SHORT COMMUNICATION

Genomic screening of Fabry disease in young stroke patients: the Taiwan experience and a review of the literature

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Background and purpose: Fabry disease is an X-linked disease, and enzyme-based screening methods are not suitable for female patients.

Methods: In total, 1000 young stroke patients (18–55 years, 661 with ischaemic stroke and 339 with hypertensive intracerebral hemorrhage) were recruited. The Sequenom iPLEX assay was used to detect 26 Fabry related mutation genes. The frequency of Fabry disease in young stroke was reviewed and compared between Asian and non-Asian countries.

Results: Two male patients with ischaemic stroke were found to have a genetic mutation of IVS4+919G>A. There was no α-galactosidase A (*GLA*) gene mutation in female patients. The frequency in Asian stroke patients was 0.62% (male vs. female 0.63% vs. 0.58%) with 0.72% for ischaemic stroke and none for hemorrhagic stroke, compared to 0.88% (0.77% vs. 1.08%) with 0.83% for ischaemic stroke and 1.40% for hemorrhagic stroke reported in western countries.

Conclusion: IVS4+919G>A is the *GLA* mutation in Taiwanese young ischaemic stroke patients. Fabry disease is more frequent among non-Asian patients compared to Asian patients.

Introduction

Fabry disease, a panethnic X-linked lysosomal disease, is characterized by accumulation of glycosphingolipids, mainly globotriaosylceramide and its deacylated form globotriaosylsphingosine (Lyso-Gb3), in many types of cells [1,2]. Although enzyme-based screening has been implemented for Fabry disease, it may fail to detect up to one-third of heterozygous females [2]. Recently, genetic screening has been developed for the high-risk population in both male and female patients [3]. A Sequenom iPLEX assay has been designed and validated to identify α-galactosidase A (*GLA*) gene mutations and variants with 100% accuracy and sensitivity in Taiwan [4].

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The aim of the present study was to apply the Sequenom iPLEX assay to screen 26 hotspot mutations and variants of *GLA*, which are involved in Taiwanese Fabry disease, in a cohort of young stroke patients.

Methods and materials

The Stroke Registry in Chang Gung Healthcare System in Taiwan [5] has established a blood sample bank collecting important DNA. The study protocol was in compliance with the Helsinki Declaration and approved by the institutional review board of Linkou Chang Gung Memorial Hospital.

Young stroke patients were aged 18–55 years and included 488 males and 173 females with ischaemic stroke [17 males and four females with transient ischaemic attack (TIA)] and 272 males and 67 females with hypertensive intracerebral hemorrhage (HICH).

EUROPEAN JOURNAL OF NEUROLOGY

Table 1 The frequency of Fabry disease in young stroke screening in Asian and non-Asian countries

Country	Age	Stroke subtype	Number of patients	% mutation in gender	% mutation in stroke subtype
Asia	18–91	IS (CI + TIA) 2081, HS 339	2420 (1902 males + 518 females)	Total 0.62% [12 males (0.63%), and three females (0.58%)]	IS (CI + TIA) 15 (0.72%), HS 0%
Non-Asian	15–70	IS (CI + TIA) 9431 including cryptogenic IS (CI + TIA) 1586, HS 571, others 403	10 405 (6512 males + 3893 females)	Total 0.88% [50 males (0.77%), 42 females (1.08%)]	IS (CI + TIA) 78 (0.83%), cryptogenic IS (CI + TIA) 36 (2.27%), HS 8 (1.40%)

CI, cerebral infarction; HS, hemorrhagic stroke; IS, ischaemic stroke; TIA, transient ischaemic attack.

Informed consent for the genetic analysis was obtained from all recruited patients.

Ischaemic stroke subtypes were classified according to our previous study [6]. Hypertension-related HICH was considered when the hematoma was located in the basal ganglion (putamen, thalamus), cerebellum and pons. The definitions of vascular risk factors and clinical survey for young stroke were mentioned in our previous study [6].

The 26 Fabry disease *GLA* mutations and their protein nomenclature are listed in Table S1. These mutations/variants included 21 *GLA* mutation single-nucleotide polymorphisms reported in Taiwan [4] and five *GLA* benign variants previously detected in patients with stroke [4]. The protocol of MassARRAY-based mutation genotyping using the assay Designer v.4.0 software (Agena Bioscience, San Diego, CA, USA) is mentioned after Table S1. A leukocyte *GLA* activity assay was determined according to the previous method [1]. The diagnosis of Fabry disease was based on a leukocyte *GLA* activity <4.76 nmol/mg protein/h.

Results

The clinical features of the young stroke patients are presented in Table S2. Two males with stroke onset at 49 and 50 years, respectively, were detected. Both had an intronic *GLA* mutation of IVS4+919G>A, and their *GLA* enzyme activity was 3.77 and 7.15 nmol/mg protein/h, respectively.

No Fabry disease was found in patients with TIA or HICH, and also none in female patients. The frequency of Fabry disease in our cohort was 0.2% for all stroke patients with 0.3% for ischaemic stroke and 0% for HICH.

Discussion

The present study used a Sequenom iPLEX assay as the first-line determination of *GLA* mutation, further confirmed with plasma *GLA* enzyme activity assay. The Sequenom iPLEX assay with nation-specific hot-spot mutation genes is suggested as a cost-effective screening method for large series.

In the review of stroke screening (Tables 1 and S3), the overall frequency was 0.62% in 2420 Asian strokes with 0.63% in male subjects, 0.58% in female subjects, 0.72% in 2081 ischaemic strokes and 0% in 339 hemorrhagic strokes. The overall frequency was 0.88% in 10 405 non-Asian strokes with 0.77% in males and 1.08% in females. There were 9431 ischaemic strokes with 1586 cryptogenic strokes, 571 hemorrhagic strokes and 403 others. The frequency of Fabry disease was 0.83% in ischaemic stroke with 2.27% in cryptogenic stroke and 1.40% in hemorrhagic stroke.

The incidence of late-onset IVS4+919G>A mutation is reported as 1:1600 in Taiwanese newborns [7]. Patients carrying IVS4+919G>A and classical Fabry mutations could be distinguished from healthy controls by an elevated plasma lyso-Gb3 [8]. Although similar magnetic resonance imaging findings for both classical Fabry disease and the subtype with IVS4+919G>A mutation [9], patients with IVS4+919G>A have a higher frequency of deep white matter hyperintensities and a higher incidence of infarctions and pulvinar signs than healthy controls [7]. Lyso-Gb3 also correlated with age and left ventricular mass index in Fabry patients with IVS4+919G>A mutation [8].

Our study has two limitations: (i) the Sequenom iPLEX assay cannot cover the full range of Fabry mutations since Fabry disease has more than 800 mutations affecting both males and females; (ii) measurement of lyso-Gb3, a plasmatic biomarker of Fabry disease, was not performed.

Conclusion

IVS4+919G>A mutation gene can be seen only in Taiwanese newborn and young ischaemic stroke patients and is rarely detected in other Asian and non-Asian countries. There is less gender difference in

Caucasians, but few Fabry disease occurrences are found in female Asians.

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Disclosure of conflict of interest

T.H.L. and A.P.B. have received honoraria for presentations and board meetings from Sanofi Genzyme; A.P.B. is a member of the European Advisory Board of the Fabry Registry, which is sponsored by Sanofi Genzyme. The other authors declare no conflict of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. α-galactosidase A (*GLA*) gene mutations and the protein nomenclature.

Table S2. Baseline demographics and clinical features of young stroke patients.

Table S3. The frequency of Fabry disease in young stroke screening in different countries.

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