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Belgian Fabry Study

Prevalence of Fabry Disease in a Cohort of 1000 Young Patients With Cerebrovascular Disease

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Background and Purpose—Data on the prevalence of Fabry disease in patients with central nervous system pathology are limited and controversial. In this study, we assessed the prevalence of Fabry disease in young patients presenting with cerebrovascular disease in Belgium.

Methods—In this national, prospective, multicenter study, we screened for Fabry disease in 1000 patients presenting with ischemic stroke, transient ischemic attack, or intracranial hemorrhage; unexplained white matter lesions; or vertebro-basilar dolichoectasia. In male patients, we measured α -galactosidase A (α -GAL A) activity in dried blood spots. Female patients were screened for mutations by exonic DNA sequencing of the α -GAL A gene.

Results— α -GAL A activity was deficient in 19 men (3.5%), although all had normal α -GAL A gene sequences. Enzymatic deficiency was confirmed on repeat assessment in 2 male patients (0.4%). We identified missense mutations in 8 unrelated female patients (1.8%): Asp313Tyr (n=5), Ala143Thr (n=2), and Ser126Gly (n=1). The pathogenicity of the 2 former missense mutations is controversial. Ser126Gly is a novel mutation that can be linked to late-onset Fabry disease.

Conclusion— α -GAL A deficiency may play a role in up to 1% of young patients presenting with cerebrovascular disease. These findings suggest that atypical variants of Fabry disease with late-onset cerebrovascular disease exist, although the clinical relevance is unclear in all cases. (*Stroke*. 2010;41:863-868.)

Key Words: Fabry disease ■ cerebrovascular accident ■ white matter lesions ■ dolichoectasia ■ α -galactosidase A ■ lysosomal storage disorders ■ Belgium

Fabry disease (Anderson-Fabry disease; Online Mendelian Inheritance in Man No. 301500) is an X-linked lysosomal storage disorder caused by mutations in the α -galactosidase A (α -GAL A) gene (*GLA*), leading to α -GAL A deficiencies.¹ Consequently, neutral glycosphingolipids accumulate in many tissues and cell types, including the vascular epithelium, cornea, kidneys, and heart. Male hemizygotes are generally more severely affected than are heterozygous females, but disabling clinical features and disease progression often also occur in female patients.² Patients with the classic

phenotype of Fabry disease usually present in childhood with pain crises, acroparesthesia, hypohidrosis, gastrointestinal symptoms, angiokeratoma, and corneal abnormalities. Severe morbidity and mortality follow in adult life due to renal failure, cardiac involvement, and stroke.³ Atypical variants with late-onset manifestations in the cardiac, neurologic, and renal systems have been described.⁴⁻¹³

Estimates on the prevalence of classic Fabry disease vary from 1 in 40 000 to 1 in 117 000.¹⁴ Given the nonspecific presenting symptoms and delayed presentation, atypical vari-

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ants of Fabry disease might be underdiagnosed.¹⁵ Epidemiologic studies have reported the condition in 0.2% to 1.2% of patients with end-stage renal disease^{4–8} and in 1% to 6.3% of patients with unexplained hypertrophic cardiomyopathy.^{9–11} The prevalence of Fabry disease in young patients with cryptogenic stroke was reported to be as high as 4.9% in men and 2.4% in women.¹³ This finding, however, was not reproduced in a smaller, retrospective study.¹⁶ White matter lesions and vertebrobasilar dolichoectasia are commonly found in patients with Fabry disease,¹⁷ but literature data on the prevalence of Fabry disease in patients with these neuroimaging findings are nonexistent.

The aim of the Belgian Fabry Study (BeFaS) was to prospectively assess the frequency of Fabry disease in young Belgian patients with neurologic hallmarks of this disease, namely, stroke, unexplained white matter lesions, and vertebrobasilar dolichoectasia.

Methods

Patients and Procedures

Thirty-three clinical neurology departments throughout Belgium participated in this national, prospective, multicenter study, BeFaS. From March 2007 to October 2008, 1000 patients aged 18 to 60 years, consecutively presenting at a participating neurology department with stroke (ischemic stroke, transient ischemic attack, or intracranial hemorrhage), unexplained white matter lesions, or vertebrobasilar dolichoectasia were enrolled. Unexplained white matter lesions were defined as the presence of lesions in the deep or subcortical white matter on magnetic resonance imaging or computed tomography for which no etiology was found after expert neurologist evaluation. Patients who were unable to provide informed consent, who were already diagnosed with Fabry disease, or in whom this disease was previously excluded by appropriate enzymatic or genetic analyses were excluded from participation in BeFaS. The study was conducted in accordance with the revised Declaration of Helsinki (1998) and in agreement with the guidelines of the ethics committees of ZNA Antwerp, the University of Antwerp, and the ethics committees of each of the participating clinical neurologic centers.

After informed consent was obtained, demographic data, cardiovascular risk factors, presence of signs and symptoms of Fabry disease, and clinical and neuroimaging data were registered in a database by using web-based case report forms. Assessment of clinical signs suggestive of Fabry disease was optional. Diagnosis or exclusion of cornea verticillata was performed by ophthalmologists, screening for angiokeratoma was done by routine clinical examination, and presence of acroparesthesia was obtained by anamnesis. This symptom was regarded to be present if a history of burning or tingling sensation in the extremities was reported by the patient or a proxy.

Enzymatic and Genetic Testing

A blood sample was obtained for production of blood spots for measurement of α -GAL A activity in male patients and for genetic analyses of *GAL* in female patients, as described previously.¹⁸ In men, screening for Fabry disease can reliably be performed by enzymatic analysis, but this technique is affected by a high risk of false-negative results in women. Genetic testing therefore is the method of choice in the latter.² All diagnostic tests were performed blinded to case identity. Male patients with abnormal enzymatic activity (<3.0 ng/h per mL) were additionally examined by gene sequencing and repeat analysis of α -GAL A activity in a subset of patients. In female patients with an abnormal result on gene sequencing, repeat gene sequencing was performed.

Statistical Analysis

Data were analyzed with the use of Microsoft Excel (version 2007; Microsoft Corp, Redmond, Wash) and the SPSS 15.0 software package for Windows (SPSS Inc, Chicago, Ill). The 2-tailed unpaired *t* test was used to compare continuous variables between 2 groups. A 1-way ANOVA was applied for comparison of continuous variables when there were >2 groups, and the χ^2 test was used for noncontinuous variables.

Role of the Funding Source

The sponsor of the study had no role in the study design, data collection, data analysis, data interpretation, or the writing of the report. The corresponding author had full access to all data and had final responsibility for the decision to submit for publication.

Results

Clinical Data

The mean age (\pm SD) in the cohort of 1000 patients participating in BeFaS was 47.7 ± 9.1 years, and 547 participants (54.7%) were male. Magnetic resonance imaging of the brain was obtained in 80.2% of patients. Cerebral computed tomography was performed in the remaining patients. Classic cerebrovascular risk factors, including arterial hypertension, diabetes mellitus, dyslipidemia, smoking, and coronary disease, were present in 38.7%, 9.7%, 38.2%, 55.9%, and 7.6% of patients, respectively. In 16.3% and 4.4% of participants, a history of previous stroke or renal disease was found. Family history for stroke, myocardial infarction before the age of 60 years, and dialysis was present in 19.7%, 13.0%, and 1.3%. Clinical signs suggestive of Fabry disease were reported in a minority of patients: cornea verticillata was found in 0.1% and angiokeratoma in 0.9%. Dyhidrosis, episodic pain, and acroparesthesia were reported by 4.6%, 7.0%, and 13.3% of patients. Evidence of hypertrophic cardiomyopathy was found on echocardiography in 12.0%. White matter lesions were found in 35.0% of patients.

The main clinical criterion for inclusion in BeFaS was stroke ($n=842$ patients), 573 of whom were diagnosed with ischemic stroke, 220 with transient ischemic attack, and 49 with intracranial hemorrhage (33 and 16 patients with intracerebral or subarachnoid hemorrhage, respectively). One hundred fifty-three patients were included for unexplained white matter lesions on neuroimaging but only 2 patients for vertebrobasilar dolichoectasia. Specification of the inclusion criterion was missing on the web-based inclusion form for 3 patients.

Evidence of acute cerebral infarction was present on neuroimaging in 570 patients with ischemic stroke (99.5%). The infarct was located in the anterior circulation in 335 patients (58.8%), in the posterior circulation in 152 patients (26.7%), and in both the anterior and posterior circulation in 83 patients (14.6%). Stroke etiology was classified according to TOAST criteria¹⁹ as atherothrombotic in 143 patients (25.1%), cardioembolic in 125 (21.9%), and lacunar in 99 (17.4%). A specific cause for the ischemic stroke was present in 69 patients (12.1%): cervicocephalic arterial dissection ($n=37$), vasculitis ($n=11$), hereditary coagulopathy ($n=6$), antiphospholipid syndrome ($n=3$), radiotherapy-induced vasculopathy ($n=2$), amphetamine-induced vasculopathy ($n=2$), moyamoya syndrome ($n=2$), migrainous stroke ($n=2$), cere-

Table 1. Baseline Clinical Data, Cerebrovascular Risk Factors, and Signs and Symptoms Suggestive of Fabry Disease, According to the Inclusion Criterion

	Stroke			Unexplained White Matter Lesions (n=153)	Vertebrobasilar Dolichoectasia (n=2)	P Value
	Ischemic Stroke (n=573)	TIA (n=220)	Intracranial Hemorrhage (n=49)			
General data						
Age at presentation, y	48.2 (9.0)	47.0 (9.3)	48.8 (7.9)	46.4 (9.8)	56.0 (2.8)	0.065
Male	350 (61.1%)	122 (55.5%)	122 (55.5%)	44 (28.8%)	1 (50.0%)	<0.001
MRI-based neuroimaging	451 (78.7%)	182 (82.7%)	20 (40.8%)	147 (93.6%)	2 (100%)	<0.001
Classic cerebrovascular risk factors						
Arterial hypertension	241 (42.1%)	91 (41.4%)	19 (38.8%)	34 (22.2%)	2 (100.0%)	<0.001
Diabetes mellitus	72 (12.6%)	18 (8.2%)	1 (2.0%)	5 (3.3%)	1 (50.0%)	<0.001
Dyslipidemia	235 (41.0%)	106 (48.2%)	6 (12.3%)	34 (22.2%)	1 (50.0%)	<0.001
Smoking	353 (61.6%)	119 (54.1%)	31 (63.3%)	55 (36.0%)	1 (50.0%)	<0.001
Coronary disease	48 (8.4%)	18 (8.2%)	2 (4.1%)	7 (4.6%)	1 (50.0%)	0.075
Signs and symptoms suggestive of Fabry disease						
White matter lesions	153 (26.7%)	31 (14.1%)	12 (24.5%)	153 (100.0%)	1 (50.0%)	<0.001
Previous stroke	87 (15.2%)	44 (20.0%)	9 (18.4%)	21 (13.7%)	0 (0.0%)	0.442
Renal disease	26 (4.5%)	8 (3.6%)	3 (6.1%)	6 (3.9%)	0 (0.0%)	0.930
Hypertrophic cardiomyopathy	75 (13.1%)	34 (15.5%)	5 (10.2%)	6 (3.9%)	0 (0.0%)	<0.001
Family history of stroke	103 (18.0%)	59 (26.8%)	6 (12.3%)	25 (16.3%)	1 (50.0%)	0.017
Family history of MI <60 y	69 (12.0%)	41 (18.6%)	4 (8.2%)	13 (8.5%)	1 (50.0%)	0.012
Family history of dialysis	8 (1.4%)	3 (1.4%)	1 (2.0%)	1 (0.7%)	0 (0.0%)	0.942
Cornea verticillata	0 (0%)	0 (0%)	0 (0%)	1 (0.7%)	0 (0.0%)	0.002
Angiokeratoma	7 (1.2%)	2 (0.9%)	0 (0%)	0 (0%)	0 (0.0%)	0.061
Dyshidrosis	24 (4.2%)	11 (5.0%)	6 (12.2%)	5 (3.3%)	0 (0.0%)	0.119
Pain episodes	23 (4.0%)	18 (8.2%)	2 (4.1%)	26 (17.0%)	1 (50.0%)	<0.001
Acroparesthesia	54 (9.4%)	34 (15.5%)	8 (16.3%)	37 (24.2%)	0 (0.0%)	<0.001

TIA indicates transient ischemic attack; MRI, magnetic resonance imaging; and MI, myocardial infarction.

bral venous thrombosis (n=2), hemolytic uremic syndrome (n=1), and paraneoplastic coagulopathy (n=1). In 134 patients (23.5%), stroke etiology was cryptogenic. The prevalence of intracranial hemorrhage in the reported population was only 5.8% of all stroke patients. This may be explained by the fact that for patients with intracranial hemorrhage, routine medical practice in many participating centers is often provided by neurosurgeons rather than neurologists. Sixteen of 49 patients with intracranial hemorrhage (32.7%) had subarachnoid hemorrhage. The location of intracerebral hemorrhage was lobar in 13 patients (39.4%), involved basal ganglia in 11 (33.3%), brain stem in 5 (15.2%), and thalamus in 4 (12.1%). A cerebral vascular anomaly was present in 21 patients (aneurysm, cavernous malformation, and arteriovenous malformation, respectively, in 15, 4, and 2 patients). Other causes of intracranial hemorrhage included alcohol abuse in 2 patients and hypertension in 1 individual. Unexplained white matter lesions and dolichoectasia were found in patients who underwent neuroimaging for indications like headache, dizziness, seizures, or syncope. Table 1 shows baseline clinical data and signs and symptoms of Fabry disease, according to the inclusion criterion. In contrast to patients who were enrolled because of unexplained white

matter lesions, stroke patients were predominantly male, less frequently received magnetic resonance–based neuroimaging, displayed more classic cerebrovascular risk factors, and more often had hypertrophic cardiomyopathy and a positive family history of stroke or myocardial infarction at a young age. A detailed stratification of the study population by entry condition, sex, age, and cerebrovascular history is presented in Table 2.

Diagnostic Test Results

As shown in the Figure, α -GAL A activity was assessed on dried blood spot in 545 of 547 male patients and was below the normal cutpoint of 3.0 nmol/h per mL in 19 patients. Exonic DNA sequencing of *GLA* was performed in all these patients but failed to identify a mutation. Additionally, a repeat dried blood spot was obtained for 9 patients; α -GAL A activity was found to be normal in 7. However, in 1 patient, a complete enzyme deficiency was observed, and a severe deficiency (0.3 ng/h per mL) was found in another (Table 3).

GLA sequencing results were available for 448 of 453 female patients. Two and 3 samples were lost due to a technical error or during shipment, respectively. A missense mutation was identified in 8 unrelated female patients:

Table 2. Study Population Stratified by Entry Condition, Sex, Age, and Cerebrovascular History

Entry Condition	Sex	Age at Presentation, Mean \pm SD, y	Cerebrovascular History
Stroke	Male	49.2 (8.5)	81 (16.2%)
	Female	46.0 (9.4)	59 (17.5%)
Ischemic stroke	Male	49.6 (8.6)	52 (14.9%)
	Female	45.9 (9.1)	35 (15.7%)
TIA	Male	48.4 (8.2)	20 (16.4%)
	Female	45.3 (10.3)	24 (24.5%)
Intracranial hemorrhage	Male	47.6 (8.5)	9 (32.1%)
	Female	50.4 (6.7)	0 (0.0%)
Unexplained white matter lesions	Male	47.7 (9.3)	10 (22.7%)
	Female	45.9 (10.0)	11 (10.1%)
Vertebrobasilar dolichoectasia	Male	57.0 (0.0)	0 (0.0%)
	Female	55.0 (0.0)	0 (0.0%)

TIA indicates transient ischemic attack.

Asp313Tyr in 5, Ala143Thr in 2 subjects, and a novel mutation, Ser126Gly, in 1 subject (Table 4). The presence of the mutation was confirmed by repeating the exonic DNA sequencing.

Discussion

Cerebral micro- and macroangiopathies are hallmarks of Fabry disease.^{17,20} It is hypothesized that lipid deposits in vascular endothelial and smooth muscle cells cause oxidative stress, vascular dysfunction, vessel occlusion, and tissue ischemia.²¹ These pathophysiological mechanisms are associated with an increased risk of premature stroke, progressive white matter lesions, and dolichoectasia as major clinical and neuroimaging correlates.¹⁷ Recent literature data indicate that stroke frequently occurs before the diagnosis of Fabry disease has been made and in the absence of other key signs of this disease.²²

In contrast to the considerable number of studies on the presence of Fabry disease in patients with renal or cardiac

disorders,^{4–11} only 2 studies reported on the prevalence of Fabry disease with neurologic hallmarks for this condition.^{13,16} Both studies focused on young patients with cryptogenic stroke. In a large cohort of 721 patients diagnosed with cryptogenic stroke, the prevalence of Fabry disease was found to be as high as 4.9% in men and 2.4% in women.¹³ We were unable, however, to reproduce this finding in a retrospective study of 103 cryptogenic stroke patients.¹⁶ Fabry disease is associated with cerebral micro- and macroangiopathy.^{17,20} Moreover, cardioembolic phenomena²³ and coagulopathy²⁴ may be common in this disorder. Limiting screening for Fabry disease to patients without these conditions might therefore induce selection bias. For this reason, inclusion criteria in BeFaS were not restricted to patients presenting with cryptogenic stroke. Other screening projects for Fabry disease in ischemic stroke patients have recently been finalized (M.A. Wozniak et al and M. Viana-Baptista et al; personal communications) or are currently ongoing. Despite being identified as relevant indicators for cerebral vasculopathy in Fabry disease, epidemiologic studies in patients with stroke of all causes, unexplained white matter lesions, or dolichoectasia have not been done before. Assessment of the clinical signs suggestive of Fabry disease was not performed in a standardized manner and may limit generalizability of these findings. For instance, the remarkably high prevalence of acroparesthesia in our population may be secondary to overreporting.

In the present study, deficient α -GAL A activity was present in 3.5% of male patients, and a mutation in *GAL* was found in 1.8% of female patients. Diagnosis of Fabry disease, however, is not straightforward in all patients. The absence of a mutation in all male patients with this enzyme deficiency suggests that the dried blood spot analysis may have been false-positive in some patients. However, repeat assessment α -GAL A activity in a subset of 9 patients confirmed the enzyme deficiency in a patient aged 49 years with ischemic stroke secondary to carotid artery dissection and in another patient aged 54 years with a cryptogenic transient ischemic

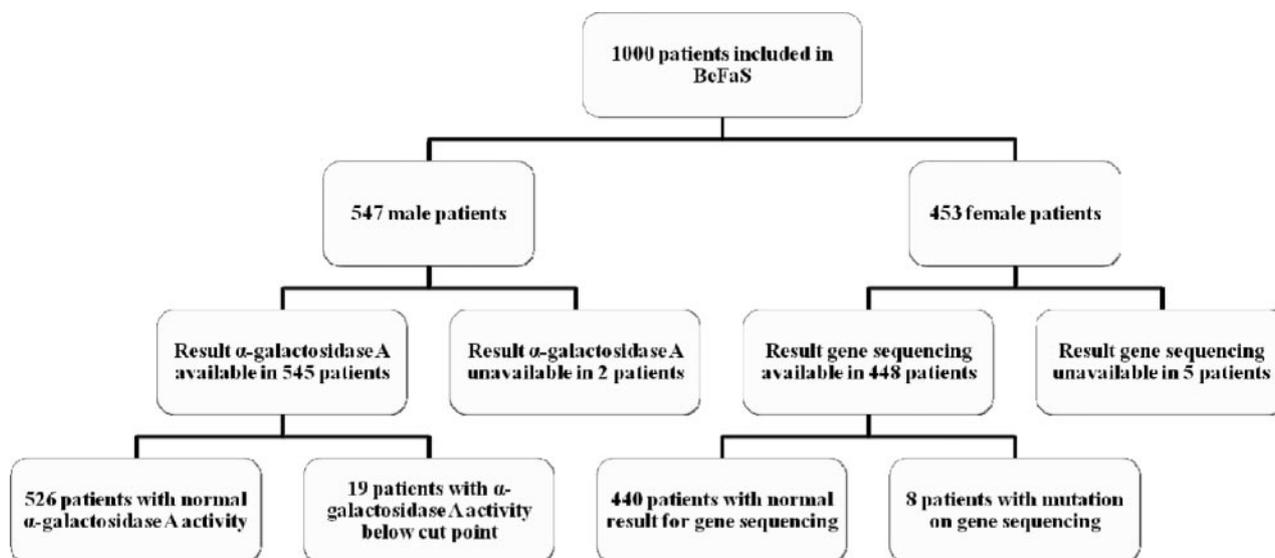


Figure. Flowchart for the BeFaS.

Table 3. Diagnostic Assessments in 19 Male Patients With α -GAL A Deficiency

Case	Age, y	α -GAL A Value, nmol/h per mL	Exonic DNA Sequencing	Repeat α -GAL A Value, nmol/h per mL	Inclusion Criterion	Etiology	Cerebrovascular History
1	60	2.1	Normal	21.7	Intracerebral hemorrhage	Hypertension	Negative
2	48	2.7	Normal	NA	Ischemic stroke	Cryptogenic	Negative
3	42	1.1	Normal	29.1	Ischemic stroke	Cryptogenic	Negative
4	49	1.0	Normal	1.5	Ischemic stroke	Carotid dissection	Negative
5	45	0.0	Normal	26.5	Ischemic stroke	Cryptogenic	Negative
6	53	1.8	Normal	26.3	TIA	Cardioembolic	Negative
7	54	2.8	Normal	NA	Ischemic stroke	Atherothrombotic	Negative
8	48	1.8	Normal	NA	White matter lesions	Cryptogenic	Negative
9	54	0.8	Normal	NA	Intracerebral hemorrhage	Hemangioma	Negative
10	31	2.7	Normal	3.4	Ischemic stroke	Cryptogenic	Negative
11	54	2.0	Normal	NA	Ischemic stroke	Atherothrombotic	Stroke
12	49	0.0	Normal	NA	Ischemic stroke	Cryptogenic	Negative
13	39	0.0	Normal	NA	Ischemic stroke	Cardioembolic	Negative
14	55	0.0	Normal	NA	TIA	Cryptogenic	Negative
15	52	1.5	Normal	NA	TIA	Cryptogenic	Negative
16	55	1.6	Normal	34.0	TIA	Cryptogenic	Negative
17	54	0.0	Normal	0.0	TIA	Cryptogenic	Negative
18	46	1.6	Normal	NA	Ischemic stroke	Cryptogenic	Negative
19	59	3.0	Normal	7.0	TIA	Cardioembolic	Negative

NA indicates not available; TIA, transient ischemic attack.

attack (cases 4 and 17, Table 3). Magnetic resonance imaging showed acute cerebral ischemia in the anterior circulation in both patients, and the family history was remarkable for stroke in 1 patient. An atypical variant of Fabry disease cannot be excluded in these patients, and additional diagnostic assessments are ongoing. We found the Asp313Tyr mutation in 5 female patients. This mutation has been identified in classically affected males as the single mutation²⁵ or in the *cis* position with another missense mutation.^{26,27} On the other hand, this mutation was also reported in 0.45% of normal X chromosomes, and *in vitro* studies favor the classification of Asp 313Tyr as a polymorphism.²⁷ The mutation Ala143Thr has previously been reported in patients with the late-onset variant of Fabry disease,^{5,15} but Ser126Gly appears to be a novel mutation that may also be linked to late-onset Fabry disease. The discovery of a previously unreported mutation is not surprising, because most families have a private mutation and new mutations are frequently found with the diagnosis of an index case.²⁸

Our results suggest that Fabry disease may play a role in up to 1% of young patients presenting with cerebrovascular disease. However, current knowledge on the complex pathogenesis of this condition is incomplete and does not always allow a definitive diagnosis in every patient, especially in cases of late-onset Fabry disease. This illustrates the need for additional basic and epidemiologic research. In an ongoing research project, we are focusing on patients with abnormal screening results identified in the BeFaS and aim to obtain more certainty about the diagnosis of Fabry disease through careful clinical, biochemical, and histological examinations. In addition, studies with larger cohorts of high-risk patients are ongoing and may help to formulate definite answers for these patients.

Summary

We report on a national, prospective, multicenter study on the prevalence of Fabry disease in 1000 young patients pres-

Table 4. Diagnostic Assessments in 8 Female Patients With a Mutation in the *GAL*

Case	Age, y	Gene Sequencing	Inclusion Criterion	Etiology	Cerebrovascular History
1	43	Asp313Tyr	TIA	Cryptogenic	Negative
2	52	Asp313Tyr	TIA	Cryptogenic	Negative
3	41	Asp313Tyr	White matter lesions	Cryptogenic	Negative
4	35	Asp313Tyr	White matter lesions	Cryptogenic	Negative
5	53	Asp313Tyr	TIA	Cryptogenic	Stroke
6	43	Ala143Thr	Ischemic stroke	Carotid dissection	Negative
7	51	Ala143Thr	Ischemic stroke	Carotid dissection	Negative
8	41	Ser126Gly	Ischemic stroke	Cryptogenic	Negative

TIA indicates transient ischemic attack.

enting with cerebrovascular disease. Our results suggest that α -GAL A deficiency may play a role in up to 1% of young patients presenting with cerebrovascular disease. Although the clinical relevance is not straightforward in all cases, these findings suggest that atypical variants of Fabry disease with late-onset cerebrovascular disease exist and that their genetic background is poorly understood.

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Disclosures

P.P. De Deyn, R. Brouns, F. Eyskens, and V. Thijs have received compensation from Shire Belgium for serving on the BeFaS scientific advisory committee. V. Thijs is a Clinical Investigator of the FWO Flanders. The other authors have no conflicts of interest to declare.

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