Clinical and genetic spectrum in Chinese families with Fabry disease: a single-centre case series

Xin Chen†, Hezhi Li†, Hongtao Liao, Xianzhang Zhan, Zhian Zhong, Qianhuan Zhang, Lie Liu, Yuanhong Liang, Hai Deng, Xianhong Fang, Yumei Xue, Shulin Wu and Yang Liu*

Guangdong Cardiovascular Institute, Guangdong Provincial People’s Hospital, Guangdong Academy of Medical Sciences, 106 Zhongshan Rd, Guangzhou, 510080, China

Abstract

Aims  Fabry disease (FD) is an X-linked genetic disease caused by mutations in the GLA gene that leads to deficient activity of lysosomal enzymes, accumulation of globotriaosylceramide in multi-organ systems, and variant clinical manifestations. We aimed to detail the clinical and genetic spectrum of FD in Chinese families.

Methods and results  Five male probands with unexplained left ventricular hypertrophy and their family members were investigated. Genetic screening was available in 11 subjects of the 5 families, 10 of whom proved to be carriers of either GLA gene mutation, including 3 previous reported missense mutations (c.128G > A, c.811G > A, c.950T > C), 1 novel missense mutation (c.37G > C), and 1 novel deletion mutation (c.1241delT). A total of 17 patients were definitely or possibly diagnosed of FD, given their clinical manifestations and hereditary nature of FD. Echocardiography demonstrated normal cardiac structure and function in six female patients. Electrocardiographic pre-excitation occurred in 80% (4/5) of men and 16.7% (1/6) of women. Six patients (6/14, 42.9%) had chronic kidney disease with decreased renal function and all were male (6/7, 85.7%). Six patients presented with acroparesthesia, hypohidrosis, or both. Three female patients and two male patients experienced sudden death, and one male patient with the mutation (c.128G > A) died of progressive heart failure, between 41 and 66 years of age.

Conclusions  We reported five unrelated families of FD with different GLA mutations. Clinical manifestations were highly heterogeneous between male and female patients even within the same family. Female patients showed relatively low risks of structural heart disease and renal insufficiency. However, the long-term outcomes might be adverse in both sexes. Our study underlines the importance of molecular screening of the GLA gene for early identification and clinical decision making in patients with FD.

Keywords  Fabry disease; GLA gene; Left ventricular hypertrophy; Renal insufficiency

Introduction

Fabry disease (FD, OMIM #301500) is a rare X-linked genetic disease caused by mutations in the GLA gene that encodes α-galactosidase A (α-Gal A). FD is an inborn lysosomal storage disease, and its incidence ranges from 1/40 000 to 1/117 000 in the general population. The deficient α-Gal A leads to intra-lysosomal accumulation of glycosphingolipids in multiple organs, causing variant clinical symptoms of different systems, including peripheral and automatic neuropathy, cerebrovascular and cardiovascular disease, renal disease, and ocular disease. According to differences in residual α-Gal A activity and clinical manifestations, FD is categorized into severe, early onset ‘classical type’ and mild, late onset ‘non-classical type’. Cardiac involvement of FD typically manifests as a hypertrophic phenotype; therefore, it is often misdiagnosed as hypertrophic cardiomyopathy (HCM). The reported prevalence of FD is up to 12% in European and American patients diagnosed with HCM. In the present study, we detail the clinical phenotypes of five different
Chinese families with FD caused by two novel mutations and three previous reported missense mutations.

Methods

Study subjects

Five unrelated male probands (43 to 63 years of age at presentation) with unexplained left ventricular hypertrophy and their family members were enrolled in our study. All subjects were evaluated on the basis of medical history, physical examination, laboratory tests, 12-lead electrocardiogram (ECG), and transthoracic echocardiography if possible. The study was approved by the ethics committees of Guangdong Provincial People’s Hospital and performed in accordance with the Declaration of Helsinki. Informed and written consent was obtained from all the study subjects.

Molecular genetic analysis

Genomic DNA was extracted from peripheral blood lymphocytes using a commercial kit (QIAGEN Co., Ltd., CA, USA) for genetic screening with next-generation sequencing. All sequencing and genetic analyses have been detailed in our previous study. In brief, genetic screening was available in 11 subjects and performed at presentation between the ages of 12 and 63 years, 10 of whom proved to be carriers of either GLA gene mutation. Candidate variants in five probands were identified by the next-generation sequencing and further validated by direct Sanger sequencing. Their family members only underwent direct Sanger sequencing to confirm the identified mutations. Seven patients probably carried the GLA mutation, given their clinical manifestations and hereditary nature of FD. A total of 17 patients was proved or likely diagnosed of FD.

Histological analysis

The proband in Family A underwent endomyocardial biopsy because of refractory and progressive heart failure. Cardiac tissue specimens were biopsied from the right interventricular septum via the right internal jugular vein. Specimens were fixed in 10% formalin for staining with haematoxylin and eosin (H&E) and Masson’s trichrome, or in 2.5% glutaraldehyde for transmission electron microscopy (JEM1400-Plus, JEOL Ltd., Tokyo, Japan) and toluidine blue staining.

Results

GLA mutations in probands

Four missense mutations and one deletion mutation in GLA were identified in five probands (Figure 1). A missense mutation consisting of a G-to-A transition in Exon 1 (c.128G > A) was found in Family A, leading to the substitution of an aspartic acid for glycine at Residue 43 (p.Gly43Asp). A novel single-base deletion mutation in Exon 7 (c.1241delT) was identified in Family B, which predicted a frameshift mutation and a putatively truncated protein (p.Leu414fsX4). A missense mutation consisting of a G-to-A transition in Exon 6 (c.811G > A) was found in Family C, leading to the substitution of a serine for glycine at Residue 271 (p.Gly271Ser). Another missense mutation consisting of a T-to-C transition in Exon 6 (c.950T > C) was identified in Family D, predicting the substitution of a threonine for isoleucine at Residue 317 (p.ile317Thr). A novel missense mutation consisting of a G-to-C transition in Exon 1 (c.37G > C) was found in Family E, leading to the substitution of a proline for alanine at Residue 13 (p.Ala13Pro). This proband had significantly elevated levels of lyso-GL-3 (19.22 ng/mL, normal range: <1.11 ng/mL).

Clinical features in probands

Clinical recognition in four patients occurred by virtue of cardiac symptoms (exertional dyspnoea, palpitation, and chest pain) and in one patient due to renal insufficiency. Four of them presented with acroparesthesia, two with hypohidrosis and four were diagnosed with Stage IV or V chronic kidney disease. The onset of symptoms occurred between the ages of 20 and 55 years. One patient was referred to our hospital because of a non-ST-elevation myocardial infarction. He underwent percutaneous coronary intervention of the occluded left circumflex artery. Two patients developed atrial fibrillation and/or atrial flutter and one of them also experienced aborted cardiac arrest due to ventricular tachyarrhythmia. One patient received an implantable cardioverter-defibrillator due to bradycardia and HCM at the age of 59 years.

The surface 12-lead ECGs were strikingly abnormal in all probands. The QRS morphology of lead V1 was an R pattern in two patients and rS in the remaining patients. Four patients had ventricular pre-excitation patterns with short PR intervals, initial QRS slurring (delta waves), or both (Figure 2). All these patients showed markedly increased standard and/or precordial lead voltages with deep negative T-waves. The electrophysiological study in a proband with electrocardiographic pre-excitation excluded the presence of an atrioventricular accessory pathway. The details of electrophysiological study are provided in the Supporting Information.
Echocardiography showed left ventricular hypertrophy and diastolic dysfunction in all five probands (*Figure 3*, *Table 1*). The maximum septal wall thickness was 16–23 mm. The left ventricle tended to thin and dilate in one patient over 2 years of follow-up. Cardiac function progressively deteriorated in two patients with reduced left ventricular ejection fraction (40–45%) during the follow-up period, one of whom died of heart failure at the age of 66 years while awaiting heart transplantation. Left ventricular outflow tract obstruction was present in one patient (Valsalva-induced pressure gradient, 74 mmHg). He underwent alcohol septal ablation to relieve the symptoms.

*Figure 2* Electrocardiogram of patient II:1 in Family D presented short PR (116 ms) intervals, positive delta waves in V1–2, and negative delta waves in leads I and V3–6 during sinus rhythm.
Moderate to severe mitral and tricuspid valve regurgitation was present in two patients (Figure 3). Cardiac magnetic resonance imaging of the proband in Family A demonstrated delayed gadolinium enhancement in the inferolateral wall of the left ventricle, suggesting potential myocardial fibrosis (Figure 4).

GLA mutations and clinical features in family members

In Family A, DNA was available from five offspring of the proband. All four daughters were carriers of the mutation. One daughter (III/2) complained of acroparesthesia for several years and another (III/5) had recurrent dizziness. Three daughters experienced episodes of syncope. Except for the daughter (III/5) presenting with short PR intervals (110 ms), ECGs and echocardiograms in mutation-positive family members were normal. The mother (I/2) of the proband experienced sudden death at the age of 60 years. She probably carried the GLA mutation, given that her son was clinically and genetically affected.

In Family B, DNA was also available from the proband’s niece (III/1). She was heterozygous for the same mutation and had elevated lyso-GL-3 level (6.71 ng/mL, normal range: <1.11 ng/mL) but was asymptomatic and presented with normal cardiac examination and renal function at the age of 12 years. The mother (I/2) of the proband complained of exertional dyspnoea. His brother (II/1) was diagnosed with nephritis in the third decade with hypohidrosis and recurrent oedema. He died suddenly at the age of 41 years. The mutation was therefore inferred in subjects I/2 and II/2 of Family B, given their clinical manifestations and X-linked hereditary nature of this disease.

In Family C, the mother (I/2) of the proband had a history of asthma. She presented with exertional dyspnoea in the fifth decade of her life and suffered sudden death at the age of 60 years. In Family E, the mother (I/2) of the proband experienced sudden death at the age of 60 years. His brother (II/1) was diagnosed with end-stage renal failure at the age of 50 years with need of dialysis and died suddenly at the age of 53 years. The proband’s daughter (III/1) was asymptomatic and had normal ECGs and echocardiograms. She should be a carrier given X-linked hereditary pattern of FD. However, she refused to perform genetic screening and assess plasma lyso-GL-3 levels. The clinical characteristics of these patients were detailed in Table 1.

Histological analysis

The proband of Family A underwent endomyocardial biopsy for pathological examination. Light microscopy revealed mild disarray of myocardial fibres with prominent cardiomyocyte atrophy and focal interstitial fibrosis [Figure 5(A) and 5(B)]. Toluidine blue staining revealed diffusely distributed glycosphingolipid vacuoles in cardiomyocytes [Figure 5(C) and 5(D)]. Transmission electron microscopy showed prominent myofibrillar dissolution with excess accumulation of...
Table 1 Clinical and demographic findings in 16 patients with Fabry diseases

| Family | Subject | Age (years)/gender | Genetically affected | Clinical presentations | CKD/stages | Echocardiogram | CKD, stages | LVEF, % | Arrhythmia | FU/age (years) | Death/age (years) |
|--------|---------|-------------------|---------------------|-----------------------|------------|----------------|-------------|---------|-------------|----------------|------------------|
| A      | I:2     | NA/F              | NA                  | SD                    | NA         | NA             | NA          | NA      | NA          | NA              | NA               |
| A      | II:2    | 63/M              | +                   | Oedema, HF, acroparesthesia, hypohidrosis | +/IV | 19, 9.4, 50, 7.9, 8.4 | 54 (64 years) | 54, 40 | VP, AFL/AF, VT, SSS | 3/66 | +/66 |
| A      | III:2   | 33/F              | +/-                | Syncope               | —          | 8.2, 7.4, 43, — | —          | 73 | NA | 3/36 | — |
| A      | III:3   | 31/F              | +                   | Syncope               | —          | 10.3, 7.2, 43, — | —          | 67 | — | 3/34 | — |
| A      | III:4   | 30/F              | +                   | Syncope               | —          | 8.6, 8, 41, — | —          | 77 | — | 3/33 | — |
| A      | III:5   | 29/F              | +                   | Syncope               | —          | 9.5, 6.5, 42, — | —          | 70 | VP | 3/32 | — |
| B      | I:2     | 73/F              | NA                  | Exertional dyspnoea   | NA | — | — | 71 | NA | 1/74 | — |
| B      | II:2    | NA/M              | NA                  | SD, oedema, hypohidrosis | +/V | NA, NA, NA, NA, NA, NA, NA | NA | NA | — | +/41 |
| B      | II:3    | 43/M              | +                   | Oedema, acroparesthesia, hypohidrosis | +/V | 17, 17, 51, — | — | 71 | NA | 3/46 | — |
| B      | III:1   | 12/F              | +                   | —                     | 7 | 7 | 46, — | 60 | — | 1/13 | — |
| C      | I:2     | NA/F              | NA                  | SD, exertional dyspnoea | NA | NA | NA | NA | NA | NA | — | +/63 |
| C      | II:1    | 63/M              | +                   | Acroparesthesia, ACS  | +/V | 22, 16, 44, 2.2 | — | 67 | VP | 1/64 | — |
| D      | II:1    | 51/M              | +                   | Exertional dyspnoea   | — | 23, 22, 36, 3.9 | — | 58 | VP | 2/53 | — |
| E      | I:2     | NA/F              | NA                  | SD, stroke            | NA | NA | NA | NA | NA | NA | — | +/61 |
| E      | II:1    | NA/M              | NA                  | SD                    | +/V | NA, NA, NA, NA, NA, NA, NA | NA | NA | — | +/53 |
| E      | II:2    | 63/M              | +                   | Exertional dyspnoea, HF, syncope, oedema, acroparesthesia, angiokeratomas | +/V | 15, 15, 48, — | — | 54, 45 (64 years) | VP, AFL/AF, SSS, AVB | — |

ACS, acute coronary syndrome; AFL/AF, atrial flutter/atrial fibrillation; AVB, atrioventricular block; CKD, chronic kidney disease; F, female; FU, follow-up; HF, heart failure; IVS, intra-ventricular septum; LVDd, left ventricular diastolic diameter; LVEF, left ventricular ejection fraction; LVPW, left ventricular posterior wall; M, male; MR, mitral regurgitation; NA, not available; SD, sudden death; SSS, sick sinus syndrome; TR, tricuspid regurgitation; VP, ventricular pre-excitation patterns with short PR intervals, initial QRS slurring (delta waves), or both; VT, ventricular tachycardia.
amorphous vacuoles and central vacuolar degeneration of myocytes called ‘zebra bodies’ (Figure 5(E) and 5(F)).

**Discussion**

This study has several important findings relevant to the clinical management of FD. First, we expanded the phenotypic and genetic spectrum of GLA-related disorders. We identified five GLA mutations in five unrelated families, including three previously reported missense mutations (c.128G > A, c.811G > A, and c.950 T > C), a novel missense mutation (c.37G > C), and a novel deletion mutation (c.1241delT). Although three missense mutations have been reported previously in genetic studies, neither a detailed description of the clinical characteristics nor phenotypes of family members associated with these mutants were available.4,6-9 Second, phenotypic expression of FD was highly heterogeneous in Chinese families. Male patients demonstrated significantly cardiac structural and electrocardiographic abnormalities and renal involvement. Third, female patients were less likely than male patients to develop severe disorders; however, regular cardiac evaluations were still critical because of potential risk of sudden death. Fourth, our study highlighted the importance of genetic screening of the GLA gene for early identification of patients with FD.

The clinical manifestations of our patients demonstrated high heterogeneity even within family members carrying the same GLA mutation. Male patients with FD presented with 100% penetrance in our study, whereas female patients showed incomplete penetrance with variable expressivity from asymptomatic to severe. Five male probands demonstrated a high prevalence of left ventricular hypertrophy (5/5, 100%), whereas six female patients who underwent echocardiography demonstrated normal cardiac structure and function. Electrocardiographic pre-excitation occurred in 80% (4/5) of men and 16.7% (1/6) of women. Six patients (6/14, 42.9%) had reduced renal function and all were men (6/7, 85.7%). The potential mechanism for this phenotypic difference may be associated with the X-linked nature of inheritance and skewed X-chromosome inactivation.10

Of the 17 patients definitely or possibly diagnosed with FD, 3 female patients (subject I/2 in Family A, subject I/2 in Family C, and subject I/2 in Family E) and 2 male patients (subject II/2 in Family B and subject II/1 in Family E) died suddenly from unknown causes and 1 male patient (subject II/2 in Family A) died of progressive heart failure, by 41 to 66 years of age. Although female patients were affected less severely than male patients, our data suggested that FD could also lead to sudden death in female patients, suggestive of the importance of regular cardiology evaluations and long-term follow-up in these patients.

Electrocardiographic abnormalities typically present with short PR intervals and later atrioventricular block in patients with FD.11,12 In our study, electrocardiographic pre-excitation was present in four male probands and one heterozygous female. An electrophysiology study of the proband in Family A ruled out the presence of atrioventricular accessory pathways, which was consistent with published data.13 The previous study indicated that the short PR interval in FD can result from accelerated conduction in the atrioventricular node. However, enhanced atrioventricular node conduction cannot explain the presence of QRS slurring mimicking ventricular pre-excitation. Our recent study on Danon disease, another lysosomal storage disorder, proved that ventricular pre-excitation was because of the presence of fasciculoventricular connections, rather than abnormal atrioventricular bypass tracts or accelerated atrioventricular node conduction.5,14 Both FD and Danon disease are caused by lysosomal dysfunction as a consequence of deficiency of a single enzyme or lysosome-associated membrane protein.15 Therefore, we speculate that a similar mechanism with Danon disease may also be responsible for the electrocardiographic abnormalities in patients with FD. Previous cardiac histopathology of FD verified that all cardiac tissues, including the
conducting system, were involved and contained glycosphingolipid deposits, suggesting of pre-excited ECG caused by fasciculoventricular connections possible because of the disruption of the His bundle and Purkinje fibre insulation. Additionally, the high incidence of progression from short PR intervals to atrioventricular block in patients with FD also supports that the ventricular pre-excitation pattern is related to a completely infranodal connection in accordance with the fasciculoventricular pathway.

Early identification of FD is extremely important for patient care because rapid clinical deterioration leads to cardiac death. Clinical heterogeneity, rarity of the disease, and early cardiac presentation similar to HCM increase the risk of delayed diagnosis of FD. Our cases suggest that extracardiac manifestations, especially renal insufficiency, provide important clues to further distinguish left ventricular hypertrophy associated with GLA mutations from those caused by defects in other disease-causing genes in males.

Limitations and future perspectives

In the present study, not all family members underwent molecular screening for the GLA gene, and seven patients were diagnosed with FD on the basis of their clinical manifestations and X-linked hereditary nature of this disease. Only one proband and one female patient checked their lyso-GL-3 levels in our study. The post-mortem examination was not performed in the cases of sudden death; therefore, a definite relationship between sudden death and FD was not established in these cases. None of the patients received enzyme replacement therapy with recombinant α-Gal A. On the one hand, it was commercially not available before in China. On the other hand, the delayed diagnosis of FD in our probands made them miss the best time for specific therapy of FD cardiomyopathy. Future multicentre or national studies are needed to obtain the clinical and genetic characteristics of FD patients in China as well as their response to specific therapy.
Conclusions

We described five unrelated families with GLA mutations that cause FD. The clinical manifestations were highly heterogeneous even within the same family. Male patients demonstrated a high prevalence of left ventricular hypertrophy and renal dysfunction, whereas female patients showed relatively low risks for structural heart disease and kidney failure. Our study underlines the importance of molecular screening of the GLA gene for early identification and clinical decision making in patients with FD.

Acknowledgements

We are grateful to the family members for their participation in the study.

Conflict of interest

All authors report no conflicts.

References

1. El-Abassi R, Singhal D, England JD. Fabry's disease. J Neurol Sci 2014; 344: 5–19.
2. Brady RO, Gal AE, Bradley RM, Martensson E, Warshaw AL, Laster L. Enzymatic defect in Fabry's disease: ceramidetrihexosidase deficiency. N Engl J Med 1969: 276: 1163–1167.
3. Elliott P, Baker R, Pasquale F, Quarta G, Ebrahim H, Mehta AB, Hughes DA. Prevalence of Anderson-fabry disease in patients with hypertrophic cardiomyopathy: the european Anderson-fabry disease survey. Heart 2011; 97: 1957–1960.
4. Sachdev B, Takenaka T, Teraguchi H, Tei C, Lee P, McKenna WJ, Elliott PM. Prevalence of Anderson-fabry disease in male patients with late onset hypertrophic cardiomyopathy. Circulation 2002; 105: 1407–1411.
5. Liu Y, Chen X, Wang F, Liang Y, Deng H, Liao H, Zhang Q, Zhang B, Zhan X, Fang X, Shehata M, Wang X, Xue Y, Wu S. Prevalence and clinical characteristics of Danon disease among patients with left ventricular hypertrophy and concomitant electrocardiographic preexcitation. Mol Genet Genom Med 2019: e638.
6. Garman SC, Garboczi DN. The molecular defect leading to fabry disease: structure of human α-galactosidase. J Mol Biol 2004; 337: 319–335.
7. Shabbeer J, Yasuda M, Benson SD, Desnick RJ. Fabry disease: identification of 50 novel alpha-galactosidase a mutations causing the classic phenotype and three-dimensional structural analysis of 29 missense mutations. Hum Genomics 2006; 2: 297–309.
8. Shabbeer J, Yasuda M, Luca E, Desnick RJ. Fabry disease: 45 novel mutations in the alpha-galactosidase a gene causing the classical phenotype. Mol Genet Metab 2002; 76: 23–30.
9. Dobrovolsky R, Dvorakova L, Ledvinova J, Magage S, Palecek T, Bultas J. Cardiac involvement in fabry disease. Acta Paediatr Suppl 2002; 91: 15–20.
10. Frustaci A, Morgante D, Russo MA, Scopolletti F, Grande C, Verardo R, Franciosa P, Chimienti C. Pathology and function of conduction tissue in fabry disease cardiomyopathy. Circ Arrhythm Electrophysiol 2015; 8: 799–805.
11. Jastrzebski M, Bacior B, Dimitrow PP, Kavecka-Jaszcz K. Electrophysiological study in a patient with fabry disease and a short PQ interval. Europace 2006; 8: 1045–1047.
12. Liu Y, Wang F, Chen X, Liang Y, Deng H, Liao H, Rao F, Wei W, Zhang Q, Zhang B, Zhan X, Fang X, Nair S, Shehata M, Wang X, Xue Y, Wu S. Right ventricular pathways responsible for ventricular preexcitation in patients with Danon Disease. Circulation 2019; 114: 6704.
13. Nair V, Belanger EC, Veinot JP. Lysosomal storage disorders affecting the heart: a review. Cardiovasc Pathol 2019; 28: 12–24.
14. Becker AE, Schoorl R, Balk AG, van der Heide RM. Cardiac manifestations of Fabry's disease. report of a case with mirtal insufficiency and

Funding

Dr. Wu receives research grants from the Medical Science and Technology Foundation of Guangdong Province (No. 2019B020230004). Dr. Liu (Y.L.) receives research grants from the National Natural Science Foundation of China (No. 81970288).

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Tracings obtained from the electrophysiological study of the proband in Family A. Atrial pacing demonstrated no change in the degree of ventricular pre-excitation and QRS morphologies, suggestive of antegrade conduction via atrioventricular node rather than atrioventricular accessory pathway. Ventricular pacing demonstrated 2:1 and concentric VA conduction.
electrocardiographic evidence of myocardial infarction. *Am J Cardiol* 1975; 36: 829–835.

17. Baig S, Edward NC, Kotecha D, Liu B, Nordin S, Kozor R, Moon JC, Geberhiwot T, Steeds RP. Ventricular arrhythmia and sudden cardiac death in Fabry disease: a systematic review of risk factors in clinical practice. *Europace* 2018; 20: f153–f161.

18. Shah JS, Hughes DA, Sachdev B, Tome M, Ward D, Lee P, Mehta AB, Elliott PM. Prevalence and clinical significance of cardiac arrhythmia in Anderson-Fabry disease. *Am J Cardiol* 2005; 96: 842–846.