</i> 1996 <sup>[16]</sup>	138	104	2,44 (1,31-4,55)
Total	7709	17284	1,11 (1,02-1,25)

**Table 2: Results of published studies of the association between the prothrombin G20210A polymorphism and ischemic stroke. Odds ratios for the outcome compared carriers of the A allele with those with wild type (G/G). CI indicates confidence interval.**

FII			
Publication	Cases	Controls	OR
Pezzini <i>et al</i> <sup>[17]</sup>	125	149	2.03 (0.47-8.66)
Szolnoki <i>et al</i> <sup>[6]</sup>	689	652	1.18 (0.32-4.43)
Smiles <i>et al</i> <sup>[18]</sup>	182	453	1.25 (0.46-3.39)
Gomez Garcia <i>et al</i> <sup>[19]</sup>	49	87	2.36 (0.60-9.23)
Hankey <i>et al</i> <sup>[20]</sup>	219	205	1.91 (0.56-6.43)
Lopaciuk <i>et al</i> <sup>[21]</sup>	100	238	0.95 (0.18-4.99)
Egan <i>et al</i> <sup>[22]</sup>	38	635	0.60 (0.03-10.26)
Voetsch <i>et al</i> <sup>[23]</sup>	114	119	2.15 (0.52-8.80)
Margaglione <i>et al</i> 1999 <sup>[24]</sup>	202	1036	1.20 (0.59-2.43)
Ridker <i>et al</i> 1999 <sup>[25]</sup>	259	1774	1.10 (0.57-2.10)
Iniesta <i>et al</i> 1999 <sup>[26]</sup>	124	202	0.40 (0.04-3.64)
Casas <i>et al</i> <sup>[12]</sup>	3028	7131	1.44 (1.11-1.86)
Nowak-Gottl <i>et al</i> 1999 <sup>[27]</sup>	148	296	4.7 (1.4-15.6)
Berge <i>et al</i> 2007 <sup>[28]</sup>	367	482	2.25 (0.61-8.90)
Total	5644	13459	1.57(1.23-2.89)

**Table 4: Results of published studies of the association between the methylenetetrahydrofolate reductase C677T polymorphism and ischemic stroke. Odds ratios for the outcome compared individuals homozygous for the T allele (T/T) with those heterozygous individuals (C/T) plus wild type (C/C). CI indicates confidence interval.**

MTHFR			
Publication	Cases	Controls	OR
Roshan Ariyaratnam <i>et al</i> 2007 <sup>[5]</sup>	1859	2380	1.18 (0.90-1.56)
Roshan Ariyaratnam <i>et al</i> 2007 <sup>[5]</sup>	478	541	1.34 (0.87-2.06)
Pezzini <i>et al</i> <sup>[17]</sup>	125	149	1.51 (0.82-2.79)
Szolnoki <i>et al</i> <sup>[6]</sup>	689	652	1.38 (0.94-2.00)
Pezzini <i>et al</i> <sup>[35]</sup>	31	36	1.19 (0.27-5.19)
Madonna <i>et al</i> <sup>[29]</sup>	132	262	1.42 (0.84-2.38)
McIlroy <i>et al</i> <sup>[36]</sup>	63	71	0.56 (0.05-6.29)
Lopaciuk <i>et al</i> <sup>[21]</sup>	100	238	1.11 (0.54-2.30)
Topic <i>et al</i> <sup>[37]</sup>	56	124	17.57 (2.11-146.58)
Voetsch <i>et al</i> <sup>[23]</sup>	114	119	1.31 (0.62-2.81)
Eikelboom <i>et al</i> <sup>[38]</sup>	215	205	1.04 (0.57-1.90)
Gross <i>et al</i> <sup>[39]</sup>	84	112	1.29 (0.59-2.85)
Casas <i>et al</i> <sup>[12]</sup>	3387	4597	1.24 (1.08-1.42)
Casas <i>et al</i> <sup>[12]</sup>	6324	7604	1.26 (1.14-1.40)
Total	13657	17090	1.23(0.61-1.47)

investigated, with 12 studies that included 10674 cases and 33430 controls. Carriers of the factor ApoE E4 allele were 0.95 times to develop stroke (95% CI, 0.77-1.14;  $P=0.002$ ) [Table 5].

## Comments

In this comprehensive meta-analysis, 3 (47%) of the 5 candidate polymorphisms analyzed significantly increased the risk of stroke. In 24 of these studies (*ACE* I/D, factor V Leiden, *MTHFR* C677T, prothrombin

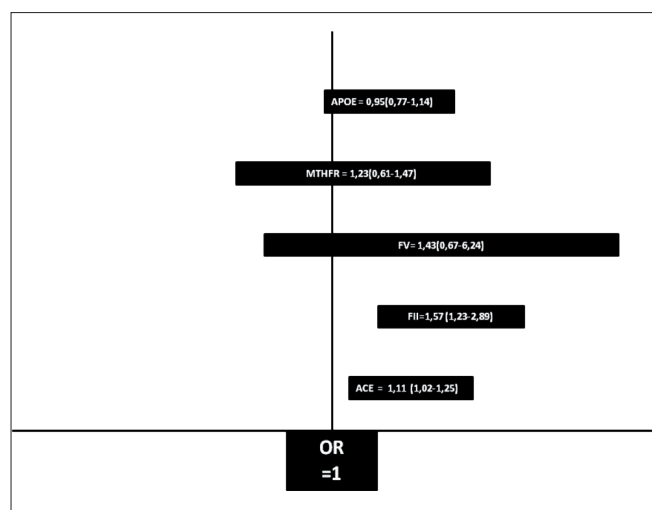
**Table 3: Results of published studies of the association between the factor V Leiden mutation and ischemic stroke. Odds ratios for the outcome compared carriers of the Gln506 allele vs wild type (Arg/Arg). CI indicates confidence interval.**

FV			
Publication	Cases	Controls	OR
Pezzini <i>et al</i> <sup>[17]</sup>	125	149	1.45 (0.43-4.88)
Szolnoki <i>et al</i> <sup>[6]</sup>	689	652	1.54 (1.03-2.30)
Madonna <i>et al</i> <sup>[29]</sup>	132	262	0.81 (0.33-2.00)
Juul <i>et al</i> <sup>[30]</sup>	231	7907	0.92 (0.56-1.52)
Zunker <i>et al</i> <sup>[31]</sup>	471	112	0.95 (0.38-2.38)
Lopaciuk <i>et al</i> <sup>[21]</sup>	100	238	0.71 (0.19-2.62)
Hankey <i>et al</i> <sup>[20]</sup>	219	205	2.40 (0.74-7.79)
Voetsch <i>et al</i> <sup>[23]</sup>	114	119	0.73 (0.23-2.38)
Casas <i>et al</i> <sup>[12]</sup>	4588	13798	1.33 (1.12-1.58)
Iniesta <i>et al</i> 1999 <sup>[26]</sup>	124	202	0.98 (0.23-4.16)
Margaglione <i>et al</i> 1999 <sup>[24]</sup>	202	1036	4.03 (2.46-6.60)
Grossmann <i>et al</i> 2002 <sup>[32]</sup>	93	186	3.19 (1.38-7.39)
Lalouschek <i>et al</i> <sup>[33]</sup>	468	468	8.8 (2.00-38)
Slooter <i>et al</i> 2005 <sup>[34]</sup>	193	767	1.8 (0.9-3.6)
Total	7749	26101	1.43(0.67-6.24)

**Table 5: Results of published studies of the association between the apolipoprotein E polymorphism and ischemic stroke. Odds ratios for the outcome compared carriers of the ε4 allele with those with the ε3 and ε2 alleles. CI indicates confidence interval.**

APOE			
Publication	Cases	Controls	OR
Roshan Ariyaratnam <i>et al</i> 2007 <sup>[5]</sup>	419	477	2.18 (1.52-3.13)
Roshan Ariyaratnam <i>et al</i> 2007 <sup>[5]</sup>	495	1304	1.51 (0.93-2.45)
McIlroy <i>et al</i> <sup>[36]</sup>	64	71	0.93 (0.38-2.24)
Topic <i>et al</i> <sup>[37]</sup>	56	124	2.45 (0.91-6.55)
MacLeod <i>et al</i> 2001 <sup>[40]</sup>	266	225	0.70 (0.47-1.04)
Frikke-Schmidt <i>et al</i> 2001 <sup>[41]</sup>	286	8938	1.12 (0.87-1.44)
Catto <i>et al</i> 2000 <sup>[24]</sup>	515	289	0.88 (0.64-1.21)
Margaglione <i>et al</i> 1998 <sup>[15]</sup>	108	1000	0.32 (0.16-0.66)
Kessler <i>et al</i> 1997 <sup>[42]</sup>	220	223	1.18 (0.76-1.84)
Sudlow <i>et al</i> 2006 <sup>[43]</sup>	5961	17965	1.11 (1.01-1.22)
Banerjee <i>et al</i> 2007 <sup>[44]</sup>	988	1441	1.47 (1-2.15)
Pezzini <i>et al</i> 2004 <sup>[45]</sup>	124	147	2.29 (1.10-4.76)
Baum <i>et al</i> 2006 <sup>[46]</sup>	246	336	1.8 (1.2-2.7)
McCarron <i>et al</i> 1999 <sup>[47]</sup>	926	890	1.68 (1.36-2.09)
Total	10674	33430	0.95(0.77-1.14)

G20210A, and apolipoprotein E), the number of cases included per gene was more than 1000, allowing more precise estimates to be made of the effect of these genes than from any single study. Most candidate genes assessed in stroke thus far have been evaluated initially for their potential role in ischemic disease. Therefore, up to now, most genetic studies have focused on genes involved in thrombosis and coagulation (factor V Leiden, prothrombin, and *MTHFR*, also genes regulating other well-established risk factors for stroke (e.g., hypertension, diabetes mellitus, and hyperlipidemia (*ACE* and apolipoprotein E) are studied.



**Figure 1: summary of the results of the five genes studies in the meta analysis (ACE, MTHFR, FVL, FII AND APOE)**

The limits of the box presents the extreme values of OR

The factor V Leiden mutation causes activated protein C resistance. Activated protein C limits clot formation by proteolytic inactivation of factors Va and VIIIa, and the single point mutation in the gene for factor V (1691G→A) studied predicts replacement of arginine by glycine at position 506 in the activated protein C cleavage site. After activation, the mutated factor V is less efficiently degraded by activated protein C than normal factor V, resulting in increased thrombin generation and a hypercoagulable state, with a 5-10-fold increased risk of thrombosis in heterozygotes and a 50-100-fold increased risk in homozygotes which may explain the increased risk of stroke in carriers of this mutation observed in this study (OR=1.43 (0.67-6.24)). In our study, the risk factor is varying from 0.19 found by Lopaciuk *et al.*<sup>[21]</sup> to 38 found in the series of Lalouschek *et al.*<sup>[33]</sup> Those results can be explained by the large difference of the A1691G allelic frequency in several populations.

A sequence variation in the 3'-untranslated region of the prothrombin gene (G20210A), which alters messenger RNA stability is associated with elevated prothrombin levels and thrombin formation and may similarly lead to a procoagulant state. A carrier frequency around 3.0% has been detected in southern Europe, whereas the prothrombin variant is very rare in non-Caucasians (OR=1.57 (1.23-2.89)), the extreme values of OR are unregistered in same the work of Egan *et al.*<sup>[9]</sup> 2000 (OR (0.03-10.26)).

Plasma and intracellular levels of ACE have been shown to be partly determined by the presence of the ACE I/D polymorphism in healthy individuals and in patients with stroke. Individuals homozygous for the D allele have a 56% increase in ACE activity compared with I allele homozygotes. 121 Angiotensin-converting enzyme converts angiotensin I to angiotensin II, which is known to be involved in vascular hypertrophy, vasoconstriction, and atherosclerotic processes (OR=1.11 (1.02-1.25)). In the 12 works studied in our meta-analysis, the OR is sensibly varying between 0.5 and 2.

Long-term differences of 5 µmol/L in the serum concentration of homocysteine are associated with a 59% increase in the risk of stroke. The C677T mutation in the MTHFR gene, which encodes an amino acid substitution (A222V), renders the enzyme thermolabile and reduces metabolism of homocysteine (OR=1.23 (0.61-1.47)). The MTHFR remains the most controversial genetics factor with an average of OR from 0.05 in McIlroy *et al.*<sup>[36]</sup> to 146.58 in the work of Topic *et al.*<sup>[37]</sup> Carriers of the apolipoprotein E ε4 allele, which affects serum cholesterol, and which has been associated with a moderate increase in the risk of coronary heart disease, are at a substantially higher risk of stroke (OR=0.95 (0.77-1.14)).

The results of our meta-analysis about the five stroke genes are summarized in Figure 1.

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