

The spectrum of neurological manifestations of Fabry disease in a large Turkish family with *c.[680G>A] p.[R227Q]* mutation

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Abstract

Background & Objectives: Fabry disease (FD) is a rare lysosomal storage disease with X-linked recessive inheritance caused by a mutation in the α -galactosidase A gene (*GLA*) (X14448.1) on chromosome X, leading to α -galactosidase (α -Gal A) (EC: 3.2.1.22) enzyme deficiency. In this report, we present the genetic mutations, clinical features and the neurological involvement of a large Turkish family with FD diagnosed in our clinic during the etiological investigation of a young index patient with recurrent ischemic stroke episodes. **Methods:** We evaluated 20 members (9 male, 11 female) of a large Turkish family including the index patient. All of them were investigated with a detailed medical history, systemic and neurological examination. Enzyme activity of α -Gal A and *GLA* gene mutation were tested using dried blood spot (DBS) method. The normative value of α -Gal A enzyme activity was above the cut-off value of 1.2 μ mol/h. **Results:** The α -Gal A activity was low in 13 cases (5 male, 27.7%). A total of 13 patients (4 male patients out of 5 males with low enzyme activity and 9 female patients) were found to have a *c.[680G>A] p.[R227Q]* missense mutation in the *GLA* gene. Ischemic stroke, renal disorder, cardiomyopathy, neuropathic pain, acroparesthesia, hearing loss, ocular involvement, angiokeratoma, hypohydrosis and hyperhydrosis were the clinical manifestations of FD in the affected family members.

Conclusion: The clinical and genetic features of this large Turkish family with FD support an association between the neurological phenotype and the *c.[680G>A] p.[R227Q]* mutation. Since FD is treatable, it is recommended to perform enzymatic and genetic studies among family members.

Key words: Fabry Disease, *GLA* mutation, family screening, pedigree

INTRODUCTION

Fabry's disease (FD) is a rare hereditary lysosomal storage disease with X-linked recessive inheritance. Mutation of the α -galactosidase A gene (*GLA*) (X14448.1) causes altered α -galactosidase (α -Gal A) (EC: 3.2.1.22) activity leading the lysosomal accumulation of neutral glycosphingolipids inside the various cell types.¹ Localization of glycosphingolipid depositions determines the clinical features of the FD. Deposits could be accumulated in endothelial cells, smooth muscle cells of the blood vessels, renal epithelial cells, pericytes, myocardial cells, neurons of the spinal cord and dorsal root ganglia.^{1,2} Therefore, multiple organs get damaged concurrently in FD patients. Renal, cardiac and cerebrovascular disorders generally occur with the progression of the disease.¹

In accordance to the inheritance pattern of

the disease, the males with the hemizygous *GLA* mutations developed the FD. The heterozygous females can also show the clinical symptoms of the disease from mild to severe.³ Up to date, approximately 2000 *GLA* gene mutations have been reported.⁴ In general, the same genetic mutation is shared among the members of the families.⁵

In this report, we present the genetic mutations, clinical features and the neurological involvement of a large Turkish family with FD diagnosed in our clinic during the investigation of a young patient with recurrent ischemic stroke.

METHODS

Patients and clinical investigation

Twenty participants including the index patient and 19 members of his large family were enrolled

in this study. In each individual, medical history, physical examination, α -Gal A enzyme activity, and *GLA* mutation analysis were performed. Those with a mutation and having relevant symptoms, were further investigated by a detailed systemic and neurological examination, ophthalmological and audiological evaluations, electrocardiography (EKG), echocardiogram (ECHO), electroneuromyography (ENMG), Electroencephalogram (EEG), magnetic resonance imaging (MRI), magnetic resonance angiography (MRA), carotid ultrasonography, urinalysis, renal ultrasonography and blood analysis.

Gal A activity analysis

Measurement of enzyme activity in dried blood spot samples was performed via DBS method using filter paper containing DBS as a source of DNA.⁶ Four drops of the blood were transferred to a filter paper, allowed to dry at room temperature, and kept at 2–4 °C until analysis. Patients with values <1.2 $\mu\text{mol/l/h}$ were considered to have low α -Gal A activity.

GLA mutation on genetic analysis

The genetic screening of the *GLA* mutation was performed in patients with low α -Gal A activity by using the DBS cards. However, the genetic study was performed in women even if the enzyme activity was normal. DNA analysis of the *GLA* gene was used to confirm the diagnosis of FD. The gene encoding *GLA* is found on *Xq22.1* (chrX:101,397,803-101,407,925 10,123 bp) and spans 13kb of genomic DNA (7 exons, cDNA of 1290 bases). The *GLA* gene encodes a 429 amino acid protein, of which the first 31 residues form a lysosomal signal peptide. The coding sequences and flanking intronic sequences (minimum of 20 base pairs) of exons 1-7 of the *GLA* gene are amplified from purified genomic DNA and sequenced in the forward and reverse directions. Sequencing of a single exon is available for targeted mutation analysis. Patient sequences are compared to the reference DNA sequence.⁷

A written informed consent was obtained from the patients for publication of this report and any accompanying images.

Statistical analysis

Statistical analysis of our study was performed by using statistical package for social sciences (SPSS) program (18.0 windows; SPSS Inc., Chicago, IL, USA). Descriptive statistics were

calculated for all continuous and categorical variables. Continuous data were described by mean \pm standard deviation (SD) or median and interquartile range (IQR), categorical data are presented as numbers or percentages.

RESULTS

A 23-year-old male patient (index patient, IV.1) presented to the emergency department with left-sided hemiparesis. His past medical history was notable for cerebrovascular disease (left-sided hemiparesis and paresthesia, improved without sequel) 4 years earlier, followed by herpes simplex virus (HSV) I encephalitis 1 months later. There consanguineous marriage between his parents (first cousins). The brain diffusion-weighted and apparent diffusion coefficient (ADC) MRI of this patient revealed an acute infarction in the right globus pallidus (Figure 1). There were no abnormalities in routine laboratory tests including hemogram, serum glucose and electrolyte levels, lipid profile, hepatic and renal function tests. Further studies were performed to determine the underlying cause of stroke. Electrocardiography (ECG) showed normal sinus rhythm. Carotid ultrasonography (USG) and ECHO were also normal. Carotid MRA did not reveal any pathological findings. The markers for vasculitis were negative. Prothrombin mutation G20210A and factor V Leiden mutation were not present. The levels of antithrombin III, protein C, and protein S were within normal limits. However, he had a homozygous mutation in *methylenetetrahydrofolate reductase (MTHFR) C677* gene. The serum homocysteine was normal (9 $\mu\text{mol/L}$; nv: 5-15). He was referred to the hematology service, whose opinion was that the patient had low risk for thrombosis, and did not recommend anticoagulant treatment. Assessment of the enzyme α -Gal A and gene analysis with DBS method showing a very low enzymatic activity (0.1 nmols/h/mL; nv>1.2) and a hemizygous missense mutation of the *c.[680G>A] p.[R227Q]* gene. The patient was diagnosed as having FD.

A subsequent screening for FD was performed on 20 members of the family (9 males, 45%). The mean age of the study patients was 36.7 \pm 16.67 (range 8-73 years). The pedigree is shown in Figure 2 and relevant clinical, enzymatic and genetic data are given in the Table 1. The mean α -Gal A enzyme activity of the 20 cases was 1.21 \pm 1.12nmols/h/mL. The molecular analysis of the *GLA* gene revealed the known *c.[680G>A] p.[R227Q]* mutation in 13 patients aged from 8

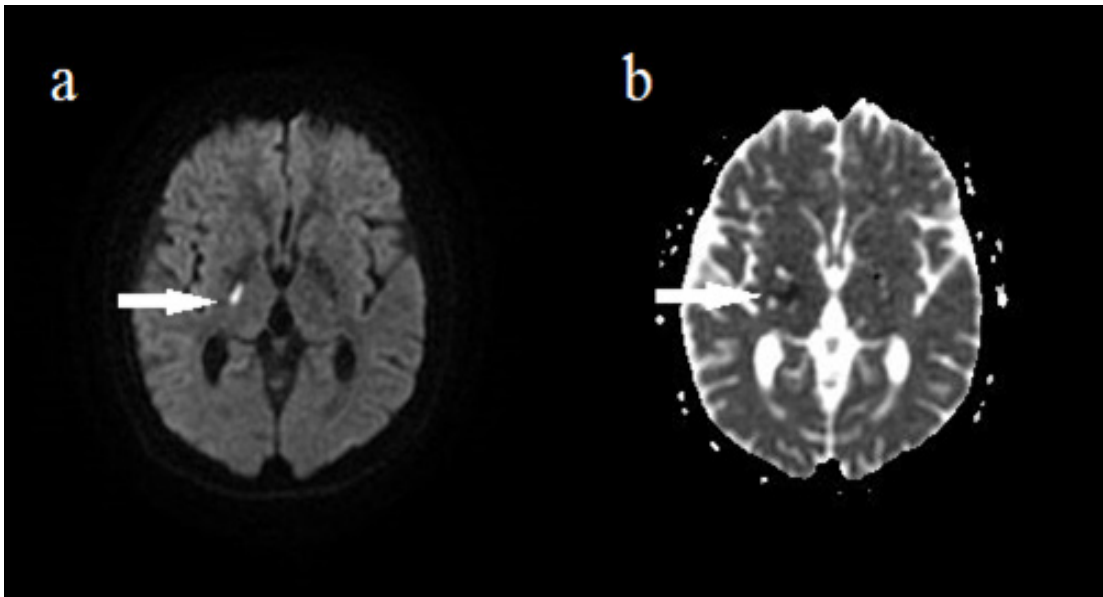


Figure 1. Brain MRI of the index patient. Axial diffusion-weighted image (a) shows diffusion restriction (arrow) in the right globus pallidus. The corresponding ADC image (b) shows decreased ADC, as evident by the dark signal (arrow) in the region of reduced diffusion.

to 44 years (mean 30.46 ± 11.6). Besides the index patient, 3 hemizygous males and 9 symptomatic heterozygous females were identified. The mean

α -Gal A enzyme activity of the cases with *GLA* mutation was measured as 0.66 ± 0.64 nmols/h/mL.

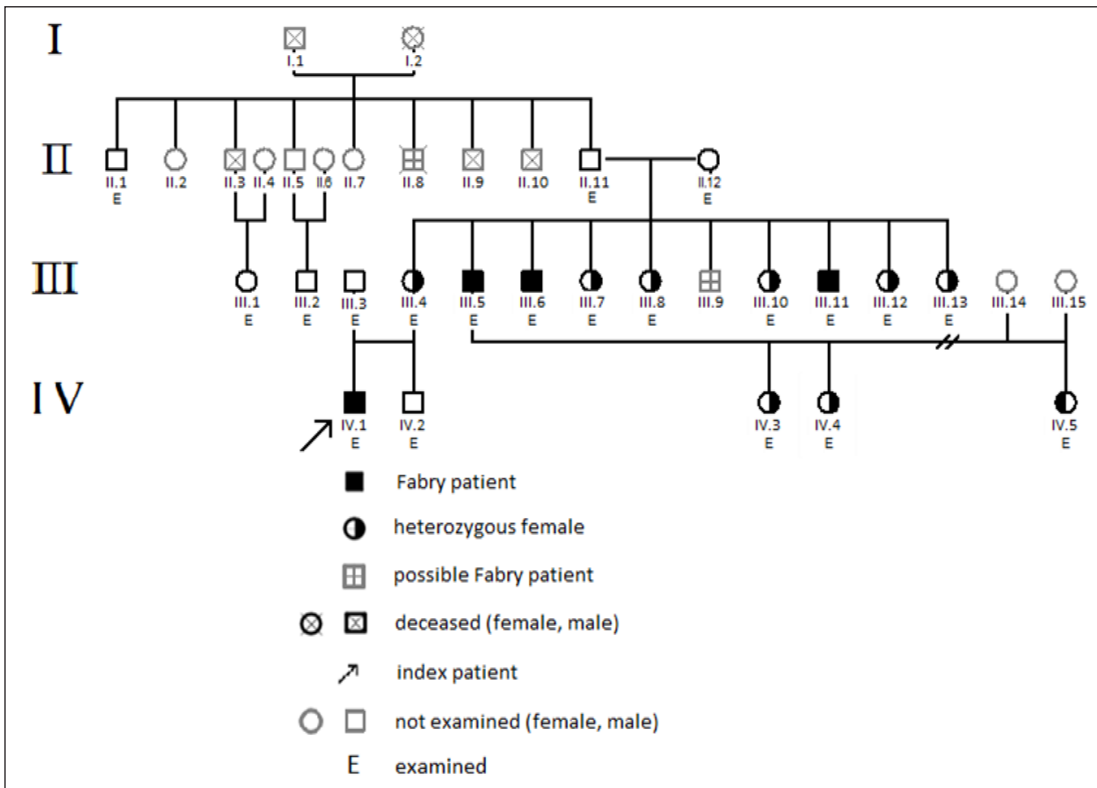


Figure 2. Pedigree of the Fabry disease

Table 1: Demographic, clinical features, α -Gal A enzyme activities and GLA gene mutation results of the cases

Case	Age	Sex	GLA Mutation	α -Gal A Enzyme activity	Stroke	Cardiac	Renal	Ocular	Dermatological				Other neurological				
									AK	Hyperh	Hypoh	HL	NP	AP	ES	HL	
II.1	73	M	-	3.8	+	+	-	+	-	-	-	-	-	-	-	-	-
II.11	62	M	-	2.2	-	-	-	-	-	-	-	-	-	-	-	-	-
II.12	61	F	-	2.1	-	-	-	-	-	-	-	-	-	-	-	-	-
III.1	47	F	-	3.7	+	-	-	-	-	-	-	-	-	-	-	-	-
III.2	34	M	-	0.7	-	-	-	-	-	-	-	-	-	+	-	+	-
III.3	45	M	-	1.6	-	-	-	-	-	-	-	-	-	-	-	-	-
III.4	44	F	Heterozygous	0.9	-	-	+	-	-	-	-	-	-	+	-	-	+
III.5	43	M	Hemizygous	0.1	+	+	+	-	-	-	-	-	-	+	+	+	-
III.6	42	M	Hemizygous	0	-	-	+	+	-	-	-	-	-	+	+	+	-
III.7	41	F	Heterozygous	0.9	-	-	+	+	-	-	-	-	-	+	+	+	-
III.8	40	F	Heterozygous	0.9	-	+	+	+	-	-	-	-	-	+	+	+	-
III.10	34	F	Heterozygous	0.6	-	-	-	-	-	-	-	-	-	-	-	-	-
III.11	32	M	Hemizygous	0.1	-	-	-	-	-	-	-	-	-	-	+	-	-
III.12	30	F	Heterozygous	0.7	-	-	-	-	-	-	-	-	-	-	-	-	-
III.13	21	F	Heterozygous	0.6	-	-	-	-	-	-	-	-	-	+	-	-	-
IV.1	24	M	Hemizygous	0.1	+	-	-	-	-	-	-	-	-	-	-	-	-
IV.2	16	M	-	1.5	-	-	-	-	-	-	-	-	-	-	-	-	-
IV.3	20	F	Heterozygous	2.5	-	-	-	-	-	-	-	-	-	+	-	+	-
IV.4	17	F	Heterozygous	0.7	-	-	-	-	-	-	-	-	-	+	-	+	-
IV.5	8	F	Heterozygous	0.5	-	-	-	-	-	-	-	-	-	+	-	+	-

AK: Angiokeratoma, Hyperh: Hyperhidrosis, Hypoh: Hypohidrosis, NP: Neuroapathic Pain, AP: Acroparesthesia, ES: Epileptic Seizure, HL: Hearing loss

The enzyme level was low in 13 members (8 females, 5 males) of the family. Four of 5 males with low enzyme activity and 9 females were found to have a known missense mutation of the *c.[680G>A] p.[R227Q]* gene. Although a female case (IV.3) had the *GLA* mutation, her enzyme activity was within normal limits. She had hyperhidrosis and acroparesthesia. On the other hand, the enzyme activity of a male (III.2) with no *GLA* mutation was low. He had a history of epileptic seizure, cognitive impairment and neuropathic pain. Whole gene sequence analysis did not reveal any mutation in this male case with a different clinical picture from other siblings.

DISCUSSION

Anderson-Fabry disease was first described by two separate scientists in 1898.^{8,9} In general population, the incidence of the disease was reported to range from 1:476,000 to 1:117,000.¹⁰ A prevalence study revealed the frequency of FD as 0.015 per 100,000 live births in Turkey.¹¹ In a study among Turkish patients undergoing hemodialysis, the prevalence of FD was determined to be 0.1 – 0.3%.¹²⁻¹⁴ Because of the atypical or oligosymptomatic forms of the disease, precise incidence and prevalence of FD are still not known.¹⁵ Although the average delay from onset of symptom to the time of diagnosis is 12-16 years, it may take up to 20 years in some cases. In a recent paper, more than 25% of FD patients are reported to be misdiagnosed.¹⁶ Measurement of α -Gal A enzyme activity in males and molecular genetic testing of *GLA* in females are the reliable methods for diagnosing FD.^{17,18}

Mutations detected in the classical type of FD are large splicing defects, missense or nonsense mutations, while the variant (mild) type is characterized by splicing defects or missense mutations.² Missense mutation or substitution refers to a change in one amino acid in a protein, arising from a point mutation in a single nucleotide. In case of FD, missense mutations in *GLA* gene render the gene nonfunctional. As a result of the X-linked recessive inheritance pattern, FD typically affects males, whereas, heterozygous females may show high variability in clinical manifestations which is attributed to random X-chromosome inactivation. The levels of activity of α -Gal A enzyme were helpful for the diagnosis but did not have prognostic value. In male patients, the diagnosis of FD could be made by detecting low levels of α -Gal A activity in plasma or white blood cells. As the carrier

females may have low or normal enzyme activity, molecular genetic testing is a more preferred method in the diagnosis of FD in females.^{1,19} In our study, the enzyme level was low in 13 members (8 females, 5 males) of the family. Four of 5 males with low enzyme activity and 9 females were found to have a known missense mutation of the *c.[680G>A] p.[R227Q]* gene.¹⁹ Although a female case (IV.3) had the *GLA* mutation, her enzyme activity was within normal limits. She had hyperhidrosis and acroparesthesia. These findings showed the importance of searching for *GLA* gene mutation in female members of the family even if they had normal enzyme levels.

FD may cause various clinical manifestations. In classical form with deficient activity of α -*GLA*, the symptoms of the disease typically begin to occur in childhood. Acroparesthesias, angiokeratomas, hypohidrosis, hearing loss and corneal dystrophy are common initial symptoms. In general, prominent skin symptoms occur in adolescence. Renal failure, cardiac or neurological diseases mostly occur in adulthood. In later-onset variant form with residual enzyme activity, cardiac disease and/or renal failure develop after the fourth decade. It is recognized that central and peripheral neurological complications are frequently seen in the course of FD, and cerebrovascular diseases are often the first cause of hospitalization.^{1,2,16} Our index case had none of the classical initial symptoms of the disease and was diagnosed to have FD upon investigation for recurrent stroke episodes at a young age. As another risk factor for stroke, homozygous *MTHFR* gene mutation was not considered as major etiology in this case, because of his normal serum homocysteine level.

The major cause of early morbidity and mortality in FD is the young onset cerebrovascular disease. Neuronal accumulation of globotriaosylceramide (Gb-3) in the cerebral cortex and brain stem, vascular occlusion, endothelial dysfunction and a prothrombotic state have been proposed for the development of stroke.^{20,21} Our index patient had two stroke episodes when he was 19 and 23 years old. Family screening identified the FD diagnosis in his 43-year-old maternal uncle (III.5), who also had a minor stroke episode previously. A history of ischemic stroke was also present in two more cases (II.1, III.1) that were not diagnosed with FD. Notwithstanding that the affected females manifesting neurological symptoms, none of them had cerebrovascular disease. A homozygous *MTHFR C677* gene mutation has been identified in our index patient. To the best of our knowledge, a link between factor V Leiden mutation, stroke

and the FD has been reported; but a connection between *GLA* and *MTHFR* mutations has not been established.²² Our index patient also had a history of HSV I encephalitis. In Fabry patients with cerebrovascular complications, the occurrence of concomitant aseptic meningitis has been reported previously.²³ The close occurrence of HSV I encephalitis and stroke in FD has not been reported previously.

The symptoms of early neurological involvement may include acroparesthesia, neuropathic pain, hearing loss, and tinnitus. Seven of our patients (III.2, III.4-8, III.11) had neuropathic pain, 7 had (III.5-8, IV.3, IV.4, IV.5) acroparesthesia and 2 had hearing loss (III.4, III.5).

Cardiac involvement in FD consists of left ventricular hypertrophy, mitral valve regurgitation, coronary artery disease, cardiomyopathy, and arrhythmia.^{24,25} Two FD cases (III.5, III.8) in our study had cardiomyopathy. One case of age of 73 years (II.1) had coronary artery disease and myocardial infarction. It has been said that renal manifestations are usually nonspecific. Asymptomatic proteinuria and hematuria episodes, which are often the first signs of renal involvement, may be missed. Renal symptoms mostly appear in the third decade in half of the FD patients, but the severity of renal disease worsen with advancing age. In all of the FD patients, an end-stage renal failure will be expected by the age of 55.²⁶ Five of our patients (III.4-8) had renal involvement. However, only one patient (III.5) progressed to end-stage requiring hemodialysis. Corneal verticillate (the most common and earliest sign), whirlpool-shaped corneal opacities, cataract, conjunctival and retinal dilatation and tortuosity may occur as the ocular involvement of the FD.²⁷ In our study, 3 patients (III.6-8) had ocular involvement of FD. One case (II.1) had age-related cataract. Diffuse angiokeratoma is a characteristic, but a nonspecific sign of the FD and is generally the earliest sign in affected males. It is observed in 66 % of males and 33 % of females. Other common skin lesions are telangiectasia, lymphedema, hyperhydrosis and hypohydrosis.^{28,29} One patient (III.7) in our study had angiokeratomas, 3 had (III.5-7) hypohydrosis and 6 had (III.1, III.3, III.8, IV.3-5) hyperhydrosis.

Medical or surgical treatment of the symptoms provides benefit for the patients. Enzyme replacement therapy (ERT) developed for FD is a promising treatment option. ERT may prevent the Gb-3 accumulation and progression of the disease before irreversible organ damage occurs.³⁰⁻³² In our study, 5 patients have been treated with ERT.

Although various clinical manifestations in the families with different *GLA* gene mutations are reported, the genotype–phenotype correlations of FD are not confirmed. In 2009, Mehta *et al.* reported the clinical outcomes of 1,453 FD patients from 19 countries participating in the Fabry Outcome Survey (FOS). The most frequently reported signs and symptoms were neurologic, affecting 68% of the patients.³³ Stroke was reported in 23% of FD patients and the mean age of onset was 33.7 years. However, researchers did not report a correlation between the neurologic manifestation of FD and the type of genetic mutation. In 2010, Brouns *et al.* reported that Ser126Gly and Ala143Thr mutations were associated with stroke as atypical variants of FD with late-onset cerebrovascular disease.³⁴ In 2014, Schelleckes *et al.* reported that 13 of 15 FD patients carrying the *GLA* haplotype -10C>T [rs2071225], IVS2-81_-77delCAGCC [rs5903184], IVS4-16A>G [rs2071397], and IVS6-22C>T [rs2071228] had neurological manifestations such as neuropathic pain, allodynia and stroke.³⁵ In our study, all of the newly diagnosed relatives have been carrying the same mutation '*c.[680G>A] p.[R227Q]*' and frequently (76.9%) had neurological symptoms. Besides, other organ system involvements were not prominent in our patients. Therefore, we hypothesize that this mutation may be related to the neurological phenotype of FD. However, further studies with larger populations are needed to confirm this association.

In this study, we presented the spectrum of neurological manifestations in a large Turkish family of FD with *c.[680G>A] p.[R227Q]* mutation detected during the investigation of a young stroke patient. FD should be screened among young stroke patients particularly in those with multiorgan involvement. Our study demonstrates the importance of early diagnosis, constructing a detailed pedigree and providing a genetic counseling for the FD patients. Since FD is a treatable disorder, performing the enzymatic and genetic tests is valuable in the family members. We proposed that the hemizygous missense mutation of the *c.[680G>A] p.[R227Q]* gene as a predictor of the neurological phenotype of FD.

DISCLOSURE

Financial support: None

Conflict of interest: None

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