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Monogenic, Polygenic, and MicroRNA Markers for Ischemic Stroke

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Abstract

Ischemic stroke (IS) is a leading disease with high mortality and disability, as well as with limited therapeutic window. Biomarkers for earlier diagnosis of IS have long been pursued. Family and twin studies confirm that genetic variations play an important role in IS pathogenesis. Besides DNA mutations found previously by genetic linkage analysis for monogenic IS (Mendelian inheritance), recent studies using genome-wide associated study (GWAS) and microRNA expression profiling have resulted in a large number of DNA and microRNA biomarkers in polygenic IS (sporadic IS), especially in different IS subtypes and imaging phenotypes. The present review summarizes genetic markers discovered by clinical studies and discusses their pathogenic molecular mechanisms involved in developmental or regenerative anomalies of blood vessel walls, neuronal apoptosis, excitotoxic death, inflammation, neurogenesis, and angiogenesis. The possible impact of environment on genetics is addressed as well. We also include a perspective on further studies and clinical application of these IS biomarkers.

Keywords Biomarkers · Genetics · Ischemic stroke · MicroRNA · Monogenic · Polygenic

Introduction

Stroke is one of the leading causes of mortality and disability, annually affecting 16.9 million people worldwide [1]. Approximately 80–90% of cases are ischemic stroke (IS) [2, 3]. Cerebral lesions due to ischemia and hypoxia can cause acute, irreversible neuronal death. The short therapeutic window and poor prognosis bring a sense of urgency to developing earlier diagnostic strategies and preventive treatment [4–8]. Recent revolutionary advances in microarray and computational methodologies have facilitated high-throughput screening of biomarkers in complex diseases, as evidenced by recent successes in gene discovery for coronary artery disease [9]. Here, we summarize the potential genetic markers including DNA mutations, single nucleotide polymorphisms (SNP), and microRNAs that have been discovered so far in both single-gene and sporadic IS patients.

IS Subtypes, Heredity, and Modes of Inheritance

According to Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria, IS is classified into five subtypes: large

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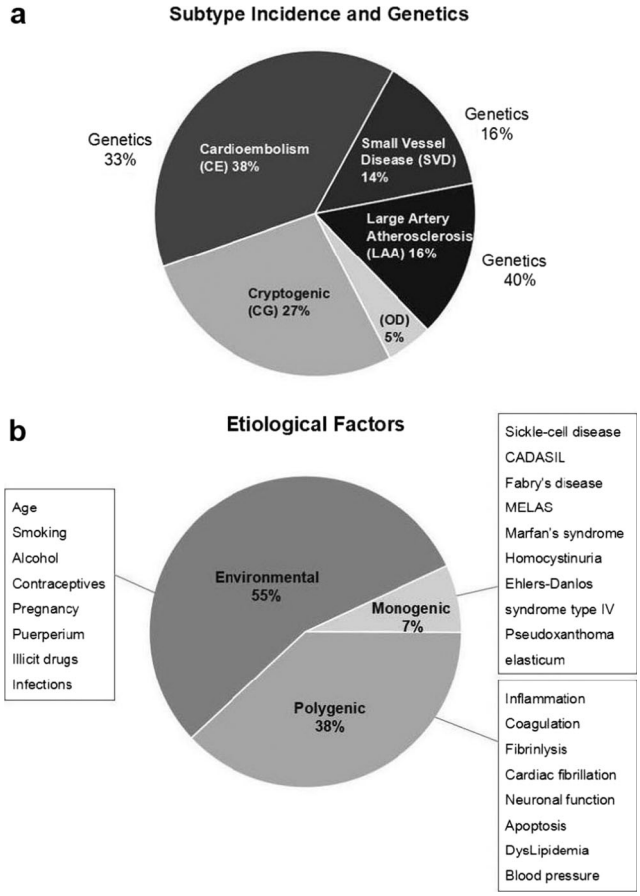

Fig. 1 Etiology, subtype and heredity of ischemic stroke (IS). **a** Incidence and genetics of different IS subtypes. The percentages in the boxes indicate incidences and those outside the boxes denote genetics; OD: other determined etiology. **b** Etiological factors of IS. The percentages in the boxes indicate incidences and the content in the text boxes indicates risk factors. These investigative data are extracted from references studying on European residents [11–14]

artery atherosclerosis (LAA), small-vessel disease (SVD), cardioembolic stroke (CES), other determined etiology (ODE), and cryptogenic ischemia stroke (CIS) [10]. It is reported that the distribution of these 5 subtypes among UK residents of European background is 16, 14, 38, 5, and 27%, respectively [11] (Fig. 1a).

The heredity of IS has been confirmed by twin and family-history studies [15] and extended by genome-wide association studies (GWAS) [12]. In general, hereditary factors cause IS by three pathways, i.e., monogenic, polygenetic, and epigenetic modes. Although monogenic IS has high penetrance, it only occurs in 7% of IS patients [13]. In contrast, about 38% of IS incidences result from genetic polymorphisms in multiple genes with each risk allele making minor effects to the pathogenesis. The various combinations of these risk alleles lead to different heredity of stroke subtypes, which is reported to be 40% for LAA, 33% for CES, and 16% for SVD (Fig. 1a) [12]. These results are consistent with the current common consensus that stroke is not a disorder but rather a syndrome. The involvement of epigenetic alterations in environmentally triggered phenotypes and diseases has generated a lot of interest [16] and this is also true for IS where environmental risk factors such as aging, smoking, alcohol and other such factors account for non-genetic etiologies of IS incidence [14] (Fig. 1b).

Genetic Markers for Monogenic IS

There are dozens of established monogenic disorders to date that can cause IS as the predominant clinical phenotype or as part of a systemic disease. The majority of monogenic forms of stroke are associated with single IS subtypes, although a few can present with more than one subtype [17]. Genetic linkage analysis has identified most of the pathogenic genes [18] as presented in Table 1 [19–49], which can act as robust diagnostic bases for clinical suspicion in young adults with stroke due to unknown causes [50]. According to targeted components of these genetic defects, most monogenic IS is caused by structural deficiencies of blood vessel walls due to developmental or regenerative anomalies (Fig. 2). Of note, the severity and onset age of IS phenotype are affected by genetic diversity and life styles [19].

Genetic Markers for Polygenic IS

Studies to identify genetic markers for common sporadic IS have used linkage-association analysis, genome-wide association (GWS) analysis and rare variation association analysis (RVA) based on next-generation genetic sequencing (NGGS) [51]. The use of these innovative approaches has already identified a number of novel variants associated with risk of IS (Table 3) and has provided important insights into the genetic basis of this disease.

Genetic Markers Discovered by Linkage-Association Studies

Linkage-association studies are based on the initial linkage analysis in stroke families to scan for susceptibility genes and subsequent association analysis in unrelated stroke individuals using microsatellite markers. Recent linkage-association studies have found two predisposed genes: arachidonate 5-lipoxygenase activating protein (ALOX5AP) and phosphodiesterase 4D (PDE4D), for common stroke and IS respectively [52, 53].

The ALOX5AP gene encodes a major regulator for 5-lipoxygenase that catalyzes the oxidation of arachidonic acid to leukotrienes A4 (LTA4), which is released by inflammatory cells at injured sites and thus plays an important role in atherosclerosis and other vascular damage [54]. Three SNPs (SG13S114A/T, SG13S89A/G and SG13S32A/C) of

Table 1 Genetic markers for monogenic cause of ischemic stroke

Genes	Gene products	Diseases	Mode of inheritance	Mutaition Loci	IS Subtype	No. of Mutations	Mechanisms	IS onset rates*	Age of IS Presentation	Refs
NOTCH3	Notch3	CADASIL	AD	19p13.2-p13.1	SVD	230	The Notch3 ectodomain misfolding and accumulation lead to intimal hyperplasia.	51–74%	46 ± 9.7 years	[14, 16–23]
HTRA1	HtrA serine peptidase/protease 1	CARASIL	AR	10q25	SVD	11	De-repression of TGF-β1 and fibrous intimal proliferation.	50%	20–30 years	[20, 24, 25]
TREX1	TREX1 3′-5′ exonuclease	RVCL	AD	3p21.31	SVD-like	5 frame-shift mutations	Lose of perinuclear localization and apoptosis induction leading to intimal hyperplasia	97%	40–50 years	[26–28]
COL4A1/COL4A2	Procollagen Type VI α1	HANAC, ARALES	AD	13q24	SVD	14	Interruption and thickening of capillary basement membrane.	6%	35–50 years	[29–31]
α-GAL A	α-galactosidase A	Fabry disease	X-linked	Xq22.1	LAA, SVD	245	Accumulation of globotriaosylceramide in lysosomes of vascular endothelial cells and smooth muscle cells.	Male: 6.0%; Female: 3.7%	40–55 years	[32–34]
HBB	β-hemoglobin	Sickle-cell anemia	AR	11p15.4	LAA, SVD		Defective Beta-chain hemoglobin molecule.	3.2%	< 45 years	[35, 36]
FBN1	Fibrillin-1	Marfan syndrome	AD	15q21.1	CES	637	Deficiency of fibrillin-1 disrupts the elastic lamina of aorta.	2.5%	< 40 years	[37–39]
CBS	Cystathione β-synthase	Homocystinuria	AR	21q22.3	LAA, SVD	48	High homocysteine leading to vascular endothelial injuries.	13%	childhood	[40–42]
NF1	Neurofibromin	Neurofibromatosis type 1	AD	17q11.2	LAA, SVD	553	Increasing mitogenic signaling and leading to cellular proliferation or differentiation	0.49%	< 30 years	[43–46]
MTLL1	MELAS	$tRNALeu^{(UUR)}$	Maternally inherited	mtDNA	IS-like	5	Protein synthese obstacle affect energy metabolism, reducing oxidation stress tolerance	84–99%	2–40 years	[47–49]

* IS onset rates in patients carrying monogenic mutations

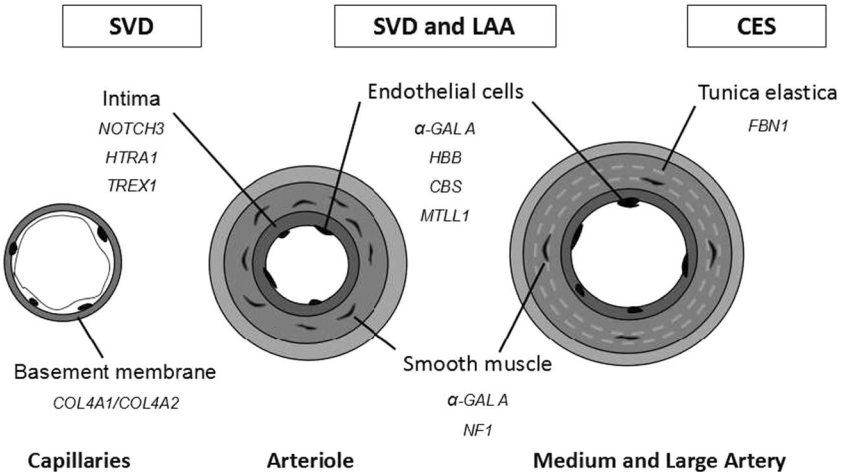

Fig. 2 Targeted components of blood vessels by genetic defects in monogenic ischemic stroke. The genetic mutations affecting integrity of basement membrane in capillaries and intima elastica in arterioles can lead to cerebral small vessel diseases (SVD); activation of endothelial cells and smooth muscle cells in arterioles and large arteries by dysfunctional expression of growth factors or metabolic enzymes can cause cerebral SVD and large artery atherosclerotic (LAA) stroke; mutations in FBN1 leading to deficient tunica elastica are the main causes of cardioembolic stroke (CES) in Marfan syndrome. The italic scripts in the figure denote genetic markers for monogenic ischemic stroke; the lines indicate the targeted components of blood vessel walls by genetic markers

ALOX5AP with a relationship to IS incidence have been extensively studied in Asians and Caucasians; however, conflicting results have been reported across different ethnic backgrounds [55]. Recent meta-analysis of the previous studies indicated SG13S114T as a risk factor of IS in the Caucasian population but a protective factor of LAA stroke in the Chinese population [55, 56].

The PDE4D gene encodes a cAMP-specific 3′, 5′-cyclic phosphodiesterase 4D that can degrade the signal transduction molecule cAMP in multiple cell types, including vascular cells [57]. Linkage-association studies identified an association of SNP 83 and AC008818-1 polymorphism in the PDE4D gene with carotid stroke in an Icelandic population [47]. But these results have not been completely replicated in other ethnicities. Recent meta-analysis concluded that the allele 0 of AC008818-1 was associated with only 1.12-fold risk increase of IS in Caucasians [58] and SNP83 polymorphism was associated with 1.69-fold risk increase of CE subtype of IS under the dominant model [59].

Genetic Markers Discovered by GWAS

Earlier studies utilizing GWAS focused on genetic variants identified in other cardiovascular diseases. Variants in two genes associated with atrial fibrillation (AF), PITX2 and ZFHX3, were found to be independent risk factors for IS. Further analysis determined that these associations were specific to CE stroke, which was confirmed by several subsequent studies [60–65]. Furthermore, a genetic variant in CDKN2A/CDKN2B located at chromosome 9p21 that has a widely reported association with myocardial infarction and coronary artery disease [66] was associated with LAA stroke across multiple cohorts [62, 67, 68].

The first widely replicated novel genetic association with IS subtype was the 7q21 locus near histone deacetylase 9 (HDAC9), which was found to be associated especially with the LAA stroke (OR 1.42) [60]. This association was replicated in the same study in additional patients from Europe, America, and Australia and in subsequent studies [62, 65]. Further GWAS analyses have reported that the genetic variants located at chromosome 6p21.1 and MMP12 and TSPAN2 were associated with LAA stroke, but not other subtypes of stroke among white populations [62, 65, 69, 70]. A genetic variant near ABCC1 was associated with CG-type IS in European and African populations [62].

Several GWAS studies performed in Asian populations identified an association of CELSR1 with IS in Japanese and the LAA subtype of IS in the Chinese Han population [71, 72]. A GWAS conducted in a group of Japanese IS patients identified a genetic variant in PRKCH especially associated with SVD stroke [73]. This result was replicated in both an independent Japanese cohort and a Chinese population [73, 74]. Additionally, a genetic variant at chromosome 14q13.3 nearing PTCSC3 was also reported to be associated with LAA stroke in the Chinese Han population [75]. A SNP locus (rs505922) in ABO gene increases the risk of LAA, CE and overall IS in Europeans while another locus in the same gene seems to be a protective factor for LAA stroke in the Chinese Han population [76, 77].

The first genetic variants to be associated with all types of IS were reported to lie in the chromosome 12p13 region

near NINJ2 [78]. However, this association has not been replicated in large case–control meta-analyses [61, 62, 65, 79]. The first association of a genetic variant with all types of IS to be replicated convincingly was reported with a locus at 12q24 near ALDH2 [62, 80]. Recently, a novel variant near FOXF2 was also found to be associated with overall stroke [81].

Genetic Markers Based on Imaging Phenotypes

White Matter Hyperintensities (WMH) WMH on T2-weighted MRI are reported to be a risk factor and predictor of lacunar stroke in prospective community populations [82, 83]. Severe confluent WMH are often found in patients with the SVD stroke subtype [84]. Twin and family history studies suggest a heritability of 55 to 80% to WMH [85] and common SNPs contribute between 13 and 45% to the heritability [86]. Previous candidate gene studies investigated variants in 19 genes and found associations between WMH extent and polymorphisms in APOE, MTHFR, ACE and ATG genes [87]. In 2011, the CHARGE Consortium performed the first GWAS on WMH in the general population and identified 6 SNPs mapping to a locus on chromosome 17q25 [88]. A recent meta-analysis of community populations and of stroke patients indicated 6 common genetic polymorphism loci that were associated with WMH at the genome-wide level: rs72934505 (NBEAL1); rs941898 (EVL); rs962888 (C1QL1); rs9515201 (COL4A2); rs7214628 (TRIM65); rs78857879 (EFEMP1) [89].

Carotid Intima-Media Thickness (CIMT) The CIMT based on high-resolution carotid duplex ultrasound is a surrogate marker of subclinical atherosclerosis and a strong predictor of stroke [90]. Family studies have demonstrated IMT heritability ranging from 30% to 65% [91]. A larger meta-analysis conducted by the CHARGE collaborators in over 31,000 European subjects has identified three novel carotid IMT associations that reach genome-wide significance ($p < 1 \times 10^{-8}$): rs11781551 (ZHX2); rs445925 (APOC1, APOE, APOC2, APOC4); rs6601530 (PINX1) [92]. The first large-scale GWAS of carotid IMT in a non-European population found one suggestive association ($p = 2.3 \times 10^{-7}$): rs17356664 (19q13, EXOC3L2, MARK4) [93]. While the first GWAS in an Asian population identified two novel loci that were significantly associated with CIMT at the genomic level ($p < 1 \times 10^{-7}$): rs36071027 (EBF1) and rs975809 (PCDH15) [94].

MicroRNA Markers for IS

miRNAs are small endogenously expressed noncoding RNAs approximately 22 nucleotides in length that regulate gene expression, mainly at the post-transcriptional level. The human genome encodes over 1000 miRNAs, each of which may regulate the level of hundreds of mRNAs [95]. Therefore, miRNAs are likely to be involved in many biologic and pathogenic processes [96, 97, 98]. The discovery of circulating miRNAs in peripheral blood, which are unexpectedly stable [99], has allowed the recent completion of several studies in human stroke patients that have confirmed the differential expression of specific miRNAs following stroke and have addressed their potential use as diagnostic and prognostic markers (Table 2).

MiRNA Profiling

The miRNA profiling studies have been conducted in succession in whole blood [100, 101], plasma [102–104], serum [105], peripheral blood cell [106], and cerebral spinal fluid [107] samples. There are substantial differences in miRNA expressions in young IS patients [101], IS with lower stroke risk [103], IS with post-stroke depression [104], or patients with other neurological diseases [107] compared with normal healthy people or control IS patients. Differences also exist between pre-stroke samples and post-stroke samples within various times after onset [100] (Table 3). Considering the different time periods examined in reference to injury and recovery and different sample types, these studies have led to little overlap in terms of consistency of finding.

Candidate MiRNA Markers

Besides miRNA profiling, several miRNAs documented to involve in apoptosis, excitotoxic neuronal death, inflammation, neurogenesis, and angiogenesis have showed significant dysregulation in IS patients (Table 3).

Apoptosis Apoptosis is the main cause of neuronal death in the penumbra region following acute brain infarction [2, 132–134]. In addition, the apoptosis of endothelial cells caused by hyperlipoidemia, hyperhomo-cystinemia, and hypertension could increase risk of stroke especially for LAA stroke. Increasing evidences support the involvement of miRNAs in the regulation of these apoptotic processes before and after IS [135].

MiR-16 belongs to cluster miR-15/miR-16 that has been well documented as an apoptosis-related miRNA [136]. It has been identified as tumor-suppressor gene and downregulation of miR-16 contributes to cancer development [137]. In contrast, miR-16 is increased in the serum of patients with critical limb ischemia and stroke [109]. The serum expression levels of miR-15a and miR-16 are found to be elevated 8.3- and 42-fold respectively in IS patients compared to controls. Diagnostic efficiency analysis by receiver operating characteristic (ROC) curves revealed areas under the curves (AUCs) of 0.698, and 0.82 for these two miRNAs respectively [108].

Table 2 Genetic markers for polygenic cause of ischemic stroke by genome-wide associated studies and meta-analysis

Genes	Chr	Marker loci	Minor alleles	Subtypes	Ethnic	Case (n)	OR	*P* value	Gene product	Gene functions	Shared diseases	Refs
PITX2	4q25	rs2200733	T	CE	Europe	1498	1.52	5.8×10^{-12}	Paired like homeodomain 2	Involving in left-right asymmetry during development.	AF	[60–63]
			T		Europe[c]	2322	1.32	5.1×10^{-8}				
			T		SiGN[a]	7418	1.36	8.1×10^{-30}				
		rs1906591	A		Europe	1376	1.48	1.2×10^{-7}				
HDAC9	7p21.1	rs11984041	A	LAA	Europe	1780	1.42	1.9×10^{-11}	Histone deacetylase 9	Involving in heart and muscle tissue development.	CAD, ICA	[61, 62, 65]
			A		SiGN[a]	4595	1.23	4.5×10^{-8}				
		rs2107595	A		Metastroke	2167	1.39	2.0×10^{-16}				
TSPAN2	1p13.2	rs12122341	G	LAA	SiGN[a]	4964	1.19	1.3×10^{-9}	Tetraspanin 2	Regulating cell development, activation, growth and motility.	Migraine, lung cancer	[62]
ABCC1	16p13.1	rs74475935	G	CIS	SiGN[a]	5861	4.63	4.7×10^{-11}	ATP-binding cassette C1	Functioning as an organic anion transporter	Cancer	[62]
ZFHX3	16q22.3	rs7193343	T	CE	Europe	1454	1.22	2.1×10^{-4}	Zinc finger homeobox protein 3	Regulating myogenic and neuronal differentiation.	AF	[62, 64, 65]
			T		SiGN[a]	7418	1.16	8.9×10^{-9}				
		rs879324	A		Metastroke	2365	1.25	1.5×10^{-7}				
CDKN2A/B	9p21.3	rs23832073	G	LAA	Europe	961	1.16	8.3×10^{-3}	Cyclin-dependent kinase inhibitor	Regulating CDK4 and p53 in cell cycle G1 progression	CAD, MI, ICA	[62, 67, 68]
			G		SiGN[a]	2346	1.09	8.1×10^{-3}				
		rs2383206	G		Chinese[b]	122	2.09	2.0×10^{-3}				
6p21	6p21.1	rs556621	T	LAA	Europe	2136	1.21	4.7×10^{-8}			Cancer	[62, 65, 69]
			T		Metastroke	4601	1.03	5.3×10^{-5}				
			T		SiGN[a]	2346	1.11	2.6×10^{-3}				
MMP12	11q22.2	rs660599	T	LAA	Europe	6778	1.18	2.6×10^{-8}	Matrix metallo-proteinase 12	Involving in the breakdown of extracellular matrix	CAD, cancer	[70]
CELSR1	22q13.31	rs6007897	G	IS	Japanese	750	1.85	4.7×10^{-4}	Cadherin EGF LAG sevenpass G-type receptor 1	Involving in contact-mediated communication in early embryo genesis	Spina bifida	[71, 72]
			G	LAA	Chinese	422	2.84	2.0×10^{-3}				
		rs4044210	G	IS	Japanese	748	1.78	1.0×10^{-3}				
				LAA	Chinese	422	2.93	1.0×10^{-3}				
PRKCH	14q23.1	rs2230500	A	SVD	Japanese	1628	1.40	5.1×10^{-7}	protein kinase Cη	Involving in vascular endothelium injury	HS	[73, 74]
			A	IS	Chinese	873	1.31	5.8×10^{-3}				
PTCSC3	14q13.3	rs934075	G	LAA	Chinese-Han	444	1.41	4.0×10^{-9}	Papillary thyroid carcinoma susceptibility candidate 3	Noncoding RNA involving in carcinoma development through modifying relative gene expression.	Thyroid tumors	[75]
		rs2415317	C				1.39	3.1×10^{-8}				
		rs1952706	T				1.37	2.9×10^{-8}				
ABO	9q34.2	rs505922	C	LAA	Europe	2113	1.23	1.0×10^{-3}	Glycosyltransferases	Converting H antigen into A or B antigen of blood group.	CAD, MI, VTE	[76, 77]
			C	CES	Europe	2326	1.13	2.0×10^{-4}				
		rs532436	A	IS	Europe	16,820	1.09	4.3×10^{-8}				
		rs2073824	A	LAA	Chinese	644	0.61	1.7×10^{-4}				
NINJ2	12p13.33	rs12425791	A	IS	Europe	1161	1.29	1.1×10^{-9}	Nerve injury induced protein 2	Promoting neurite outgrowth and nerve regeneration after nerve injury.	Prostate cancer	[61, 62, 70,
			A		Asian[b]	8626	1.08	2.5×10^{-2}				

Table 2 (continued)

Genes	Chr	Marker loci	Minor alleles	Subtypes	Ethnic	Case (n)	OR	*P* value	Gene product	Gene functions	Shared diseases	Refs
		rs11833579	A		Europe[c]	9407	1.00	0.98				78; 79]
			A		Metastroke	12,389	1.00	0.81				
			A		SiGN[a]	16,851	1.02	0.22				
ALDH2	12q24.12	rs10744777	T	IS	Europe	15,448	1.10	1.2×10^{-9}	Aldehyde dehydrogenase 2	Involving in alcohol metabolism	CAD, MI, HT	[62, 80]
					SiGN[a]	16,851	1.07	4.2×10^{-9}				
FOXF2	6p25	rs12204590	A	Stroke	SiGN[a]	19,816	1.08	1.48×10^{-8}	Forkhead box F2		ARS	[81]

SiGN Stroke Genetics Network, stroke cases were from American, European and African; *CAD* coronary artery disease, *MI* myocardial infarction, *VTE* venous thromboembolism, *CSA* coronary spastic angina, *HT* hypertension, *LAA* large artery atherosclerosis, *SVD* cerebral small vessel disease, *CIS* cryptogenic ischemia stroke, *ICA* intracranial aneurysm, *CES* cardioembolic stroke

[a] Cases including European, African and Americas using GWAS

[b] Meta-analysis only

[c] Replication study in population of Europen ancestry

Moreover, plasma miR-16 concentration in IS patients is higher than that in hemorrhagic stroke (HS) patients. The odds ratio (OR) for discriminating HS and IS with miR-16 is 9.75, suggesting miR-16 may be a good discrimination marker between HS and IS [110].

MiR-21 is initially reported to have a protective role in ischemia reperfusion-induced cardiocyte apoptosis via inhibiting the phosphatase and tensin homolog (PTEN)/Akt-dependent pathway [138]. Plasma miR-21 and miR-24 are significantly lower in acute cerebral infarction (ACI) patients within 24 h onset than in the controls, and a negative correlation is revealed between miR-21, miR-24 and the National Institutes of Health Scales Score (NIHSS). Therefore, plasma miR-21 and miR-24 are suggested as potential early stage markers of acute cerebral infarction [111]. Moreover, the data of N2A neuroblastoma cells following oxygen glucose deprivation (OGD) and reoxygenation indicates that miR-21 may have an anti-apoptotic effect, while miR-24 may have a pro-apoptotic effect [111].

MiR-29b belongs to the miR-29 family that is reported to target both pro- and anti-apoptotic BCL-2 family members [135]. It is markedly induced during neuronal maturation and functioned as a strong inhibitor of neuronal apoptosis through targeting BH3-only genes [139]. The levels of miR-29b from the infarct site and blood are both decreased in middle cerebral artery occlusion (MCAO) mice. Overexpression of miR-29b by gene transfer to mice brains reduces infarct volume, edema, and blood–brain barrier (BBB) disruption via downregulating aquaporin-4 (AQP-4) [112]. Clinical studies show that the level of miR-29b in white blood cells in stroke patients within 72 h after stroke onset is significantly lower compared with the controls, and is negatively associated with NIHSS scores and brain infarct volume, suggesting miR-29b as a circulating biomarker to predict stroke outcomes [112].

Inflammation The inflammatory response is not only an important etiological factor in IS, but also plays a pivotal role in early ischemia-hypoxia injuries and long-term neuronal recovery. It has been reported that inhibition of inflammatory cells reduces ischemic brain injury [140], but also increases the risk of diminished neurological outcome and death [141]. Studies on IS patients have disclosed significant changes of several miRNAs targeting inflammation factors: Let-7, miRNA-124, miRNA-145, miRNA-181.

Let-7 is a miRNA family containing 12 members in humans, which are highly abundant regulators of gene expression in the CNS [142]. Extracellular let-7 activates the RNA-sensing Toll-like receptor (TLR) and induces neurodegeneration [143]. Clinical studies show that the circulating let-7b is at a higher level in IS patients than healthy volunteers up through 24 weeks and the AUC of let-7b at 24 h, 1, 4 and 24w is 0.93, 0.92, 0.92 and 0.91 respectively [113]. Serum let-7e is also

Table 3 MicroRNA markers in patients with ischemic stroke

Category	Source[a]	Time[e]	Markers[f]	Refs	Category	Markers	Changes[g]	AUC	Target genes	Refs
Profiling	Whole blood	21–72 h	Up: miRNA-125b-2, 27a, 422a, 488, and 627.	[100]	Apoptosis	miRNA-15a	+8.3	0.70	Bcl-2, Akt-3, NF-κB	[108]
	Whole blood (Young[b])	6–18 m	Up: miRNA-25, 181a, 513a-5p, 550, 602, 665, 891a, 923, 933, 939, 1184, 1246, 1261, 1275, 1285, 1290, let-7e; Down: 15b, 126, 142-3p, 186, 519e, 768-5p, 1259, let-7 f.	[101]		−16	+42.0	0.82	Bcl-2, VEGF-A	[108, 109, 110]
						−21	+3.3	NA	Bcl-2	[111]
						−24	+4.9	NA	XIAP	111]
						−29b	–	NA	AQP-4	[112]
					Inflammation	miRNA-let-7b	+7.5	0.93	LOX-1	[113]
	Plasma	24 h	Up: miRNA-106b-5P, 4306; Down: 320e, 320d.	[102]		-let-7e	+1.5	0.86	CRP	[100, 114]
	Plasma (Low risk[c])	2–24 m	Up: miRNA-1258, 125a-5p, 1260, 1273, 149, 220b, 23a, 26b, 29b-1, 302e, 488, 490-3p, 506, 659, 890, 920, 934; Down: 25, 34b, 483-5p, 498.	[103]		-let-7c-5p	−1.9	NA	Caspase 3	[115]
						-let-7 f	–	NA	IL-6	[116]
						-let-7i	−2.1	NA	CD86, CXCL8, HMGB1	[105, 117]
						−124	+1.3	0.76	hs-CRP, MMP-9	[118]
	Plasma (Depression[d])	2w	Up: miRNA-22-3p, 4476, 486-5p, 92a-3p; Down: 1185- 1-3p, 1234-5p, 1247-3p, 133a, 183-3p, 187-5p, 3184-3p, 3202, 3615, 3667-5p, 4310, 4714-5p, 4716-3p, 4738-3p, 4769-5p,548a, 5571-5p, 629-3p, 636, 665, 887.	[104]		−145	+2.1	NA	KLF4/5	[119, 120, 121]
						−221	+10.4	NA	PTEN, AKT	[122]
					Excitotoxicity	miRNA-223	+3.5	NA	GluR2, NR2B	[123]
						−107	+2.8	0.96	GLT-1	[124]
						−128b	+2.1	0.90	SP1, GLT-1	[124]
	Serum	24 h	Up: miRNA-32-3p, 106-5p, 1246; Down: 532-5p.	[105]		−125b	+2.5	0.95	NR2A	[125, 126]
	PBC	72 h	Up: miRNA-363, 487b; Down: miRNA-122, 148a, 19a, 320d, 4429, let-7i.	[106]	Neurogenesis and angiogenesis	miRNA-9	–	NA	hs-CRP, MMP-9	[118, 127]
						−210	−1.4	0.80	EFNA3	[128, 129, 130]
	CSF	72 h	Up: miRNA-let-7c, 221-3p.	[107]		−126	−8.3	0.92	VCAM-1, DLK1	[113, 131]

AKT protein kinase B, *Akt-3* AKT serine/threonine kinase 3, *AQP-4* aquaporin-4, *Bcl-2* B cell lymphoma-2, *CSF* cerebral spinal fluid, *CXCL8* C-X-C motif chemokine ligand 8, *DLK-1* delta-like 1 homolog, *EFNA3* Eph family receptor interacting proteins-A3, *GLT-1* glutamate transporter-1, *GluR2* glutamate receptor 2, *HMGB1* high mobility group box-1, *hs-CRP* high-sensitive C-reactive protein, *IGF-1* insulin-like growth factor-1, *KLF4/5* kruppel-like factor 4/5, *IL-6* interleukin 6, *LOX-1* lectin-like oxidized low-density lipoprotein receptor-1, *MMP-9* matrix metalloprotein-9, *NF-KB* nuclear factor kappa-B, *NR2A* NMDA receptor 2A, *NR2B* NMDA receptor 2B, *PBC* peripheral blood cell, *PT* prothrombin, *PTEN* phosphatase and tensin homolog deleted on chromosome ten, *SP1* transcription factor sp1, *TLR* toll-like receptor, *VCAM-1* vascular cell adhesion molecule 1, *VEGF-A* vascular endothelial growth factor A, *XIAP* x-linked inhibitor of apoptosis protein, *NA* not available

[a] Sample source

[b] Young IS patients

[c] Low IS risk people

[d] IS patients with depression

[e] Deteting time from IS onset

[f] miRNA markers (up: upregulated expression; down: downregulated expression)

[g] References refer to supplemental materials

[h] Changing magnifications ("+" upregulated expression, "– " downregulated expression)

significantly increased in IS patients within 24 h onset and is positively associated with serum C reactive protein (CRP) levels. It has been demonstrated that let-7e had a specificity up to 73.4% and a sensitivity of 82.8% for IS diagnosis at the acute stage [114]. In contrast, the content of let-7c-5p is significantly reduced in the plasma of IS patients and the ipsilateral cortex and striatum of MCAO mice at 24 h reperfusion, which concurs with increased activation of microglia [115]. While another member of let-7 family, let-7f, is reported to be significantly downregulated in sera of massive cerebral infarction without hemorrhagic transformation (HT) patients within 48 h of a stroke compared with controls, leading to an increased serum IL-6 level [116]. Additionally, let-7i is reported to be decreased in circulating leukocytes of patients with acute IS within 72 h and to be associated with increased expression of its mRNA targets including CD86, CXCL8, HMGB1, indicating its involvement in leukocyte activation, recruitment and proliferation [117].

MiR-124 is the most abundant miRNA of the CNS and is commonly recognized as a modulator on polarization of activated microglia and infiltrating macrophages towards the anti-inflammatory M2 phenotype during focal cerebral ischemia [144]. The serum level of miR-124 in IS patients is significantly decreased within 24 h after IS onset, which is negatively correlated with infarct volume, plasma high-sensitivity C-reactive protein (hs-CRP) and MMP-9 levels [118]. The miR-124 expression is also found in atheromatous plaque of acute IS patients compared with intact tissue [145]. It seems that miR-124 is suppressed in acute IS thus facilitating inflammation and brain injury.

MiR-145 is recognized as a marker and modulator of vascular smooth muscle cell phenotype [146]. Serum miR-145 is significantly upregulated within 24 h after stroke onset, which is strongly and positively correlated with hs-CRP [119, 120]. It is also found to be significantly overexpressed in symptomatic versus asymptomatic human carotid plaques [121]. The antagomir to miR-145 has been found to be neuroprotective in vivo [147], indicating the potential use of miR-145 as a candidate biomarker or therapeutic target for stroke. In contrast, serum miR-221 is significant decreased in IS patients at 1 and 7 days after stroke onset and is negatively associated with hs-CRP. However, it is found to be significantly overexpressed in symptomatic versus asymptomatic human carotid plaques [120–122].

Excitotoxicity Excitotoxicity is an important trigger of neuronal damage in early-stage of cerebral ischemia. It stems from excessive accumulation of excitatory amino acids such as glutamate, which leads to toxic increases in intracellular calcium and zinc [148, 149]. Recent data indicated that specific miRNAs such as miRNA-223, miRNA-107, and miRNA-125b regulate glutamate neurotransmission and excitotoxicity during stroke [150].

miR-223 expression is increased in circulating blood samples of patients with acute IS, and the severity and volume of infarct is lesser in patients who had higher expression of miR-223 [151]. Overexpression of miR-223 is found to attenuate neuronal loss after excitotoxic insult by lowering the level of glutamate receptor subunits GluR2 and NMDA receptor 2B (NR2B) in brain [123]. However, several studies have shown that AMPAR lacking GluR2 subunit increases neuronal vulnerability to excitotoxicity [152, 153]. In addition, the circulating levels of miR-107 and miR-128b are increased 2.78- and 2.13-fold respectively in IS patients in comparison to the healthy volunteers and positively correlated with the severity of stroke as defined by NIHSS classes. The AUC for circulating miR-107 and miR-128b is 0.97 and 0.903 respectively [124]. At the same time, the elevation of both miR-107 and glutamate in IS patients are accounted at least partially by suppression of GLT-1 expression [154].

On the other hand, miR-125b exhibits maximum expression levels within the acute phase of stroke in humans [104, 125]. Together with evidence that the NR2A expression in hippocampal neurons is negatively regulated through its 3'UTR by FMRP, miR-125b, and Argonaute 1 [126], it can be inferred that overexpression of miR-125b after IS can downregulate the level of NR2ARs, reduce post-stroke excitotoxicity and protect cell from death.

Neurogenesis and Angiogenesis Angiogenesis and neurogenesis are crucial processes for brain tissue repair and remodeling after brain injury. MiR-9 has a key effect on differentiation of oligodendrocyte progenitor cells and myelinogenesis [155]. There is apparently disordered expression of miR-9 in patients with Alzheimer's disease or Huntington's disease [156]. miRNA-9 is downregulated in OGD neurons and MCAO mice brain and application of miR-9 agomir can restore the neurological scores and reduce infarct volume, brain water content, and behavioral impairments by promoting the repair of myelin sheath and suppressing neuronal apoptosis [127]. Serum level of miR-9 is reported to be negatively correlated with blood hsCRP, MMP-9 levels, infarct volume and NIHSS scores in acute IS patients with lesion volume > 4 cm^3 indicating that the protective role of miR-9 is also associated with inhibition of neuroinflammation [118].

MiR-210 is a pleiotropic hypoxiamir activated by hypoxia inducible factor-alpha (HIF-α) for hypoxic induction [157]. It is the only miRNA that is upregulated under hypoxia in several cell types in vitro [158]. Intracerebral injection of miR-210 could substantively promote endothelial cell proliferation and new microvessel formation, and increase the number of neural progenitor cells in the subventricular zone of normal adult mouse brain via the vascular endothelial growth factor (VEGF) pathway [159]. Furthermore, miR-210 is significantly upregulated in the adult rat ischemic cerebral cortex,

leading to enhanced Notch1 signaling [160]. miR-210 in human atherosclerotic plaques is four-fold higher than that in control arteries [128]. However, acute IS patients show significantly decreased blood miR-210 level at 4 and 7 days of stroke onset compared to healthy controls and patients with higher circulating blood miR-210 display better clinical outcomes, indicating blood miR-210 is a sensitive biomarker for clinical prognosis in acute cerebral ischemia [129, 130].

MiR-126 has been found to be specifically and highly expressed in human endothelial cells and enhances the proangiogenic actions of VEGF and FGF and promotes blood vessel formation by repressing the expression of Spred-1, an intracellular inhibitor of angiogenic signaling [129]. Targeted deletion of miR-126 in mice displays leaky vessels, hemorrhage and defective cardiac neovascularization following myocardial infarction [131]. A miRNA profiling study discloses that miR-126 is downregulated in young stroke patients. The AUC of plasma miR-126 at 24 h, 1, 4 and 24w is 0.92, 0.94, 0.93, and 0.92 respectively after symptoms onset [113].

Conclusion

Overall, the current evidences support that genetic factor is an important etiology of IS. Two genes, TREX1 and MTLL1, can serve as markers for monogenic IS due to high IS incidence in mutation carriers and have potential usefulness for IS prediction in people under 45 years of age. For polygenic IS, the use of GWAS techniques has identified a large number of markers. Four loci—PITX2, ZFHX3, HDAC9, and 12q24.12—have been repeatedly identified to exceed genome-scale significance ($p < 5.0 \times 10^{-8}$) in residents with European background. However, findings of several prospective studies indicate that improvements in prediction ability by genetic risk score (GRS) are limited [131, 160, 161]. It seems that polygenic IS is associated with excessive susceptible genes, each only exerting a minor effect, leading to its vulnerability to environmental, nutritional, and other non-genetic factors.

Considering the high heredity of polygenic IS and the present low predictive ability of GRS, novel genetic markers still need to be explored by using improved method. One possible improvement is to focus on rare genetic variations by using next generation gene sequencing technique. Based on the present finding that indicates “different subtypes, different variants”, refined clinical subtyping is also necessary. A recent study [162] showed significant enrichment in low-frequency variants (allele frequency < 5%) for both LAA and SVD, and an enrichment of higher frequency variants (allele frequency 10 and 30%) for CE (all $p < 10^{-5}$). Larger IS samples and more detailed stratification sampling are also needed to exclude the interference of non-inherited elements and to allow for well-powered studies that link genotype to phenotype. Prediction models may have to be changed to personalized medicine due to the complex nature of polygenic IS. The possible impact of environment on epigenetic regulation needs to be validated in large scale human studies to help understand the effects of extrinsic factors on IS over time and across generations.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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