Original Article

Monogenic basis of young-onset cryptogenic stroke: a multicenter study

Wei-Zhuang Yuan[1#], Liang Shang[2#], Dai-Shi Tian[3], Shi-Wen Wu[4], Yong You[5], Cheng-Lin Tian[6], Bo Wu[7], Jun Liu[8], Qin-Jian Sun[9], Qing Liu[1], Wei-Hai Xu[1]

[1]Department of Neurology, State Key Laboratory of Complex Severe and Rare Diseases, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences (CAMS) and Peking Union Medical College (PUMC), Beijing, China; [2]Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (CAMS) and Peking Union Medical College (PUMC), Beijing, China; [3]Department of Neurology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China; [4]Department of Neurology, The Third Medical Center of General Hospital of the Chinese People's Armed Police Force, Beijing, China; [5]Department of Neurology, The First Affiliated Hospital of University of South China, Hengyang, China; [6]Department of Neurology, Chinese PLA General Hospital, Beijing, China; [7]Department of Neurology, West China Hospital, Sichuan University, Chengdu, China; [8]Department of Neurology, Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China; [9]Department of Neurology, Shandong Provincial Hospital, Jinan, China

Contributions: (I) Conception and design: Q Liu, WH Xu; (II) Administrative support: WH Xu; (III) Provision of study materials or patients: All authors; (IV) Collection and assembly of data: All authors; (V) Data analysis and interpretation: Q Liu, WZ Yuan, L Shang; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to the work.

Correspondence to: Qing Liu; Wei-Hai Xu. Department of Neurology, State Key Laboratory of Complex Severe and Rare Diseases, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences (CAMS) and Peking Union Medical College (PUMC), Shuaifuyuan1, Dongcheng District, Beijing 100730, China. Email: drliuqing@126.com; xuwh@pumch.cn.

Background: The prevalence of stroke in young adults is increasing. We investigated the monogenic basis of young adult cryptogenic stroke patients.

Methods: This multicenter study enrolled cryptogenic stroke patients under 55 years old, and individuals with nonstroke diseases were included as controls. Targeted next-generation sequencing (NGS) was applied with a custom-designed gene panel that included 551 genes. Rare variants were classified into 2 groups: pathogenic variants and variants of unknown significance.

Results: A total of 153 individuals, including 30 (21 males, 70%; mean age 36.1±10.2 years) in the disease group and 123 (59 males, 48.0%; mean age 40.4±13.1 years) in the control group, were recruited. In the disease group, 32 rare variants were identified. Among these individuals, 18 pathogenic variants in 16 patients were detected, with a 53.3% (16/30) diagnostic yield of monogenic causes for cryptogenic stroke. None of these mutations were observed in the control group. Among the mutant genes, the most prevalent were Notch receptor 3 (*NOTCH3*), protein kinase AMP-activated noncatalytic subunit gamma 2 (*PRKAG2*), and ryanodine receptor 2 (*RYR2*). Genes associated with cardiogenic diseases showed the highest mutation frequency (10/18, 55.6%) followed by genes associated with small-vessel diseases (SVDs) and coagulation disorders. None of the patients with mutations had evident abnormalities in the heart or other systems checked by routine tests. For the imaging phenotype–genotype association analysis, infarctions in both the anterior and posterior cerebral circulation were only observed in patients with genes related to cardiogenic disease.

Conclusions: In this study, pathogenic variants were identified in nearly half of the young-onset cryptogenic stroke patients, with genes related to cardiogenic diseases being the most frequently mutated. This may have implications for future clinical decision-making, including the development of finer and more sensitive examinations.

Keywords: Stroke in young adults; ischemic stroke; genetic

Submitted Jul 25, 2021. Accepted for publication Dec 31, 2021.
doi: 10.21037/atm-21-3843
View this article at: https://dx.doi.org/10.21037/atm-21-3843

Introduction

Despite a decline in older adults, the stroke incidence in young adults is increasing (1). Stroke at younger ages is catastrophic because patients can be disabled for long periods and suffer severe loss of functionality at the peak of their most productive years. Even after extensive tests, the etiology of stroke in young adults is not determined in 25% to 50% of cases, a situation referred to as cryptogenic stroke (2-4). Clinical genetic studies have the potential to discover the underlying biological mechanisms that cause cryptogenic stroke, and an increasing number of genes concerning hereditary cerebrovascular diseases have been reported upon. Genetic factors may account for up to 50% of an individual's risk of developing a stroke in the future (5). We investigated young-onset cryptogenic stroke as a clinical entity, as knowledge of its genetic aspects may help to elucidate the mechanisms of this type of stroke and contribute to the development of medical practices in the future.

Next-generation sequencing (NGS) is now widely used in clinical practice and research due to its efficiency and relatively low cost. Instead of screening the whole genome or exome, targeted NGS (tNGS) aimed at limited and selective genes may detect variants, thus providing substantial advantages of analysis time, cost, targeting, and read coverage or depth (6). In the current study, we designed a gene panel comprising 551 genes for cryptogenic stroke screening in young adults. We present the following article in accordance with the STROBE reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-21-3843/rc).

Methods

Study design and patients

In this prospective, multicenter study, patients under 55 years of age with cryptogenic stroke within the previous 6 months were enrolled. Cryptogenic stroke was defined as an ischemic stroke not attributed to a definite source of large-vessel atherosclerosis, cardioembolism, or small-vessel disease (SVD), according to the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) classification in the presence of extensive cardiac, vascular, hematologic, and serological evaluation (7). Stroke patients with incomplete evaluation or evidence of more than 1 competing cause were not included in this study. Ethnic- and age-matched individuals with neurological or nonneurological disorders were included as controls when cerebral infarction or hemorrhage was ruled out.

The study protocol was approved by the review board of Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and participating centers (No. ZS-1554) and was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Written informed consent was obtained from all patients.

Evaluation, sample collection, and genetic testing

To identify stroke etiologies, comprehensive examinations were conducted before enrollment, including (I) carotid duplex; (II2) transcranial Doppler ultrasound bubble test (in all patients) or transesophageal echocardiogram (in 2 patients); (III) magnetic resonance angiography or computed tomography angiography; (IV) ultrasonic cardiogram, regular electrocardiogram (ECG), or 24-hour ECG monitoring; (V) relevant blood tests. After enrollment, the clinical data, including age, sex, conventional risk factors, history of cardiovascular diseases, and family medical records, were recorded on standardized case report forms. Conventional risk factors included hypertension, diabetes mellitus, hypercholesterolemia/hyperlipidemia, cigarette smoking, cardiovascular diseases, and arrhythmia, including atrial fibrillation (AF). Other risk factors were also included to explore rare risk factors, including tumors, sleep disorders, trauma, infections, drug or alcohol intake, strenuous activity, or the Valsalva maneuver before stroke onset. A review of concurrent diseases was performed, including of autoimmune disease, thyroid disease, hematological system disorders, and other disorders. Positive family history was recorded if there was a stroke in first-degree relatives.

tNGS and interpretation

Multiple genes associated with stroke etiology were included

in the gene panel design (refer to available online: https://cdn.amegroups.cn/static/public/atm-21-3843-1.pdf). We incorporated a total of 551 genes related to arterial structure abnormalities, cardiogenic pathways, coagulating deficits, and metabolic causes, such as hereditary hypertension, hyperlipidemia, and hyperhomocysteinemia. Genes were included according to the following criteria: (I) causative or postulated to be susceptible genes in stroke patients and (II) related to stroke or exhibiting stroke-related traits, such as cardiac arrhythmia and structural diseases. These genes could be classified into 8 categories: (I) SVD; (II) non-SVD arterial diseases; (III) coagulation and anticoagulation imbalance; (IV) metabolic disorders; (V) congenital heart diseases; (VI) hereditary cardiomyopathy; (VII) arrhythmic disease; and (VIII) miscellaneous.

Genomic DNA was extracted from peripheral blood leukocytes using a QIAamp DNA Blood Mini Kit (Qiagen Inc., Valencia, CA, USA) according to standard protocols. An enriched genomic library was sequenced on the HiSeq2000 platform (Illumina Inc., San Diego, CA, USA) with 90-bp paired-end reads. Variants with a read depth ≥10 and genotype quality ≥20 were retained as quality-control metrics. The achieved average depth and coverage were above 100× and 96.5%, respectively. Sequence alignments were conducted in reference to UCSC hg19 (*Homo sapiens*) using the open-source Burrows-Wheeler-Alignment software tool v. 0.7.17 and then reformatted using the SAMtools software package v. 1.9 (8). Variant frequencies were initially determined in the Genome Aggregation Database (gnomAD) and Exome Aggregation Consortium (ExAC), to remove common single nucleotide polymorphisms (SNPs). Only nonsynonymous, splicing, and frameshift variants with a minor allele frequency (MAF) of less than 0.5% across all population databases were selected for further analysis. Variant annotation and filtering were performed by Mutation Taster (http://www.mutationtaster.org), SIFT (http://sift.jcvi.org/), and PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/). Sanger sequencing was performed to validate the identified mutations. The criteria used to define the variants were based on the inheritance pattern of disease genes and the recommendations of the American College of Medical Genetics (ACMG) (9).

Rare variants were categorized into 2 subgroups for practical application: pathogenic and unknown significance. Pathogenic variants met the ACMG standards (9). Variants with unknown significance (VUS) were defined as (I) variants that did not match the ACMG criteria for pathogenic variants that might include mismatched inheritance patterns and (II) variants of patients from unverifiable family history or unavailable segregation analysis. The profile and spectrum of mutant genes were summarized. Genotype–phenotype analysis was performed in the mutation group stratified by stroke lesion number and location.

Statistical analysis

Quantitative data were described as mean ± standard deviation, while qualitative data were described as counts with percentage. Data were analyzed with SPSS v. 23.0 software (IBM Corp., Armonk, NY, USA).

Results

Clinical data

A total of 51 patients from 15 hospitals were initially enrolled as the disease group from October 2017 to November 2018. We excluded 4 patients with intracerebral hemorrhages, 1 patient with transient ischemic attack, and 16 nonstroke patients. Finally, 30 patients (21 males, 70%; mean age 36.1±10.2 years) were eligible for enrollment as disease group participants. Of these, 7 patients (23.3%) and 17 (56.7%) patients had risk factors and a family history of stroke, respectively; 12 patients were aged under 35 years, 9 patients were 35–44 years, and 9 were 45–55 years; 8 and 16 patients had posterior or anterior cerebral infarction, respectively; 5 patients had infarctions located in both the anterior and posterior regions, whereas the other 1 patient had a cerebral border zone infarction; as for cardiac evaluation, transesophageal echocardiography was conducted in 2 patients, and a patent foramen ovale was found in 1 patient.

A total of 123 patients (59 males, 48.0%) were enrolled as the control group. The mean age of the control group was 40.4 years (the standard deviation was 13.1). Within the control group, 68 patients were diagnosed with amyotrophic lateral sclerosis, 10 had follicular thyroid carcinoma, 19 had idiopathic tremors, 7 had genetic muscular diseases, 7 had hereditary dystonia, 5 had hereditary spastic paraplegia, 3 had hereditary peripheral neuropathy, 2 had mitochondrial disease, 1 had familial epilepsy, and 1 had paroxysmal dystonia. Risk factors, which were found in 13% (n=16) of the control group, included hypertension (in 4 patients), hyperlipidemia (in 5 patients), and smoking (in 8 patients).

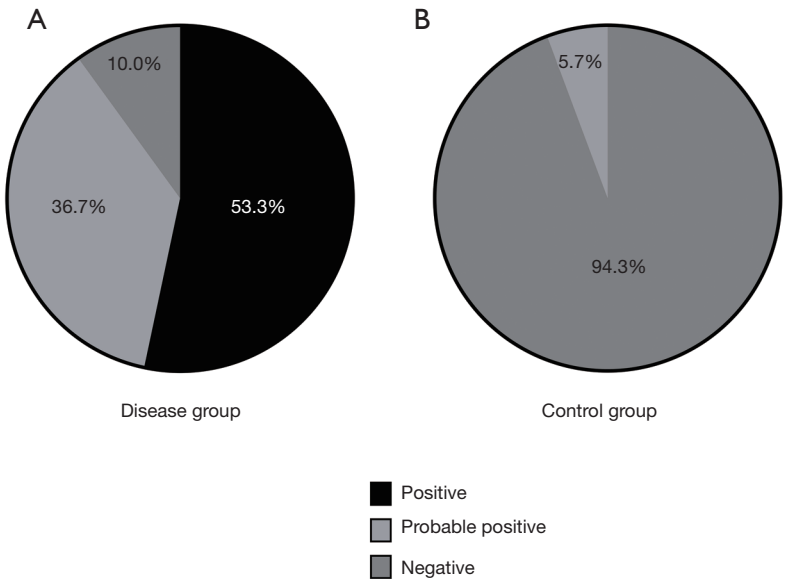

Figure 1 Diagnostic yield in (A) the disease group and (B) the control group.

Genetic analysis

A total of 32 rare variants in 24 genes in 27 patients (27/30, 90%) were identified in the disease group and 12 rare variants in 7 patients (7/123, 5.7%) were detected in the control group (*Figure 1A*,*1B*, available online: https://cdn.amegroups.cn/static/public/atm-21-3843-1.pdf). Among them, 18 pathogenic variants in 16 patients (16/30, 53.3%) were found in the stroke group according to the ACMG criteria, but none of these mutations were found in the 123 control individuals (available online: https://cdn.amegroups.cn/static/public/atm-21-3843-1.pdf). Variants that did not match the inheritance pattern or needed further segregation or functional analysis to confirm their pathogenicity were included in the list of unknown significance. These variants might have included previously reported missense variants in genes, such as Titin (*TTN*, listed in https://cdn.amegroups.cn/static/public/atm-21-3843-1.pdf). The clinical details of the patients with these variants are also reported in https://cdn.amegroups.cn/static/public/atm-21-3843-1.pdf.

The majority of monogenic disease genes were related to cardiogenic disorders. Of the mutation spectrum, there were 9 patients with 10 pathogenic variants in the genes of hereditary cardiomyopathy or congenital heart disease, 4 in genes related to SVD (22.2%), and 4 in genes related to coagulation and anticoagulation imbalances (22.2%; *Figure 2A*). These mutations added up to a total of 10 mutations in genes related to cardiac disease, which accounted for 55.6% (10/18) of genetic causes.

The Notch Receptor 3 *(NOTCH3*) was the most frequently mutated gene in the patient group. Three mutations in *NOTCH3* were detected in 3 patients (10%), followed by 2 pathogenic variants in the ryanodine receptor 2 (*RYR2*) and protein kinase AMP-activated noncatalytic subunit gamma 2 (*PRKAG2*). Other mutant genes are listed in *Table 1* and shown in *Figure 2A*.

Loss of function (LoF) mutations were detected in junctophilin 2 (*JPH2*), *TTN*, fibrinogen alpha chain (*FGA*), protein C *(PROC*), and Von Hippel-Lindau tumor suppressor (*VHL*). Except for the mutation in *PROC* (29), all the others were novel. Two *RYR2* mutations were found, one of which was reported with functional evidence for pathogenicity (p. T1107M) (15). The other variant, p. D4195A, was absent in the 123 control individuals. This variant was located in the region conserved across species, and 2 adjacent mutations (p. C4193W, p. T4196A) were reported to be pathogenic (32,33). One rare galactosidase alpha variant (p. L21V) identified showed high conservation, and a different missense change was previously reported (p.L21P) (34). Two mutations were related to thrombophilia, including *PROC* and protein S *(PROS1*), which previous *in vitro* studies suggest might affect anticoagulant functions (30,31).

In addition, 3 patients had more than 1 rare variant. Patient 44 had 2 LoF mutations in *FGA* and *TTN*. Patient

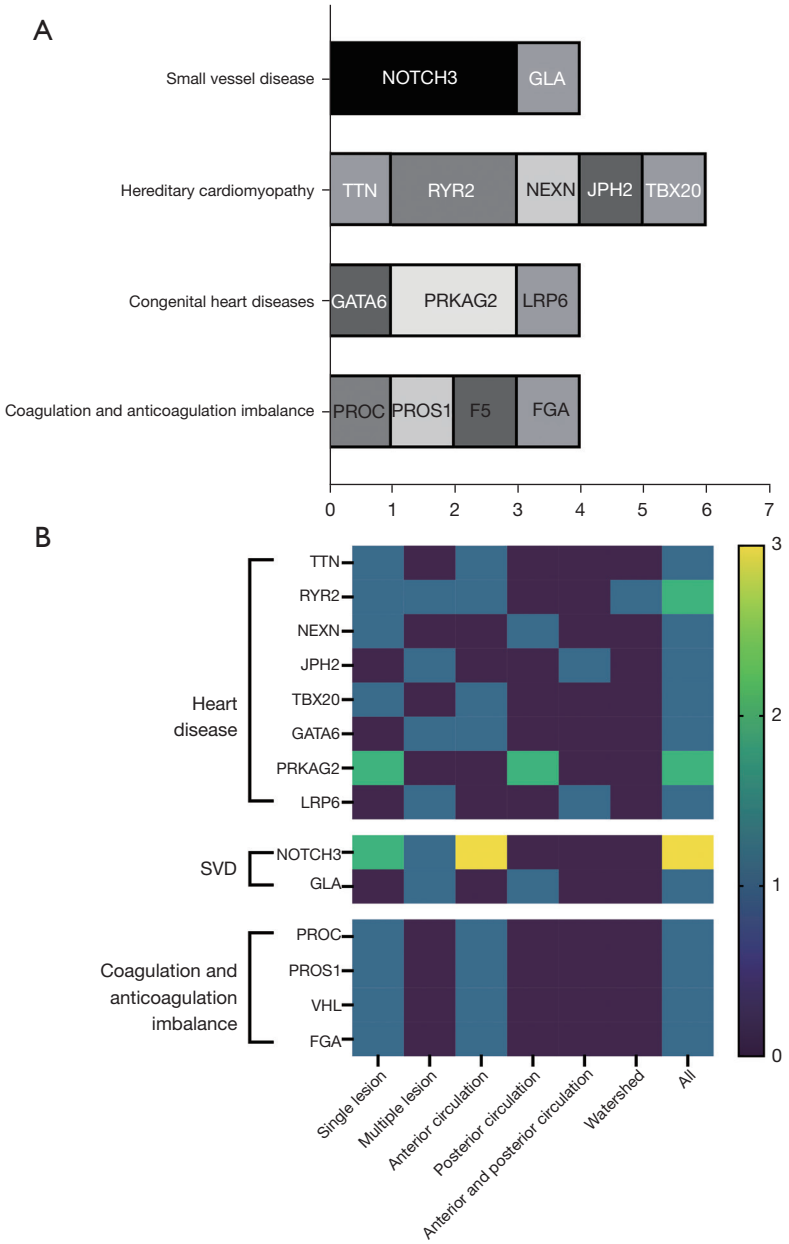

Figure 2 Pathogenic gene variant number in (A) the different disease categories and (B) by phenotype. *NEXN*, nexilin; *JPH2*, junctophilin 2; *TTN*, titin; *RYR2*, ryanodine receptor 2; *FGA*, fibrinogen alpha chain; *TBX20*, T-box transcription factor 20; *GATA6*, GATA binding protein 6; *LRP6*, low-density lipoprotein receptor–related protein 6; *PROC*, protein C; *PROS1*, protein S; *VHL*, Von Hippel-Lindau tumor suppressor; *GLA*, galactosidase alpha; *NOTCH3*, Notch receptor 3; *PRKAG2*, protein kinase AMP-activated noncatalytic subunit gamma 2; SVD, small vessel disease.

4 had 2 *PRKAG2* variants, both previously reported to be damaging (22-24). Patient 21 had a LoF mutation in phospholipase A2 group VII and a rare variant of unknown significance in *TTN* (see available online: https://cdn.amegroups.cn/static/public/atm-21-3843-1.pdf).

Phenotype-genotype analysis

When stratified into single versus multiple lesions, *NOTCH3 and PRKAG2* were the most prevalent genes with a single infarction. For multiple infarctions, the mutated

Table 1 Phenotype and genotype of patients with pathogenic gene variants

Patient number	Sex	Age	Risk factor	Family history	MRI characteristics	Gene symbol	Inheritance	Refseq	Nucleotide change (Protein change)	Mutation	Status	Exon/Intron ID	SNP ID	Frequency	Disease abbreviation	Categories
7	F	29	No	Yes	Bilateral multiple cortexes and subcortical infarction in the anterior and posterior circulation	*JPH2*	AD	NM_020433	c.G880T (p.E294X)	Nonsense	Novel	Exon 2	–	–	CMH17	Hereditary cardiomyopathy
22	F	45	No	No	Unilateral single cortical infarction in watershed territory	*RYR2*	AD	NM_001035	c.C3320T (p.T1107 M)	Missense	Reported (10-15)	Exon 28	Rs200236750	0.000323[e]	CPVT	Hereditary cardiomyopathy
30	M	35	No	Yes	Unilateral multiple cortex infarction in the anterior circulation	*RYR2*	AD	NM_001035	c.A12584C (p.D4195A)	Missense	Novel	Exon 90	–	–	CPVT	Hereditary cardiomyopathy
44	M	17	No	No	Unilateral single subcortical infarction in anterior circulation without the involvement of the internal capsule and basal ganglia region	*TTN*	AD	NM_001256850	c.T54027G (p.Y18009X)	Nonsense	Novel	Exon 249	–	–	Dilated cardiomyopathy	Hereditary cardiomyopathy
						FGA	AD	NM_000508	c.C607T (p.Q203X)	Nonsense	Reported (16)	Exon 5	Rs200299414	0.0000082[e]	Familial visceral amyloidosis and Hypofibrinogenemia	Coagulation and anticoagulation imbalance
46	M	35	No	No	Unilateral multiple cortex infarction in the anterior circulation	*TBX20*	—	NM_001077653	c.G1001A (p.R334Q)	Missense	Reported (17)	Exon 7	–	–	FDC	Hereditary cardiomyopathy
1	M	24	No	Yes	Unilateral single cortical infarction in the posterior circulation	*NEXN*	AD	NM_144573	c.C835T (p.R279C)	Missense	Reported (18)	Exon 8	Rs146245480	0.0012[c], 0.0005945[e]	FHC	Hereditary cardiomyopathy
23	M	46	No	Yes	Bilateral multiple cortex infarction in the anterior circulation	*GATA6*	AD	NM_005257	c.G551A (p.S184N)	Missense	Reported (19,20)	Exon 2	Rs387906816	0.001[c], 0.000841[e]	ASD9 and TOF	Congenital heart diseases
47	F	41	No	Yes	Unilateral multiple cortexes and subcortical infarctions in the anterior and posterior circulation	*LRP6*	AD	NM_002336	c.A246T (p.K82N)	Missense	Reported (21)	Exon 2	Rs199693693	0.0002636[e]	ADCAD2	Congenital heart diseases
4	F	36	Hypertension	Yes	Unilateral single pons infarction	*PRKAG2*	AD	NM_016203	c.G298A (p.G100S)	Missense	Reported (22)	Exon 3	Rs79474211	0.01458[c], 0.0081323[e]	PRKAG2 syndrome	Congenital heart diseases
						PRKAG2	AD	NM_016204	c.G130A (p.A44T)	Missense	Reported (23,24)	Exon 2	Rs144857453	0.001[c], 0.0002465[e]	PRKAG2 syndrome	Congenital heart diseases
2	M	40	No	Yes	Unilateral single subcortical infarction in the territory of one perforating arteriole in the anterior circulation and severe white matter hyperintensities	*NOTCH3*	AD	NM_000435	c.C505T (p.R169C)	Missense	Reported (25,26)	Exon 4	Rs28933696	–	CADASIL	Small-vessel diseases
12	M	54	No	No	Unilateral single subcortical infarction in the territory of one perforating arteriole in the anterior circulation and severe white matter hyperintensities	*NOTCH3*	AD	NM_000435	c.C368G (p.C123S)	Missense	Novel	Exon 4	–	–	CADASIL	Small-vessel diseases
14	F	35	No	Yes	Unilateral single subcortical infarction in the territory of one perforating arteriole in the anterior circulation	*NOTCH3*	AD	NM_000435	c.A502T (p.C168S)	Missense	Novel	Exon 4	–	–	CADASIL	Small-vessel diseases
38	M	44	No	No	Unilateral single subcortical infarction in the territory of one perforating arteriole in the anterior circulation	*PROC*	AD	NM_000312	c.572_574delAGA (p.K192del)	Codon deletion	Reported (27-30)	Exon 7	–	0.00066[e], 0.004[g]	Protein C deficiency	Coagulation and anticoagulation imbalance
50	F	48	No	Yes	Unilateral single subcortical infarction in the territory of one perforating arteriole in the anterior circulation	*PROS1*	AD	NM_000313	c.A1095C (p.N365K)	Missense	Reported (31)	Exon 10	Rs199469491	0.0006[c], 0.0014666[e]	Protein S deficiency	Coagulation and anticoagulation imbalance
25	M	26	Smoking	Yes	Unilateral single cortical and subcortical infarction in anterior circulation without the involvement of the internal capsule and basal ganglia region	*VHL*	AD	NM_000551	c.G106T (p.E36X)	Nonsense	Novel	Exon 1	–	–	ECYT2	Coagulation and anticoagulation imbalance
10	M	37	Smoking, diabetes mellitus, hypertension, and hyperlipidemia	Yes	Bilateral multiple cortical infarctions in the posterior circulation	*GLA*	XR	NM_000169	c.C61G (p.L21V)	Missense	Novel	Exon 1	–	–	Fabry disease	Small-vessel diseases

[c], ClinVar frequency; [e], ExACAF frequency; [g], 1000-genome frequency. MRI, magnetic resonance imaging; *NEXN*, nexilin; *JPH2*, junctophilin 2; *TTN*, Titin; *RYR2,* ryanodine receptor 2; *FGA,* fibrinogen alpha chain; *TBX20*, T-box transcription factor 20; *GATA6*, GATA binding protein 6; *LRP6*, low-density lipoprotein receptor-related protein 6; *PROC*, protein C; *PROS1*, protein S; *VHL*, von Hippel-Lindau tumor suppressor; *PRKAG2*, protein kinase AMP-activated noncatalytic subunit gamma 2; *NOTCH3*, Notch receptor 3; *GLA*, galactosidase alpha; CMH17, cardiomyopathy hypertrophic 17; CPVT, catecholaminergic polymorphic ventricular tachycardia; FDC, familial dilated cardiomyopathy; FHC, familial hypertrophic cardiomyopathy; ASD9, atrial septal defect type 9; TOF, tetralogy of Fallot; ADCAD2, autosomal dominant coronary artery disease 2; PRKAG2, protein kinase AMP-activated noncatalytic subunit gamma 2; ECYT2, familial erythrocytosis-2; CADASIL, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; AR, autosomal recessive inheritance; AD, autosomal dominant inheritance; XR, X-linked recessive dominant inheritance; M, male; F, female.

genes were related to SVD or heart disease; however, only in patients whose lesions affected both the anterior and posterior cerebral circulation areas were cardiogenic disease genes found (i.e., *JPH2* and low-density lipoprotein receptor–related protein 6; *Figure 2B*).

Four variants related to cerebral small vessel disease were detected, and they accounted for 23.5% of the gene results. Three pathogenic novel variants in *NOTCH3* were detected (patients 2, 12, 14), which were associated with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). Three patients were admitted for lacunar infarction, of whom 2 had multiple hyperintensity lesions of cerebral white matter on magnetic resonance imaging (MRI). Both patient 38 and patient 50 showed single lenticular artery territory infarction with mutations in *PROC* and *PROS1*. Unfortunately, these coagulation test results were not recorded.

Discussion

The application of genetic screening in stroke has been limited in previous studies. Genetic testing for stroke is usually performed for a specific disorder and is mainly based on clinical experience and judgment (35,36). Some clinical features may suggest the existence of genetic disorders, such as angiokeratomas (suggesting Fabry disease) and hyperelastic skin (suggesting Ehlers-Danlos type IV). However, accurate and efficient diagnosis is difficult when these specific features are absent. Stroke etiology in young patients is often missed by less experienced clinicians, which can lead to treatment delay (37).

The prospective study, the Lombardia GENS project, investigated 209 patients for 5 monogenic diseases associated with stroke and had a diagnostic yield of 7% (38,39). However, the study only screened for 5 genes, and 91% of patients only received tests for 1 of the disorders. An NGS gene panel was also applied to stroke patients. An ongoing, large-sample prospective study, the Small Vessel and Lacunar (SVE-LA) project, designed a screening panel covering 43 genes with monogenic genes, such as *NOTCH3*, and polygenic genes, such as interleukin 6 (*IL-6*) (40). However, the genes and patients' clinical phenotype were restricted to SVD, and the number of genes was also limited. Recently, Ilinca *et al.* (41) developed 3 stroke gene panels intended for comprehensive usage. The panels covered genes related to monogenic stroke (120 genes), genes related to stroke (62 genes), and genes related to stroke susceptibility (32 genes). However, these gene panels have not yet been applied in clinic.

The current multicenter study, to our knowledge, is the first to develop and apply a comprehensive genetic screening of cryptogenic stroke in young adults. Compared to previous studies, our diagnosis yield was much higher likely due to the expanded investigation of stroke-related causes.

We revealed that cardiogenic disease genes play a predominant role in young adult stroke patients. Half of the detected mutations were cardiac-associated genes, including hereditary cardiomyopathy and congenital heart diseases with structural abnormalities. Most of our detected cardio-related genes have been reported to be associated with dysrhythmia or structural abnormality (*TTN*, *RYR2*, *NEXN*, *JPH2*, *TBX20*, *GATA6*, *PRKAG2*) (42-51). Unlike various pathophysiologies of large-artery atherosclerosis and small-artery occlusion, cardiogenic stroke is more commonly related to cerebral embolism due to hemodynamic abnormalities (AF, cardiac structural abnormalities). The pathophysiology of dysrhythmia is complex and includes features such as electrical remodeling, structural remodeling, and autonomic nervous system changes (47). In accordance with our result, a recent study found a statistically increased proportion of LoF variance of *TTN* in early-onset idiopathic AF (51). Other genes, such as *RYR2* and *JPH2*, involved in Ca^{2+} handling or gap junction formation have been associated with arrhythmias (47). The underlying mechanisms might be intracellular Ca^{2+} overload by sarcoplasmic reticulum release events (50). Furthermore, one of our *RYR2* mutations was reported in a dysrhythmia patient with functional evidence for pathogenicity (15).

The predominance of cardiogenic disease genes indicated that cryptogenic stroke caused by cardiogenic sources might be underestimated to a large extent. In the current study, none of our patients showed heart abnormalities in routine examination, which might be explained by the following reasons. First, in a previous report, only 3% of AF cases were diagnosed using 24-hour ECG in cryptogenic stroke, but the detection rate increased to 30% with 36-month monitoring (52). Elevated P terminal force v1, a marker of early atrial pathological change, was also related to embolic stroke (53). Second, recent studies have found associations between subtle left (and right) atrial dysfunction, left ventricular systolic and diastolic dysfunction, and left ventricular noncompaction and early-onset cryptogenic stroke. These associations were demonstrated only by using advanced 4D echocardiography and cardiac MRI, with apparently normal routine cardiac investigations in

all patients, suggesting a very early stage of cardiac disease. Therefore, the current cardiac condition was possibly just one contributing mechanism among the yet unexplored ones (54-58). Undoubtedly, highly sensitive methods or biomarkers for the early detection of cardiogenic stroke remain to be developed or implemented clinically.

Dissecting genetic etiology may help clinical decision-making in preventing recurrent strokes in currently cryptogenic patients. Two recent large randomized control studies, the NAVIGATE ESUS (Rivaroxiban Versus Aspirin in Secondary Prevention of Stroke and Prevention of Systemic Embolism in Patients With Recent Embolic Stroke of Undetermined Source) trial and RE-SPECT ESUS (the Randomized, Double-Blind, Evaluation in Secondary Stroke Prevention Comparing the Efficacy and Safety of the Oral Thrombin Inhibitor Dabigatran Etexilate Versus Acetylsalicylic Acid in Patients With Embolic Stroke of Undetermined Source) trial, showed that cryptogenic stroke did not benefit from anticoagulation (59). This indicates cryptogenic stroke is a heterogeneous entity. Thus, these advanced methods, such as genetic testing, are expected to guide clinical treatments in the future. Finding a mutation associated with large-vessel disease may warrant placing the patient on preventative antiplatelet therapy or at least knowing the choices available. If there is a mutation associated with arrhythmia, a patient with recurrent stroke will likely start taking antiarrhythmia medication despite this not being indicated by the ECG results.

This study had several limitations. First, the sample size in this study was insufficient, and the selection bias might have been introduced during patient recruitment. Although rare genetic risk factors were likely to be present in young adult patients, the main reasons for young-onset stroke in the Chinese population were still due to conventional risk factors, and atherosclerosis accounted for 57% of total strokes (60). Our strict inclusion criteria for cryptogenic stroke further limited the study population, and so a larger-sample study is needed to validate our findings. Second, family validation and functional analysis would be preferred. We failed to collect samples from patients' family members, which reduced the rate of mutation detection. Rare variants in patient 34 (with *HTRA1* variant) and patient 19 (*NPHS1*) would require segregation analysis to confirm the pathogenicity. Determining whether the variants were damaging in patient 49 (*PROS1*), patient 35 (*F5*), patient 15 (*TREX1*), and patient 6 (*AKAP9*) would require *in vitro* functional tests. Third, the patients in this study were of Chinese origin, and the incidence of genetic variants may vary greatly in different races (61). The results should be extrapolated to other racial and ethnic populations with caution. Finally, whole exome sequencing (WES) might be a better choice in future studies, but tNGS was an efficient and economical platform when the study was designed. However, WES is becoming cost-effective and may achieve equally satisfactory data quality as tNGS. With the accumulation of more screened genes, WES may further expand the mutant gene spectrum of young-onset cryptogenic stroke.

Conclusions

Our study suggests that genetic factors may play an important role in young adult cryptogenic stroke patients, and further studies in large cohorts are required. Genes related to cardiogenic diseases were the most frequently mutated, which may indicate that more sensitive examinations are needed in the future. Genetic screening for cryptogenic stroke may better inform clinical decision-making.

Acknowledgments

The authors appreciate the data collection and judgment provided by Dong Sun (Department of Neurology, Wuhan University Zhongnan Hospital), Le Fang (Department of Neurology, Japanese Friendship Hospital, Jilin University), and Dan Xie (Department of Neurology, Beijing Friendship Hospital, Capital University of Medical Sciences).
Funding: This study was supported by the National Science Fund for Distinguished Young Scholars (No. 8202500477) and the Chinese Academy of Medical Sciences (CAMS) Innovation Fund for Medical Sciences (CIFMS) (No. 2016-I2M-1-002).

Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-21-3843/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-21-3843/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-21-3843/coif). Wei-hai Xu serves as an unpaid Associate Editor-in-Chief

of *Annals of Translational Medicine* from June 2019 to May 2024. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the review board of Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and participating centers (No. ZS-1554). Informed consent was granted by all individual participants.

References

1. Kissela BM, Khoury JC, Alwell K, et al. Age at stroke: temporal trends in stroke incidence in a large, biracial population. Neurology 2012;79:1781-7.
2. Naess H, Nyland HI, Thomassen L, et al. Long-term outcome of cerebral infarction in young adults. Acta Neurol Scand 2004;110:107-12.
3. Yesilot Barlas N, Putaala J, Waje-Andreassen U, et al. Etiology of first-ever ischaemic stroke in European young adults: the 15 cities young stroke study. Eur J Neurol 2013;20:1431-9.
4. Smajlović D. Strokes in young adults: epidemiology and prevention. Vasc Health Risk Manag 2015;11:157-64.
5. Bevan S, Markus HS. Genetics of common polygenic ischaemic stroke: current understanding and future challenges. Stroke Res Treat 2011;2011:179061.
6. Kunii M, Doi H, Ishii Y, et al. Genetic analysis of adult leukoencephalopathy patients using a custom-designed gene panel. Clin Genet 2018;94:232-8.
7. Adams HP Jr, Bendixen BH, Kappelle LJ, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. Stroke 1993;24:35-41.
8. Etherington GJ, Ramirez-Gonzalez RH, MacLean D. bio-samtools 2: a package for analysis and visualization of sequence and alignment data with SAMtools in Ruby. Bioinformatics 2015;31:2565-7.
9. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405-24.
10. Lau K, Van Petegem F. Crystal structures of wild type and disease mutant forms of the ryanodine receptor SPRY2 domain. Nat Commun 2014;5:5397.
11. Tang Y, Tian X, Wang R, et al. Abnormal termination of Ca2+ release is a common defect of RyR2 mutations associated with cardiomyopathies. Circ Res 2012;110:968-77.
12. Tester DJ, Medeiros-Domingo A, Will ML, et al. Cardiac channel molecular autopsy: insights from 173 consecutive cases of autopsy-negative sudden unexplained death referred for postmortem genetic testing. Mayo Clin Proc 2012;87:524-39.
13. Methner DN, Scherer SE, Welch K, et al. Postmortem genetic screening for the identification, verification, and reporting of genetic variants contributing to the sudden death of the young. Genome Res 2016;26:1170-7.
14. Jabbari J, Jabbari R, Nielsen MW, et al. New exome data question the pathogenicity of genetic variants previously associated with catecholaminergic polymorphic ventricular tachycardia. Circ Cardiovasc Genet 2013;6:481-9.
15. Medeiros-Domingo A, Bhuiyan ZA, Tester DJ, et al. The RYR2-encoded ryanodine receptor/calcium release channel in patients diagnosed previously with either catecholaminergic polymorphic ventricular tachycardia or genotype negative, exercise-induced long QT syndrome: a comprehensive open reading frame mutational analysis. J Am Coll Cardiol 2009;54:2065-74.
16. Fang Y, Dai BT, Wang XF, et al. Identification of three FGA mutations in two Chinese families with congenital afibrinogenaemia. Haemophilia 2006;12:615-20.
17. Qian L, Mohapatra B, Akasaka T, et al. Transcription factor neuromancer/TBX20 is required for cardiac function in Drosophila with implications for human heart disease. Proc Natl Acad Sci U S A 2008;105:19833-8.
18. Wang H, Li Z, Wang J, et al. Mutations in NEXN, a Z-disc gene, are associated with hypertrophic cardiomyopathy. Am J Hum Genet 2010;87:687-93.

19. Lin X, Huo Z, Liu X, et al. A novel GATA6 mutation in patients with tetralogy of Fallot or atrial septal defect. J Hum Genet 2010;55:662-7.
20. Kodo K, Yamagishi H. GATA transcription factors in congenital heart defects: a commentary on a novel GATA6 mutation in patients with tetralogy of Fallot or atrial septal defect. J Hum Genet 2010;55:637-8.
21. Xu Y, Gong W, Peng J, et al. Functional analysis LRP6 novel mutations in patients with coronary artery disease. PLoS One 2014;9:e84345.
22. Zhang BL, Xu RL, Zhang J, et al. Identification and functional analysis of a novel PRKAG2 mutation responsible for Chinese PRKAG2 cardiac syndrome reveal an important role of non-CBS domains in regulating the AMPK pathway. J Cardiol 2013;62:241-8.
23. Wilson KD, Shen P, Fung E, et al. A Rapid, High-Quality, Cost-Effective, Comprehensive and Expandable Targeted Next-Generation Sequencing Assay for Inherited Heart Diseases. Circ Res 2015;117:603-11.
24. Li Z, Huang J, Zhao J, et al. Rapid molecular genetic diagnosis of hypertrophic cardiomyopathy by semiconductor sequencing. J Transl Med 2014;12:173.
25. Joutel A, Vahedi K, Corpechot C, et al. Strong clustering and stereotyped nature of Notch3 mutations in CADASIL patients. Lancet 1997;350:1511-5.
26. Joutel A, Corpechot C, Ducros A, et al. Notch3 mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia. Nature 1996;383:707-10.
27. Iijima K, Nakamura A, Kurokawa H, et al. A homozygous protein C deficiency (Lys 192 del) who developed venous thrombosis for the first time at adulthood. Thromb Res 2010;125:100-1.
28. Tang L, Wang HF, Lu X, et al. Common genetic risk factors for venous thrombosis in the Chinese population. Am J Hum Genet 2013;92:177-87.
29. Ichiyama M, Ohga S, Ochiai M, et al. Fetal hydrocephalus and neonatal stroke as the first presentation of protein C deficiency. Brain Dev 2016;38:253-6.
30. Tang L, Lu X, Yu JM, et al. PROC c.574_576del polymorphism: a common genetic risk factor for venous thrombosis in the Chinese population. J Thromb Haemost 2012;10:2019-26.
31. Tang L, Jian XR, Hamasaki N, et al. Molecular basis of protein S deficiency in China. Am J Hematol 2013;88:899-905.
32. Ohno S, Hasegawa K, Horie M. Gender Differences in the Inheritance Mode of RYR2 Mutations in Catecholaminergic Polymorphic Ventricular Tachycardia Patients. PLoS One 2015;10:e0131517.
33. Tester DJ, Arya P, Will M, et al. Genotypic heterogeneity and phenotypic mimicry among unrelated patients referred for catecholaminergic polymorphic ventricular tachycardia genetic testing. Heart Rhythm 2006;3:800-5.
34. Lukas J, Scalia S, Eichler S, et al. Functional and Clinical Consequences of Novel α-Galactosidase A Mutations in Fabry Disease. Hum Mutat 2016;37:43-51.
35. Sharma P, Yadav S, Meschia JF. Genetics of ischaemic stroke. J Neurol Neurosurg Psychiatry 2013;84:1302-8.
36. Majersik JJ, Skalabrin EJ. Single-gene stroke disorders. Semin Neurol 2006;26:33-48.
37. Sultan S, Elkind MS. The growing problem of stroke among young adults. Curr Cardiol Rep 2013;15:421.
38. Bersano A, Markus HS, Quaglini S, et al. Clinical Pregenetic Screening for Stroke Monogenic Diseases: Results From Lombardia GENS Registry. Stroke 2016;47:1702-9.
39. Bersano A, Baron P, Lanfranconi S, et al. Lombardia GENS: a collaborative registry for monogenic diseases associated with stroke. Funct Neurol 2012;27:107-17.
40. Bersano A, Zuffardi O, Pantoni L, et al. Next generation sequencing for systematic assessment of genetics of small-vessel disease and lacunar stroke. J Stroke Cerebrovasc Dis 2015;24:759-65.
41. Ilinca A, Samuelsson S, Piccinelli P, et al. A stroke gene panel for whole-exome sequencing. Eur J Hum Genet 2019;27:317-24.
42. Kang S. Low-density lipoprotein receptor-related protein 6-mediated signaling pathways and associated cardiovascular diseases: diagnostic and therapeutic opportunities. Hum Genet 2020;139:447-59.
43. Porto AG, Brun F, Severini GM, et al. Clinical Spectrum of PRKAG2 Syndrome. Circ Arrhythm Electrophysiol 2016;9:e003121.
44. Raghuram N, Marwaha A, Greer MC, et al. Congenital hypothyroidism, cardiac defects, and pancreatic agenesis in an infant with GATA6 mutation. Am J Med Genet A 2020;182:1496-9.
45. Chen Y, Xiao D, Zhang L, et al. The Role of Tbx20 in Cardiovascular Development and Function. Front Cell Dev Biol 2021;9:638542.
46. Beavers DL, Landstrom AP, Chiang DY, et al. Emerging roles of junctophilin-2 in the heart and implications for cardiac diseases. Cardiovasc Res 2014;103:198-205.
47. Rommel C, Rösner S, Lother A, et al. The Transcription Factor ETV1 Induces Atrial Remodeling and Arrhythmia. Circ Res 2018;123:550-63.

48. Liu C, Spinozzi S, Chen JY, et al. Nexilin Is a New Component of Junctional Membrane Complexes Required for Cardiac T-Tubule Formation. Circulation 2019;140:55-66.
49. Søndergaard MT, Liu Y, Brohus M, et al. Diminished inhibition and facilitated activation of RyR2-mediated Ca2+ release is a common defect of arrhythmogenic calmodulin mutations. FEBS J 2019;286:4554-78.
50. Zhu H, Xue H, Jin QH, et al. Increased expression of ryanodine receptor type-2 during atrial fibrillation by miR-106-25 cluster independent mechanism. Exp Cell Res 2019;375:113-7.
51. Choi SH, Weng LC, Roselli C, et al. Association Between Titin Loss-of-Function Variants and Early-Onset Atrial Fibrillation. JAMA 2018;320:2354-64.
52. Carmona-Puerta R, Castro-Torres Y. Atrial fibrillation and cryptogenic stroke. What is the current evidence? Role of electrocardiographic monitoring. J Arrhythm 2018;34:1-3.
53. Yaghi S, Boehme AK, Hazan R, et al. Atrial Cardiopathy and Cryptogenic Stroke: A Cross-sectional Pilot Study. J Stroke Cerebrovasc Dis 2016;25:110-4.
54. Pöyhönen P, Kuusisto J, Pirinen J, et al. Right atrium and cryptogenic ischaemic stroke in the young: a case-control study. Open Heart 2021;8:e001596.
55. Pirinen J, Kuusisto J, Martinez-Majander N, et al. Evidence of subtle left ventricular systolic dysfunction in cryptogenic stroke in the young. Echocardiography 2021;38:271-9.
56. Pöyhönen P, Kuusisto J, Järvinen V, et al. Left ventricular non-compaction as a potential source for cryptogenic ischemic stroke in the young: A case-control study. PLoS One 2020;15:e0237228.
57. Pirinen J, Kuusisto J, Järvinen V, et al. Diastolic function in young patients with cryptogenic stroke: A case-control pilot study. Clin Physiol Funct Imaging 2020;40:336-42.
58. Pirinen J, Järvinen V, Martinez-Majander N, et al. Left Atrial Dynamics Is Altered in Young Adults With Cryptogenic Ischemic Stroke: A Case-Control Study Utilizing Advanced Echocardiography. J Am Heart Assoc 2020;9:e014578.
59. Hart RG, Sharma M, Mundl H, et al. Rivaroxaban for Stroke Prevention after Embolic Stroke of Undetermined Source. N Engl J Med 2018;378:2191-201.
60. Zhang Y, He L. Risk factors study of ischemic stroke in young adults in Southwest China. Sichuan Da Xue Xue Bao Yi Xue Ban 2012;43:553-7.
61. National Research Council (US) Panel on Race E, Health in Later Life. Understanding Racial and Ethnic Differences in Health in Late Life: A Research Agenda. Washington (DC): National Academies Press (US); 2004.

Cite this article as: Yuan WZ, Shang L, Tian DS, Wu SW, You Y, Tian CL, Wu B, Liu J, Sun QJ, Liu Q, Xu WH. Monogenic basis of young-onset cryptogenic stroke: a multicenter study. Ann Transl Med 2022;10(9):512. doi: 10.21037/atm-21-3843

