

## Review

# Genetics of stroke

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Stroke is the second most common cause of death and the most common cause of disability in developed countries. Stroke is a multi-factorial disease caused by a combination of environmental and genetic factors. Numerous epidemiologic studies have documented a significant genetic component in the occurrence of strokes. Genes encoding products involved in lipid metabolism, thrombosis, and inflammation are believed to be potential genetic factors for stroke. Although a large group of candidate genes have been studied, most of the epidemiological results are conflicting. Studies of stroke as a monogenic disease have made huge progress, and animal models serve as an indispensable tool to dissect the complex genetics of stroke. In the present review, we provide insight into the role of *in vivo* stroke models for the study of stroke genetics.

**Keywords:** genetics; stroke; ischemic stroke; hemorrhagic stroke

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## Introduction

Stroke is the second most common cause of death and disability in developed countries<sup>[1]</sup>. Ischemic stroke is the most common, with an estimated incidence of approximately 80%<sup>[2]</sup>. A less common subtype of stroke is hemorrhagic stroke, which occurs due to subarachnoid hemorrhage (SAH) and/or intracerebral hemorrhage (ICH). In general, stroke is a heterogeneous, multi-factorial disease caused by the combination of certain risk factors and genetic factors<sup>[3]</sup>. Conventional risk factors, including hypertension, cigarette smoking, diabetes, atrial fibrillation and obesity, account for much of the risk of stroke<sup>[3]</sup>. Unfortunately, these risk factors explain only approximately 60% of cases<sup>[4]</sup>. There may be other important factors, including genetic factors, that have not yet been identified. Genetic background, suggested by various studies in families, twins and animal models, might contribute to a predisposition to stroke<sup>[5]</sup>.

There are two major approaches to find genes related to stroke: the candidate gene approach and the genome-wide approach (GWA)<sup>[6]</sup>. The former has become more popular recently. Genes encoding proteins involved in lipid metabolism, thrombosis, atherosclerosis and others are thought to be potential genetic factors for stroke. The GWA entails genotyping several hundreds of thousands of genetic variants and is a powerful tool to identify new susceptibility genes and provide new insights into the pathogenesis and prevention of stroke.

However, if no biologically plausible gene or no annotated genes can be identified in the regions of interest, the interpretation of GWAs remains obscure<sup>[7]</sup>.

In this review, we summarize epidemiologic evidence for a genetic component to the occurrence of ischemic and hemorrhagic stroke. The monogenic and multi-factorial aspect, respectively, are discussed in the two types of stroke. Animal models and the potential clinical application of genetics in stroke treatment are also addressed in this manuscript.

## Ischemic stroke

Normally, ischemic stroke is divided into small-vessel, large-vessel and cardio-embolic subtypes. Evidence shows that genetic factors are more important in small- and large-vessel stroke than in cardio-embolic stroke<sup>[8, 9]</sup>. Some intermediate phenotypes also exhibit high heritability, such as carotid intima-medial wall thickness (IMT) and white-matter lesions (WML)<sup>[3]</sup>. Single-gene and multi-factorial stroke are both included.

## Single-gene disorders with ischemic stroke

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an adult-onset, inherited autosomal dominant disease characterized by recurrent strokes and progressive dementia, with or without migraine-like headaches, seizures, and pseudobulbar palsy<sup>[10]</sup>. Notch3 mutations, located on chromosome 19p12, have been identified as a genetic cause of CADASIL<sup>[11]</sup>. Notch3 is a highly conserved trans-membrane receptor of the Notch

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family that is involved in signaling events that control cell fate decisions during embryonic development<sup>[12]</sup>. Most of the mutations are missense mutations and result in an odd number of cysteine residues within one of the epidermal growth factor (EGF)-like domains; these mutations may cause ineffective signaling and/or accumulation of Notch3 molecules at extra-cellular spaces, which leads to toxicity at tissues expressing Notch<sup>[13]</sup>. Notch3 expression is restricted to smooth muscle cells (SMCs)<sup>[13]</sup>, and thus, progressive degeneration of SMCs appears to be important in the pathogenesis of CADASIL. The ultrastructural study of SMCs demonstrated deposits of granular osmiophilic material (GOM) of unknown nature near the basement membrane of SMCs<sup>[14]</sup>, which seems to be a degeneration product of SMCs. Hundreds of families with Notch3 mutations have been described, with the majority being of European origin<sup>[10]</sup>. Relatively fewer mutation sites have been found in Asian populations. CADASIL might have been overlooked in Asia because of the higher baseline occurrence of vascular dementia<sup>[15]</sup>. Racial differences in the contribution of Notch3 mutations may also be a factor. Heterozygous mutations in Notch3 have been demonstrated in more than 90% of families with CADASIL in some Caucasian studies<sup>[13]</sup>. However, in Japanese studies<sup>[15]</sup>, the mutations were found in less than 25% of CADASIL families, and in one large family, investigators did not see any significant linkage to marker genes on chromosome 19<sup>[15]</sup>. These results suggest the existence of other risk genes in Asian populations. Notch3 is a receptor for a ligand, and the ligands are thought to be Delta and Jagged, as in other Notch molecules. Therefore, mutations in ligand genes may cause a similar condition.

Fabry disease is an X-linked lysosomal storage disorder caused by deficiency of  $\alpha$ -galactosidase A (GLA), which results in the storage of globotriaosylceramide (Gb3) in various organs, particularly in the myocardium, renal epithelium, skin, eye, and vasculature<sup>[16]</sup>. Fabry's disease is surprisingly common in young stroke patients<sup>[16]</sup>. Most patients carry missense or nonsense mutations in the coding region of GLA. Stroke in patients with Fabry disease occurs typically by the third or fourth decade of life and manifests as both large-artery disease and small-vessel disease, with a preference for the posterior circulation<sup>[3]</sup>. Most strokes appear to be related to *in situ* thrombosis of a small-caliber blood vessel with Gb3 deposits. In Fabry disease, however, many heterozygous women present with the full spectrum of disease manifestations but with a later onset of symptoms, a slower rate of progression, and a higher phenotypic variability than men<sup>[3]</sup>. Affected males are diagnosed readily by determining the level of GLA activity in plasma or peripheral leukocytes. Affected females have normal to very low levels of GLA activity, and therefore, genetic diagnosis in females is essential. The Multiplex Ligation-dependent Probe Amplification (MLPA), a recent technique that can easily detect rearrangements, would be well suited for routine detection of deletions and duplications<sup>[17]</sup> and would therefore improve GLA mutational screening.

Sickle-cell disease (SCD) is an autosomal recessive disorder caused by a mutation in hemoglobin and is referred to as

hemoglobin S (HbS)<sup>[3]</sup>. The mutation is a valine to glutamic acid substitution in the 6th amino acid position in the  $\beta$ -globin chain. The geographic origins of HbS lie in regions of the world with endemic malaria, where the heterozygote condition confers relative resistance to malaria and thus confers a survival advantage. Generation of HbS is a monogenic event, however, the phenotype of SCD is multi-genetic<sup>[18]</sup>. Other genes not linked to the  $\beta$ -globin locus participate in relevant pathological events that are controlled by many genes<sup>[18]</sup>. For example, a 158C/T mutation upstream of the gene that enhances fetal hemoglobin (HbF) expression has an inhibitory effect on the polymerization of HbS. Additionally, it has been found that under steady state conditions, SCD patients show evidence of ongoing inflammation and exhibit polymorphisms in the vascular cell adhesion molecule (VCAM), P-selectin and IL-4 receptor gene, which is also associated with stroke risk in SCD<sup>[19]</sup>. SCD is the most common cause of stroke in children. A critical component in the pathogenesis of SCD is that sickled red blood cells tend to adhere to the endothelium, thus favoring thrombus formation and vascular occlusion<sup>[3]</sup>. The Stroke Prevention in Sickle Cell Disease (STOP) trial<sup>[20]</sup> showed that long-term transfusion therapy dramatically reduces the risk of a first stroke in high-risk patients. However, due to adverse side effects, transfusion therapy remains a challenge.

Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) is a maternally inherited syndrome caused by mutations in mitochondrial DNA<sup>[3]</sup>. Studies have shown that the mutational spectrum is broad. About 80% of MELAS patients exhibit a heteroplasmic mutation in the dihydrouridine loop of the tRNA Leu (UUR) gene at nucleotide position 3243<sup>[21]</sup>. Another T-to-C transition at nucleotide position 3271 occurred as a secondary common mutation<sup>[3]</sup>. The pathogenic mtDNA mutations can result in dysfunction of mitochondrial oxidative phosphorylation (OXPHOS)<sup>[22]</sup>, impairing respiratory capacity of the cell and ATP synthesis. Fornuskova *et al*<sup>[22]</sup> found that the 3243A→G frontal cortex mitochondria showed a marked loss of the complex IV holoenzyme, accompanied by accumulation of assembly intermediates. A similar phenomenon was described in yeast ATP synthase mutants<sup>[23]</sup>. These results disclose new aspects of OXPHOS deficiencies in the brain, particularly in the case of ATP synthase. In addition to diminished energy provisions, an insufficient discharge of mitochondrial membrane potential, leading to reactive oxygen species (ROS) production, was proposed as the underlying pathogenic mechanism of ATP synthase deficiency<sup>[24]</sup>. With regard to mutation screening, urinary sediments, because of a higher proportion of mutant loads, could be an alternative method for the detection of mutant load in MELAS patients<sup>[25]</sup>.

### Common multi-factorial stroke

#### Inflammation

Ischemic brain injury is characterized by acute local inflammation and changes in concentrations of inflammatory cytokines<sup>[26]</sup>, especially in the atherosclerosis subtype.

C-reactive protein (CRP) is an inflammatory molecule that is

frequently deposited in atherosclerotic lesions in human and animal models<sup>[27]</sup>. In acute-phase reactions, CRP concentrations can increase up to a thousand times, and the association between elevated CRP concentrations and progression of atherosclerosis is well documented<sup>[28]</sup>. Current studies have found the 1059G/C polymorphism in exon 2 of the CRP gene to be an independent genetic risk factor for ischemic stroke<sup>[29]</sup> and atherosclerosis<sup>[30]</sup> in older Japanese individuals. However, the association of this variant with cerebrovascular events could not be replicated in younger Chinese subjects<sup>[31]</sup>, which suggests that the effect of the GC genotype might be associated with age. Interestingly, in this study, the CRP 1059G/C single-nucleotide polymorphism (SNP) was also found to be a genetic determinant for the difference between intra- and extra-cranial cerebral atherosclerosis. Unlike the GG genotype, CRP expression in extra-cranial cerebral arteries is high in individuals with the GC genotype individuals. One possible explanation might be that this variant could influence the endogenous expression of CRP in vessels.

Interleukin-6 (IL-6) is an important mediator of inflammatory events. Elevated plasma levels of IL-6 are associated with an increased risk and worse outcome of acute vascular events. A common G/C promoter polymorphism in the IL-6 gene has been shown to affect basal IL-6 levels, with the C allele associated with lower plasma IL-6 levels<sup>[32]</sup>. However, epidemiological data on the association of the IL-6 gene polymorphism with ischemic stroke, subtypes and intermediate phenotypes are conflicting. Pola *et al*<sup>[33]</sup> observed an increased risk of ischemic stroke in carriers of the IL-6 GG genotype (OR=8.6), which is in agreement with the results of Stefan Greisenegger<sup>[34]</sup>. Rundek *et al* also revealed that the G allele confers an increased risk of IMT<sup>[35]</sup>. However, several population-based, case-control studies<sup>[36]</sup> failed to confirm these associations. One study found the CC genotype to be uniquely associated<sup>[37]</sup> with lacunar stroke, suggesting a particular susceptibility of small, deep penetrators of cerebral arteries to IL-6-mediated inflammatory damage. Further studies are needed to confirm the genotype-phenotype association.

Other inflammatory genes, such as interleukin (IL)-1 $\alpha$ <sup>[36]</sup>, tumor necrosis factor- $\alpha$ <sup>[38]</sup>, IL-10<sup>[39]</sup>, intercellular adhesion molecule 1 (ICAM1)<sup>[40]</sup>, and P-selectin<sup>[41]</sup>, have been implicated as susceptibility factors by several independent investigators. However, some studies have not produced positive associations<sup>[42]</sup>.

### Hemostatic system

Genes involved in the hemostatic mechanism are logical candidate genes in prothrombotic conditions of stroke. For example, the mutation of factor V R506Q (FV) makes it resistant to protein C<sup>[43]</sup>; the gene polymorphisms of prothrombin G20210A is associated with the levels and activity of fibrinogen and prothrombin<sup>[44]</sup>. The gene polymorphisms of platelet surface glycoprotein (GP), such as P1A1/A2 and the Kozak sequence<sup>[45]</sup>, change the structure or function of platelet surface receptors. However, the association between these gene polymorphisms and ischemic stroke are controversial<sup>[46, 47]</sup>.

In addition, plasminogen activator inhibitor-1 (PAI-1) plays a key role in fibrinolytic homeostasis. A common single base pair insertion, (5G)/deletion (4G), which occurs 675 bp upstream from the transcription start site of the PAI-1 gene, has been reported to alter levels of plasma PAI-1, with the 4G allele associated with increased circulating PAI-1 protein levels<sup>[48]</sup>. Considerable data support the view that increased plasma PAI-1 activity is responsible for the decreased fibrinolytic activity in patients with stroke<sup>[48]</sup>. However, epidemiological data on the role of the PAI-1 4G/5G polymorphism in ischemic stroke are still controversial, with some studies<sup>[49]</sup> finding that 4G/4G confers an increased risk of stroke and others finding no association<sup>[50]</sup>. Another group demonstrated a protective effect of the 4G allele against ischemic stroke (OR=0.176), which may be through a mechanism unrelated to fibrinolysis, and instead involves altered plaque stabilization and/or antagonism of tissue-type plasminogen activator (tPA) effects<sup>[51]</sup>.

### Lipid metabolism

Apolipoprotein E (apo E), a ligand for various cell-surface receptors, modulates the metabolism of atherogenic lipoprotein particles and participates in the process of cellular incorporation of specific lipoproteins. The APOE  $\epsilon 2/\epsilon 3/\epsilon 4$  polymorphism has been shown to have an impact on total cholesterol, LDL-cholesterol and apoE plasma levels. Total and LDL cholesterol levels were the highest in apoE  $\epsilon 4$  stroke patients and the lowest in  $\epsilon 2$  subjects<sup>[52]</sup>. The role of this polymorphism in ischemic stroke, however, is inconclusive. A study in China showed a 2.1-fold significantly increased risk of ischemic stroke in Apo  $\epsilon 4$  carriers<sup>[53]</sup>. These results were replicated in a Tunisia study<sup>[51, 54]</sup>, where Apo  $\epsilon 4$  was found to be higher in the stroke patients (0.370 *vs* 0.181;  $P < 0.001$ ). Furthermore, there have been reports on the role of the  $\epsilon 4$  allele as a prognostic genetic marker for the atherothrombotic subtype, lacunar infarcts<sup>[55]</sup> and carotid plaques<sup>[56]</sup>. The  $\epsilon 2$  allele has been reported to be associated with lower risk of carotid atherosclerosis<sup>[56]</sup> and white matter disease<sup>[57]</sup>. However, several population-based studies<sup>[58]</sup> have shown no association between Apo  $\epsilon 4/\epsilon 2$  and stroke or stroke subtypes. In summary, the findings on the exact role of the ApoE gene in the risk of stroke are not clear.

The paraoxonase (PON) family has been demonstrated to prevent lipid peroxidation and consequently exerts anti-atherosclerotic effects. Alteration of enzyme activity due to polymorphisms in the PON genes may influence the development of atheromas and thus affect stroke risk<sup>[59]</sup>. Q192R, a common variant of the PON1 gene, is the major determinant of paraoxonase activity<sup>[60]</sup>. Several studies have shown that rare variants are associated with increased risk of ischemic stroke<sup>[61]</sup> and IMT<sup>[62]</sup>. However, several researchers<sup>[63]</sup> failed to replicate these data.

### Renin-angiotensin-aldosterone system

Angiotensin converting enzyme (ACE), an important member of the renin-angiotensin system, is involved in the develop-

ment of hypertension, atherosclerosis and cardiovascular disease. A deletion (D)/insertion (I) polymorphism of a 287-bp fragment of intron 16 of the ACE gene has been identified as a genetic factor for ischemic stroke<sup>[64]</sup>. Homozygous presence of D alleles is associated with higher plasma ACE activity. Case-control studies<sup>[65, 66]</sup> revealed that ACE D/D genotypes contribute to ischemic stroke incidence, and this may be due to the effect on essential hypertension. In one study<sup>[67]</sup>, the association between the ACE D allele and lacunar infarction was positive in the normotensive patients, but the association diminished in hypertensive patients, suggesting an independent effect of ACE D alleles on the lacunar infarction. However, regarding the role of ACE polymorphisms in ischemic stroke, the results are uncertain, with several studies failing to confirm the association<sup>[68]</sup>.

Angiotensinogen (AGT), another important member in the renin-angiotensin system, has a methionine to threonine substitution at amino acid 235 (M235T) in which the T allele has been reported to elevate the serum level of AGT, causing an increase in blood pressure<sup>[69]</sup>. As a consequence, van Rijn *et al*<sup>[69]</sup> found an association between the AGT TT genotype and atherosclerosis and white matter lesions (WML). In addition, the AGT 235T allele has been reported to contribute to salt sensitivity<sup>[70]</sup>. However, the effect of the AGT gene polymorphism on the risk of ischemic stroke remains controversial<sup>[71]</sup>.

#### **Hyperhomocysteinemia**

Hyperhomocysteinemia is an independent risk factor for ischemic stroke. A key enzyme, 5,10-methyltetrahydrofolate reductase (MTHFR), is involved in homocysteine metabolism. A common functional polymorphism of MTHFR, C677T, has been found to be associated with differences in homocysteine concentration, with about 1.93  $\mu\text{mol/L}$  between TT and CC homozygotes<sup>[72]</sup>. The MTHFR T allele is thought to be associated with thermolabile MTHFR and reduced enzyme activity. In a meta-analysis<sup>[72]</sup>, the OR for TT homozygotes as an independent risk factor for ischemic stroke was 1.26. Furthermore, the observed increase in risk of stroke among individuals homozygous for the MTHFR T allele was close to that predicted from the differences in homocysteine concentration conferred by this variant. This concordance is consistent with a causal relationship between homocysteine concentration and stroke. In terms of stroke subtypes, the T allele of the MTHFR gene is significantly associated with atherothrombotic stroke and WML in Japanese elderly individuals<sup>[73, 74]</sup>. However, some studies have produced controversial results<sup>[75]</sup>. Racial-ethnic differences in folate metabolism and localized dietary differences may account for the disparity. In addition, there may be an interaction between the HDL-C and MTHFR genotypes in atherothrombotic strokes. The authors believe that Hcy auto-oxidation supports the oxidation of LDL through generation of the superoxide anion radical. Furthermore, it has been reported that VLDL and LDL demonstrate a high binding capacity for Hcy. These findings may indicate synergistic interaction between MTHFR and lipid metabolism in the development of atherothrombotic stroke (Table 1).

#### **Matrix metalloproteinases (MMPs)**

Vascular remodeling is an essential phenomenon in the development of atherosclerotic changes in the arterial wall. MMPs with proteolytic activity against extracellular matrix (ECM) components<sup>[76]</sup> regulate the accumulation of extracellular matrix during tissue injury, and thus, MMPs regulate the growth of the atherosclerotic plaque<sup>[77]</sup>. Human atheroma samples display increased MMP-3 and MMP-9 expression in plaque shoulder regions and regions of foam cell accumulation<sup>[78]</sup>.

The 5A/6A polymorphism in the MMP-3 promoter region (-1612) is a widely studied gene locus. Most findings support the hypothesis that individuals homozygous for the 6A allele have low stromelysin concentrations in their arterial walls due to low gene transcription, and the resulting low proteolytic activity might favor deposition of the extracellular matrix, which would lead to a more rapid development and progression of an atherosclerotic plaque<sup>[79]</sup>. However, other findings do not support this hypothesis<sup>[80]</sup>.

#### **Phosphodiesterase 4D (PDE4D)**

PDE4D, which had previously been identified through GWAs, was identified as the first novel gene to predispose to ischemic stroke independently of conventional risk factors. PDE4D degrades second-messenger cAMP<sup>[81]</sup>, a key signaling molecule, and leads to increased proliferation and migration of vascular smooth-muscle cells *in vitro*, which is a hallmark of atherosclerosis<sup>[81]</sup>. In association analyses of an Icelandic population<sup>[82]</sup>, several SNPs in PDE4D were associated with the combined phenotype of cardioembolism (CE) and large-artery atherosclerosis, suggesting a mechanism of accelerated atherosclerosis. Subsequently, Bevan *et al*<sup>[83]</sup> performed a study in a non-Icelandic European population and found a possible association with CE stroke, but the lack of an association found with carotid IMT and plaque would suggest a mechanism other than accelerated atherosclerosis. However, the role of PDE4D is inconclusive, with some investigators<sup>[84, 85]</sup> replicating the association and some not<sup>[86]</sup>. Despite extensive genotyping of the PDE4D locus, the disease-causing genetic variant has not been identified thus far.

#### **ALOX5AP and leukotriene pathway**

ALOX5AP encoding 5-lipoxygenase activating protein (FLAP), an important component of the leukotriene pathway, is another gene that has been discovered through GWAs. A haplotype spanning ALOX5AP, HapA, was shown to be associated with a 1.7-fold increased risk of stroke in the Icelandic population<sup>[87]</sup>. The association between HapA and ischemic stroke was subsequently replicated in the Scottish population, which shares a common Gaelic ancestry with the Icelandic population<sup>[88]</sup>. However, negative results have also been demonstrated<sup>[84]</sup>.

Other genetic variations involved in leukotriene biosynthesis, such as the genes encoding LTA4 hydrolase, LTC4 synthase, and the LTB4 receptor, were found to confer an

**Table 1.** The association studies between selected candidate genes and ischemic stroke.

Gene	Polymorphism	Reference	Studydesign	Study population	Results
IL-6	174G/C	33	CC	119IS/133C	OR(GG)=8.6
		34	CC	214IS/214C	OR(GG)=3.2
		35	Linkage	854 members of 224 white British families	CC associated with 4.8% increase in carotid IMT
		36	CC	176S/212C North Indian	No association
PAI-1	4G/5G	49	Nested cc	Population-based surveys 2cohorts:113IS(A),275IS(B) Northern Sweden	OR(4G)=1.87-A OR(4G)=1.56-B
		51	CC	135IS/135C	OR(4G)=0.176
		50	CC	190IS/185C	NO association
APOE	ε2/ε3/ε4	51	CC	216S/282C Tunisia	(S:C)ε4-37%:18.1%;ε3-54.6%:73.6%
		54	CC	107IS/101C Yakut	OR(ε2)=0.35 ;OR(ε2/ε3)=0.28
		53	CC	226IS/201C China	OR(ε4/ε4)=2.1
		56	CC	5856 French	OR(ε4)=2.12;OR(ε2)=0.79 for IMT
		57	CC	334IS/TIA	OR(ε2)=2.9 for WML
		58	CC	100IS/96C Greek	No association
PON1	Q192R	61	CC	108IS/78C	OR(RR)=3.434
		63	CC	350IS/242C	NO association
ACE	I/D	65	Nested cc	275IS/549C	OR(D)=1.58
		66	Meta	76 studies published in mainland China	OR=1.87 for IS
		67	CC	129IS/27C Japan	OR(D)=2.57 for LI, not for ATI/CE
		68	CC	108IS/79C	No association
AGT	M235T	69	CC	>7000 Rotterdam	OR(TT)=1.25 for carotid plaques
		71	CC	928S/602C	NO association
MTHFR	C677T	72	Meta	111 studies published in 2003	OR(TT)=1.26 for IS
		73	Cohort	178SBI/1543C	OR(TT)=1.72 for SBI, OR(TT)=1.58 for WML
		74	CC	48ATI/38LI/9CE/241C	OR(TT)=3.87 for ATI
		75	CC	203S/55C	NO association

(CC=case-control study. C=controls. S=stroke. IS=ischemic stroke. IMT=intima-media thickness. TIA=transient ischaemic attack. LI= lacunar infarction. ATI= atherothrombotic infarction. CE= cardiac embolism. WML=white-matter lesions. SBI=silent brain infarction. OR=odd ratio)

increased risk of ischemic stroke<sup>[86, 88, 89]</sup>. However, these associations still need to be confirmed by further studies.

#### Other candidate pathways and genes

Research on the DNA damage and repair pathway<sup>[90]</sup>, cytochrome P450<sup>[91]</sup>, COX-2<sup>[92]</sup>, and endothelial nitric oxide synthase (eNOS)<sup>[21]</sup> in ischemic stroke has been reported; however, there was no reliable evidence of association, and further studies thus are required.

#### Hemorrhagic stroke

Hemorrhagic stroke includes two etiologically and pathologically different main disease categories: SAH and ICH. SAH usually results from the rupture of a cerebral arterial aneurysm<sup>[93]</sup>. ICH can be divided into primary ICH (PICH) and

secondary ICH. PICH accounts for 78%-88% of ICH cases and is mainly caused by chronic hypertension and spontaneous small vessel rupture due to amyloid angiopathy (CAA)<sup>[94]</sup>.

#### Single-gene disorder with hemorrhagic stroke

Two types of hereditary cerebral hemorrhage with amyloidosis (HCHWA) have been described: the Icelandic type (HCHWA-I) and the Dutch type (HCHWA-D). Both are autosomal dominant forms of amyloidosis restricted to the small vasculature of the brain and are clinically characterized by recurrent strokes, leading to an early death. In spite of their clinico-pathological similarities, the amyloid fibrils are structurally different.

HCHWA-D is an autosomal dominant disease caused by a glutamine to glutamic acid substitution at amino acid position

22 of codon 693 of the amyloid beta precursor protein (A $\beta$ -PP) gene located on chromosome 21<sup>[95]</sup>. The main symptoms are (recurrent) hemorrhagic stroke and dementia in the 5th or 6th decade of life<sup>[96]</sup>. About two thirds of patients with HCHWA-D experience fatal ICH, and the remaining one third present with vascular dementia. The mutation produces an aberrant A $\beta$  species (A $\beta$  E22Q) and causes severe meningocortical vascular A $\beta$  deposition<sup>[97]</sup>, which affects the proteolytic degradation of A $\beta$  and its transport across the blood-brain barrier<sup>[98]</sup>. Additionally, emerging studies provide additional evidence for a role of A $\beta$ -PP as a modulator of coagulation in *in vitro* studies. It is hypothesized that local binding of A $\beta$ -PP to FXIa and possibly also to factor IXa in cerebral amyloid-loaded vessels may favor the development of ICH<sup>[99]</sup>.

HCHWA-I, also termed hereditary cystatin C amyloid angiopathy, is an autosomal dominant disorder caused by a mutation in the gene that encodes for the cysteine protease inhibitor, cystatin C<sup>[100]</sup>. The mutation is a leucine to glutamine amino acid substitution at position 68 (C68Q), which allows the formation of amyloid deposits mainly in the arterial walls in the brain<sup>[101]</sup>. The amino acid substitution promotes an improperly folded, variant cystatin C molecule<sup>[102]</sup> that is more amyloidogenic. About 17% of strokes in Icelandic patients younger than 35 years are due to HCHWA-I<sup>[99]</sup>. Most patients with HCHWA-I experience their first stroke before the age of 30, and most patients die before age 50.

## Common multi-factorial stroke

### Hemostatic system

Emerging evidence points to a significant influence of coagulation factor gene polymorphisms on the incidence of hemorrhagic stroke. Among these, the effect of a FXIII Val34Leu polymorphism on ICH has been widely discussed. However, the role of this polymorphism in ICH is still controversial. Previous reports found that the 34Leu allele predisposes individuals to an increased risk for PICH in an Italian cohort (OR=1.70)<sup>[103]</sup>. In addition, fibrinogen concentration was found to modify the effect of the polymorphism, such that carriers of the 34Leu allele with a high concentration of fibrinogen may be at risk of hemorrhage<sup>[104]</sup>. Contradictory results, however, have also been reported<sup>[47]</sup>.

### Lipid metabolism

CAA is caused by the accumulation of amyloid beta-protein (A $\beta$ ), and the major clinical manifestation of CAA is stroke due to a lobar hemorrhage<sup>[94]</sup>. APOE  $\epsilon$ 4 was also found to be associated with CAA. A complex relationship between APOE  $\epsilon$ 4/ $\epsilon$ 2 and hemorrhagic stroke due to CAA (CAAH) is emerging<sup>[105]</sup>. In a study of elderly Japanese individuals<sup>[106]</sup>, APOE  $\epsilon$ 4 carriers showed significantly higher CAA severity than non- $\epsilon$ 4 carriers ( $p=0.0058$ ). Another pathological study has demonstrated that APOE  $\epsilon$ 2 is over-represented among patients with CAAH<sup>[107]</sup>. In addition, the  $\epsilon$ 2 allele is specifically associated with CAA-associated microangiopathic changes, such as fibrinoid necrosis and concentric splitting of the vessel wall<sup>[107]</sup>. Generally, it should be noted that the APOE  $\epsilon$ 4 allele

contributes more to CAA in the elderly, whereas the APOE  $\epsilon$ 2 allele contributes more to CAAH.

### Alpha-1 antichymotrypsin (ACT)

ACT, a serine proteinase inhibitor, has been implicated in the pathogenesis of hemorrhagic stroke. The TT genotype of the ACT signal peptide A/T polymorphism has been reported to confer susceptibility to PICH<sup>[108]</sup>. In analyses restricted to normotensive patients, the association became stronger. The ACT TT genotype may facilitate proteolytic rupture of vessels that harbor amyloidotic changes or play a role in another form of nonhypertensive cerebral angiopathy<sup>[108]</sup>. In a Chinese study, a significant gene-environment interaction, referred to as a super multiplicative type 4 interaction, was shown between both ACT AT and TT genotypes and primary hypertension, which is not fully consistent with other results<sup>[109]</sup>. On the other hand, some results support the data, suggesting a lack of association between the ACT A/T polymorphism and PICH<sup>[110]</sup>.

### Other association studies

The association between genetic variants of cytokines (IL-6, TNF)<sup>[111]</sup>, MMP-3<sup>[79]</sup> and the ER alpha gene (ESR1)<sup>[112]</sup> and hemorrhagic stroke has been explored, but the findings are inconclusive<sup>[113]</sup>. In addition, there is mounting evidence<sup>[114]</sup> that an eNOS T786C SNP predicts susceptibility to post-SAH vasospasm, which is a major cause of morbidity and mortality after SAH.

### Animal model

To claim causality for a genotype-phenotype relationship, the functionality of a candidate variant must be appropriately and convincingly confirmed *in vitro* and *in vivo*, the latter usually in animal models.

Spontaneously hypertensive stroke-prone rats (SHR-SP), which are an inbred animal model, resemble stroke in humans in many aspects, such as the dependence on a special permissive dietary regimen (low in potassium and protein, high in sodium) and elevated blood pressure. Conversely, spontaneously hypertensive rats (SHR) remain stroke-free, despite a similar degree of hypertension after exposure to the same diet. The F2 generation from experimental crossbreeding of SHR-SP and SHR can identify genetic loci predisposing specifically to stroke and exclude the confounding factor of blood pressure. The quantitative trait locus, termed Str1/Str2/Str3, identified through a genomic screening approach with quantitative locus mapping in F2 crosses, strongly affected the latency to stroke in SHR-SP fed a stroke-permissive diet<sup>[115]</sup>. Furthermore, Str2 co-localized with the gene for atrial natriuretic peptide (ANP), a hormone with vasoactive properties.

Other animal models of stroke have been generated through genetic engineering. For example, PON1 knockout mice have high amounts of oxidized LDL and are more susceptible to atherosclerosis<sup>[116]</sup>, whereas transgenic mice overexpressing human PON1 have decreased atherosclerotic lesion formation<sup>[117]</sup>. CADASIL knock-in mutant mice have also

provided valuable information on genetic factors involved in stroke predisposition in human beings.

### Potential clinical application of genetics of stroke

Gene therapy for stroke is one of the most promising frontiers. Theoretically, gene therapy could deliver critical proteins to lesions or bestow on recipient tissue a desired enzymatic or neurotrophic activity to prevent progression and promote regression. In the ApoE mouse, plaque progression was arrested and regression was induced after transfection with the wild-type ApoE gene<sup>[118]</sup>. Significant experimental gene therapy studies have led to currently active clinical trials using direct intracerebral delivery of viral vectors. As with any gene therapeutic strategy, issues about patient selection, route of delivery, vector selection, and appropriate clinical measures attend these nascent efforts<sup>[119]</sup>. However, the translation into clinical application is difficult to realize.

In addition, genetic tests are needed to assess the clinical utility of selecting drugs and doses routinely used to treat patients with stroke or at risk for stroke. For example, genotype-guided warfarin therapy for anticoagulation in elderly patients with AF was reported to be potentially cost-effective, and its benefits were closely related to efficacy in preventing bleeding events<sup>[120]</sup>. However, in general, the clinical usefulness of genetics testing was low, such as the activated protein C resistance/factor V Leiden mutation test<sup>[121]</sup>. Many tests were performed based on unsubstantiated or vague indications. Furthermore, normal test results led to unwarranted refrain from giving advice about antithrombotic measures, leading to potential harm to the patient.

### Perspectives and limitations

Since stroke is a complex disease comprising multiple risk factors and multi-gene involvement, unraveling the genetics of stroke represents a unique challenge. Although a large group of candidate genes have been studied, the epidemiological results have varied. The reasons for these contradictory results may be limited sample size, diversity in study design and endpoints, differences in genetic background, and selection of control populations. Thus, greater rigor and consistency in future studies are warranted to elucidate the role of genetic variations in stroke; such work will provide a rationale for preventing strokes with small-molecule therapy and diagnostic tools for predicting risk and the molecular subtype of a past or future stroke<sup>[122, 123]</sup>.

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