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Frequency of Unrecognized Fabry Disease Among Young European-American and African-American Men With First Ischemic Stroke

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Abstract

Background and Purpose—The cause of initial ischemic stroke in up to 30% of young patients remains unclear. Fabry disease, due to deficient α-galactosidase A (α-Gal A) activity, is a vascular endothelial glycosphingolipid storage disease typically presenting in childhood. With advancing age, patients develop renal, cardiac, and cerebrovascular disease and die prematurely. A European study suggested an increased prevalence of unrecognized Fabry disease in patients with cryptogenic stroke. We hypothesized that α-Gal A deficiency is a rare cause of initial early-onset ischemic stroke in men.

Methods—The Stroke Prevention in Young Men Study enrolled >550 men (15 to 49 years) with first ischemic stroke in the Baltimore–Washington area in 2004 to 2007. Frozen plasma samples were assayed for α-Gal A activity, and DNA from patients with consistently low plasma α-Gal A activities were sequenced.

Results—The study sample consisted of 558 men (42% African-American; median age 44 years). Stroke was cryptogenic in 154 men (40% African-American). In 10 patients with low plasma α-Gal A activities, DNA sequencing identified alterations in the α-Gal A gene in 2 patients. The polymorphism, D313Y, which results in low plasma enzyme activity, but near normal levels of cellular activity was seen in one European-American male. The Fabry disease-causing A143T mutation was seen in an African-American male with cryptogenic stroke (0.18% of all strokes: upper 95% CI=0.53%; 0.65% of cryptogenic strokes: upper 95% CI=1.92%).

Conclusions—In this biracial population, unrecognized Fabry disease is a rare but treatable cause of initial ischemic stroke in young men.

Keywords

brain infarction; genetic diseases; genetic screening; Fabry disease; stroke; X-linked

Fabry disease, due to deficient α-galactosidase A (α-Gal A) activity, is a vascular endothelial glycosphingolipid storage disease that has 2 major subtypes. The classic

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phenotype typically presents in affected males in childhood or early adolescence with angiokeratoma, acroparesthesias, hypohidrosis, gastrointestinal cramping and diarrhea, and corneal changes. With advancing age, affected males develop renal, cardiac, and cerebrovascular disease and die prematurely in the fourth or fifth decades of life.[1] Affected males with the later-onset phenotype do not have the early manifestations of the classic phenotype and present later in life with cardiac and/or renal disease.[2,3] In a series of >37 000 Italian newborn males, the incidence of mutations causing classic Fabry disease was approximately one in 37 000, whereas one in approximately 4600 males had mutations predicting the later-onset phenotype.[4] Furthermore, screening has identified previously unrecognized α-Gal A deficiency in approximately 0.20% to 1.0% of hemodialysis patients[5–8] and in 3% to 4% of patients with left ventricular hypertrophy or hypertrophic cardiomyopathy.[3,9–11] This suggests that many patients with the later-onset phenotype Fabry disease are not diagnosed.

Recently, a German study reported previously unrecognized α-Gal A deficiency in 4.9% of 432 young men with initial and recurrent cryptogenic ischemic strokes.[12] However, Brouns et al[13] in a smaller study of 64 Belgian men with cryptogenic strokes did not identify any patients with α-Gal A deficiency. Thus, the prevalence of unrecognized Fabry disease among young patients with ischemic strokes remains unclear, particularly among patients with first stroke or strokes attributed to other causes. We report our findings on the frequency of undiagnosed Fabry disease in young men presenting with an initial ischemic stroke in a multiracial American population.

Methods

Patient Population

The Stroke Prevention in Young Men Study is a population-based case–control study initiated to examine risk factors for ischemic stroke in young men. Study recruitment and data collection were conducted between 2003 and 2008. Cases were men, aged 15 to 49 years, hospitalized with a first cerebral infarction identified by discharge surveillance from one of 51 hospitals in the greater Baltimore–Washington area and direct referral from regional neurologists. The methods for discharge surveillance, chart abstraction, case adjudication, and assignment of probable and possible underlying causes have been described elsewhere.[14,15] Recruitment within 3 years of stroke was required for enrollment of cases. Control subjects were men free of a history of stroke identified by random-digit dialing and were frequency-matched to the cases by age and geographic region of residence.

α-Gal A Enzyme Screening and Mutation Analysis

Frozen plasma samples were assayed for α-Gal A activity16 with the addition of 117 mmol/L of α-N-acetylgalactosamine in the reaction mixture to inhibit α-N-acetylgalactosaminidase (α-Gal B) activity.17 Plasma samples with <30% of mean normal activity (15.6±6.2 nmol/hr/mL plasma, n=200 males) were reassayed. DNA from patients with consistently low plasma α-Gal A activities were sequenced as previously described to identify specific α-Gal A gene mutations and to confirm the diagnosis of Fabry disease.[18,19] The α-Gal A promoter (−1000 to ATG), all exons, and intron/exon boundaries were sequenced. This study was approved by the Institutional Review Boards of University of Maryland School of Medicine and Mount Sinai School of Medicine.

Results

The study sample consisted of 558 men (301 European-American, 235 African-American, 22 other ethnicities; median age 44 years). Stroke was cryptogenic in 28% (154 of 558) of

men (92 European-American; 58 African-American; 4 other ethnicities). The α-Gal A activities in the plasma samples ranged from 0.71 to 78.3 with a mean and median of 18.6 and 14.4 nmol/hr/mL, respectively. Based on a cutoff of 30% of the normal mean α-Gal A activity (<4.65 nmol/hr/mL), plasmas from 10 subjects were reassayed. These patients had initial enzyme activities from 0.71 to 4.62 nmol/hr/mL. The repeat assays ranged from 0.36 to 6.8 nmol/mL/hr (Table 1). Genomic DNA was isolated from their leukocytes, which had been stored frozen, and their α-Gal A genes were sequenced. Two patients had α-Gal A gene alterations (Table 1). In a European-American male, the common polymorphism, D313Y, was identified. The D313Y polymorphism results in low plasma enzyme activity,[20,21] but >60% of normal levels in leukocytes or when expressed in COS-1 cells[21] and is not associated with clinical disease. Sequencing identified the second alteration as a previously identified Fabry disease-causing mutation, A143T,[22] in an African-American male who had a cryptogenic stroke (0.18% of all strokes: upper 95% CI=0.53%; 0.65% of cryptogenic strokes: upper 95% CI=1.92%). This patient had not previously been diagnosed with Fabry disease.

Discussion

To date, there have been 2 published studies of the frequency of unrecognized Fabry disease among patients with cryptogenic strokes. Rolfs et al[12] reported that 4.9% of 432 males and 2.5% of 289 females with cryptogenic strokes had unrecognized Fabry disease. Subsequently, Brouns et al[13] reported that Fabry disease was not identified among 64 Belgian males with cryptogenic strokes. Contrary to the German report, our study indicates that Fabry disease is rare in young adults with first ischemic stroke of undetermined cause.

It is likely that the wide discrepancy in reported prevalence of Fabry disease between our study and the German study is due to differences in study populations (Table 2). The German report[12] suggested that unrecognized Fabry disease occurred in almost 5% of 18- to 55-year-old men with an otherwise undetermined stroke etiology. In that study, 10% of all cryptogenic stroke cases had multiple cerebrovascular events and 46.7% (10 of 21) of men with a cryptogenic stroke and a Fabry mutation had multiple cerebrovascular events. It is not clear if the German study included only patients with ischemic stroke or included a small percentage of patients with primary intracerebral hemorrhage.

In contrast, our study included only first ischemic stroke cases. When the German study is reanalyzed to include only first ischemic stroke cases, the prevalence of Fabry disease is lower. The German data can be disaggregated into a prevalence of 2.17% patients with Fabry disease among 367 men with first ischemic stroke and a prevalence of 24.3 among 41 men with recurrent ischemic stroke. This lower rate is substantially closer to the rate we report. In addition, the mutations associated with the German cases were not reported, so it is possible that some of the cases may have had the D313Y polymorphism with low plasma enzyme activity, which has an allele frequency of approximately 0.5% among European-Americans but with near normal cellular enzyme activity.[20,21]

Our study is the first to look at unselected patients with first ischemic stroke. We had hypothesized that the metabolic defect of Fabry disease could be synergistic with other risk factors. In addition, Fabry disease may be associated with cardiomyopathy and the stroke would have been classified as cardioembolic rather than cryptogenic. However, there were no cases of Fabry disease in strokes of known etiology in our study.

The diagnosis of Fabry disease in young patients with stroke is important for several reasons. Although it is not known whether enzyme replacement therapy will prevent ischemic stroke recurrence, it is known to delay or prevent other manifestations of Fabry

disease, particularly renal and cardiac complications.[23,24] Equally important are the potential benefits for family members. Institution of enzymatic replacement therapy in presymptomatic individuals could potentially eliminate the manifestations of Fabry disease.

The results of our study suggest that the yield for screening is lower for first compared with recurrent cryptogenic ischemic stroke. Although our study did not identify any particular features that would enhance the yield of screening, it remains prudent to consider screening patients with clinical features suggestive of Fabry disease. Because Fabry disease is an X-linked disease, family history should be obtained regarding the (1) mother's brothers; (2) patient's brothers; (3) patient's sons; and (4) patient's sister's male children. Female carriers can also manifest disease symptoms.[25,26] The occurrence of early or idiopathic end-stage renal disease, proteinuria, cardiac disease, ischemic stroke, hypohidrosis, acroparesthesias, and/or angiokeratomas should suggest the diagnosis of Fabry disease. Genetic testing should be undertaken for the patient and at-risk family members. Affected individuals should be referred for further evaluation and therapeutic intervention.

Conclusion

Our study suggests the yield of screening young men with an initial ischemic stroke regardless of etiology for Fabry disease is low regardless of etiology. The yield of screening in recurrent cryptogenic ischemic stroke in young adults remains unclear. There is a need for a large sample size replication of the findings of the German study, which suggested a prevalence of 24.3% for unrecognized Fabry disease among men with recurrent cryptogenic stroke. Because Fabry disease is a treatable condition and the diagnosis has implications for other family members, the decision to screen for Fabry disease should be made on an individual basis.

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Table 1

The Summary of Mutation Analysis in Patients With Consistently Low α-Gal A Activity

Male Patients With Stroke	α-Gal A Activity†		Mutation Analysis
	Initial Assay‡	Confirmatory Assay‡	
1	2.08	1.00	A143T
2	4.62	6.80	WT*
3	2.84	3.09	WT
4	2.03	2.23	WT
5	0.71	0.36	D313Y
6	2.94	3.20	WT
7	4.06	2.69	WT
8	1.01	1.66	WT
9	3.04	1.67	WT
10	3.75	3.50	WT

*Wild type.

†nmol/hr/mL protein; normal mean±SD: 15.6 nmol/hr/mL protein; normal mean=same for all patients.

‡All values are means of duplicate enzyme assays.

Table 2

Prevalence of Fabry Disease in Young Men With Cryptogenic Ischemic Stroke

	First Ischemic Stroke			Recurrent Ischemic Stroke		
	Total No.	No. With Fabry Disease	Prevalence	Total No.	No. With Fabry Disease	Prevalence
Rolfs *	367	8	2.17	41	10	24.3
Brouns †	46	0	0	9	0	0
Wozniak	154	1	0.65	N/A	N/A	

* Estimated from reference 12 using the following 4 assumptions: (1) total of 432 men with any stroke, assuming the same rate of hemorrhage as entire sample (5.5%), then 408 men with any ischemic stroke; (2) assuming the same rate of recurrent stroke as in the entire sample (10%), then 367 men had an initial ischemic stroke; (3) 10 men with Fabry disease had recurrent stroke and 11 with initial stroke; (4) given the high mortality rate of hemorrhage, assuming that 3 men with Fabry disease and hemorrhagic stroke were all initial strokes, then 8 men with Fabry disease had initial ischemic stroke.

† Dr R. Brouns[13] and personal communication (Dr R. Brouns, 2009).