Prevalence of Fabry Disease in Young Patients with Stroke in Argentina

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Background: Fabry disease (FD) is an underdiagnosed cause of stroke in young adults, but the frequency of this association is largely unknown. We estimated the prevalence of FD in a nationwide cohort of young adults who had stroke and transient ischemic attack (TIA) in Argentina. *Methods:* This was a prospective, multicenter study of stroke and FD in young adults (18-55 years) conducted in Argentina between 2011 and 2015. Patients were enrolled if they had had a TIA or an ischemic or hemorrhagic stroke within the previous 180 days. FD was diagnosed by measuring α-galactosidase A activity (males) and through genetic studies (females). *Results:* We enrolled 311 patients (54% men, mean age: 41 years). Ischemic events occurred in 89% of patients (80% infarcts, 9% TIA) and hemorrhagic strokes in 11%. One female (.3% of the total group, 1% of the cryptogenic ischemic strokes) had the pathogenic mutation c.888G>A/p.Met296Ile /Exon 6 on the *GAL* gene. Her only other manifestation of FD was angiokeratoma. Eighteen females had nonpathogenic intronic variations: c.-10C>T, c.-12G>A, or both. Two patients had the nonpathogenic mutation D313Y, while a third had the likely benign mutation S126G. *Conclusions:* FD was identified in 1 patient (.3%) in this first Latin American study. The patient presented with a late-onset oligo-symptomatic form of the disease. A large number of nonpathogenic mutations were present in our cohort, and it is essential that they not be mistaken for pathogenic mutations to

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Received December 8, 2016; revision received June 3, 2017; accepted September 24, 2017.

The AISYF study was sponsored by a research Grant from Shire Human Genetic Therapies.

R.R. has received speaker and scientific consulting fees from Novartis, Gador, Shire, Amicus, and Sanofi Genzyme. P.B. has received speaker's fees from Shire. L.L.C. has received speaker's fees from Shire and Pfizer. P.R. has received speaker or scientific consulting fees from Shire, Alexion, BioMarin, and Sanofi Genzyme. The remaining authors have nothing to disclose.

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1052-3057/$ - see front matter

https://doi.org/10.1016/j.jstrokecerebrovasdis.2017.09.045

avoid unnecessary enzyme replacement treatment. **Key Words:** Fabry disease—stroke—young—cerebrovascular disease—mutations.

Introduction

Stroke is a leading cause of death and disability worldwide.[1] From 5% to 10% of all strokes occur in patients under 45 years old, generating considerable morbidity and mortality in this age group. The crude annual incidence rate of stroke in young adults is estimated at between 8 and 19 per 100,000,[2] whereas mortality rates range between 2.9% at 1 year and 26.8% after 20 years.[3] The causes of stroke in this group are very diverse but remain undetermined in up to 40% of patients after extensive workup.[4] Moreover, only limited information exists regarding stroke in young adults in Latin America.[5-9]

Fabry disease (FD) is an X-linked lysosomal storage disorder characterized by decreased or absent activity of lysosomal enzyme α-galactosidase A (α-GAL A); it has been identified as an underdiagnosed etiology of stroke in the young.[10] The prevalence of CVA in FD patients identified in the Fabry Outcome Survey (FOS) was 11% in males and 15% in females, a prevalence 12 times higher than observed in a comparable non-Fabry population.[11] In the global Fabry Registry, 6.9% of males and 4.3% of females with FD had had an ischemic or hemorrhagic stroke. Furthermore, 50% of males and 38% of females had their stroke before the diagnosis of FD was made.[12] Among patients with FD and no history of stroke or transient ischemic attack (TIA), 44% had silent brain infarcts on brain magnetic resonance imaging (MRI).[13] Rolfs et al reported that 4.9% of males and 2.4% of females with cryptogenic stroke younger than 55 years suffered from FD, which corresponds to approximately 1.2% of young stroke patients overall.[10]

Several other studies screening for FD in young stroke patients throughout the world have yielded conflicting results.[14-22] We herein report the Argentinean Initiative for the Study of Young patients with Stroke and Fabry disease (AISYF Study), the first national, multicenter, prospective study to investigate the association between stroke and FD in young Latin American adults.

Methods

Study Design and Patient Selection

We performed a prospective, multicenter, nationwide study of ischemic and hemorrhagic stroke and FD in young adults in Argentina. The study was conducted at 22 centers across the country, coordinated by the Department of Neurology at Hospital Británico de Buenos Aires between January 2011 and December 2015. Patient enrollment required written informed consent.

We ultimately enrolled 311 patients between the ages of 18 and 55 years with either a TIA (defined as a transient episode of neurological dysfunction caused by focal brain, spinal cord, or retinal ischemia, without acute infarction),[23] an acute ischemic stroke (defined as a focal neurological deficit due to infarction of central nervous system tissue),[23] or an intracerebral hemorrhage (defined as a focal neurological deficit associated with focal collection of blood within the brain parenchyma).[24] Patients were enrolled within 180 days of their cerebrovascular event. We excluded patients with ischemic stroke following subarachnoid hemorrhage, cancer, or trauma, as well as patients with a hemorrhagic stroke due to a vascular malformation (e.g., aneurysm, arteriovenous malformation, or cavernous hemangioma) or when suspected to be related to cancer, trauma, or anticoagulation. We also excluded patients with either an epidural or subdural hemorrhage.

Stroke Subtype Classification and Etiological Workup

After informed consent was obtained from the patients, demographic data, cardiovascular risk factors, the presence of signs and symptoms of FD, and clinical and neuroimaging data were registered in a database. Head computed tomography or MRI had been performed in all patients before enrollment. After enrollment, all patients underwent comprehensive etiological investigations, including brain and vascular imaging, electrocardiography, echocardiography, extensive laboratory testing, and FD enzymatic and genetic studies. Information on comorbidities and vascular risk factors were collected prospectively using a standardized prespecified case report form. All variables analyzed were checked for completeness, range, and outliers. Trial of ORG 10172 in acute stroke (TOAST) criteria were used to define the clinical subtypes of ischemic stroke.[25] Intracerebral hemorrhage was categorized by location.[24,26] Demographic information and comorbid conditions of our patients will be reported separately.

Diagnosis of Fabry Disease

Measurement of α-GAL A activity (in all males) was determined using dried blood spots on filter paper[27] and performed at the Instituto de Estudios Inmunológicos y Fisiopatológicos (IIFP) Facultad de Ciencias Exactas, Universidad Nacional de La Plata-Consejo Nacional de Investigaciones Científicas y Técnicas, La Plata, Argentina. When deficient activity was detected, confirmation of the enzymatic result was performed in leukocytes from whole blood. Sequencing of the *GLA* gene was per-

formed in all females and males with deficient enzyme activity.

The first 43% of our patients were analyzed at the Albrecht-Kossel-Institute, Medical Faculty of the University Rostock, with identical procedures used in the Stroke in young Fabry patients (SIFAP) study. The remaining 57% of the samples, analyzed in IIFP, used identical genetic evaluation as the first half, based on sequence analysis of coding regions of 7 exons along with intron–exon boundaries of the *GAL* gene.

Results

A total of 311 patients were enrolled (54% men, mean age: 41years). Ischemic strokes had occurred in 89% of the patients (infarcts 80%, TIA 9%) and hemorrhagic strokes in 11%.

Stroke subtypes based on the TOAST classification system[25] were: atherosclerosis of large vessels, n = 33 (12.3%); cardiac embolic source, n = 20 (7.5%); small artery occlusion, n = 31 (11.5%); other determined cause, n = 73 (27.1%); and undetermined cause: 2 or more etiologies, n = 16 (5.9%); negative investigation, n = 55 (20.4%) and incomplete investigation, n = 41 (15.2%). There were 19 patients with recurrent strokes and 17 with recurrent TIA.

The epidemiological characteristics of this group will be reported separately.

We identified only 1 patient (.3% of the total sample, 1% of the patients with cryptogenic ischemic strokes) with a pathogenic mutation: c.888G>A / p.Met296Ile / Exon 6. A second patient was identified with a previously considered pathogenic mutation c.376A>G / p.Ser126Gly / Exon 3. In addition, 21 females had nonpathogenic mutations including c.-10C>T (10 patients); c.937G>T and p.Asp313Tyr (D313Y) (2 patients); c.-12G>A (8 patients); and both c.-10C>T and c.-12G>A (1 patient).[28,29]

Patient with Pathogenic Mutation c.888G>A / p.Met296Ile / Exon 6

A 48-year-old woman was referred to us with acute left hemiparesis. Risk factors included hypertension and smoking. Brain MRI revealed bilateral hyperintense lesions in the corona radiata (Fig 1). Electrocardiography and echocardiography were normal, as were trombophilic studies. The patient had no neuropathic pain or cornea verticillata but had angiokeratomas. Enzymatic studies for FD were normal, and a genetic study identified a G to A transition at nucleotide 888 in exon 6 of the coding sequence, which predicted the substitution of isoleucine for methionine at residue 296 (Met296Ile). On family history, both the father and paternal grandmother of the patient had suffered a stroke in their 50s. She also had a paternal uncle with end-stage renal disease of unclear etiology. Evaluation of her uncle and the rest of her family identified 10 additional patients with FD; 3 are currently on enzyme replacement treatment (ERT) (Fig 2 and Table 1).

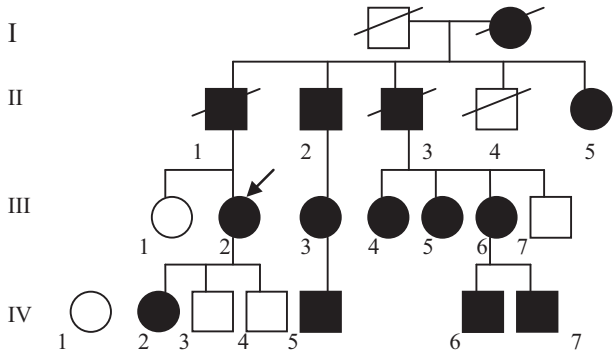

Figure 1. *Pedigree of the family with the p.Met296Ile mutation. Square: male; Circle: female; Filled: affected; Arrow: proband.*

Patient with a Mutation of Unclear Significance c.376A>G / p.Ser126Gly / Exon 3

A 26-year-old female presented with acute left hemiplegia and dysarthria. She had a medical history of migraine, use of oral contraceptives, and recent neck manipulations. On admission, she had dysarthria, left hemiplegia, and hemianopsia. Brain MRI revealed a large, right frontoparietal infarction with dissection of the right internal carotid artery. Within 24 hours, the patient's level of

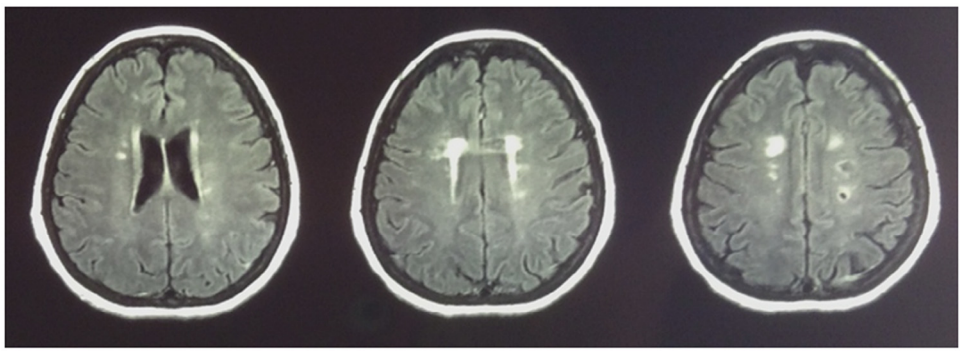

Figure 2. *Brain magnetic resonance imaging of patient with p.Met296Ile mutation. Fluid attenuation inversion recovery. Deep small-vessel disease and confluent white matter lesions.*

Table 1. *Family with FD and a pathogenic mutation c.888G>A / p.Met296Ile: Clinical manifestations*

N	Age/ gender	Nephro	Cardio	Angiok	Hypoh	A Pain	Dizzin	Hypoac	C Vert	TIA/ CVA	N Pain	QST	Brain MRI lesions
1	48/F	no	yes	yes	yes	no	no	no	no	yes	no	N	yes
2	47/F	no	no	no	no	yes	no	no	no	no	no	N	no
3	70/M	yes	yes	no	no	no	no	no	no	no	no	SFN	yes
4	69/F	no	no	yes	no	no	no	no	no	no	no	SFN	no
5	38/F	no	no	yes	no	yes	no	no	no	no	yes	N	no
6	43/F	no	yes	yes	yes	no	no	no	no	no	no	SFN	no
7	41/F	no	no	no	no	no	no	no	no	no	no	N	no
8	18/M	no	no	no	no	no	no	no	no	no	no	N	no
9	15/M	no	no	no	no	no	no	no	no	no	no	N	no
10	37/F	no	no	no	no	no	no	no	no	no	no	SFN	no
11	3/M	no	no	no	no	no	no	no	no	no	no	N	no

Abbreviations: A Pain, abdominal pain; Angiok, angiokeratoma; Cardio, cardiomyopathy; C Vert, cornea verticillata; Dizzin, dizziness; F, female; Hypoac, hypoacusia; Hypoh, hypohidrosis; MRI, magnetic resonance imaging; M, Male; N pain, neuropathic pain; N, normal; Nephro, nephropathy; QST, quantitative sensory testing; SFN, small-fiber neuropathy.

consciousness declined due to intracranial hypertension; consequently, an urgent right craniectomy was performed, after which her mental status improved. Four days later, she became comatose, and a new brain MRI and angiography demonstrated cerebral venous thrombosis. Prothrombotic tests were performed, and anticardiolipin antibodies and Factor V Leiden were identified. The patient was anticoagulated. FD was evaluated as part of the AISYF protocol: α-GAL A enzyme activity, both in dried blood spots and leukocytes, was normal, whereas genetic testing revealed a heterozygous mutation in the *GLA* gene: S126G. Both renal and cardiac function of the patient were normal. Moreover, she had no signs of classical FD, like neuropathic pain, cornea verticillata, or angiokeratomas. Her MRI revealed neither the pulvinar sign nor an enlarged basilar artery. Her father (55 years old) also was found to have normal α-GAL A activity in leukocytes, and the same hemizygous mutation was identified; however, he also lacked any signs or symptoms compatible with FD. Our patient progressively improved. Four years later, at the time of her last follow-up assessment, she had persistent left hemiparesis but was able to work.

Discussion

There is limited information regarding strokes among young adults in Latin America. Moreover, in large studies, the etiology of stroke remained undetermined in 16%-32%; none of these studies included FD in their evaluation.[5,6]

We identified a low prevalence of FD in young adults with stroke: .3% of the total group and 1% of the group with cryptogenic stroke (including patients with both: stroke of undetermined etiology and those with an incomplete study). The Met296Ile mutation of our proband was associated with late-onset FD; and rather than demonstrating the classical phenotype, this patient presented only with stroke and angiokeratomas. The identification of 10 additional patients in her family, who also had oligosymptomatic manifestations, underlines the usefulness of screening programs in stroke patients. There is increasing evidence that patients with stroke identified through screening studies usually have mild symptoms of the disease and that the cerebral event occurs in the absence of severe cardiac or kidney involvement.[12,20,30] MRI evidence of previous asymptomatic lesions, as our patient had, as well as recurrent stroke before diagnosis, seems to be common in this group.[13,20] The Met296Ile mutation was originally identified in a patient with late-onset FD, accompanied by cardiomyopathy and hemorrhagic stroke.[31]

A second important finding of the current study is that *GLA* gene mutations of unclear significance are common in young patients with stroke, emphasizing the challenge of any screening study to ascertain which mutations are pathogenic. This is essential not only to correctly identify patients with FD, but also to avoid unnecessary treatments. The intronic mutations we found, as well as the D113Y mutation, are currently considered nonpathogenic; whether they represent a risk factor for stroke is still unclear[20,22] Therefore, we did not pursue further investigations in those patients or relatives for FD signs or symptoms.[28,29]

The S126G mutation is a previously reported missense mutation that results from an A to G transition at cDNA position 376 in exon 3 of the *GLA* gene, which leads to changing the amino acid serine in position 126 of the protein into glycine. This mutation has been reported to be pathogenic in stroke patients.[16,20] In a *GAL* genetic test report, Altarescu et al described the S126G mutation in a patient with neuropathic pain as the sole clinical manifestation and low enzymatic activity.[32] However,

in 2002, the same group reported this mutation in a 12-year-old patient with no renal syndrome and the lack of any enzymatic testing.[33] More recently, in an extensive review on *GLA* gene mutations, the S126G mutation was described as a benign mutation.[28]

The Belgian Fabry study included a total of 1000 patients with either a first stroke (TIA, ischemic stroke, or intracranial hemorrhage) or unexplained white matter lesions or basilar artery dolichoectasia. The authors identified missense mutations in eight nonrelated females (D313Y: 5 subjects, A143T: 2 subjects, S126G: 1 subject).[16] However, in a later, more in-depth analysis of the index patients and their relatives in which they evaluated 6 carriers (2 of them male), among those with the S126G mutation, all 6 were found to have had completely normal laboratory studies, including normal α-GAL A enzyme activity in plasma and normal plasma and urinary GB3 and Lyso GB3. None of the carriers had signs or symptoms of classic FD. The authors concluded they could not prove the pathogenic nature of the S126G mutation.[34] Rolfs et al recently reported the results of the SIFAP study, a multicenter observational study that included 5023 patients with stroke. Definitive FD was detected in .5% (n = 27). In 3 of these patients, Rolfs et al identified the S126G mutation and suggested that it was associated with stroke as the only manifestation of FD.[20] Nevertheless, a recent review on screening for FD concluded that none of the individuals identified as Fabry patients in the SIFAP study could be categorized because of insufficient data.[35] Moreover, the association between a given mutation and stroke in screening studies might be the result of selection bias.[35,36] Lukas et al reported about 60% residual in vitro activity of the S126G mutation and stated that the pathogenic value of this mutation remains unclear.[37] In keeping with the threshold theory, only decreased enzyme activity below the critical threshold value would result in storage of the corresponding lipid substrate. Decreased enzyme activity above the calculated threshold value should not influence the turnover rate of the substrate. This is because, above this threshold, there is no linear relationship between enzyme activity and turnover, and pathological storage occurs only below this level. With the exception of acid ceramidase, a decrease in enzyme activity of more than 20% relative to normal cells—a typical range for heterozygote carriers of inherited diseases—has no impact on the turnover rate.[38] Because our proband had no other manifestations of FD and her father was asymptomatic and had normal enzymatic values of α-GAL A, we concluded that S126G was not a pathogenic mutation in these two individuals.

The frequency of FD among patients with stroke is still a controversial issue. In addition to the SIFAP study, several screening evaluations of young patients with stroke, including over 3000 patients from around the world, have been published following the original German study.[10,20] Inclusion criteria varied between these studies, including young patients with stroke that were either ischemic or hemorrhagic or both, and the presence of hyperintense MRI lesions. Among these studies, 4 failed to identify any patients with *GAL* gene pathogenic mutations.[14,18,19,22] Among the remaining studies, 11 patients were identified with a D313Y mutation,[15,16] 6 had the R118C mutation,[15] 3 had the A143T mutation,[16,17] and 1 retrospective study identified a single patient with a combination of intronic splice variants.[21] Some of these same mutations were described as pathogenic in the SIFAP study.[20] Nevertheless, the D313Y mutation is currently not considered clinically relevant for FD.[28,29]

A pedigree analysis was performed in 6 patients with the R118C mutation. The investigators reviewed the clinical, biochemical, and histopathologic data obtained from 22 individuals of Portuguese and Spanish origin with this same mutation, and not only questioned the pathogenic significance of this variant but also recommended not treating these patients.[39] One patient with this mutation was identified in a recent screening study for Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy and FD in 900 patients younger than 70 years old with a lacunar stroke. No clinical data were available; consequently, the significance of this finding is unclear.[40]

The A143T mutation was considered pathogenic in some recently published studies.[17,20] However, no family data were provided, and no additional signs or symptoms of FD were reported. Conversely, an in-depth study of 12 patients with the A143T mutation, including either a heart or kidney biopsy in 3, revealed the lack of either classical signs of FD or Gb3 accumulation. The authors suggested the possibility of selection bias.[36]

Similarly, 4 members of nonrelated families with A143T were reported. The mother had left ventricular hypertrophy and stroke, but a cardiac biopsy was negative for FD.[41]

More recently, a multicenter Italian study evaluated 350 young patients with stroke but failed to identify pathogenic FD mutations,[42] whereas a single-center study also in Italy identified 3 patients with FD among 108 young stroke patients. All had recurrent stroke with an oligosymptomatic phenotype (p.Arg301Gly, p.Leu415Arg, p.Gly183Alafs17).[30] Like our patient, they lacked the classical FD phenotype but presented with recurrent strokes and a family history of stroke or either cardiac or kidney involvement. Similarly, a recent study in Turkey evaluated 54 patients with cryptogenic stroke. The α-GAL A activity was low in 3 patients. c.680G>A / p.R227Q missense mutation was identified in 2 male patients with recurrent strokes—1 presented with the classical form of FD and the other only presented with stroke. No mutation was identified in the third patient.[43]

The prevalence we identified in our study is similar to the low prevalence of FD reported by the SIFAP[20], the largest study on the subject and is within the range of

0% to .5% reported by most studies evaluating FD in young patients with strokes.[14,17-22]

Although the frequency of FD among young adults with stroke is low, the prevalence is higher when patients with cryptogenic cerebrovascular accident are evaluated. The late onset of symptoms and limited manifestations of FD make their identification difficult outside of screening studies. Moreover, once identified, these patients and their affected relatives might benefit from ERT. Recent studies suggest that ERT in FD patients may reduce the burden of cerebrovascular lesions.[44,45]

Conclusions

In our sample of 311 young adults presenting with a stroke or TIA, we identified FD in a single patient (.3% of the sample and 1% of the patients with cryptogenic ischemic strokes). Our study confirms that these patients can present with a late-onset oligo-symptomatic phenotype. Moreover, screening young patients with cryptogenic strokes allows identification not only of patients with FD but also of their affected relatives. *GLA* gene nonpathogenic mutations are commonly detected in stroke-screening studies and must not be mistaken for pathogenic mutations. We believe that the S126G mutation should be currently considered a nonpathogenic mutation and that these patients should therefore not be treated with ERT.

Appendix

Participating centers

Hospital Británico
Reisin Ricardo
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Miñarro Diego
Fernández Pardal Manuel
Belén Tillard
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De Francesco Laura
Tkachukt Verónica
Hospital Presidente Peron
Humanchio Janina
Fundación Favaloro
Riccio Patricia
Sposatto Luciano
Klein Francisco
Muñoz Francisco
Gonzalez Toledo Eugenia
FLENI
Ameriso Sebastián
Pujol Virginia
Povedano Guillermo
Hospital Posadas
Zinnerman Alberto
Martínez Alejandra
Conti Eugenia
Messina Juan
Hospital Roffo
Casas Parera Ignacio
Tafur José
Sanatorio Mitre
Gatto Emilia
Persi Gabriel
INAREPSMar del Plata
Bendersky Eduardo
Montero Lorena
Ateca Alicia
Hospital Zubizarreta
Allegri Ricardo
Bartoloni Leonardo
Hospital Tornú
Di Egidio Mariana
Ciardi Celina
Alleva Alejandro
CEMIC
Rivero Alberto
Romano Marina
Hospital Durand
Cairola Patricia
Academia Nacional de Medicina
Sanchez LucerosAnalía
Meschengesier Susana
Hospital de Vicente López
Halfon Javier
Schubaroff Pablo
Hospital Español
Calandra Cristian
Hospital San Martín
Pedersoli Luis Martín
Zuccolo Laura
Hospital Municipal de Campana
Reynoso Félix
Sanatorio Parque Tucumán
Nofal Pedro
Leri Mónica
CENAC Rosario
Bruera Guadalupe
Sanatorio Parque Rosario
Lorena Jure
Sanatorio Adventista del Plata
Sotelo Andrea

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