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Fabry disease screening in young patients with acute ischemic stroke in Korea

Yunjung Choi[1], Taedong Ok[1,2], Kyung-Yul Lee[1,3]

[1]*Department of Neurology, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Korea*
[2]*Department of Neurology, National Health Insurance Service Ilsan Hospital, Goyang, Korea*
[3]*Severance Institute for Vascular and Metabolic Research, Yonsei University College of Medicine, Seoul, Korea*

Background: Fabry disease is an X-linked lysosomal storage disorder that results from a mutation in the α-galactosidase A (*GLA*) gene. It shows multiple organ involvement, including cerebrovascular disease. Since Fabry disease has a prevalence of approximately 4% in young patients with cryptogenic stroke, screening for this condition is recommended for young stroke patients. This study aimed to investigate the prevalence of Fabry disease in young acute ischemic stroke patients in Korea, the distribution of *GLA* gene mutations, and the subtypes of ischemic stroke.

Methods: This study included 211 young patients with acute ischemic stroke or transient ischemic attack. To screen for Fabry disease, α-galactosidase A (α-Gal A) enzyme activity was measured and DNA sequencing analysis of the *GLA* gene was performed.

Results: None of the patients exhibited low α-Gal A enzyme activity or had a pathogenic *GLA* mutation, but 18 nonpathogenic *GLA* gene variants were detected, including c.-10C>T in 16 patients, c.-33C>T in one patient, and c.196G>C in one patient. The mean α-Gal A enzyme activity in 14 male patients with the c.-10C>T variant was 5.17±1.19, which was significantly lower than that of male patients with the normal genotype (7.47±3.48, P<0.05). The distribution of stroke subtypes in patients with *GLA* gene polymorphisms was not significantly different from that in patients with a normal genotype.

Conclusions: This study demonstrates that Fabry disease is rare in young patients with ischemic stroke or transient ischemic attack in Korea, and we suggest that routine screening for Fabry disease may not be necessary for ischemic stroke patients.

Keywords: alpha-Galactosidase; Fabry disease; Ischemic stroke

INTRODUCTION

Fabry disease is an X-linked lysosomal storage disorder that results from mutations in the α-galactosidase A (*GLA*) gene, leading to a deficiency of the enzyme α-galactosidase A (α-Gal A). A deficiency in α-Gal A results in intralysosomal accumulation of globotriaosylceramide (Gb3) in various types of cells, leading to structural damage caused by cellular dysfunction [1,2]. These changes initially affect the microvasculature, but as the disease worsens, medium-sized to large arteries undergo arterial remodeling and intima-media thickening. Multiple organs, including the heart, kidney, and cerebrovascular system, are affected, resulting in multiorgan dysfunction [1]. The pathophysiological mechanisms are not fully understood, although they may be explained by vasculopathy, which involves defects in the blood vessel wall, altered blood components, and altered blood flow. This vasculopathy can induce cerebrovas-

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Correspondence to Kyung-Yul Lee, MD
Department of Neurology, Gangnam Severance Hospital, Yonsei University College of Medicine, 211 Eonju-ro, Gangnam-gu, Seoul 06273, Korea.
Email: kylee@yuhs.ac

cular complications, which are leading causes of morbidity and early mortality in Fabry disease patients [1]. Cerebral infarction and transient ischemic attack (TIA) are the most common neurological manifestations of Fabry disease [3].

There are around 600 variants in the *GLA* gene, which codes for α-Gal A, but a clear genotype-phenotype correlation is not always observed [1]. A diagnosis of Fabry disease can be made by screening for low α-Gal A activity, with residual enzyme activity of less than 1% suggesting classical Fabry disease and less than 30% to 35% suggesting late-onset Fabry disease [4]. Dried blood spot (DBS) samples are often utilized to analyze α-Gal A activity. In males, the diagnosis can be confirmed by undetectable or severely decreased α-Gal A activity in DBS, plasma, and leukocytes, but female heterozygotes require genetic testing, as they may have normal activity due to random X-chromosome inactivation. Therefore, mutational analysis of the *GLA* gene is required for the diagnosis in females.

An early diagnosis is important because Fabry disease can currently be treated with enzyme replacement therapy. Therefore, screening for Fabry disease is recommended in high-risk groups, such as patients with unexplained left ventricular hypertrophy or hypertrophic cardiomyopathy; patients with unexplained renal failure, proteinuria, or microalbuminuria; and young patients with unexplained stroke [1,5,6]. According to a German study, the prevalence of Fabry disease in a cohort of patients aged 18 to 55 years with cryptogenic stroke was approximately 4% (28 of 721) [3]. Since around 27% of ischemic strokes are thought to be cryptogenic, this percentage may correspond to approximately 1% to 2% of young stroke patients. Therefore, it was strongly suggested that clinicians should consider Fabry disease in young patients with cryptogenic stroke. In a subsequent cohort study of young patients with acute cerebrovascular disease, including ischemic stroke, TIA, and hemorrhagic stroke between the ages of 18 to 55 years from 15 European countries, 0.5% (27 of 5,023) were confirmed to have definite Fabry disease, and 0.4% (18 of 5,023) were suspected of having probable Fabry disease [7]. In contrast to Western countries, a Japanese study reported only one patient (0.17%) with Fabry disease among 588 patients with ischemic or hemorrhage stroke [8], and Fabry disease was not detected in 357 young Chinese patients with ischemic stroke [9]. These findings indicate that the prevalence of Fabry disease in Asian populations with ischemic stroke may be lower than in Western countries. However, the prevalence of Fabry disease in ischemic stroke patients in Korea has not yet been reported.

In addition, previous studies analyzing the etiologies of ischemic stroke in Fabry disease patients according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification have found the highest frequency for undetermined causes, followed in descending order by cardioembolic, other determined causes, and small vessel occlusion [10,11]. Some studies have also suggested that specific *GLA* gene variants might be associated with an increased risk of certain stroke subtypes in Fabry disease patients [8,9].

Our study investigated the prevalence of Fabry disease in young ischemic stroke patients in Korea and the distribution of *GLA* mutations. We also aimed to investigate the α-Gal A activity levels according to *GLA* mutations and the correlation between stroke subtypes and *GLA* mutations.

METHODS

Ethics statements

This study was approved by the Institutional Review Board of Severance Hospital, Yonsei University Health System (No. 3-2022-0091). The requirement for written informed consent was waived due to the retrospective nature of the study.

Subjects

We collected clinical data from young ischemic stroke patients admitted to the neurology department between February 2017 and December 2022. The inclusion criteria were patients between 18 and 55 years of age, patients diagnosed with ischemic stroke including TIA, and patients who consented to the Fabry disease screening test.

We selected 211 patients who fulfilled the inclusion criteria. Information on baseline characteristics was collected, including age, sex, stroke type (ischemic stroke or TIA), history of previous stroke or TIA, comorbidities (hypertension, diabetes mellitus, hyperlipidemia, smoking, atrial fibrillation, coronary heart disease, and peripheral arterial occlusive disease), medication (antithrombotics, anticoagulants, and statins), and stroke subtype. The TOAST classification was used to classify the ischemic stroke subtype [12].

Screening for Fabry disease

To screen for Fabry disease, α-Gal A activity was measured using DBS samples and liquid chromatography-tandem mass spectrometry. Plasma α-Gal A activity ≤2.35 μmol/hr/L was defined as abnormally low and used as the cutoff for diagnosing Fabry disease. Genetic testing for Fabry disease was performed by DNA analysis of the *GLA* gene (chromosomal locus, Xq22). Confirmation tests were performed by polymerase chain reaction and sequencing using genomic DNA isolated from EDTA-treated whole blood.

The diagnosis of Fabry disease was defined as abnormally low α-Gal A activity for male patients and a confirmed pathogenic *GLA* gene mutation for female patients. Mutations were classified into five subtypes according to the 2015 American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) guidelines [13] for the interpretation of sequence variants., which suggested using the terms "pathogenic," "likely pathogenic," "uncertain significance," "likely benign," and "benign" to describe variants identified in Mendelian disorders.

Statistical analysis

Continuous variables are presented as means with standard deviations, and categorical variables are presented as frequencies and percentages. The two-tailed t-test was used for continuous variables, and the Fisher exact test was used for categorical variables. The Mann-Whitney test was used as a nonparametric method. A P-value <0.05 was considered statistically significant. All analyses were performed using IBM SPSS ver. 25 (IBM Corp).

RESULTS

The baseline characteristics of the 211 patients who met the inclusion criteria are shown in Table 1. The mean age of the participants was 45.15±7.47 years and 170 (80.6%) were male. Of the patients, 191 presented with ischemic stroke and 20 patients with TIA. None of the patients had a pathogenic *GLA* mutation and abnormal α-Gal A activity.

Table 2 shows α-Gal A activity according to the *GLA* genotype and sex in 200 patients, excluding 11 patients who did not undergo *GLA* genetic testing. A total of 18 nonpathogenic *GLA* gene variants were detected, including c.-10C>T in 16 patients (7.58%), c.33C>T in one patient (0.47%), and c.196G>C in one patient (0.47%). None of the patients with *GLA* gene variants had α-Gal A levels below the cutoff value, but the mean enzyme activity in male patients with the c.-10C>T polymorphism was significantly lower than that of male patients with the normal genotype (5.17±1.19 vs. 7.47±3.48, P=0.002).

We also investigated the association between TOAST stroke subtype and *GLA* gene polymorphisms (Table 3). The frequency of stroke subtype according to the *GLA* gene polymorphism was not statistically significant (P=0.441). Because the number of patients with the c.-10C>T polymorphism was relatively higher than that of patients with other polymorphisms, they were compared to the wild type. However, there was no statistically significant difference in

Table 1. Baseline characteristics of 211 young acute ischemic stroke patients

Characteristic	Value
Age (yr)	45.15±7.47
Male sex	170 (80.6)
Stroke type	
Ischemic stroke	191 (91.5)
TIA	20 (9.5)
History of previous stroke	9 (4.3)
History of previous TIA	7 (3.3)
Comorbidities	
Hypertension	101 (47.9)
Diabetes mellitus	45 (21.3)
Hyperlipidemia	73 (34.6)
Smoking (any)	136 (64.5)
Atrial fibrillation	4 (1.9)
Coronary heart disease	3 (1.4)
PAOD	4 (1.9)
Medication	
Antiplatelet	13 (6.2)
Anticoagulant	7 (3.3)
Statin	24 (11.4)
TOAST classification	
Large artery atherosclerosis	30 (14.2)
Small vessel occlusion	49 (23.2)
Cardioembolism	47 (22.3)
Stroke of other cause	39 (18.5)
Stroke of undetermined causes	46 (21.8)

Values are presented as mean±standard deviation or number (%).
TIA, transient ischemic attack; PAOD, peripheral arterial occlusive disease; TOAST, Trial of Org 10172 in Acute Stroke Treatment.

the frequency of stroke subtypes between patients with the c.-10C>T polymorphism and those with the wild-type genotype (P=0.601).

DISCUSSION

Classical or late-onset Fabry disease based on α-Gal A activity was not found in young ischemic stroke patients in Korea. Contrary to studies from Western countries, Fabry disease has rarely been reported in Asian ischemic stroke patients. The possibility of ethnic differences can be considered first. Additionally, the results of Western studies may have been different because those studies included patients with hemorrhagic stroke and other cerebrovascular diseases. In a recent nationwide study in the Czech Republic, out of 986 stroke patients (including those with ischemic and hemorrhagic stroke), only two patients (0.2%) with ischemic stroke were identified as having pathogenic *GLA* gene variants, and both were under 50 years of age [14]. This suggests that the prevalence of Fabry disease may not be as high as reported in previously published studies.

Over 600 different mutations have been identified in the *GLA* gene. The c.644A>G (p.N215S) variant is the most prevalent variant associated with late-onset cardiac Fabry disease in European populations [15]. In a screening of Taiwanese neonates, the c.936+919G>A (IVS4+919G>A) variant, which is associated with the late-onset cardiac type of Fabry disease, had a high prevalence of approximately 1 in 1,250 [16]. In 117 Japanese Fabry disease patients, the most common pathogenic mutations were c.888G>A (p.M296I), c.335G>A (p.R112H), and c.936+919G>A, which are found in the late-onset form; c.679C>T (p.R227X) and c.334C>T (p.R112C), which are found in the classic form; and c.902G>A (p.R301Q), which is detected in both the classic and late-onset forms [17]. Other studies have suggested that the S126G and A143T variants may be related to a stroke-only phenotype of Fabry disease [7].

Although we did not find any pathogenic *GLA* mutations, 18 patients had three nonpathogenic *GLA* gene variants. According to the ACMG guidelines, the c.-10C>T and c.33C>T variants were reported to be benign. Both variants are known as single-nucleotide polymorphisms (SNPs) located in the 5' untranslated region of exon 1 in the *GLA* gene. The c.196G>C (p.E66Q) polymorphism is in the coding region; it is a missense mutation in which a

Table 2. Level of α-galactosidase activity according to *GLA* gene polymorphisms

GLA gene polymorphism	No. of patients	α-galactosidase activity (μmol/hr/L)
Wild type	182 (91.0)	7.33±3.51
Male	145	7.47±3.48
Female	37	7.29±3.53
c.-10C>T	16 (8.0)	5.46±1.43
Male	14	5.17±1.19
Female	2	7.53±1.60
c.33C>T	1 (0.5)	9.19
Male	1	9.19
Female	0	-
c.196G>C	1 (0.5)	3.16
Male	0	-
Female	1	3.16
Total	200 (100)	7.07±3.39

Values are presented as number (%), mean±standard deviation, or number only. Eleven patients who did not undergo *GLA* genetic testing were excluded.
GLA, α-galactosidase A.

Table 3. Frequency of stroke subtypes according to *GLA* gene polymorphisms

GLA gene polymorphism	Stroke subtype (TOAST)					P-value
	LAA	SVO	Cardioembolism	Other	Undetermined	
Normal (n=182)	25 (13.7)	42 (23.1)	41 (22.5)	34 (18.7)	40 (22.0)	0.441
c.-10C>T (n=16)	1 (6.3)	5 (31.3)	4 (25.0)	1 (6.3)	5 (31.3)	
c.33C>T (n=1)	1 (100)	0	0	0	0	
c.196G>C (n=1)	0	0	0	1 (100)	0	
Total (n=200)	27 (13.5)	47 (23.5)	45 (22.5)	36 (18.0)	45 (22.5)	

Values are presented as number (%).
GLA, α-galactosidase A; TOAST, Trial of Org 10172 in Acute Stroke Treatment; LAA, large artery atherosclerosis; SVO, small vessel occlusion.

glutamate residue is substituted with glutamine at codon 66 due to a single base sequence change on exon 2. Various studies have inconsistently described this as a likely benign or uncertain significance, and conflicting interpretations exist regarding its pathogenicity. However, previous case reports revealed this mutation in cardiac and renal variants of Fabry disease in patients with hypertrophic cardiomyopathy or hemodialysis, respectively, suggesting that it may be a pathogenic mutation [18,19]. Female patients with the c.196G>C variant were previously reported to have a family history of various clinical features, ranging from asymptomatic large artery atherosclerosis to acute ischemic stroke [20]. This variant also showed multiple small vessel occlusions and a high incidence of intracerebral hemorrhage in elderly male patients [21].

Togawa et al. [22] found lower α-Gal A activity in the plasma of men with the c.196G>C variant due to decreased stability of the enzyme affected by the mutation (E66Q at the protein level), but comparatively high residual enzyme activity was observed in white blood cells, and there was no accumulation of Gb3 in fibroblasts and no histological evidence of Fabry disease in biopsied skin tissues. Five patients with this variant were also found in a cohort of 28 Korean families with Fabry disease, but the clinical characteristics of these patients were insufficient for the diagnosis of Fabry disease. When Fabry disease screening was performed in Korean and Japanese populations, this mutation was observed at a relatively high frequency (0.5%–1.0%) compared to other mutations [22,23]. Overall, this mutation is considered to be a functional polymorphism rather than a pathogenic mutation. Therefore, the evidence for an association between the c.196G>C variant and Fabry disease is still unclear and requires further study [24].

In our study, the α-Gal A levels were within the normal range in all patients, but the mean enzyme activity of male patients with the c.-10C>T variant was significantly lower than that of male patients with the normal genotype. In male patients with cryptogenic stroke, some intronic haplotypes in α-Gal A have been associated with decreased *GLA* expression [25]. In particular, previous studies have reported that patients with the c.-10C>T variant showed decreased *GLA* gene expression and relatively low α-Gal A activity [4]. In a *GLA* SNP screening study conducted on 100 healthy Portuguese individuals reported by Oliveira et al. [26], the mean α-Gal A activity was significantly lower in carriers of the c.-10C>T variant, at approximately 25%. Additionally, among Brazilian male patients with suspected Fabry disease or at high risk for Fabry disease, the mean enzyme activity was reduced by approximately 21% in patients with this variant [27].

A report showed that the frequency of stroke due to other causes and undetermined causes according to the TOAST classification was higher in patients with the c.-10C>T variant [9]. However, we found no correlation between *GLA* mutations and the TOAST classification in this study. We speculate that the small number of patients with *GLA* mutations was insufficient to yield statistical significance.

There are several limitations to our study. First, we acknowledge that our results may have been limited by the small sample size. Second, our study lacks information on the history of other organ involvement in Fabry disease. Despite the high prevalence of renal involvement in Fabry disease, chronic kidney disease was not included in the list of comorbidities. It would have also been helpful to include the typical features of Fabry disease, such as cutaneous or ophthalmic symptoms and neuropathic pain. Third, this study did not include other biomarkers such as globotriaosylsphingosine (lyso-GL-3). Plasma lyso-GL-3 is known to be involved in the cerebrovascular system and has high levels in patients with Fabry disease. However, it is only correlated with disease severity in female patients and may be normal in heterozygous females [1]. However, when combined with α-Gal A activity, analyzing lyso-GL-3 levels improves the detection rate compared to testing enzyme activity alone, and this method can be used to screen for Fabry disease in females [28]. Since lyso-GL-3 has recently emerged as a valuable pathologic biomarker, it would be helpful to include it in future investigations.

Screening for Fabry disease has been recommended for young patients with ischemic stroke because it accounts for about 1% to 2% of cryptogenic stroke causes. Our study found that the prevalence of Fabry disease in young ischemic stroke patients in Korea was lower than has been reported in Western countries. In conclusion, Fabry disease in young ischemic stroke patients is rare in Korea and routine screening tests may not be necessary. The clinical significance of nonpathogenic variants, including c.-10C>T and c.196G>C, needs to be established by additional research with large numbers of patients.

ARTICLE INFORMATION

Ethics statements

This study was approved by the Institutional Review Board of Severance Hospital, Yonsei University Health System (No. 3-2022-0091). The requirement for written informed consent was waived due to the retrospective nature of the study.

Conflicts of interest

The authors have no conflicts of interest to declare.

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Author contributions

Conceptualization: all authors; Data curation: all authors; Formal analysis: YC; Investigation: YC, TO; Methodology: all authors; Project administration: all authors; Software: YC; Supervision: KYL; Validation: YC, KYL; Visualization: YC; Writing–original draft: YC, KYL; Writing–review & editing: all authors. All authors read and approved the final manuscript.

ORCID

Yunjung Choi, https://orcid.org/0000-0003-1323-7421
Taedong Ok, https://orcid.org/0000-0003-2901-5778
Kyung-Yul Lee, https://orcid.org/0000-0001-5585-7739

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