

## ADVANCES IN THE GENETIC BASIS OF ISCHEMIC STROKE NOVINE NA POLJU GENETIKE ISHEMIJSKOG MOŽDANOG UDARA

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**Summary:** As one of the leading causes of death within both the developed and developing world, stroke is a worldwide problem. About 80% of strokes are ischemic. It is caused by multiple genetic factors, environmental factors, and interactions among these factors. There is a long list of candidate genes that have been studied for a possible association with ischemic stroke. Among the most widely investigated genes are those involved in haemostasis, inflammation, nitric oxide production, homocysteine and lipid metabolism, renin-angiotensin-aldosterone system. Combined linkage/association studies have demonstrated that genes encoding PDE4D and ALOX5AP confer risk for stroke. We review the studies of these genes which may have potential application on the early diagnosis, prevention and treatment ischemic stroke patients.

**Keywords:** allele, candidate genes, ischemic stroke, polymorphism

**Kratak sadržaj:** Kao jedan od vodećih uzroka smrtnosti kako u razvijenim tako i u zemljama u razvoju, moždani udar je svetski problem. Oko 80% svih moždanih udara čini ishemijski moždani udar. U patogenezi moždanog udara važnu ulogu imaju genetski faktori, faktori sredine i njihova interakcija. Veliki broj gena-kandidata povezuje se sa ishemijskim moždanim udarom. Geni kandidati koji se ispituju u moždanom udaru dele se u nekoliko grupa u zavisnosti od toga da li utiču na hemostazu, inflamaciju, sintezu azot monoksida, metabolizam homocisteina i lipida, renin-angiotenzim-aldosteron sistem. Kombinovanjem linkidž/asocijacionih studija utvrđeno je da geni koji kodiraju PDE4D i ALOX5AP doprinose riziku od dobijanja moždanog udara. U daljem tekstu biće prikazani rezultati studija koje su se bavile ispitivanjem ovih gena, a koji bi mogli da imaju potencijalnu primenu u ranoj dijagnostici, prevenciji i lečenju pacijenata sa ishemijskom bolešću mozga.

**Ključne reči:** alel, geni kandidati, ishemijski moždani udar, polimorfizam

### Introduction

Stroke is among the leading causes of death in the world and common cause of disability. Its incidence is rising with increasing life expectancy, although about 20% of strokes occur before the age of 65.

There are two main clinical phenotypes of stroke: ischemic stroke which is responsible for 80–90% of stroke events, and hemorrhagic stroke which is responsible for the remaining 10–20%. Ischemic stroke is due to a complete occlusion of a cerebral artery, which

might be caused by a local atherosclerotic process in the brain or by an embolic or cardiogenic event. Ischemic stroke has further subphenotypes, which include large vessel and small vessel occlusive disease. Genetic causes of stroke range from classic Mendelian (a single gene leads to disease) to complex (multiple genes contribute to risk for stroke in combination with other genetic and/or environmental factors).

The first approach to stroke genes focuses on the rare Mendelian forms of stroke. This approach uses the method of linkage mapping of large families that display a Mendelian pattern of inheritance, followed by the resequencing of coding exons within the linkage peak for highly penetrant missense or nonsense mutations. The Mendelian approach has resulted in discovering several monogenic stroke genes.

The basis for human studies into putative causative genes involved in ischemic stroke has taken the form of the candidate gene approach. This involves

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selecting a functionally relevant gene to study, genes that might be involved in the development of the intermediate phenotype (e.g. atherosclerosis and carotid intima-media thickening) or the medical risk factors (e.g. hypertension, hyperlipidemia and diabetes). The next step is identification of an SNP or an SNP haplotype that shows a significantly higher frequency (increased susceptibility, increased risk) or a lower frequency (decreased susceptibility, protective effect against disease) in a population of patients than in a population of matched, normal control subjects (case-control studies). To date, a large number of candidate genes have been investigated for association to stroke on the basis of their effect on processes such as hemostasis, inflammation, nitric oxide synthesis, on the renin angiotensin-aldosterone system, homocystein or lipid metabolism (1, 2).

Recently, there has been increasing interest on combined linkage/association approaches. Using this approach, putative genes directly associated with common polygenic stroke have been identified. A recent study carried out on an Icelandic population demonstrated that the PDE4D gene encoding for phosphodiesterase 4D, and ALOX5AP gene encoding 5-lipoxygenase activating protein (FLAP) are the susceptibility factors for ischemic stroke (3, 4).

Significant advances have been recently made in identifying disease-causing genes and susceptibility genes for ischemic stroke. This review highlights the most important discoveries made in past few years.

### **Association studies in ischemic stroke: coagulation and fibrinolytic system**

Various coagulopathies can cause stroke. Genes related to the coagulation system are logical candidates for genetic susceptibility studies. Protein S and protein C deficiency are rare causes of early onset ischemic stroke. Other coagulation factors have been studied in large cohorts. The most investigated candidate genes (mutations) affecting haemostasis in patients with ischemic stroke were: factor V R506Q (factor V Leiden), factor VIIR353Q, factor XIIIIV34L, prothrombin G20210A,  $\beta$ -fibrinogen 148C/T, plasminogen activator inhibitor-1 4G/5G and platelet glycoprotein receptors (Gpla/Ila, Gplb, GplIb/IIIa, GPVI) genes.

Association of the factor V Leiden mutation with ischemic stroke has been by far the most investigated. The most case-control studies have failed to find an association. From 2001–2004 four meta-analysis revealed the association of factor V Leiden mutation and ischemic stroke; only two of them found a positive association (5–7). Wu and Tsongalis (8) reported an odds ratio for adult cerebrovascular events of 1.43 (95% CI, 1.03–1.97). In recent meta analysis (9) with 26 studies that included 4588 cases and 13798 controls, carriers of the factor V Gln506 allele were 1.33

times more likely to develop stroke (95% CI, 1.12–1.58;  $P=0.001$ ). Except the most common studies polymorphism in the codon 353 in exon 6 of factor VII gene, four polymorphisms in this factor (–401G/T, –402G/A, 5' F7, IVS7) were analyzed in a few association studies in ischemic stroke. Funk et al. (10) found that individuals carrying rare allele –402G/A have increased factor VII plasma levels and an increased risk for developing transient ischemic attack and acute ischemic cerebrovascular events before the age of 60 years. Meta analysis of Casas et al. (9) on a small sample size (545 cases and 504 controls), pooled from 3 studies (–323I/D (A1/A2) polymorphism) confirmed the negative association of this polymorphism with ischemic stroke risk (OR 1.11 (95% CI, 0.83–1.48)). Lack of the association between polymorphism in the factor XIII gene (Val34Leu) and ischemic stroke is evident in numerous studies (11–14). A large, well-matched case-control study of cerebral infarction reported a major protective effect of Leu34, with interactions with smoking that modified risk of stroke (15). No significant association were observed for factor XIII genes with large data sets in meta analysis of Casas et al. (9) which included 6 studies with 2166 cases (OR 0.97; 95% CI 0.75–1.25;  $P=0.80$ ). More than 30 studies examined the association between prothrombin G20210A mutation and ischemic stroke, almost with negative results. Casas et al. (9) pooled the data from 19 studies which have analyzed prothrombin G20210A mutation in total of 3028 cases and 7131 controls. The summary OR under a fixed-effects model showed that carriers of the mutation were 1.44 times more likely to develop stroke (95% CI, 1.11–1.86;  $P=0.006$ ). Meta analysis of Kim et al. (16) has confirmed this association in younger patients. The studies which examined the relationship between  $\beta$ -fibrinogen gene –148C/T polymorphism and ischemic stroke gave almost positive results. Recently, meta-analysis (1223 patients and 1433 controls) of relationship between this gene polymorphism and stroke has showed that there was 32% increased risk of cerebral infarction for the genotypes (C/T+T/T) compared with the wild C/C homozygotes. Thus, the allele T might be a genetic risk factor to increase susceptibility to cerebral infarction at the protein and genetic levels (17). Polymorphisms of the gene encoding beta-fibrinogen have been shown to correlate with either large-vessel stroke or carotid IMT, which is consistent with plasma levels of fibrinogen correlating with risk for future stroke. Recently published studies examined the other polymorphism in the proximal promoter region of beta-fibrinogen gene (–455G/A) have not shown a consistent association to stroke (18). The relationship between 4G/5G polymorphism of the PAI-1 gene and stroke is unclear. It has been reported that the 4G/4G genotype confers an increased risk of stroke, but other investigator have reported the same genotype to be neutral or even protective in terms of stroke risk. The newest meta-analysis of Casas et al. (9) which pooled the results obtained from 4 studies (842 cases, 1189

controls) has noticed the positive association of 4G/5G polymorphism in promoter of the PAI-1 gene (OR, 1.47; 95% CI, 1.13–1.92;  $P=0.004$ ). Recently, in the meta-analysis (11 studies; 2562 cases/3560 controls) Attia et al. (19) obtained the pooled ORs for PAI-1 4G/4G versus 5G/5G and 4G/5G vs 5G/5G (OR (95%CI): 0.89 (0.66–1.20), 0.99 (0.85–1.15), respectively). Tsantes and coauthors (20) performed a meta-analysis of eighteen studies (15 case-control and three cohort studies, 3104 cases/4870 controls) about PAI-1 4G/5G polymorphism and risk of ischemic stroke. It failed to demonstrate a significant association between the 4G/5G polymorphism and ischemic stroke (OR 0.848 (95%CI 0.662–1.087)). The role of platelet glycoprotein receptor polymorphisms has also been studied extensively in patients with ischemic stroke. Meta-analysis of Nikolopoulos and coauthors (7 independent studies) (21) do not support positive association between the C807T polymorphism and stroke neither in the group of patients as a whole nor in any relevant subgroup does indicating that increase in GPIIb/IIIa surface levels do not contribute to an increased thrombotic risk. In meta-analysis, Casas et al. (9) analyzed 5 polymorphisms in genes for platelet glycoprotein receptors. Genetic marker associated with an increase in the risk of stroke, but for which the data set was much smaller was GPIb ((Thr/Met or human platelet antigen (HPA) type 2 (HPA2) (564 cases; OR, 1.55; 95% CI, 1.14–2.11;  $P=0.006$ )) with no evidence for heterogeneity in meta-analysis. Meta-analysis of 3 studies of GPIbA Kozak sequence gave positive results (350 cases; OR, 1.88; 95% CI, 1.28–2.76;  $P=0.001$ ), but studies were highly heterogeneous. No significant associations were observed in three meta-analysis: GPIbA (VNTR) of 816 cases, glycoprotein IIb IIIa Leu33Pro or HPA1 (1467 cases; OR, 1.11; 95% CI, 0.95–1.28;  $P=0.20$ ).

Only few newer studies examined the association of polymorphisms in tissue-type plasminogen activator (t-PA) gene, thrombin activable fibrinolysis inhibitor (TAFI), von Willebrand (vWF) gene, protein Z gene, annexin A5 and ischemic stroke, but with conflicting results (19, 22–34).

### **Association studies in ischemic stroke: homocystein metabolism**

There is an evidence of the association of high plasma homocysteine levels with ischemic stroke. Many studies (more than 60 in last 10 years) have examined the genes encoding 5,10-methyltetrahydrofolate reductase (MTHFR) as an enzyme involved in homocysteine metabolism, but the majority have produced negative results.

The modest association (11 studies) between C677T variant and ischemic stroke was observed (OR 1.46; 95% CI, 1.19–1.79), as the association between

MTHFR TT mutation and ischemic stroke (OR, 1.20; 95% CI, 1.02–1.41) in meta-analysis of Kim and coauthors (35). Statistically significant association of MTHFR polymorphism and ischemic stroke were identified in Casas et al. (9) meta-analysis of 30 studies (6324 cases and 7604 controls). Individuals homozygous for the T allele had an odds ratio for stroke of 1.26 (95% CI, 1.14–1.40) compared with those homozygous for the C allele. Cronin et al. (36) performed a systematic review and meta-analysis of published 32 studies (6110 cases / 8760 controls) about association of MTHFR 677T allele with risk of ischemic stroke/TIA. It included cohort, case-control and cross-sectional studies. MTHFR 677C T polymorphism was associated with increased risk of stroke in a graded, dose-dependant manner (T allele pooled OR 1.17; 95%CI 1.09–1.26, TT genotype pooled OR 1.37; 95%CI 1.15–1.64). Two meta-analyses are recently published. Ariyaratnam and coauthors (37) in 2007 performed a literature based review of genetic association studies in stroke in persons of non-European descent. A total of seven studies (1859 cases/2380 controls) among Chinese populations evaluating the C677T variant in the *MTHFR* gene were identified. A summary OR of 1.18 (95% CI 0.90–1.56) was observed for individuals homozygous for the T allele compared with C-allele carriers (i.e., CT + CC). Also, three Korean studies with a total of 478 cases and 541 controls provided an OR of 1.34 (95% CI 0.87–2.06). A pooled analysis of Chinese and Koreans samples in meta-analysis among persons of non-European descent provided an overall OR of 1.22 (95% CI 0.98–1.52). Meta-analysis of Banerjee et al. (38) included ten studies of the association of MTHFR with ischemic stroke in Asian population (Japanese, Chinese, Korean, Indian, and Mongolian) and found a significant association of C677T polymorphism of MTHFR and ischemic stroke.

Few studies examined the associations of mutations in homocysteine metabolism-related enzyme genes including cystathionine  $\beta$ -synthase (CBS) 844ins68 (68-bp insertion at exon 8) and methionine synthase (MS) A2756G transition (results in aspartic acid being changed to a glycine residue) and ischemic stroke, all with negative results (39–41).

### **Association studies in ischemic stroke: rennin angiotensin-aldosteron system**

Numerous studies have examined genes that are thought to be involved in hypertension, most commonly angiotensin-converting enzyme (ACE), angiotensinogen (AGT) and angiotensin AT1 receptors, without consistent replication of association to stroke or carotid IMT. Compared with more than 50 studies about the association of ACE I/D polymorphism, only 20 studies examined the association of angiotensinogen and angiotensin AT1 receptor polymorphisms and ischemic stroke.

A meta-analysis has evaluated the risk of stroke in 1196 subjects versus 722 controls from seven studies. It was concluded that the ACE genotype conferred a small but modest effect, with an odds ratio of 1.31 (95% CI 1.06–1.62), according to a dominant model of inheritance. A weaker association was seen under a recessive model (42). In a meta-analysis of Casas et al. (9) including 2990 predominantly white patients and 11 305 controls, the DD genotype was shown to confer a small but significant risk of ischemic stroke (odds ratio 1.21; 95% CI 1.08–1.35). Recently published meta-analysis (37) investigated the association of ACE I/D in three ethnic groups of non-European descent (a total of 3572 Chinese individuals, 1601 Japanese individuals, and 2750 Korean individuals). The overall OR for the nine studies in the Chinese population was 1.90 (95% CI 1.23–2.93) and for six Japanese studies the OR was 1.74 (95% CI 0.88–3.42). The overall OR in the Asian group (Chinese and Japanese) was 1.82 (95% CI 1.28–2.60). Smaller meta-analysis of Banerjee et al. (38) included six studies (two Japanese, two Korean, and two Chinese) did not detect a significant association with ACE gene insertion/deletion polymorphism.

The association of common polymorphisms in exon 2 of the gene (corresponding to a change from methionine to threonine substitution at position 235 (M235T) and threonine to methionine at position 174 (T174M) mutation) with TIA or ischemic stroke were examined in some studies. Mostly, these investigations did not support those associations. A potentially interesting observation, however, is the reported association between an AGT promoter haplotype and cerebral small-vessel disease. Recent findings suggest that the A1166C polymorphism of the angiotensin II type-1-receptor gene, located at the 5' end of the 3'untranslated region of the AGTR1 gene is associated with ischemic stroke. Szolnoki and coauthors (43) have demonstrated that ACE D/D and AT1R 1166C polymorphism were associated with the development of small-vessel ischemic stroke through a mutually facilitatory interplay between them. Meta-analysis of studies of the angiotensin II type 1 receptor (AT1R) (44–47), and angiotensinogen (AGT) (44, 48–50) gene polymorphisms and risk of stroke in Chinese populations did not reveal the association (OR (95% CI) 1.19 (0.60–2.35); 1.27 (0.9–1.78), respectively).

### Association studies in ischemic stroke: lipid metabolism

Moreover, about one half of all studies (more than 60) have shown an association between ischemic stroke and the apoE  $\epsilon$ 4 allele. The apoE genotype seems to have an effect on stroke outcome as well. McCarron et al. (51) in meta-analysis of nine case-control studies (926 cases/890 controls) revealed a significantly higher apoE4 allele frequency in affected patients compared with controls (OR, 1.68; 95% CI,

1.36–2.09; P 0.001). Meta-analysis of Casas and coauthors (9), analyzed 10 studies (1805 cases/10921 controls) of the association between apoE polymorphism and ischemic stroke. Odds ratio for the outcome compared carriers of the  $\epsilon$ 4 allele with those with  $\epsilon$ 3 and  $\epsilon$ 2 alleles was 0.96 (95% CI, 0.84–1.11; P=0.60). A total of seven studies in Asians (four in Chinese (418 cases/476 controls) and three in Japanese (495 cases/1304 controls) evaluated the apoE  $\epsilon$ 4 polymorphism against the pooled  $\epsilon$ 2/ $\epsilon$ 3 were included in meta-analysis of persons of non-European descent (37). The summary OR of the Chinese studies was 2.18 (95% CI 1.52–3.13) and the pooled OR of the three Japanese studies was 1.51 (95% CI 0.93–2.45). The overall OR in the seven Asian studies was 1.77 (95% CI 1.30–2.39). Sudlow and coauthors (52) gave a systematic review of 26 studies (5018 cases and 16921 controls) about apoE genotype influence on the risk of ischemic stroke. The studies were conducted in several European countries, the United States, Brazil, Taiwan, China, Japan, Korea, and Bangladesh, and included populations of varying ethnicity. Overall pooled results suggested an association between  $\epsilon$ 4+ genotypes and ischemic stroke, particularly in large artery ischemic stroke and in Asians, but disappeared when only larger studies (200 cases; OR, 0.99; 95% CI, 0.88–1.11) or studies without control selection bias (OR, 0.99; 95% CI, 0.85–1.17) were analyzed. Meta-analysis Banerjee et al. (38) included six studies from Asian countries and detected a marginally significant association with allele  $\epsilon$ 4.

Although apoE gene polymorphism is the most frequently studied polymorphism in patients with ischemic stroke, few genetic studies studied the association between DNA polymorphisms in other apolipoprotein genes (apo AI/CIII, apoAIV, apoAV, apo B, apoH) almost with negative results. There is a growing evidence that elevated Lp(a) level has a significant role in stroke, and that PNTR polymorphism of the apo(a) gene is associated with stroke (53–57).

Several genes involved in lipid metabolism such as cholesteryl ester transfer protein (CETP), ATP-binding cassette transporter I (ABCA I), lipoprotein lipase (LPL), and paraoxonase (PON) have also been examined in the stroke populations recently. The most widely studied of all known variants of the CETP gene is the TaqI B polymorphism, which results from a nucleotide substitution at position 277 of the first intron (rs708272). Quarta et al. (58) found a protective effect of B allele for stroke. Although, several polymorphisms of the ABCA1 gene have been investigated for their association with CAD, only two published studies assessed the distribution of different polymorphisms (L158L, R219K, G316G, and R1587K) and haplotype arrangements of the ABCA I gene in ischemic stroke patients. Andrikovics et al. (59) published study in ischemic stroke on 244 Hungarian patients and suggested a protective role for the ABCA I -R219K and V771M polymorphisms. Although, few studies obtained posi-

tive results about the association of LPL polymorphisms (447 Ser/Ter, Hind III) and ischemic stroke, but the results of three published genetic studies of the association between Asn291Ser polymorphism in LPL gene and ischemic stroke (452 cases and 8879 controls) in recently published meta-analysis (9) did not support the association of this polymorphism in LPL gene and ischemic stroke. Specific polymorphisms of paraoxonases are associated with the risk of acute ischemic stroke. The PON1 Q192R (Gln192Arg) and L55M (Leu55Met) polymorphisms have been associated with risk of ischemic stroke in small studies. Analysis of different studies about two common polymorphisms of the PON2 gene (A148G (Ala148Gly) and C311S (Ser311 Cys)) and ischemic stroke risk did not support this association. The association of ischemic stroke and PON3 gene polymorphisms is not confirmed. In a recent meta-analysis of 43 studies, five largest studies estimated the per-allele risk ratio at 1.05 for PON1-192 R (B) allele and combined analyses of studies of the PON1-55 M and PON2-311 C variants showed no significant overall associations with CHD (60). The low-density lipoprotein (LDL)-receptor gene polymorphisms were investigated in 3 studies, all with negative results. Guo et al. (61) investigated the relationship between *NcoI* and *Avall* polymorphism of LDL-receptor gene in patients with atherosclerotic cerebral infarction among Han nationality in 77 patients and 113 age-matched controls. The coexistence of A-A- and N+N+ genotypes significantly increase the risk of cerebral infarction (RR 5.56, *p* 0.001). The human lectin-like oxidized low-density lipoprotein receptor 1 (OLR1/LOX-1) is the major endothelial scavenger receptor against oxidized low-density lipoprotein (Ox-LDL), which has been implicated in the pathogenesis of atherosclerosis. Polymorphism G501C in the OLR1 gene was investigated in patients with ischemic stroke (62). This study did not find significant difference in C allele frequencies between patients and controls.

### Association studies in ischemic stroke: endothelial nitric oxide

The gene encoding endothelial nitric oxide synthase is a potential candidate gene for stroke, because it is an important mediator of endothelial function. There are a number of studies, yielding conflicting results of association between Glu298Asp polymorphism in the endothelial constitutive nitric oxide synthase gene and stroke. In a comprehensive meta-analysis of Casas et al. (9), individuals homozygous for the Asp298 allele (three studies; 1086 cases and 1089 controls), compared with Glu298 carriers, did not have an increased risk of ischemic stroke (OR 0.98, 95%CI 0.76–1.26). GENIC study (63) examined the G894T variant as a risk factor in small-vessel disease and found that GG genotype is a risk factor for lacunar stroke but not other stroke subtypes. Different findings reflect differences in genetic background, because the frequency of eNOS polymorphisms has

been shown to vary markedly among different ethnic groups, or difference in environmental exposure, which has been shown to modify the influence of eNOS variants on disease risk (64, 65). In the non-Caucasian population, the studies have mainly focused on the role of the intron 4 polymorphism and have yielded contradictory results. In the Chinese population, Hou et al. (66) observed an increased risk of ischemic stroke (OR 2.13, 95%CI 1.98–4.80) for carriers of the a allele after adjusting for potential confounders. On the other hand, one in Japanese found no increase in risk of stroke for carriers of the a allele (67). In Afro-Americans, a recent, small case-control study (68) of young women reported an increased risk of stroke for the -786T>C variant (OR 2.9, 95%CI 1.3–6.4).

### Association studies in ischemic stroke: Inflammatory molecules

Among the most widely investigated genes are those involved in inflammation (interleukin 1, interleukin 6, tumor necrosis factor  $\alpha$ , toll-like receptor 4, P selectin and E-selectin, C-reactive protein).

The association of several polymorphisms of the genes for IL-1 $\alpha$  (889C/T), IL-1 $\beta$  (-511C/T) and interleukin-1 receptor antagonist (IL-1ra) (variable numbers of an 86bp identical tandem repeat, VNTR), located in a cluster on human chromosome 2, with stroke was examined in numerous recently done studies. Few studies confirmed only the association of -889C/T polymorphism in IL1 $\alpha$  gene and ischemic stroke (69–71). The most frequently examined polymorphism of TNF gene in patients with ischemic stroke is -308G/A. Decreased TNF $\alpha$  A allele frequencies in ischemic stroke patients compared with controls were noticed in majority of studies. A total of 8 studies involving 3813 subjects (1606 stroke cases and 2207 controls) were combined in meta-analysis (72). The -308A allele was not associated with ischemic stroke considering all studies (OR 0.99; 95% CI 0.70–1.41, *P*=0.96), whereas, in adult Asian subjects, the A allele was linked to a 1.6-fold decrease in ischemic stroke risk. Children and young adults from Turkey and Italy harboring the A allele were twice as likely to have ischemic stroke when compared with subjects with the GG genotype (OR 2.04, *P* = 0.004) (73, 74). Interleukin 6 G/C dimorphism at nucleotide -174 within the promoter of this gene was examined in a few case-control studies. The prevalence of CC genotype and the frequency of C allele were statistically significantly higher in patients with lacunar stroke than in asymptomatic controls. Also, Greisenegger et al. (75) evaluated the IL-6 (-174) polymorphism in 214 patients with ischemic stroke or transient ischemic attack and in 214 control subjects. The variant was associated with severe stroke in young patients with acute cerebrovascular events. In Italian study (76) the frequency of the GG genotype of -174 IL-6 G/C gene polymorphism in patients with stroke was >2 times higher than in controls

( $P=0.0001$ ). In contrast, the frequency of the CC genotype was almost 4 times higher in control subjects ( $P=0.0001$ ). Association of few polymorphisms in P selectin, E-selectin, P-selectin glycoprotein ligand-1, ICAM-1 and CRP genes with ischemic stroke was examined in patients with ischemic stroke with almost negative results. The 5A/6A polymorphism in the promoter of MMP3 gene has been shown to have an effect on MMP3 expression and is associated with an ischemic stroke. In a study of individuals with a history of ischemic stroke and a group of age- and gender-matched controls, Flex et al. (77) found that the frequency of the 5A/5A genotype was higher in the case group, and that the association of the 5A/5A genotype with increased risk of stroke was independent of classic risk factors. Although the mechanisms leading to the stroke incidences are likely to be complex, some of the incidences might have been triggered by atherosclerotic plaque rupture where increased MMP3 expression may play a role. The significant association of one polymorphism localized on intron 1 (A119C) human toll-like receptor 4 (TLR4) gene and ischemic stroke (the odds ratio of 1.19A of TLR4 in ischemic stroke was 11.71 (95% CI: 1.52–90.01)) (78).

There is a long list of candidate gene pathways and genes that have been studied for a possible association with ischemic stroke last years (gene encoding atrial natriuretic peptide (ANP) and type A natriuretic peptide receptor (NPRA) (79, 80),  $\beta$ 2- and  $\beta$ 3-adrenergic receptor (81, 82),  $\alpha$ -subunit of amiloride-sensitive epithelial sodium channels (ENaCs) (83), epoxide hydrolase (EPHX2) (84), glutathione peroxidase (GPx-3) (85), vitamin K epoxide reductase complex subunit 1 (VKORC1) (86), insulin-like growth factor I (IGF1) (87), selenoprotein S (88), endothelins (89), osteoprotegerin (90),  $\alpha$ -adducin (91), heat shock protein 70 (92), neuropeptide Y (93), growth arrest-specific gene 6 (GAS6) (94), prostaglandin-endoperoxide synthase-2 (PTGS2 or COX-2) (95), prostacyclin synthase (96), estrogen receptor  $\alpha$  (ESR1) (97), proprotein convertase subtilisin/kexin type 9 (PCSK9) (98), X-ray repair cross-complementing group 1 (XRCC1) (99). Many of them are listed in only few studies. In most cases, however, findings did not support the association of these polymorphisms and ischemic stroke and could not be replicated in subsequent studies.

### **Linkage/association studies in ischemic stroke: PDE4D and ALOX5AP**

Grouping all types of stroke together has been successful in a gene discovery project in Iceland by deCODE group, where a genome scan of 476 patients (from 179 extended Icelandic pedigrees) considered all types of stroke together and revealed linkage to chromosome 5q12. Subsequent linkage analysis and fine mapping found a strong association between PDE4D gene encoding phosphodiesterase 4D and stroke (3). Different haplotypes of PDE4D were found

to be significantly associated with combined carotid and cardiogenic strokes. Phosphodiesterase 4D can degrade the second messenger cyclic AMP (cAMP), a key signaling molecule involved in inflammatory responses of vascular cells to oxidized lipids. The second putative gene for stroke has been described by the same group. A haplotype at 13q12-13 spanning the gene ALOX5AP encoding 5-lipoxygenase activating protein (FLAP) which is involved in leukotriene synthesis and is associated with a 2-fold greater risk of stroke (4). With the news that two new stroke genes had been identified, several studies on different populations of stroke patients in last three years have been performed to uncover whether variants of PDE4D and ALOX5AP could participate in stroke in non-Icelandic populations. The results were mixed.

A case-control study of stroke patients in central Europe investigated variants in both genes. It failed to demonstrate stroke association with PDE4D haplotypes but did show a significant association with ALOX5AP variants particularly in males (100). PDE4D variants have been shown to be associated with stroke in other populations (inbred Netherlands populations-particularly small-vessel stroke), a Pakistani population, India, Japan, U.S. cohort including black and white, U.S. cohort of elderly white women) (101–105). Three European studies exclusively examined the strongest genotype and haplotype associations with ischemic stroke or the combined atherosclerotic/cardioembolic group from the Iceland study and failed to replicate these findings. In an independent large German cohort there was no association between six SNPs in PDE4D and stroke. Meta analysis of Staton and coauthors (106) found a significant association between stroke and PDE SNP 87 (pooled  $p=0.002$ ), SNP 83 (0.003) and SNP 41 (0.003). Song et al. (107) sought to extend the genetic epidemiology of PDE4D and stroke risk by examining a biracial female population of early-onset stroke, by performing novel SNP discovery and by assessing interaction with smoking. The association of rs918592 with stroke was confined exclusively to current smokers (OR 3.2,  $P=0.00014$ ), with no association observed among never-smokers (OR 0.9,  $P=0.75$ ) or former smokers (OR 1.2,  $P=0.66$ ), demonstrating a gene-environment interaction ( $P=0.03$ ). A strong dose-response relationship was also seen among current smokers. Cigarette smoking causes endothelial dysfunction and is known to modify the expression of many genes in endothelial cells. A common haplotype (HapA) in ALOX5AP is associated with a 1.7-fold increased risk of stroke in the Icelandic population. The association was stronger in men than women and was significant for both ischemic and hemorrhagic stroke. There are more than 15 published studies by independent groups attempting to reproduce and confirm the original findings of deCODE group. The association between HapA and ischemic stroke was subsequently replicated in the Scottish population, which shares a common ancestry with the

Icelandic population (108). Studies in other populations reported no association between HapA or other haplotypes and ischemic stroke (109–111). However, in a German sample several single-nucleotide polymorphisms including one out of four single nucleotide polymorphisms constituting HapA were associated with ischemic stroke.

Two genetic studies on stroke try to identify new stroke genes. SWISS (siblings with ischemic stroke study) is a large multicentered trial in the USA that collects sibling pairs affected with stroke, with the basic idea to collect genetic material and create cell lines from study subjects to perform genomwide linkage studies for ischemic stroke (112). The Framingham Heart Study Offspring cohort have not been examined for genetic influence on stroke directly but has been subjected to analysis using intermediate phenotype-carotid intima thickness and cerebral leukoariosis (113).

### Future directions

Stroke genetics is very much a forward-looking field. It may alter management of stroke through development of new approach for the prevention or rational treatment of stroke. Recently identified genes, PDE4D and ALOX5AP, code enzymes involved in specific pathways that could be targeted in stroke. Specific regulation of cAMP levels by alteration of cyclase activity or cAMP effector proteins could be additional target for rationally designed stroke therapy.

Recently, pharmacogenetics, which investigates genetically determined variations in response to drugs, has emerged as a promising research area. It is well-known that the rates of metabolism by several of the CYP450 enzymes vary because of genetically determined polymorphisms. Recent data reveal that approximately 20% of the white population carries 1 of at least 2 different CYP450 point mutations that cause sensitivity to warfarin. This suggests that CYP2C9 genotypes may someday be helpful in planning initial warfarin dosing. The polymorphism in the gene for vitamin K epoxide reductase complex 1 (VKORC1) has also been shown to strongly affect individual sensitivity to warfarin (114, 115).

Identification of a gene polymorphism profile that predicts atherosclerotic plaque formation and activation susceptibility opens new vistas of exploration for gene-directed therapy. Studies demonstrate that gene profiling can significantly influence responses to drugs such as statins. A polymorphism in the gene toll-like receptor-4 that influences innate immunity is associated with an increased beneficial effect in risk reduction of cardiovascular events in patients treated with pravastatin. Mutant allele Asp299Gly polymorphism was found to have significantly fewer cardiovascular events

in the treatment group than patients with the wild-type gene profile. These data and other studies support the concept that a comprehensive genetic profile will lead to a more efficient use of current and future medications in patients with atherosclerotic disease. Data further indicate that genetic profiling in combination with serotyping of specific organisms shows the interrelation between genetic and immune response in the increased risk for atherothrombotic stroke.

An evidence of the interaction of host genetic factors with response to acute pharmacotherapy comes from a subgroup analysis of the NINDS TPA (National Institute of Neurological Diseases and stroke tissue plasminogen activator) trial. They demonstrated a significant OR for favourable outcome in apoE2 patients who received tPA compared with apoE2-negative patients who received placebo. This study shows the potential for using genetic testing to identify a high-responder population for thrombolytic therapy, a goal that has mainly been pursued to this point based on identification of certain MRI signal characteristics.

Stroke pharmacogenomics is likely to be most widely applicable in the outpatient clinic where it could be used to tailor primary and secondary stroke prevention. Compared with other antihypertensive therapy, interaction between antihypertensive diuretic therapy and adducing gene variant was associated with a lower risk of stroke was also noticed. This study suggested more effective stroke prophylaxis based on specific genetic information.

Future stroke genetic studies may also identify variants that predict the lack of efficacy for specific treatments. This will improve the cost-effectiveness and limit the potential side-effects of drugs received by stroke patients (116).

### Conclusion

Genetics has revolutionized neurological research. Identified stroke-causing genes and other new genes which will be identified in the future, can be directly used for developing a genetic testing kit for early, accurate, and presymptomatic diagnosis of stroke. Many individuals with a high risk of developing stroke can be identified by genetic testing. It may lead to successful early prevention or delay the onset of the disease by aggressive treatment of known risk factors. Ultimately, genes for stroke can be used as targets for developing new drugs which might contribute to both prevention and treatment of stroke, and also may drive the paradigm shift in modern medicine to personalized medicine, the right medicine/therapy for right patient.

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