Check for updates

Stroke

ORIGINAL CONTRIBUTION

Systematic Genetic Assessment in Young Patients With Cryptogenic Stroke: The ES-EASY project

Loraine Mania-Pâris, MD; Antonio Vitobello, PhD; Hana Safraou, MD; Frédéric Tran Mau-Them, MD, PhD; Yannis Duffourd, MSc; Sophie Nambot, MD, PhD; Ange-Line Bruel, PhD; Anne-Sophie Denommé-Pichon, MD, PhD; Gauthier Duloquin, MD; Yannick Béjot, MD, PhD; Laurence Faivre, MD, PhD; Christel Thauvin-Robinet, MD, PhD; Quentin Thomas, MD, PhD

BACKGROUND: Up to 15% of strokes occur in young adults, with more cryptogenic cases, raising the possibility of rare causes, such as genetic diseases. Although available in everyday practice, genetic analyses are usually reserved for patients with evocative personal or family history. We aimed to assess the interest of systematic genetic analyses in young adults with cryptogenic stroke and estimate the true frequency of genetic disorders in such patients.

METHODS: We conducted a retrospective observational cohort study with additional prospective genetic testing. We screened all patients under 50 admitted to Dijon University Hospital stroke unit between 2018 and 2021. Those already genetically tested during etiological work-up were included in a first cohort (cohort 1). Among the remaining patients, those with unexplained intracerebral hemorrhage or stroke (ie, cryptogenic stroke), or a stroke subtype known to have monogenic forms, were offered exome sequencing to form cohort 2. Monogenic diagnoses were defined by likely pathogenic/pathogenic variants according to American College of Medical Genetics and Genomics criteria.

RESULTS: Among 305 patients with stroke screened, 24 had prior genetic testing (cohort 1) with exome sequencing, genome sequencing, gene panels or targeted gene analyses, leading to a molecular diagnosis in 8 (33%). Of the remaining 281 patients, 71 met eligibility criteria and 35 consented to the study (cohort 2). Exome sequencing identified pathogenic variants in 4 of them (11%). The overall diagnostic yield of genetic tests was 20.3% (12/59 patients tested across both cohorts). In total, monogenic causes explained 3.9% (12/305) of all young stroke cases. Notably, most diagnosed patients (66%) had no family history of stroke. Genetic cardiopathies and conditions conferring increased cardiovascular risk factors accounted for 50% of diagnoses.

CONCLUSIONS: Genetic analyses should be considered in all unexplained strokes or in stroke subtypes with known genetic forms (eg, Moya Moya syndrome, cardiopathy, small vessel disease), even without an evocative family history.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: cerebral hemorrhage ■ exome sequencing ■ genetic testing ■ ischemic stroke ■ risk factors ■ young adult

Stroke
Research Network

Downloaded from http://ahajournals.org by on September 30, 2025

Stroke is the second leading cause of death worldwide and remains the third leading cause of disability and death combined.[1] Its overall incidence of up to 12.2 million per year worldwide, and the fact that one in 4 people over the age of 25 will experience at least 1 stroke in their lifetime make it one of the most critical challenges of public health.[2,3]

If cerebral infarction was once largely considered to be a disease of the elderly, it is now known that young people are not spared from it. Up to 10% to 15% of

Correspondence to: Mania-Pâris Loraine, MD, Department of Neurology, Dijon University Hospital, 2 Blvd Maréchal de Lattre de Tassigny, 21000 Dijon, France. Email loraine.mania-paris@chu-dijon.fr
Supplemental Material is available at https://www.ahajournals.org/doi/suppl/10.1161/STROKEAHA.125.051020.
For Sources of Funding and Disclosures, see page XXX.
© 2025 American Heart Association, Inc.
Stroke is available at www.ahajournals.org/journal/str

ORIGINAL CONTRIBUTION

Nonstandard Abbreviations and Acronyms

CeAD	cervical artery dissection
CSVD	cerebral small vessel diseases
ES	exome sequencing
GAD	Génétique des Anomalies du Développement
ICH	intracerebral hemorrhage
MRI	magnetic resonance imaging
STREGA	Strengthening the Reporting of Genetic Association
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
TOAST	Trial of ORG 10172 in Acute Stroke Treatment

cerebral infarcts occur in patients under the age of 50, with incidence numbers only increasing since the beginning of the 21st century.[4–7] In addition, about 6% of deaths following stroke occur in patients between 15 and 49 years old.[3]

Although they share common causes, strokes in young and older people often have different etiologies. Intracerebral hemorrhages (ICH) are more likely to be caused by arteriovenous malformations, cavernomas, or cerebral venous thrombosis in the young than in the elderly.[8,9] As atherosclerosis and atrial fibrillation are mainly associated with age, they are major causes of stroke in the elderly but are less likely to be observed in young patients. On the contrary, nonatherosclerotic arteriopathies, such as cervical artery dissections (CeAD) accounting for up to 25% of strokes in young people, infectious or noninfectious vasculitis and noninflammatory vasculopathies, are more likely to be involved in cerebral infarcts in young patients.[10] Despite major advances in identifying the underlying mechanism of stroke, 22% to 52% of cerebral infarcts remain of unknown cause, and are usually referred to as cryptogenic according to TOAST (Trial of ORG 10172 in Acute Stroke Treatment) classification criteria.[11] Likewise, many ICHs remain unexplained despite extensive investigation.

Interestingly enough, cryptogenic strokes appear to be more frequent in younger (upto 50%) than in older patients (upto 35%).[12] Yet, failure to identify the causal mechanism of a stroke can lead to inappropriate and ineffective preventive treatment, resulting in a higher risk of recurrence and mortality.[13] This data raises the question of how neurologists can pursue etiological investigations once all acquired causes have been explored.

Genetic diseases may provide a partial explanation for cryptogenic strokes. There are several arguments supporting the involvement of genetic mechanisms in stroke's pathophysiology, such as the occurrence at a young age or the identification of a higher risk of stroke in patients with a family history. Furthermore, numerous polymorphisms have been identified as risk factors for stroke, alongside monogenic (Mendelian) diseases, including stroke in their phenotype.[14]

To date, about 50 monogenic diseases have been identified as able to generate stroke, encompassing every subtype of stroke and accounting for 1% to 5% of all strokes.[15,16] These are often associated with an early onset (neonatal, childhood, young adults), the absence of cardiovascular risk factors and sometimes with phenotypes that are specific to these diseases.[14] Some monogenic diseases responsible for stroke can manifest with non-neurological symptoms, such as Fabry disease which can lead to cardiac or renal failure, and skin abnormalities.[17] Others can manifest with mild to severe morphological particularities or can be associated with auto-immune diseases like Loeys-Dietz syndrome, a cause of CeAD.[18] Among these, some, such as Fabry disease, may require specific treatment or follow-up.[19]

Finally, it should be noted that some well-known diseases for which stroke is a major complication also have rare genetic forms that are rarely sought after, such as genetic forms of Moya-Moya syndrome or CeAD.

Hence, many monogenic conditions may be directly or indirectly responsible for stroke. Their rarity may lead neurologists to favor gene panel-based analyses or targeted gene testing to look for the disorders they know about, and to limit the use of such analyses to patients with evocative personal or family history. However, the extreme genetic heterogeneity of strokes may be best explored using large genomic approaches, such as exome sequencing (ES). This technique focuses on identifying rare and deleterious variants within all the protein-coding regions of the genome, providing high-resolution insights at the individual level. Thus, ES is particularly suitable for situations where patients do not have specific phenotypic features that usually guide the choice of the targeted genetic approaches previously mentioned.

The aim of our study was to evaluate the potential benefits of conducting systematic ES in patients with cryptogenic stroke and to better estimate the true frequency of monogenic diseases in young patients with stroke.

METHODS

Data Availability Statement

Anonymized data will be shared upon request from any qualified investigator.

Study Design and Participants

To better characterize the involvement of monogenic disorders in young patients with stroke, we retrospectively analyzed the medical records of all individuals aged under 50 years admitted to the Dijon University Hospital stroke unit between January 2018 and December 2021. Based on this review, we then

Stroke FIRST PROOF ONLY

Downloaded from http://ahajournals.org by on September 30, 2025

prospectively enrolled patients for whom no cause of stroke had been identified after a thorough etiological workup, to offer them to benefit from ES.

On screening of the Medical Information Department and the hospitalization rating system, we noticed that some patients had already undergone genetic testing before the initiation of the study. In this cohort (further referred to as cohort 1), patients had been referred for genetic counseling depending on the neurologist's clinical judgement and there were no standardized protocol or indications. Also, as the genetic analyses had been chosen by the referring neurologist or geneticist of the time, its nature varied from 1 patient to another (targeted gene analyses, gene panel-based analyses, ES or genome sequencing). In cohort 1, we chose not to propose ES for 2 main reasons. First, conducting broader genetic testing on patients with a previously established molecular diagnosis would be irrational, as it would not offer any additional benefit. Second, we considered that offering ES to individuals with inconclusive initial targeted gene or gene panel-based analysis would cause renewed anxiety and lead to misunderstandings. Indeed, these patients had been reassured, often several years earlier, about the absence of a clearly identified genetic cause for their stroke and that further genetic investigation was not required at that time. As such, we decided to include these patients in our analyses but in a separate cohort, to offer a comprehensive overview of the involvement of genetic disorders in the occurrence of stroke.

All patients who had not yet undergone genetic testing were then assessed for eligibility for ES (cohort 2) using data obtained from the initial retrospective review. Eligibility criteria included a diagnosis of spontaneous ICH without underlying vascular or parenchymal abnormalities, a cryptogenic cerebral infarct according to the TOAST classification[20] and a stroke mechanism potentially linked to a genetic condition, such as a cerebral small vessel disease (CSVD) in a patient with no congruent risk factors, a Moya-Moya syndrome without an identified acquired cause, or a spontaneous CeAD of undetermined origin. Exclusion criteria were severe poststroke disability that did not allow patients to give their informed consent, or an incomplete etiological screening. The etiological assessment required to classify a patient as cryptogenic stroke is detailed in the Supplemental Material (Table S1). The presence or absence of a family history of stroke was not taken into account to judge of a patient's eligibility.

Patients in cohort 2 were contacted to be offered a genetic consultation. The first consultation aimed at (1) ensuring no cause had been identified in the meantime, (2) making sure the etiological work-up was exhaustive, (3) updating personal and family history, and (4) performing a clinical exam. If all inclusion criteria were met, written consent forms were signed and blood samples were drawn. These inclusion consultations took place between June 2022 and April 2024. Patients then attended a second consultation dedicated to ES results. This study was performed within the framework of the GAD (Génétique des Anomalies du Développement) collection and approved by the appropriate institutional review board of Dijon University Hospital (DC2011-1332). This work was conducted and is reported in accordance with the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines and the STREGA studies (Strengthening the Reporting of Genetic Association) extension.[21]

Clinical Data Collection

Clinical data were retrospectively collected from the hospital medical records. Extracted variables included demographic data (age, sex), vascular risk factors (eg, hypertension, smoking, diabetes), and personal medical history. To ensure data completeness, personal and family history were systematically reviewed during the genetic consultation for patients in cohort 2.

ES Procedures

Depending on the availability of parental DNA, ES was performed in a solo (proband-only), duo (proband and 1 parent), or trio (proband and both parents) strategy. Blood was drawn at Dijon University Hospital (France). ES was performed according to previously published protocols using a SureSelect Human all exon V6 Agilent or a TWIST kit (corresponding to a Human Core Exome kit TWIST Bioscience complemented with additional probes) at the Center National de Recherche en Génomique Humaine or at Integragen.[22,23] The resulting libraries were sequenced on different sequencing platforms (Illumina Hiseq4000 or NovaSeq 6000) for paired-end reads with a target mean depth of 100×. Reads were aligned to the human genome reference sequence (GRCh37 assembly) using BWA 0.6.2 (Burrows–Wheeler Aligner). Duplicate reads were marked using Picard 1.77, and BAM files were processed using GATK 3.5 (Genome Analysis Toolkit) to realign around indels and recalibrate base quality scores.

Sequencing data was analyzed by 2 independent local biologists specialized in genetics from the GAD team (Inserm UMR1231). The results were systematically discussed at a weekly multidisciplinary meeting attended by biologists and clinicians specialized in genetics and neurology. Clinical significance was determined according to the American College of Medical Genetics and Genomics guidelines.[24] Diagnosis of a monogenic disease was defined by the identification of a variant classified as likely pathogenic (class IV) or pathogenic (class V). Genetic results were expected within 4 to 5 months after patient enrollment.

ES carries a risk of incidental findings, that is, the identification of pathogenic variants unrelated to stroke but associated with conditions for which preventive measures or active surveillance are available. In accordance with ethical guidelines, patients were given the choice to be informed or not about such incidental findings.

Statistical Analyses

Demographic and baseline data were described using counts, percentages, means, and standard deviations. Comparison of data between cerebral infarcts and ICHs was performed using the Pearson χ^2 test or Fisher exact test for categorical variables and nonparametric Mann-Whitney *U* test for continuous variables. Statistical significance was defined as $P<0.05$. All analyses were performed using R version 4.3.2.

RESULTS

Patient Cohort

Between January 2018 and December 2021, 539 patients under the age of 50 were admitted to the stroke

Downloaded from http://ahajournals.org by on September 30, 2025

STROKE FIRST PROOF ONLY

unit of Dijon University Hospital. Of these, 305 received a diagnosis of stroke: 261 cerebral infarcts (85.6%) and 44 ICHs (14.4%). The clinical characteristics of these patients are available in the Supplemental Material (Table S2). As shown in the flow chart (Figure 1), we identified 281 patients with no prior genetic testing. Of these, 210 were excluded from the study: 186 had an already identified acquired cause based on a conclusive etiological work-up, 19 had an incomplete work-up, and 5 could not be included due to either death (n=4) or severe neurological sequelae that prevented them from providing informed consent (n=1). Among the remaining eligible patients (n=71), 25 were unreachable, 8 did not attend the scheduled consultation, and 3 refused to participate. Thus, 35 could be reached and offered an ES (33 with cerebral infarcts and 2 with ICHs).

All cerebral infarcts in our cohort of young patients with stroke, grouped according to the TOAST classification are shown in Figure 2. The most common subtype was stroke of other determined cause, including CeAD or intracranial artery dissections which accounted for 18.4% (n=48) of patients. The second most common subtype was undetermined cause or cryptogenic stroke, followed by cardioembolic stroke. Patients with patent foramen ovale and aneurysm of the interatrial septum represented 18.4% (n=48) of patients. More rarely, non-atherosclerotic large vessel diseases were diagnosed, such as Moya-Moya angiopathy (1.5%; n=4) or antiphospholipid syndrome (3.1%; n=8).

The most common cause of ICH was CSVD (29.6%; n=13), followed by cavernomas (20%; n=9), arteriovenous malformations (11.36%; n=5), cerebral venous thrombosis (11.36%; n=5), and, less commonly, neoplastic lesions (2.3%; n=1) or cerebral amyloid angiopathy (2.3%; n=1).

Genetic Analyses

Before our work, 24 patients had benefited from a genetic test during their initial management (cohort 1). They were referred for a genetic consultation by the treating neurologist for various reasons: signs of severe microangiopathy on brain magnetic resonance imaging (29.2%; n=7), multiple spontaneous CeAD (20.8%; n=5), cavernomatosis on brain magnetic resonance imaging (16.7%; n=4), a diagnosis of dilated or hypertrophic cardiomyopathy on cardiac ultrasound (25%; n=6), and hyperhomocysteinemia (8.3%; n=2). Of these patients, 2 had a known family history of monogenic disease. Five had a targeted gene analysis, 7 had gene panel-based analyses, 10 had ES, and 2 had genome sequencing.

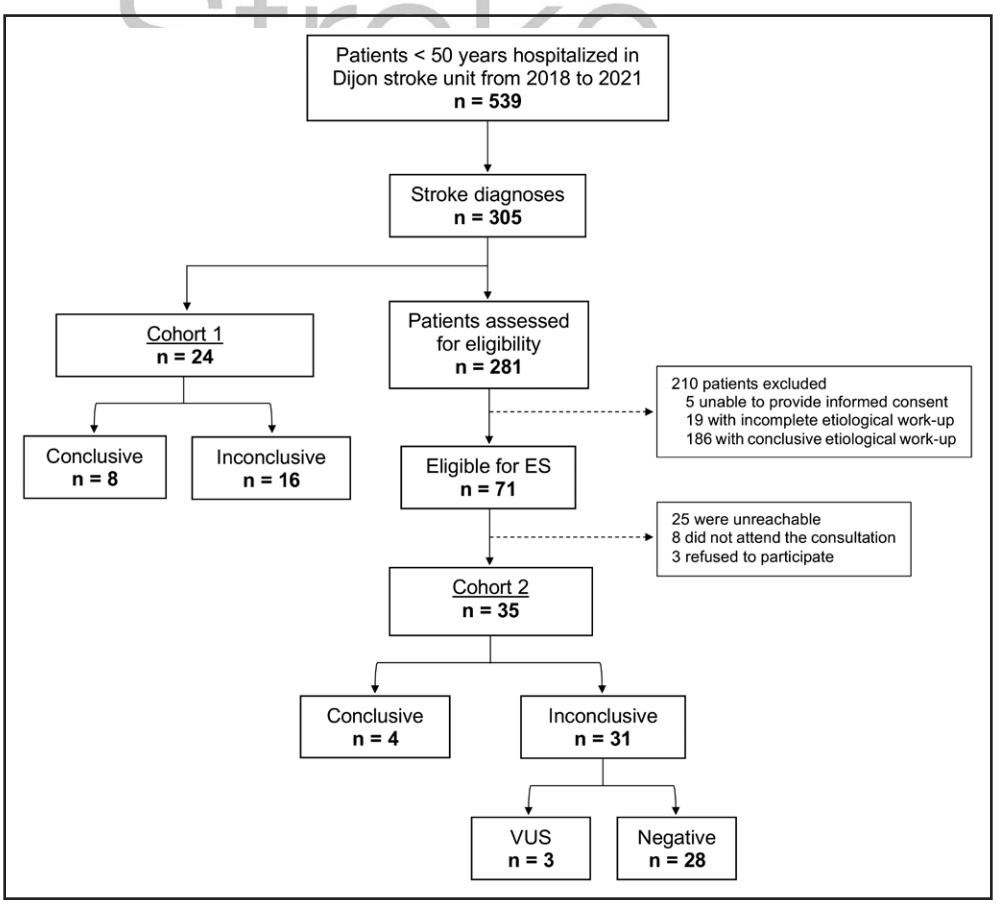

Figure 1. Flow chart.
ES indicates exome sequencing; and VUS, variants of uncertain significance.

Downloaded from http://ahajournals.org by on September 30, 2025

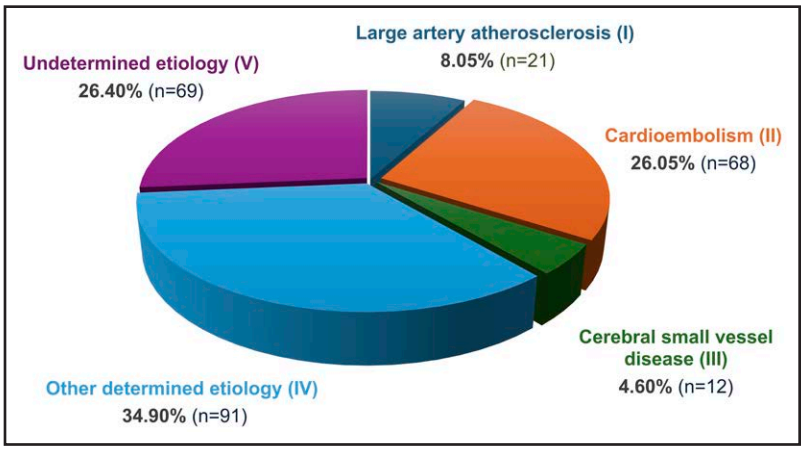

Figure 2. Cerebral infarct subtypes in our cohort were classified according to the TOAST (Trial of ORG 10172 in Acute Stroke Treatment) classification.

A molecular diagnosis was made in 8 of them (Table 1): 2 patients with homozygous *MTHFR* variants associated with hyperhomocysteinemia, a *COL3A1* variant leading to vascular Ehlers-Danlos syndrome (OMIM #130050) in a patient with spontaneous triple CeAD, a *TNNI3* variant causing familial cardiomyopathy (OMIM #613690), a *NOTCH3* variant responsible for cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy 1 (OMIM #125310), a *KRIT1* variant responsible for cavernomatosis (OMIM #116860), a familial *TTN* variant causing dilated cardiomyopathy (OMIM #604145), and a *GLA* variant responsible for Fabry disease (OMIM #301500).

Among the 71 eligible individuals, ES was done in 35 of them (cohort 2) and allowed to obtain a molecular diagnosis in 4 further patients (11.4%): a *RNF213* variant responsible for Moya Moya disease type 2 (OMIM #607151) in a patient who already had a personal history of stroke, an homozygous *NPHP1* variant causing nephronophthisis type 1 (OMIM #256100) in a patient who had severe high blood pressure, an *ABCC6* variant responsible for *pseudoxanthoma elasticum* fruste form (OMIM #177850) in a patient with brain magnetic resonance imaging features of CSVD, and finally a *TNNI3* variant responsible for hypertrophic cardiomyopathy 7 (OMIM #613690). Only one of them had a family history of monogenic disease.

Stroke

Table 1. Variants of Interest Identified in Cohort 1

	Age, y*	Gender	Stroke subtype	Gene (*OMIM)	Genomic variant (homo sapiens–GRCh38)	cDNA variant	Aminoacidic changes	Inheritance	ACMG criteria	ACMG	Final diagnosis (#OMIM)
ID-1	49	M	Ischemic	*TTN* (*188840)	chr2: g.178527479del	NM_001256850.1: c.102724del	p.(Ser34242GlnfsTer10)	Heterozygous maternally inherited	PVS1, PP5	Class V	Dilated cardiomyopathy 1G (No. 604145)
ID-2	40	F	Hemorrhagic	*KRIT1* (*604214)	chr7: g.92237769T>C	NM_194454.3: c.263-10A>G	p.(?)	Heterozygous	PM2, PP3	Class IV	Cerebral cavernous malformations type 1 (#116860)
ID-3	34	M	Ischemic	*GLA* (*300644)	chrX: g.101397964del	NM_000169.3: c.1136delA	p.(Asn379IlefsTer12)	Hemizygous	PM2, PVS1	Class V	Fabry disease (#301500)
ID-4	34	M	Ischemic	*MTHFR* (*607093)	chr1: g.11796321G>A	NM_005957.5: c.665C>T	p.(Ala222Val)	Homozygous	PM1, BA1, PP3	N/A	Hyperhomocysteinemia
ID-5	25	F	Ischemic	*COL3A1* (*120180)	Full gene deletion	…	…	Heterozygous	…	Class V	Ehlers-Danlos vascular type 4 (#130050)
ID-6	31	M	Ischemic	*TNNI3* (*191044)	chr19: g.55151859C>T	NM_000363.5: c.608G>A	p.(Gly203Asp)	Heterozygous	PM1, PM2, PM5, PP3	Class IV	Hypertrophic cardiomyopathy 7 (#613690)
ID-7	34	M	Ischemic	*NOTCH3* (*600276)	chr19: g.15192300T>C	NM_000435.3: c.341-2A>G	p.(?)	Heterozygous maternally inherited	PVS1 moderate, PM2, PP5 very strong	Class V	CADASIL type 1 (#125310)
ID-8	40	M	Ischemic	*MTHFR* (*607093)	chr1: g.11796321G>A	NM_005957.5: c.665C>T	p.(Ala222Val)	Homozygous	PM1, BA1, PP3	N/A	Hyperhomocysteinemia

ACMG indicates American College of Medical Genetics and Genomics; CADASIL, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; cDNA, complementary DNA; F, female; and M, male.

*Age at stroke onset.

Downloaded from http://ahajournals.org by on September 30, 2025

We also identified some variants of uncertain significance, offering interesting molecular candidates for 3 more patients. All variants of uncertain significance, likely pathogenic and pathogenic variants are listed in Table 2. None of the patients who presented with spontaneous intracranial artery dissection (n=1) or CeAD (n=9) were diagnosed with a monogenic disease. Our study did not reveal any secondary findings.

Overall, 59 patients had genetic tests, including 52.5% of males (n=31) and 47.5% females (n=28). Mean age at stroke onset was 39.1 years (±8.0). Family history of stroke (first or second degree) was noted in 33% (4/12) of patients for whom a molecular diagnosis was obtained, and in 8.5% (4/47) of patients for whom genetic analyses were unconclusive. Three patients with a molecular diagnosis had a family history of stroke-causing genetic disorder, accounting for 25% of them. One patient with a molecular diagnosis had a history of previous stroke. All diagnostic yields according to stroke subtypes are shown in Table 3.

Altogether, between 2018 and 2021, a genetic disorder was identified in 12 individuals, leading to an overall diagnostic yield of 20.3% (12/59).

DISCUSSION

Patients who present a stroke at a young age have a higher risk of recurrence due to their longer life expectancy. Given the higher rate of cryptogenic stroke in this population, inadequate preventive treatment and follow-up may also contribute to recurrence. The increasing clinical and fundamental evidence supporting the involvement of genetics in stroke raises the question of the interest of more systematic molecular analyses in young stroke patients. With this in mind, we conducted a retrospective observational study combined with a prospective genetic testing phase to determine the frequency and nature of genetic diseases in a cohort of patients under 50 years old who had a stroke of unknown cause despite a comprehensive work-up.

ES has been used in stroke cohorts in previous works. However, these studies had very different designs from ours and usually selected familial cases of strokes, which may have favored molecular diagnoses. As an illustration, Chang et al[25] used ES in a cohort of Taiwanese patients with stroke aged between 18 and 79 years with at least 1 family member with a history of stroke. Their diagnostic yield was 15.8% (24/152). Another study of young patients with stroke from Swedish and Finnish families (stroke onset ≤55 years old) with an average of 4.27 affected family members showed a diagnostic yield of 27.3% (6/22).[26] Conversely, our study aimed at offering a sample that was more representative of the real-life practice of a stroke unit, and to provide a reliable estimate of the true frequency of genetic diseases in patients under 50 years of age admitted to such departments. This design may explain our lower overall diagnostic yield of 20.3% (12/59), and 11.4% (4/35) when considering only patients who had ES thanks to our study (cohort 2).

Stroke FIRST PROOF ONLY

Table 2. Variants of Interest Identified in Cohort 2

	Age, y*	Gender	Stroke subtype	Gene (*OMIM)	Genomic variant (homo sapiens–GRCh38)	cDNA variant	Aminoacidic changes	Inheritance	ACMG criteria	ACMG	Final diagnosis (#OMIM)
ID-9	43	F	Ischemic	*RNF213 (*613768)*	chr17: g.80294947A>G	NM_001256071.3: c.1699A>G	p.(Met567Val)	Heterozygous	PM1, PP2, PP5	Class V	Moya Moya disease type 2 (#607151)
ID-10	44	F	Hemorrhagic	*NPHP1 (*607100)*	Del 113kb : chr2:110123348–110205013	…	…	Homozygous	…	Class V	Nephronophthisis type 1 (#256100)
ID-11	44	M	Ischemic	*ABCC6 (*603234)*	chr16: g.16219834C>T	NM_001171.6: c.333G>A	p.(Trp111Ter)	Heterozygous	PVS1, PM2, PP3, PP5	Class IV	*Pseudoxanthoma elasticum* fruste form (#177850)
ID-12	42	F	Ischemic	*TNNI3 (*191044)*	chr19: g.55156262C>G	NM_000363.4: c.221G>C	p.(Arg74Pro)	Heterozygous maternally inherited	PM1, PM2, PM5, PP3 strong	Class IV	Hypertrophic cardiomyopathy 7 (#613690)
ID-13	46	F	Ischemic	*ABCC6 (*603234)*	chr16: g.16202083A>G	NM_001171.6: c.1094T>C	p.(Leu365Pro)	Heterozygous paternally inherited	PM1, PM2, PP3 strong, BP1	Class III	*Pseudoxanthoma elasticum* fruste form (#177850)
ID-14	38	M	Hemorrhagic	*GSN (*137350)*	chr9: g.121326572A>G	NM_198252.3: c.1477A>G	p.(Met493Val)	Heterozygous maternally inherited	PM1, PM2, BP4	Class III	Amyloidosis, Finnish type (#105120)
ID-15	26	M	Ischemic	*RNF213 (*613768)*	chr17: g.80340314C>T	NM_001256071.3: c.5947C>T	p.(Arg1983Trp)	Heterozygous	PM1, BP4, BP6, BS1, BS2	Class III	Moya Moya disease type 2 (#607151)

ACMG indicates American College of Medical Genetics and Genomics; cDNA, complementary DNA; F, female; and M, male.
*Age at stroke onset.

Downloaded from http://ahajournals.org by on September 30, 2025

However, our design offers many relevant and innovative data on the genetic background of stroke. Considering all young stroke patients admitted to the Dijon stroke unit over the 4-year period, a rare monogenic disease was diagnosed in 3.9% of patients (12/305). To the best of our knowledge, this data has never been published in a previous study and provides an idea of the contribution of genetic diseases to stroke units' activity in France. In our study, patients have benefited from genetic analyses in one of 2 ways: either during their initial management before study initiation (cohort 1) or through our retrospective screening of patients with stroke under 50 years old (cohort 2). Among all patients with a molecular diagnosis, one third (4/12) received it thanks to our prospective genetic testing phase, obtaining a diagnosis sometimes up to 6 years after their stroke. In most of these cases, a genetic disease had never been suspected before, despite some patients having a family history of stroke or presenting a syndrome for which genetic forms exist (such as Moya-Moya syndrome). Furthermore, among all patients with a molecular diagnosis 66% (8/12) did not have any family history of stroke. Taken together, these data highlight how difficult it is to identify patients who may have a genetic disorder underlying their stroke.

Moreover, here again, our study may provide critical help for clinicians. Indeed, our data suggest that diagnostic yields vary greatly between all disorders that may generate a stroke. In our study, spontaneous CeAD provided an extremely low diagnostic yield (7.1%) as only one of the 14 patients with either intracranial artery dissection or CeAD was diagnosed with a genetic disorder in both cohorts 1 and 2. This finding is consistent with the literature as previous studies have shown that inherited connective tissue disorders are nearly exceptional in CeAD cohorts, and that genetic analyses are only relevant in familial forms, representing no more than 1% of all cases.[27,28] However, there are other conditions that could greatly benefit from more systematic genetic testing. Indeed, cardioembolic strokes, CSVD, Moya-Moya syndrome and ICHs reached a respective yield of 50%, 42.9%, 25%, and 20%. These findings must be considered with caution, given the modest sample sizes within our study group.

Another teaching of our study is that genetic disorders associated with stroke usually cause them in an indirect manner. Indeed, when considering genetic forms of strokes, one usually thinks of diseases, such as cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy or Fabry disease, that are known to directly generate cerebral microangiopathies as part of their phenotype. In our work, such diseases represented only 16.7% (2/12) of diagnoses. Conversely, genetic cardiopathies or diseases generating stroke through increased risk factors (arteriosclerosis, high blood pressure, hyperhomocysteinemia) represented half of patients with a molecular diagnosis (6/12).

Finally, we identified 2 patients with heterozygous *ABCC6* variants: 1 missense variants of uncertain significance in patient ID-13 and 1 likely pathogenic nonsense variant in patient ID-11. Heterozygous *ABCC6* variants have been found to be associated with an increased risk of developing a stroke.[29] Either CSVD or accelerated atherosclerosis have been described as common features of these fruste forms of *pseudoxanthoma elasticum*.[30,31] After reverse phenotyping, we found that patient ID-13 had an atypical atheroma in a dysplastic and calcified internal carotid artery and multiple falx cerebri calcifications (Figure 3). Nevertheless, carotid artery calcifications are not specific to this disease and intracranial calcifications are common findings in healthy individuals.[32] Hence, the role of this variant in the patient's condition remains to be elucidated. Further studies specifically searching for *ABCC6* variants in young patients with stroke and healthy individuals may be of interest.

There are some limitations to our study. First, the vast heterogeneity of diseases associated with stroke may have led to some being overlooked in ES data. Indeed, although our team is part of a reference center for neurogenetic disorders, some monogenic diseases related to stroke may have escaped our expertise. Second, the inclusion of patients sometimes long after stroke onset and after the completion of their early poststroke medical follow-up

Table 3. Diagnostic Yield of Genetic Testing in Relation to the Stroke Subtype (Cohorts 1 and 2)

	Genetic analyses (N=59)	Molecular diagnoses (n=12)	Diagnostic yield (%)
Cerebral infarcts	49	10	20.4%
Large artery atherosclerosis	1	0	0%
Cardioembolism	6	3	50%
CSVD	7	3	42.9%
CeAD or IAD	14	1	7.1%
Moya-Moya angiopathy	4	1	25%
Undetermined cause	17	2	11.8%
Cerebral hemorrhages	10	2	20%
Lobar hemorrhage	3	0	0%
Deep hemorrhage	7	2	28.6%

CeAD indicates cervical artery dissection; CSVD, cerebral small vessel disease; and IAD, intracranial artery dissection.

Stroke FIRST PROOF ONLY

Downloaded from http://ahajournals.org by on September 30, 2025

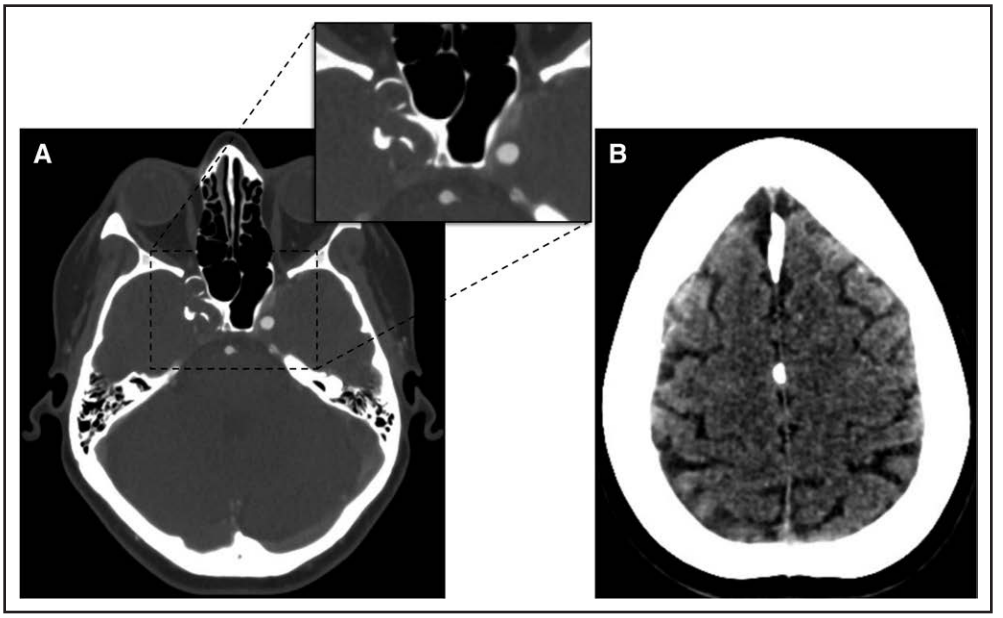

Figure 3. Cerebral images of patient ID-5 with a heterozygous *ABCC6* variant of uncertain significance.
A, Axial brain computed tomography (CT) angiography showing occlusion of a dysplastic and calcified right internal carotid artery. **B**, Axial brain CT scan showing calcifications of the falx cerebri.

at the university hospital, significantly limited recruitment. As a result, fewer than half of all eligible patients were included (49.3%), which reduced the statistical power of the study. Therefore, a prospective study enrolling consecutive patients with a negative etiological work-up and establishing a single cohort based on predefined, standardized eligibility criteria would be more likely to generate robust and informative data on the relevance of ES performed in young patients with cryptogenic stroke.

Moreover, since we chose not to perform ES in patients from cohort 1 who only had targeted gene or gene panel-based analyses, some molecular diagnoses may have been missed, potentially leading to an underestimation of the frequency of monogenic diseases among young patients with stroke.

One should also keep in mind that ES presents technical limitations, notably its inability to detect variants located in intronic or other noncoding regions, which may have generated false negative results. In addition, some strokes may have a polygenic basis, involving multiple variants or common single-nucleotide polymorphisms that modestly increase stroke risk. For instance, genome wide association studies have linked variations in the ABO locus to early onset ischemic stroke.[33] However, our study was not designed to detect such variants, and their clinical utility remains limited at this stage as they cannot be used for genetic counseling. Finally, some strokes may be associated with pathogenic variants located in genes that are not yet identified as disease-causing. Altogether, these elements make it challenging to estimate the true contribution of genetics to stroke.

In conclusion, our results highlight the importance of considering genetic disorders when investigating cryptogenic stroke, even when there are no obvious clinical indicators, such as a family history of stroke. Although some conditions appear to benefit more from genetic analyses, others such as CeAD, only exceptionally provide a molecular diagnosis. Based on our results, we recommend considering ES in all patients with a negative comprehensive work-up who have clinical characteristics suggestive of a genetic disease, or for whom tests lead to the discovery of an unexplained disorder for which genetic forms exist (eg, cardiomyopathy, Moya-Moya angiopathy, CSVD), even in the absence of a family history. Although systematic screening for monogenic diseases in all young patients with cryptogenic stroke seems premature given their rarity, it is essential to improve the screening of our patients so that they can be better referred for appropriate genetic counseling and treatment, especially in the era of growing therapies.

Stroke FIRST PROOF ONLY

Downloaded from http://ahajournals.org by on September 30, 2025

ARTICLE INFORMATION

Received March 8, 2025; final revision received August 15, 2025; accepted August 29, 2025.

Affiliations

Université Bourgogne Europe, CHU Dijon Bourgogne, Service de Neurologie, Dijon, France (L.M.-P., G.D., Y.B., Q.T.). Université Bourgogne Europe, CHU Dijon Bourgogne, Laboratoire de Génomique Médicale, FHU-TRANSLAD, Centre de recherche Translationnelle en Médecine Moléculaire—Inserm UMR1231 équipe GAD, Dijon, France (A.V., H.S., F.T.M.-T., Y.D., A.-L.B., A.-S.D.-P., L.F., C.T.-R.). Université Bourgogne Europe, CHU Dijon Bourgogne, Service de Génétique Clinique, Dijon, France (A.V., H.S., F.T.M.-T., S.N., A.-L.B., A.-S.D.-P., L.F., C.T.-R., Q.T.).

Acknowledgments

We thank the families and patients for taking part in the study. We thank the University of Burgundy center de Calcul (CCuB) for technical support and management of the informatics platform. We thank the center de Ressources Biologiques Ferdinand Cabanne (CHU Dijon) for sample biobanking.

Sources of Funding

This work was supported by grants from the Dijon University Hospital and the European Union through the FEDER programs. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the article. The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all study data and had final responsibility for the decision to submit for publication.

Disclosures

Dr Béjot reports personal fees from BMS, Pfizer, Medtronic, Amgen, Servier, NovoNordisk, Novartis, Boehringer-Ingelheim, outside the submitted work. The other authors report no conflicts.

Supplemental Material

Checklist
Tables S1–S2

REFERENCES

1. GBD 2019 Stroke Collaborators. Global, regional, and national burden of stroke and its risk factors, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet Neurol.* 2021;20:795–820. doi: 10.1016/S1474-4422(21)00252-0
2. Inserm. Accident vasculaire cérébral (AVC). Inserm, La science pour la santé. 2023. https://www.inserm.fr/dossier/accident-vasculaire-cerebral-avc/ Accessed XXX
3. Feigin VL, Brainin M, Norrving B, Martins S, Sacco RL, Hacke W, Fisher M, Pandian J, Lindsay P. World Stroke Organization (WSO): global stroke fact sheet 2022. *Int J Stroke.* 2022;17:18–29. doi: 10.1177/17474930211065917
4. Ekker MS, Verhoeven JI, Vaartjes I, Jolink WMT, Klijn CJM, de Leeuw FE. Association of stroke among adults aged 18 to 49 years with long-term mortality. *JAMA.* 2019;321:2113–2123. doi: 10.1001/jama.2019.6560
5. Yahya T, Jilani MH, Khan SU, Mszar R, Hassan SZ, Blaha MJ, Blankstein R, Virani SS, Johansen MC, Vahidy F, et al. Stroke in young adults: current trends, opportunities for prevention and pathways forward. *Am J Prev Cardiol.* 2020;3:100085. doi: 10.1016/j.ajpc.2020.100085
6. Béjot Y, Delpont B, Giroud M. Rising stroke incidence in young adults: more epidemiological evidence, more questions to be answered. *J Am Heart Assoc.* 2016;5:e003661. doi: 10.1161/JAHA.116.003661
7. Scott CA, Li L, Rothwell PM. Diverging temporal trends in stroke incidence in younger vs older people: a systematic review and meta-analysis. *JAMA Neurol.* 2022;79:1036–1048. doi: 10.1001/jamaneurol.2022.1520
8. Ruíz-Sandoval JL, Cantú C, Barinagarrementeria F. Intracerebral hemorrhage in young people. *Stroke.* 1999;30:537–541. doi: 10.1161/01.str.30.3.537
9. Broderick M, Rosignoli L, Lunagariya A, Nagaraja N. Hypertension is a leading cause of nontraumatic intracerebral hemorrhage in young adults. *J Stroke Cerebrovasc Dis.* 2020;29:104719. doi: 10.1016/j.jstrokecerebrovasdis.2020.104719
10. Hathidara MY, Saini V, Malik AM. Stroke in the young: a global update. *Curr Neurol Neurosci Rep.* 2019;19:91. doi: 10.1007/s11910-019-1004-1
11. Béjot Y, Daubail B, Giroud M. Epidemiology of stroke and transient ischemic attacks: current knowledge and perspectives. *Rev Neurol (Paris).* 2016;172:59–68. doi: 10.1016/j.neurol.2015.07.013
12. Schöberl F, Arthur Ringleb P, Wakili R, Poli S, Arne Wollenweber F, Kellert L. Juvenile stroke. *Dtsch Arztebl Int.* 2017;114:527–534. doi: 10.3238/arztebl.2017.0527
13. Perera KS, de Sa Boasquevisque D, Rao-Melacini P, Taylor A, Cheng A, Hankey GJ, Lee S, Fabregas JM, Ameriso SF, Field TS, et al; Young ESUS Investigators. Evaluating rates of recurrent ischemic stroke among young adults with embolic stroke of undetermined source. *JAMA Neurol.* 2022;79:450–458. doi: 10.1001/jamaneurol.2022.0048
14. Baird AE. Genetics and genomics of stroke: novel approaches. *J Am Coll Cardiol.* 2010;56:245–253. doi: 10.1016/j.jacc.2010.02.051
15. Terni E, Giannini N, Brondi M, Montano V, Bonuccelli U, Mancuso M. Genetics of ischaemic stroke in young adults. *BBA Clin.* 2015;3:96–106. doi: 10.1016/j.bbacli.2014.12.004
16. Yamamoto Y, Craggs L, Baumann M, Kalimo H, Kalaria RN. Review: molecular genetics and pathology of hereditary small vessel diseases of the brain. *Neuropathol Appl Neurobiol.* 2011;37:94–113. doi: 10.1111/j.1365-2990.2010.01147.x
17. Michaud M, Mauhin W, Belmatoug N, Bedreddine N, Garnotel R, Catros F, Lidove O, Gaches F. Maladie de Fabry: quand y penser? *Rev Med Interne.* 2021;42:110–119. doi: 10.1016/j.revmed.2020.08.019
18. Gouda P, Kay R, Habib M, Aziz A, Aziza E, Welsh R. Clinical features and complications of Loeys-Dietz syndrome: a systematic review. *Int J Cardiol.* 2022;362:158–167. doi: 10.1016/j.ijcard.2022.05.065
19. Hopkin RJ, Cabrera GH, Jefferies JL, Yang M, Ponce E, Brand E, Feldt-Rasmussen U, Germain DP, Guffon N, Jovanovic A, et al. Clinical outcomes among young patients with Fabry disease who initiated agalsidase beta treatment before 30 years of age: an analysis from the Fabry Registry. *Mol Genet Metab.* 2023;138:106967. doi: 10.1016/j.ymgme.2022.106967
20. Adams HP, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, Marsh EE. 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. doi: 10.1161/01.str.24.1.35
21. Little J, Higgins JPT, Ioannidis JPA, Moher D, Gagnon F, von EE, Khoury MJ, Cohen B, Davey-Smith G, Grimshaw J, et al; Strengthening the Reporting of Genetic Association Studies. Strengthening the Reporting of Genetic Association Studies (STREGA): an extension of the STROBE statement. *PLoS Med.* 2009;6:e22. doi: 10.1371/journal.pmed.1000022
22. Nambot S, Thevenon J, Kuentz P, Duffourd Y, Tisserant E, Bruel AL, Mosca-Boidron AL, Masurel-Paulet A, Lehalle D, Jean-Marçais N, et al; Orphanomix Physicians' Group. Clinical whole-exome sequencing for the diagnosis of rare disorders with congenital anomalies and/or intellectual disability: substantial interest of prospective annual reanalysis. *Genet Med.* 2018;20:645–654. doi: 10.1038/gim.2017.162
23. Thevenon J, Duffourd Y, Masurel-Paulet A, Lefebvre M, Feillet F, El Chehadeh-Djebbar S, St-Onge J, Steinmetz A, Huet F, Chouchane M, et al. Diagnostic odyssey in severe neurodevelopmental disorders: toward clinical whole-exome sequencing as a first-line diagnostic test. *Clin Genet.* 2016;89:700–707. doi: 10.1111/cge.12732
24. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, et al; ACMG Laboratory Quality Assurance Committee. 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;17:405–424. doi: 10.1038/gim.2015.30
25. Chang LH, Chi NF, Chen CY, Lin YS, Hsu SL, Tsai JY, Huang HC, Lin CJ, Chung CP, Tung CY, et al. Monogenic causes in familial stroke across intracerebral hemorrhage and ischemic stroke subtypes identified by whole-exome sequencing. *Cell Mol Neurobiol.* 2023;43:2769–2783. doi: 10.1007/s10571-022-01315-3
26. Ilinca A, Martinez-Majander N, Samuelsson S, Piccinelli P, Truvé K, Cole J, Kittner S, Soller M, Kristoffersson U, Tatlisumak T, et al. Whole-exome sequencing in 22 young ischemic stroke patients with familial clustering of stroke. *Stroke.* 2020;51:1056–1063. doi: 10.1161/STROKEAHA.119.027474
27. Debette S, Goeggel Simonetti B, Schilling S, Martin JJ, Kloss M, Sarikaya H, Hausser I, Engelter S, Metso TM, Pezzini A, et al; CADISP-plus consortium. Familial occurrence and heritable connective tissue disorders in cervical artery dissection. *Neurology.* 2014;83:2023–2031. doi: 10.1212/WNL.0000000000001027
28. Grond-Ginsbach C, Brandt T, Kloss M, Aksay SS, Lyrer P, Traenka C, Erhart P, Martin JJ, Altintas A, Siva A, et al. Next generation sequencing analysis of patients with familial cervical artery dissection. *Eur Stroke J.* 2017;2:137–143. doi: 10.1177/2396987317693402
29. De Vilder EYG, Cardoen S, Hosen MJ, Le Saux O, De Zaeytijd J, Leroy BP, De Reuck J, Coucke PJ, De Paepe A, Hemelsoet D, et al. Pathogenic variants in the ABCC6 gene are associated with an increased risk for ischemic stroke. *Brain Pathol.* 2018;28:822–831. doi: 10.1111/bpa.12620
30. Uemura M, Hatano Y, Nozaki H, Ando S, Kondo H, Hanazono A, Iwanaga A, Murota H, Osakada Y, Osaki M, et al. High frequency of HTRA1 AND ABCC6 mutations in Japanese patients with adult-onset cerebral small vessel disease. *J Neurol Neurosurg Psychiatry.* 2023;94:74–81. doi: 10.1136/jnnp-2022-329917
31. Nollet L, Campens L, Zaeytijd JD, Leroy B, Hemelsoet D, Coucke PJ, Vanakker OM. Clinical and subclinical findings in heterozygous ABCC6 carriers: results from a Belgian cohort and clinical practice guidelines. *J Med Genet.* 2022;59:496–504. doi: 10.1136/jmedgenet-2020-107565
32. Ghorbanlou M, Moradi F, Mehdizadeh M. Frequency, shape, and estimated volume of intracranial physiologic calcification in different age groups investigated by brain computed tomography scan: a retrospective study. *Anat Cell Biol.* 2022;55:63–71. doi: 10.5115/acb.21.137
33. Jaworek T, Xu H, Gaynor BJ, Cole JW, Rannikmae K, Stanne TM, Tomppo L, Abedi V, Amouyel P, Armstrong ND, et al; Cervical Artery Dissections and Ischemic Stroke Patients (CADSIP) Consortium. Contribution of common genetic variants to risk of early-onset ischemic stroke. *Neurology.* 2022;99:e1738–e1754. doi: 10.1212/WNL.0000000000201006

Downloaded from http://ahajournals.org by on September 30, 2025

STROKE FIRST PROOF ONLY