Genetics of ischemic stroke

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Stroke is the third leading cause of death worldwide and a major cause of disability in all countries. In 2005, an estimated 5.7 million people died of stroke globally and 16 million suffered first-ever strokes. The 1998 stroke incidence of 126/100,000 individuals in Saudi Arabia was lower than the rate of 650/100,000 observed in Sweden in the same year, however, the Saudi rate is expected to have risen over the past decade due to rapid ageing of the population and rising prevalence of stroke risk factors such as diabetes mellitus (DM) and obesity.

Worldwide, ischemic stroke (IS) constitutes 85-90% of all strokes, of which 60% is attributable to hypertension, DM, heart disease, elevated serum cholesterol, obesity, and cigarette smoking. Of the remaining 40% of IS, approximately half is due to other acquired conditions and the other half is caused by unknown factors.

The genetic basis of ischemic stroke. Inherited disorders may manifest with a stroke phenotype. However, disorders that exhibit classic Mendelian patterns of inheritance such as sickle cell disease (SCD) or homocysteinuria are believed to contribute to less than 1% of adult strokes in unselected populations. Yet, many studies have shown the importance of genetic factors in the etiology of IS, including multifactorial stroke associated with hypertension, smoking, and so forth. Prospective population-based studies have shown a clear association between a family history of stroke
and an increased risk of future stroke.\textsuperscript{9,10} For instance, in the Framingham Heart Study\textsuperscript{10} a positive history of stroke or transient ischemic attack in the father or mother was associated with a 2.4 and 1.4-fold increase in risk of stroke in the offspring. However, a positive family history does not necessarily imply genetic linkage since family members are commonly exposed to the same environment, some of which may influence future stroke risk from early life. Indeed, there is a clear correlation between low birth weight and increased risks of hypertension, diabetes, and stroke in later life,\textsuperscript{11} while lower social class and education in childhood may be related to higher levels of inflammatory markers in adulthood.\textsuperscript{12} Further evidence of the role of genetic factors has come from twin studies showing a 2-4 fold increase in stroke risk in monozygotic twins compared to dizygotic twins.\textsuperscript{13,14}

The past decade has witnessed intense research into the genetic basis of IS, and the molecular pathogenesis of inherited disorders associated with stroke. In this review, we present a summary of recent findings with emphasis on novel genes that influence common multifactorial stroke.

\textbf{Genetic polymorphisms and multifactorial stroke.} Several genes have been reported to influence the risk of IS, but in only a few cases were results confirmed in independent populations. These include:

\textbf{ALOX5AP and leukotrienes.} Leukotrienes (LT) are pro-inflammatory molecules expressed from macrophages, neutrophils, and mast cells.\textsuperscript{15} The gene \textit{ALOX5AP} encodes 5-lipoxygenase activating protein (FLAP), which cleaves arachidonic acid in the presence of the enzyme 5-lipoxygenase to initiate a cascade resulting in the production of several members of the LT family. \textit{LTB4}, \textit{LTC4}, and \textit{LTD4} bind to receptors to induce neutrophil chemotraction, adhesion of leukocytes to vascular endothelium and migration of smooth muscle cells.\textsuperscript{16} These processes have been shown to promote the development of atherosclerosis in humans.\textsuperscript{17} A common haplotype of ALOX5AP (HapA) is associated with a 1.7 fold increased risk of IS in Iceland,\textsuperscript{18} a finding that was later replicated in Scotland,\textsuperscript{19} and in the United Kingdom, and Germany.\textsuperscript{20} In fact, the later study has found stronger associations between certain variants of \textit{LTC4} and \textit{LTB4} receptor genes with subtypes of IS.

\textbf{Phosphodiesterase 4D.} The enzyme phosphodiesterase 4D (PDE4D) degrades cyclic adenosine monophosphate (cAMP), a signal transduction molecule present in many tissues, including vascular endothelium and inflammatory cells.\textsuperscript{21} It is coded by the PDE4D gene, also known as STRK1, located on chromosome 5q12. A study in Iceland reported a significant correlation between polymorphisms in this gene and increased incidence of IS in the Icelandic population.\textsuperscript{22} This observation was later confirmed by subsequent studies in the United Kingdom,\textsuperscript{23} United States,\textsuperscript{24,25} and Sweden,\textsuperscript{26} although not in Germany,\textsuperscript{27} perhaps due to racial and genetic variation or differences in study design.

\textbf{Soluble epoxide hydrolase.} Soluble epoxide hydrolase catalyzes epoxyeicosatrienoic (EET) acids, a group of molecules derived from arachidonic acid and involved in vascular inflammation, vascular smooth muscle tone, control of blood pressure, and atherosclerosis.\textsuperscript{28} A recent German study\textsuperscript{29} confirmed the findings of an earlier United States study, which reported significant associations between specific haplotypes in the epoxide hydrolase (EPHX2) gene and increased risks of IS,\textsuperscript{30} an effect possibly mediated through altered epoxide hydrolase activity on EETs.\textsuperscript{31}

\textbf{Atrial natriuretic peptide.} A large prospective study reported an association between some allelic variants of the atrial natriuretic peptide (ANP) gene with a 1.6-2 fold increase in risk of stroke among male US physicians.\textsuperscript{32} A subsequent Italian study reported an even stronger association among female and male Sardinians, in whom a recessive allele of ANP conferred a relative risk of 3.8 for IS, independent of other risk factors.\textsuperscript{33} Atrial natriuretic peptide plays important roles in the regulation of electrolyte and water balance and also modulates cellular growth in cardiac and vascular tissues.\textsuperscript{34,35}

\textbf{Angiotensin converting enzyme.} The angiotensin converting enzyme (ACE) converts angiotensin I to angiotensin II, a peptide involved in vascular hypertrophy, vasoconstriction, and atherosclerotic processes.\textsuperscript{36} Individuals homozygous for the D allele of the ACE gene have a 56% increase in ACE activity compared with I allele homozygotes.\textsuperscript{57} Not surprisingly, the ACE gene has been implicated as a susceptibility locus for IS. In one meta-analysis of 11 studies involving more than 14,000 Caucasians, the D/D allele of the ACE gene was found to be associated with an odds ratio of 1.21 for IS compared to D/I and I/I alleles.\textsuperscript{38} A summary of genetic polymorphisms influencing the risk of IS is displayed in Table 1.

\textbf{Hereditary multisystem disorders associated with stroke. Sickle cell disease.} This disorder results from a point mutation in the globin β-chain gene in which the replacement of thymine by adenine leads to the substitution of valine for glutamic acid at position 6 of the hemoglobin β-chain molecule. The resulting hemoglobin SS undergoes polymerization at low oxygen tension, leading to red cell sickling and hemolysis. Stroke affects 11% of individuals with SCD <20 years of age, but it is less common among older adults.\textsuperscript{39} Chronic anemia and hypoxemia in SCD leads to a markedly increased in cerebral blood flow. When the cerebral circulation is compromised by events such as an acute chest syndrome, the cerebral vasculature fails
to undergo further dilatation, resulting in ischemia.\textsuperscript{40} In line with this theory are the high prevalence of border-zone infarctions seen in patients with SCD and the 40\% increased risk of stroke in those with intracranial mean flow velocities (MFV) of 200cm/s or more on transcranial Doppler ultrasound (TCD).\textsuperscript{41} Regular TCD screening of the proximal middle cerebral artery is now recommended for all young patients with SCD. Those with abnormal MFVs should then receive monthly blood transfusion, which has been shown to reduce stroke risk by 92\% in the Stroke Prevention in Sickle Cell Anemia (STOP) trial.\textsuperscript{42}

\textit{Fabry disease.} This is an X-linked recessive lysosomal storage disorder that is caused by a deficiency of \(\alpha\)-galactosidase A, coded by the GAL A gene, leading to accumulation of globotriaosylceramide in vascular endothelium and other tissues.\textsuperscript{43} Clinical characteristics include cutaneous, ocular, renal, and neurologic abnormalities, with strokes occurring in up to 24\% of patients. In one German study, unrecognized Fabry disease was found in 4.9\% of males and 2.4\% of females presenting with cryptogenic stroke.\textsuperscript{44} Diagnosis depends on measurement of enzyme levels in leukocytes or cultured skin fibroblasts, confirmed by DNA sequence analysis. The recent introduction of recombinant human \(\alpha\)-Gal A (agalsidase beta) has altered the outlook for patients with Fabry disease, with treated patients showing significant improvements in renal function, cerebral hemodynamics and quality of life.\textsuperscript{45}

\textit{Homocysteinuria and hyperhomocysteinemia.} Homocysteine is a sulphur-containing amino acid derived from dietary methionine. It plays a vital role in cellular homeostasis, but elevated plasma levels may lead to thrombosis by its effects on the vascular wall structure and the blood coagulation system.\textsuperscript{46} The metabolism of homocysteine is dependent on folic acid, pyridoxine, cyanocobalamin and the activities of the enzymes cystathione beta-synthase (CBS) and methylene tetrahydrofolate reductase (MTHFR).\textsuperscript{47} Homozygous mutations of the CBS gene result in homocysteinuria, a pediatric syndrome characterized by a Marfanoid habitus, downward dislocation of optic lens, mental retardation, premature atherosclerosis, and early death.\textsuperscript{48} In contrast, mild to moderate hyperhomocysteinemia in adults results from mutations in the MTHFR gene, located on chromosome 1p36. Among Caucasians, C677T mutations affect up to 44\%, and A1298C mutations affect up to 13.8\% of the population.\textsuperscript{49,50} whereas in Saudi Arabia, this pattern is reversed as C677T and A1298C mutations account for 28\% and 60\% of MTHFR polymorphisms.\textsuperscript{51} The more severe C677T homozygous state is prevalent in 2.1\% of the native Saudi population.\textsuperscript{52} Many studies have confirmed an association between hyperhomocysteinemia and IS,\textsuperscript{53-55} as well as the MTHFR C677T phenotype\textsuperscript{38} and ischemic stroke.

\textit{CADASIL.} The syndrome of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy is characterized by a small vessel vasculopathy within brain parenchyma that manifests with migraine, recurrent strokes, dementia, psychiatric disturbance, and seizures.\textsuperscript{56} The disease results from

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|l|l|l|l|}
\hline
Study & Year & Location & Locus & Gene & Gene product & Remarks \\
\hline
Rubattu et al\textsuperscript{20} & 2004 & Italy & 1p & \textit{ANP} & Atrial natriuretic peptide & A recessive allele confers a RR of 3.8 for IS \\
Rubattu et al\textsuperscript{22} & 1999 & United States & 1p & \textit{ANP} & Atrial natriuretic peptide & Allele variants increase risk of stroke 1.5-fold \\
Gschwendtner et al\textsuperscript{22} & 2008 & Germany & 8p & \textit{EPHX2} & Soluble epoxide hydrolase & 3 SNPs associated with IS \\
Bevan et al\textsuperscript{20} & 2008 & United Kingdom & 13q & \textit{ALOX5AP} & FLAP & RR 1.3 - 3.0 for IS & subtypes \\
& & & & \textit{LTCA4} & LTC4 & RR 1.8 for IS, 2.2 for LAS \\
& & & & \textit{LTBR4} & LTBA receptors 1&2 & RR 1.6 for IS, 2.3 for CES \\
Lohmussaar et al\textsuperscript{27} & 2005 & Germany & 13q & \textit{ALOX5AP} & FLAP & One SNP associated with IS \\
Hegadottir et al\textsuperscript{20} & 2004 & Iceland & 5q & \textit{PDE4D} & Phosphodiesterase 4D & No association with IS \\
Gretarsdottir et al\textsuperscript{22} & 2003 & Iceland & 5q & \textit{ALOX5AP} & FLAP & Haplotypes associated with IS & myocardial infarction \\
Woo et al\textsuperscript{29} & 2006 & United States & 5q & \textit{PDE4D} & Phosphodiesterase 4D & Certain SNPs associated with LAS & CES subtypes of IS \\
Casas et al\textsuperscript{28} & 2004 & Multi-national & 17q & \textit{ACE} & Angiotensin converting enzyme & D/D allele associated with OR of 1.2 for stroke \\
\hline
\end{tabular}
\caption{Studies implicating some novel genes in multifactorial ischemic stroke.}
\end{table}
mutations in the Notch3 gene, which encodes the human Notch3 receptor, a large protein involved in cell signaling. Screening of 19 patients from 4 families from Kuwait, Sudan, and Saudi Arabia identified heterozygous and homozygous exon 3 Notch3 mutations in 3 families, while no mutation was detected in one family. A tentative diagnosis of CADASIL can be made with a skin biopsy, which shows the presence of granular osmiophilic material and positive staining with monoclonal antibody against the extracellular domain of Notch3 receptor. However, a definitive diagnosis can be made only with a DNA analysis of the Notch3 gene sequence.

**HERNS and cerebroretinal vasculopathy.** Hereditary endotheliopathy with retinopathy, nephropathy, and stroke was described in a Chinese American family over a decade ago. Clinical features include retinal macular edema and telangiectasia, renal failure, and neurologic complications including migraine-like headaches and cerebral vasculopathy. A similar disorder named cerebroretinal vasculopathy was later described in several Dutch families, in whom subsequent genetic studies revealed linkage to chromosome 3p21. Both conditions express autosomal dominant inheritance but their molecular pathogenesis remains to be determined.

**Inherited disorders of coagulation and fibrinolysis.** The procoagulant molecules prothrombin and factor V, and the natural anticoagulants protein C, protein S, and antithrombin III are plasma protein molecules that play important roles in limiting the size and extent of thrombi formed during normal hemostasis. These processes involve inhibition of factors XII, XI, IX and X by antithrombin III, degradation of factors V and VII by protein C acting in the presence of protein S, and the formation of the fibrinolytic enzyme plasmin through the actions of protein C and tissue plasminogen activator. Thus, mutations involving the genes for these factors may result in altered protein activity, hypercoagulable states and vascular thrombosis.

**Factor V Leiden and prothrombin G20210A mutation.** Factor V Leiden (FVL) is a mutated factor V gene that causes an amino acid substitution in the factor V protein, resulting in its resistance to activated protein C, while prothrombin G20210A mutation results in increased activity of prothrombin. Both conditions have been associated with increased risks of vascular thrombosis. In the Estrogen Replacement and Atherosclerosis (ERA) trial, the risk of venous thromboembolism was significantly increased in women who were carriers of the FVL mutation and taking hormone therapy compared with non-FVL women taking placebo. Likewise, significant risks of venous thrombosis have been described in association with other thrombophilic states, including the prothrombin G20210A mutation.

It has been suggested that IS in these patients may be partly due to the presence of an unrecognized patent foramen ovale, allowing a paradoxical thrombus from the right side of the heart to reach the cerebral

### Table 2 - Genetic syndromes associated with an increased susceptibility to ischemic stroke.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Inheritance</th>
<th>Locus</th>
<th>Genetic abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sickle cell disease</td>
<td>AR</td>
<td>11p</td>
<td>β-globin gene mutation causes an amino acid substitution in hemoglobin &amp; sickling of RBCs</td>
</tr>
<tr>
<td>Homocysteinuria and hyperhomocysteinemia*</td>
<td>AR, AD</td>
<td>21q</td>
<td>1p</td>
</tr>
<tr>
<td>Fabry disease</td>
<td>X-linked</td>
<td>Xq</td>
<td>Mutation in GLA, the gene for α-galactosidase</td>
</tr>
<tr>
<td>CADASIL</td>
<td>AD</td>
<td>19p</td>
<td>Mutations in Notch-3 gene which encodes Notch3 receptor, a protein involved in cell signaling</td>
</tr>
<tr>
<td>HERNS</td>
<td>AD</td>
<td>3p</td>
<td>-</td>
</tr>
<tr>
<td>Cerebroretinal vasculopathy</td>
<td>AD</td>
<td>3p</td>
<td>-</td>
</tr>
<tr>
<td>MELAS</td>
<td>AD</td>
<td>Mit-DNA</td>
<td>Mutations in tRNA gene of mitochondrial DNA</td>
</tr>
<tr>
<td>Protein C deficiency</td>
<td>AD</td>
<td>2q</td>
<td>Mutations in protein C gene</td>
</tr>
<tr>
<td>Protein S deficiency</td>
<td>AD</td>
<td>3p</td>
<td>Mutations in protein S gene</td>
</tr>
<tr>
<td>Factor V Leiden</td>
<td>AD</td>
<td>1q</td>
<td>Mutations in factor V gene leads to factor V resistance to activated protein C</td>
</tr>
<tr>
<td>AT-III deficiency</td>
<td>AD</td>
<td>1q</td>
<td>Mutations in Antithrombin III gene</td>
</tr>
<tr>
<td>PT-G20210A mutation</td>
<td>AD</td>
<td>11p-q</td>
<td>Guanine to adenine transition causes an amino acid substitution and increased prothrombotic activity</td>
</tr>
</tbody>
</table>

*Hyperhomocysteinemia may also result from environmental factors, AD - autosomal dominant, AR - autosomal recessive, AT-III - antithrombin 3, CADASIL - cerebral autosomal dominant arteriopathy with subcortical infarcts and leuko-encephalopathy, HERNS - hereditary endotheliopathy with retinopathy, nephropathy, and stroke, MELAS - mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes, Mit-DNA - mitochondrial DNA, MTHFR - 5,10-methylene tetrahydrofolate reductase, PT - prothrombin
circulation. Yet, the role of inherited thrombophilias in the etiology of arterial stroke has been controversial, with some studies showing no, or marginal associations, and other studies showing significant associations with stroke in the young and the middle-aged. A meta-analysis of 120 studies investigating genetic susceptibility to ischemic stroke was conducted in 2003 in an effort to clarify these findings. Polymorphisms involving 10 of the 32 genes studied were responsible for various thrombophilic states but only FVL and prothrombin G20210A mutation were unequivocally associated with ischemic stroke, with odds ratios of 1.33 and 1.44, respectively. Mutations involving 2 other genes (glycoprotein 1b-α and plasminogen activator inhibitor-1) showed significant associations with stroke but the dataset contained too few studies for firm conclusions. A summary of some inherited disorders predisposing to stroke is shown in Table 2.

Summary and conclusions. Recent advances in research have yielded much insight into genetic polymorphisms influencing ischemic stroke. This has helped clinicians understand the molecular pathogenesis of common multifactorial stroke as well as stroke associated with inherited disorders such as SCD and FVL. Since risk factor modification is the most effective means of stroke prevention, this knowledge may enhance prediction of disease risk and early institution of appropriate measures in susceptible individuals. Further research in this field will clearly compliment national and international efforts to reduce the global burden of stroke.

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References


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**Related topics**


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