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RESEARCH ARTICLE

Identification of a Missense Mutation in the α-galactosidase A Gene in a Chinese Family with Fabry Disease

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Abstract: ***Introduction*:** Fabry Disease (FD), the second most common lysosomal storage disorder after Gaucher disease, is characterized by variable clinical manifestations, including angiokeratoma, corneal dystrophy, recurrent episodes of extremity pain, renal impairment, cardiac complications and cerebrovascular manifestations. It is caused by mutations in the α-galactosidase A gene (gene symbol *GLA*) on chromosome Xq22, which leads to deficiency of lysosomal α-galactosidase A (α-Gal A), and subsequent accumulation of glycosphingolipids in various tissues and organs. The aim of this study is to identify the disease-causing mutation in a five-generation Chinese family with FD. A c.782G>T transversion (p.G261V) in the *GLA* gene was identified in four patients and two asymptomatic carriers by direct sequencing, and it co-segregated with the disease in the family. The variant is predicted to be disease-causing mutation and result in seriously abnormal function of α-Gal A. Four patients in this family present with classic phenotype of FD, including acroparesthesias, hypohidrosis, angiokeratomas and intermittent burning pain in extremity.

***Conclusion*:** The disease severity is similar among male and female patients. Our study extends the genotype-phenotype relationship between mutations in the *GLA* gene and clinical findings of FD, which may be helpful in the genetic counseling of patients with FD.

Keywords: Fabry disease, α-galactosidase A, Phenotype, The *GLA* gene, Mutation, p.G261V.

1. INTRODUCTION

Fabry disease (FD; MIM 301500), initially reported in 1898, is the second most common lysosomal storage disorder after Gaucher disease [1]. It is due to the deficiency of lysosomal α-galactosidase A (α-Gal A) and the subsequent accumulation of glycosphingolipids, primarily globotriaosylceramide, in various organs and tissues, including skin, eyes, intestine, heart, kidneys, peripheral and central nervous system neurons. The accumulation of glycophingolipids results in multiple clinical signs and symptoms, and substantial morbidity and mortality [2-4].

Based on the absence or presence of residual α-Gal A activity, FD patients may be divided into classic subtype and atypical (milder or later-onset) subtype [5]. The classic phenotype primarily occurs in male patients, and the microvascular lesion causes acroparesthesias, angiokeratomas, hypohidrosis, gastrointestinal abnormalities and corneal opacity in childhood. The progressive accumulation of glycosphingolipid, particularly in the vascular endothelium leads to renal impairment, cardiac complications, cerebrovascular manifestations, neurological complications and early death in adulthood [4-7]. In atypical FD, affected male patients have a late-onset phenotype with renal and cardiac complications after 30 years old [6]. Female patients show a high penetrance of FD, and clinical manifestations range from being asymptomatic to being as severe as affected male patients, although with a decade onset-delay [8-10].

FD is a relatively rare disorder, and the worldwide incidence has been estimated ranging from 1 in 40,000 to 117,000 live male births [11, 12]. However, the true incidence may be underestimated due to indeterminacy or failure to diagnose mild or atypical cases. Indeed, recent researches of FD newborn screening uncovered a notably higher frequency of 1 in 900 to 4,000 in males and 1 in 400 to 2,000 in females for atypical FD [13-16].

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FD is an X-linked disorder caused by mutations in the gene encoding α-Gal A (gene symbol *GLA*, OMIM: 300644), and more than 700 different mutations, including point mutations and complex rearrangements. Some of them that are newly described in patients with FD in the last five years, have been reported (The Human Gene Mutation Database, http://www.hgmd.cf.ac.uk/ac/) [4, 13, 14, 17-19].

Here, we report a c.782G>T transversion (p.G261V) in the *GLA* gene in a Chinese Han family with FD. The age of onset and disease severity in heterozygous female patients were similar to those of affected hemizygous males in this family.

2. MATERIALS AND METHODS

2.1. Pedigree and Subjects

A 5-generation, 21-member Chinese Han family with FD from Hunan province, China was recruited in the Third Xiangya Hospital, Central South University (Fig. **1**). Nine family members of the pedigree were involved in this study, including four affected individuals (IV:1, IV:3, IV:5 and IV:6) and five unaffected ones (III:3, III:4, V:1, V:2, and V:3). One hundred unrelated ethnically-matched individuals (male/female: 50/50, age 37.2 ± 7.3 years) without any diagnostic features of FD were recruited from the same region of Mainland China and served as normal controls. Written informed consent was obtained from each participating individual or his/her guardian, and this study had received approval from the Ethics Committee of the Third Xiangya Hospital, Central South University, P.R. of China.

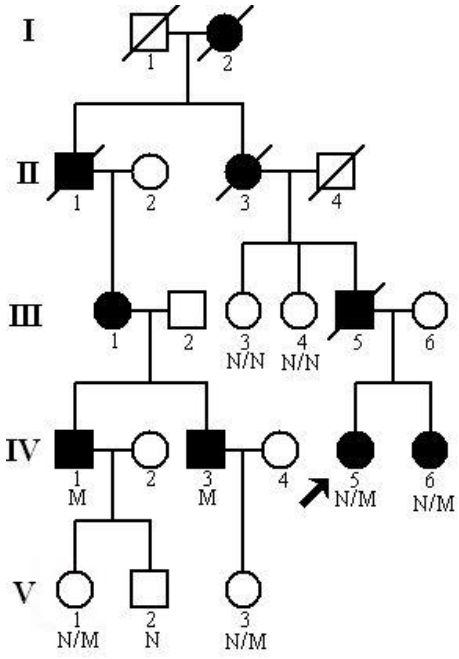

Fig. (1). Pedigree of the family with Fabry disease. *N*: normal, *M*: *GLA* c.782G>T (p.G261V) mutation. The arrow indicates proband.

2.2. Clinical Data

All affected and unaffected individuals underwent skin, ophthalmic and hearing examinations, urinalysis, urinary micro protein test, ECG, heart Doppler ultrasound. The results are summarized in Table **1**. All adult patients (IV:1, IV:3, IV:5, IV:6) had been affected by acroparesthesias, hypohidrosis, angiokeratomas and intermittent burning pain in hands and feet since 11~12 years of age. The pain was frequently exacerbated by fever, stress or changes in temperature, and typically affected the palms of the hands and soles of the feet, limiting them from participating in sports or other physical activities. The diagnosis of FD was made based on typical histological features from renal biopsy in the proband (IV:5). Renal biopsy specimens showed structural changes with deposition and vacuolation in podocytes, mesangial cells, distal tubular cells, arterial and arteriolar smooth muscle cells, glomerular/vascular endothelial cells and interstitial cells. Electron micrograph showed that cytoplasm of all kinds of renal cells was filled with osmiophilic, granular-to-lamellated membrane structures (characterized by "onion skin" or "zebra" appearance). The proband's father (III:5) died of severe renal failure at 45 years of age, and retrospective study found that his clinical manifestations was consistent with the classical symptoms of FD.

2.3. Gene Analysis

Genomic DNA was obtained from peripheral blood leukocytes using standard phenol-chloroform extraction method. The *GLA* gene was amplified in 7 fragments corresponding to all coding exons using primers in Table **2**. PCR amplification of the *GLA* gene was performed with GeneAmp 9700 thermal cycler system (Applied Biosystems, Foster City, CA, USA). The PCR was initiated with a 3 min hold at 95°C, followed by 35 cycles of 95℃ for 45 s, 58℃ for 40 s, 72℃ for 1 min, and a final extension step at 72℃ for 5 min. 8.5 μL of PCR products were treated by 0.8 U shrimp alkaline phosphatase (SAP, Fermentas) and 8 U exonuclease I (Fermentas), and analyzed directionally using Applied Biosystems 3500 Series Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) [20].

2.4. Bioinformatics Analysis of the Mutation

Multiple sequence alignments and conservation analysis were performed using the Basic Local Alignment Search Tool (http://blast.st-va.ncbi.nlm.nih.gov/Blast.cgi) [21]. Functional prediction tools, including Polymorphism Phenotyping version 2 (PolyPhen-2, http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.jcvi.org/, scores less than 0.05 are deleterious), and MutationTaster (http://www.mutationtaster.org/), were applied to evaluate the possible effects of amino acid alteration on protein structure and function [22].

3. RESULTS

A c.782G>T variant (p.G261V) in the *GLA* gene was found by sequencing the coding regions of *GLA* gene (Fig. **2**). The c.782G>T variant co-segregated with male patients and female carriers in the family, and none of the 100 ethnically-matched unrelated controls carried the variant. Our data indicate that the c.782G>T variant (p.G261V) in the *GLA* gene was the disease-causing mutation in our family. Two female patients in this family carried the heterozygous c.782G>T mutation and the two male patients were hemizygotes. All of them had classical phenotypes of FD, including acroparesthesias, hypohidrosis, angiokeratomas,

Table 1. Clinical characteristics of affected family members with Fabry disease.

—	IV:1	IV:3	IV:5	IV:6
Age (years)	39	34	26	21
Age at onset of symptoms (years)	11	12	11	12
Sex	Male	Male	Female	Female
Genotype	Hemizygote	Hemizygote	Heterozygote	Heterozygote
Proteinuria *	74 mg/day	280 mg/day	1270 mg/day	65 mg/day
Renal failure	No	No	No	No
Abnormal ECG	Yes	Yes	Yes	Yes
Left ventricular hypertrophy	Yes	Yes	Yes	Yes
Cerebrovascular events	No	No	No	No
Hypohidrosis	Yes	Yes	Yes	Yes
Acroparaesthesia	Yes	Yes	Yes	Yes
Angiokeratoma	Yes	Yes	Yes	Yes
Corneal lesion	No	No	No	No
Hearing loss	Yes	No	No	No

* Normal range: 0-150 mg/day

Table 2. Primers for the *GLA* gene.

Exon	Forward Primer (5'→3')	Reverse Primer (5'→3')	Product Size (bp)
1	GATTGGTCCGCCCCTGAG	GTTCCCGTTGAGACTCTCCA	353
2	TTGTGAAATCCCAAGGTGCC	ACAGAAGTGCTTACAGTCCTCT	315
3	AGCCTGGAATGGTTCTCTCT	GGTTCTTTGGCTCAGCTACC	330
4	GGATGACAGACTGAACCCCA	CGTTGGACTTTGAAGGAGACC	303
5	CAAGAGAAGGCTACAAGTGCC	ACCACTTTCCACAGCATCCT	394
6	CAGGATGCTGTGGAAAGTGG	AGATTTAGGCCCAAGACAAAGT	366
7	AATGCCAAACTAACAGGGCC	ATGAGCCACCTAGCCTTGAG	438

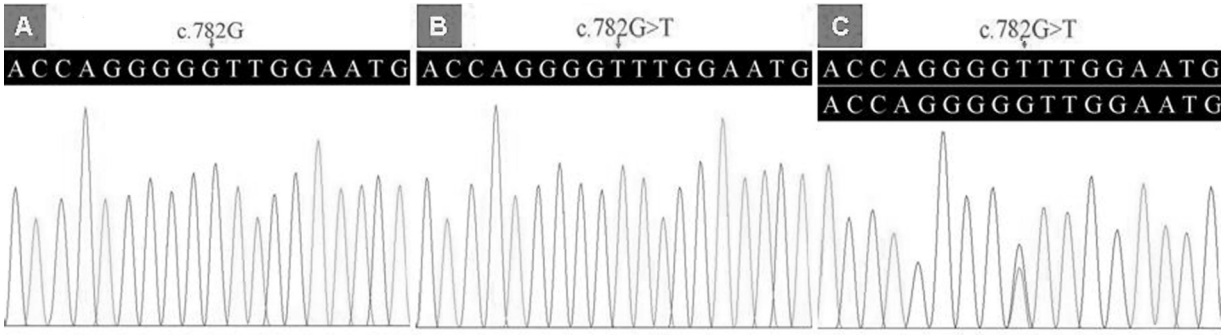

Fig. (2). Sequencing analysis of c.782G>T (p.G261V) mutation in the *GLA* gene (DNA). (**A**) Unaffected member (III:3) of the family. (**B**) Hemizygous c.782G>T (p.G261V) mutation patient (IV:1). (**C**) Heterozygous c.782G>T (p.G261V) mutation patient (IV:5).

and intermittent burning pain in hands and feet since 11~12 years of age. There were two additional female members (V:1, V:3) who carried the heterozygous c.782G>T mutation and were currently asymptomatic (6 years old and 5 years old, respectively).

The glycine at position 261 was phylogenetically conserved across vertebrates down to zebrafish (Fig. **3**). PolyPhen-2 analysis gained a score of 0.957 on the HumVar database (sensitivity, 0.63; specificity, 0.92), predicted to be probably damaging. The SIFT prediction produced a score of 0.05, indicating that the mutation was predicted to be damaging. MutationTaster predicted that the substitution was disease-causing with a probability value close to 1, indicating a high security of prediction.

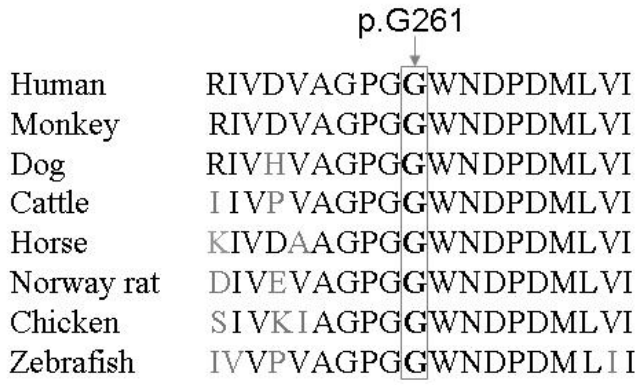

Fig. (3). Conservation analysis of α-Gal A p.G261 amino acid residue.

4. DISCUSSION

FD was independently reported by Johannes Fabry and William Anderson in 1898 [23]. In 1989, six different gene arrangements and one missense mutation associated with the *GLA* gene were found in affected males with FD based on analysis of 130 unrelated families [24]. Various mutations, including missense/nonsense mutations, splice defects, regulatory abnormalities, small deletions and insertions, small indels, gross deletions and insertions, and complex rearrangements associated with the *GLA* gene, have been discovered over the last 26 years.

The *GLA* gene, mapped to the long arm of the X chromosome (Xq22), spans 12 kb with seven exons encoding 429-amino-acid α-Gal A [25]. The α-Gal A protein exists within lysosomes in various organs and cells throughout the body. The protein predominantly hydrolyzes ceramide trihexoside, and catalyzes the hydrolysis of melibiose into galactose and glucose. Decrease or deficit of the protein may result in obstacle of the glycolipid conversion from globotriosylcera-mide (GL-3) to lactosylceramide (GL-2) [24, 26]. Pathogenic mutations in the *GLA* gene may lead to decreased or lost enzyme activity by affecting the synthesis, processing and stability of α-Gal A, or changing the hydrophobic core of the protein leading to folding defects, broken disulphide bonds or the loss of N-linked glycosylation sites, and other unknown mechanisms [17, 27]. Three segments (amino acids 162-172, 215-231, and 258-269) have a high frequency of mutation in α-Gal A [28]. The c.782G>T mutation (p.G261V) in the *GLA* gene, previously reported by Jan Lukas in 2013 [29], was identified in our Chinese Han family with FD and was absent in the 100 unrelated ethnically-matched controls. Intriguingly, another missense mutation (c.782G>A, p.G261D) involving the same nucleotide and resulting in glycine substituted by aspartic acid in position 261 was reported in 1997 [30]. These data indicate that nucleotide 782 may be a mutation hotspot. Our four patients (two males and two females) also shared similar classical clinical features to the case with c.782G>A mutation of *GLA* gene reported by Takata *et al*. [30], such as symptoms at disease onset, pain in the extremities, angiokeratoma and hypohidrosis. In our study, the p.G261V mutation was predicted to be "probably damaging" by PolyPhen-2, "damaging" by SIFT, "disease causing" by MutationTaster, consistent with the report that mutations causing the severe phenotype of FD are always located in the interior of the α-Gal A, while mutations causing the mild phenotype are inclined to be less damaging to the α-Gal A hydrophobic core [28]. The glycine at position 261 is a buried residue (Fabry database, http://fabry-database.org/) and near the active site, amino acid 266. Mutations in buried residue can create a polypeptide with folding defects, where the hydrophobic core of the protein is disrupted and the enzyme fails to fold or remain folded in the acidic environment of the lysosome [28]. The two girls (V:1, V:3) who carried the p.G261V mutation were currently asymptomatic. This is consistent with the observation that most female FD patients have the first symptoms at a median age of 13 years [31]. Various studies have shown that different genotypes of mutations in FD patients have a poor genotype-phenotype correlation; even the same mutation in one family can lead to different phenotypes, including the age of onset, disease severity, rate of progression and organ manifestations [32]. Disease manifestations in female heterozygotes are usually mild and have a slower rate of progression. Approximately 70% of female carriers have asymptomatic corneal dystrophy, 30% have minimal angiokeratomas and less than 10% have infrequent attacks of neuropathic pains [33], which partly may be due to the result of lyonisation, a process that is caused by one X chromosome in some or all cells of the female embryo being randomly inactivated [34, 35]. Interestingly, in our family, male and female patients presented with classical FD manifestations of similar severity, suggesting that skewed X chromosome may prevent the expression of the wild-type alleles and then activate the mutant alleles [36, 37]. The clinical features in our FD family are consistent with the report that the heterozygotes with inactivated healthy allele were inclined to have notably higher clinical severity score than women with random inactivation of both X-chromosomes [38].

CONCLUSION

In summary, a c.782G>T transversion (p.G261V) in the *GLA* gene was identified in a Chinese Han family with FD. To our knowledge, this is the first report of c.782G>T transversion (p.G261V) in the *GLA* gene in Asian population. Further functional studies of the *GLA* mutations and application of *in vitro* and/or *in vivo* models with genetic deficiency are warranted to facilitate a better understanding of the pathogenesis and development of targeted experimental treatments of FD.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethics approval was gained from the Ethics Committee of the Third Xiangya Hospital, Central South University, P. R. of China and written informed consent was obtained from each participating individual or his/her guardian.

HUMAN AND ANIMAL RIGHTS

Humans were used for studies that are base of this research. No Animals were used for studies that are base of this research. All human research procedures followed were in accordance with the World Medical Association Declaration of Helsinki, revised in 2013.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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