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Prevalence of Fabry Disease in Chinese Patients with Hypertrophic Cardiomyopathy*

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ABSTRACT Objective: To determine the prevalence and clinical manifestations of α-galactosidase A (GLA)mutations in chinese patients with hypertrophic cardiomyopathy (HCM). **Methods:** GLA gene was sequenced in 439 patients with HCM and 156 healthy controls. Genotype and phenotype correlation was analyzed in patients with GLA mutations. **Results:** A total of 2 pathogenic mutations in GLA gene, including 1 splicing (c.547+1G>C) and 1 missense (E66Q), was identified in 2 (0.45%) of 439 patients with HCM. All these mutations were lack in 156 healthy controls and not reported in either 1000 Genomes or Exome Sequencing Project. Bioinformatics analysis showed these mutations had damaging effect on GLA protein or destroyed the existing splicing site. E66Q were known mutations, whereas c.547+1G>C were novel. No mutation in sarcomere genes was identified in the 2 patients with GLA mutations, indicating these mutations of GLA were independent causes of cardiac hypertrophy. One neutral rare variant (V256A) was also detected in 1 female patient, who also carried a R671C mutation in MYH7. Various clinical manifestations were expressed in patients with GLA mutations, including, dyspnea in 1 patients, chest pain in 2 patients. The maximum left ventricle wall thickness was 15mm and 25mm. Resting obstruction of left ventricular outflow tract were observed in 1 patiets. Thus, the clinical manifestations of patients with GLA mutation were not distinct from the typical HCM. **Conclusions:** The prevalence of fabry disease in Chinese patients with HCM was 0.45%. Genetic testing is helpful in differentiation of the two diseases.

Key words: Hypertrophic Cardiomyopathy; Fabry Disease; Prevalence; Mutation

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Hypertrophic cardiomyopathy (HCM) is a autosomal dominant genetic disease characterized by unexplained left ventricular hypertrophy associated with nondilated ventricular chambers in the absence of another cardiac or systemic disease [1]. More than 1400 mutations in genes coding sarcomeric proteins have been identified to cause HCM, but those mutations only contribute to 50%~70% of the patients with HCM [2]. Fabry disease is an X-linked lysosomal storage disease caused by mutations in the α-Galactosidase A (GLA) gene. GLA mutations lead to lysosomal enzye α-Galactosidase A deficiency[3], result in the abnormal accumulation of glycosphingolipids and thereby cause life-threatening clinical manifestations, including renal dysfunction, stroke and left ventricular hypertrophy[4]. Many studies have shown that fabry disease may only affect cardiac tissues and mimic the phenotypic expression of HCM, which also is called phenocopy of HCM[5,6]. The prevalence of fabry disease in European and American patients with HCM ranged from 0% to 12%[3,4,9-14], but still unknown in Chinese patients.

The aims of this study were to determine the prevalence and clinical characteristics of fabry disease using exon sequencing for genetic testing in Chinese patients with HCM.

1 Materials and methods

1.1 Subjects

A total of 439 unrelated HCM patients were enrolled at Beijing Fuwai Hospital, Chinese Academy of Medical Sciences. HCM was diagnosed by an unexplained maximal left ventricular wall thickness ≥ 15 mm on echocardiography and/or cardiac magnetic resonance imaging in the absence of other cardiac or systemic diseases capable of producing that magnitude of hypertrophy.

In order to exclude common polymorphisms and neutral variants, 156 age, sex and ethnically matched healthy controls were also included for genetic testing. No cardiac or other systemic diseases were found in all of the controls by physical examination, 12-lead electrocardiogram and echocardiography.

This study was performed in accordance with the principle of the Declaration of Helsinki and approved by the Ethics Committee of Fuwai Hospital. Written informed consent was provided by all participants.

1.2 GLA gene sequencing

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Genomic DNA was extracted from peripheral blood leukocytes[7]. GLA gene was comprehensively sequenced with multiplexing targeted sequencing. All coding exons and their adjacent 5 bp intronic sequences of GLA were captured using a custom designed probe library (Agilent Technologies, Santa Clara, CA, USA) and sequenced on an Illumina GAIIx (Illumina Inc, CA, USA) to generate pair-end reads of 120 bp at each end. PCR duplications were removed by PICARD, and sequence alignment and variant calling were performed with CLC Genomics Workbench (CLC-bio, Aarhus, Denmark). Sequencing reads from each individual were mapped to the human genome (GRCh37/hg19). The coverage of GLA gene was analyzed and variants were called using the following filter parameters: coverage ≥25× and variant frequency of at least 20%.

1.3 Pathogenic determination

Common polymorphisms and potential neutral variants were firstly excluded by the presence in 156 healthy controls or with a minor allele frequency ≥ 0.5% in either the 1000 genomes project (release 20100804) or Exome Variant Server (NHLBI GO Exome Sequencing Project (ESP), Seattle, WA,http://eversusgs.washington.edu/EVS/ June 2013). The rest variants were considered to be pathogenic if fulfill any one of the following 4 criteria: 1) known mutation reported to cause fabry disease, 2) nonsense variants resulting truncated protein expression, 3) novel missense variants with damaging effect predicted by either PolyPhen-2 or SIFT, and 4) novel intronic variants destroying wild-type splice site shown by Human Splicing Finder. Novel variant was defined as absent in Human Gene Mutation Database (accessed in May of 2013). All of the pathogenic variants identified in the study were validated by bidirectional Sanger capillary sequencing.

1.4 Sarcomere genes analysis

Eight sarcomere protein encoding genes, including MYH7, MYBPC3, TNNT2, TNNI3, MYL2, MYL3, TPM1 and ACTC1, were screened previous using the same manner as GLA sequencing. The coexistence of mutations in sarcomere and GLA genes was analyzed.

2 Results

2.1 HCM cohorts

439 patients were screened in this study, included 299men and 140wowen. Among the 439 patients, the age of onset range from 0.5 y to 83.0y (43.6± 14.9y), and from 0.5y to 83.0y(43.4± 14.9y) in 299 male patients and from 2.0y to 83.0y (44.0± 16.4y) in 140 female patients. Echocardiography show that LVEDD range from 28.0mm to 65.0mm (45.1± 6.1mm), and LVEF range from 20% to 88% (66.5%± 9.3), and left atrial diameter range from 18.0mm to 71.0mm (39.9± 6.9mm). The Maximum wall thickness range from 8mm to 44mm(21.4± 5.1mm)in all patients, from 12.0mm to 44.0mm (21.6± 5.3mm) in male patients, from 8.0mm to 33.0mm (21.0± 5.3mm) in female patients. Heart function of patients show lever Ⅰ in 216 patients, lever Ⅱ in 166 patients, lever Ⅲ in 46 patients and lever Ⅳ in 11 patients.Of these 439 patients show 168 patients with obstruction, 103 patients with abnormal Q waves, 300 patients with abnormal T waves, 75 patients with family history.

2.2 GLA mutations

Table 1 Identified mutations of GLA gene in patients diagnosed as HCM.

cDNA	Protein	Type	Status	1000G	ESP	PP2*	SIFT+	HSF	Pathogenic+	Sarcomere mutations
c.196G>C	E66Q	MS	Known	0	0	Pro (0.996)	D (0.02)	-	yes	w/o
c.547+1G>T	-	SP	Novel	0	0	-	-	-33.5	yes	w/o
c.767T>C	V256A	MS	Novel	0	0	Ben (0.002)	T (0.73)	-	no	MYH7, R671C

Note: MS,mission;SP, splicing;1000G, 1000 Genomes ;ESP, Exome Sequencing Project; PP2*, PolyPhen-2P;HSF, Human Splicing Finder

A total of 1 splicing (c.547+1G>T) and 2 nonsynonymous variants of GLA gene, including E66Q and V256A, were identified in 3 patients (table 1 and Fig 1). All of these variants were individual and rare. None of them was reported in 1000 Genomes and Exome Sequencing Project, or detected in the 156 healthy controls. E66Q were known fabry disease-causing mutations [8], whereas V256A and c.547+1G>T were novel. HSF showed c.547+1G>T variants destroyed the splicing donor site of the 3rd exon. Both PolyPhen-2 and SIFT showed V256A had neutral effect on the GLA protein. Thus, c.547+1G>T were considered as novel pathogenic mutations of fabry disease, while V256A was considered as a benign rare variant. Consistently, E66Q, V256A and c.547+1G>T, but not V256A, were highly conserved cross different species (Fig 2). Furthermore, no mutations in 8 sarcomere genes were identified in the 2 patients with pathogenic GLA

mutations, while a R671C mutation in MYH7 gene was found in the patient with V256A of GLA gene.

2.3 Clinical manifestations of patients with GLA mutations

The clinical characteristics of these 3 patients are described in Table 2. In 2 FD patients, included 1 men and 1 women ,the age of onset was 34y and 37y, respectively ,and the maximal end-diastolic left ventricular wall thickness show 21 mm and 25mm. Left ventricular ejection are normal in the whole 2 patients. Various clinical manifestations were expressed included, dyspnea in 1 patient, chest pain in 2 patients. Thus, the clinical expression of patients with GLA mutations were not distinct from the typical HCM in Chinese population.

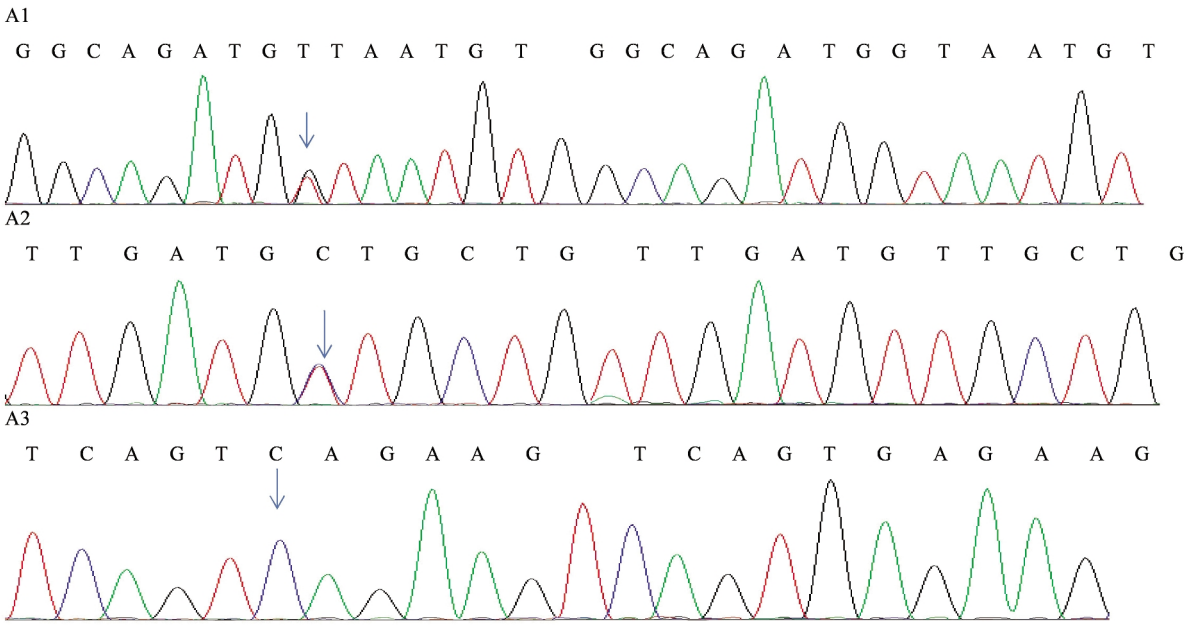

Fig. 1 The result of sequence :c.547+1G>T(A1),V256A(A2) and E66Q(A3)

Table 2 Clinical characteristics of patients with GLA mutations

patient	Age	gender	mutation	LVEF (%)	MLVWT (mm)	LVOT Obstructing (mmHg)	Abnormal Q wave	Abnormal T wave	dyspnea	chest pain	syncope
1	23	F	c.767T>C	63	15	-	-	-	-	-	+
2	34	F	c.547+1G>T	67	21	-	-	+	-	+	-
3	37	M	c.196G>C	75	25	+	+	-	+	+	-

Note: LVEF, left ventricular ejection fraction; MLVWT, maximal end-diastolic left ventricular wall thickness; LVOT, left ventricular outflow tract.-, no; +, yes.

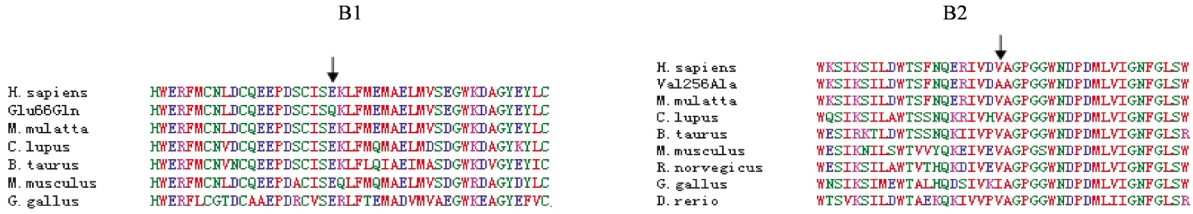

Fig. 2 Conservation was determined using Clustal W2.

Note: The E66Q(B1) and V256A(B2) substitution involves an amino acid that is highly conserved among species, respectively

3 Discussion

To the best of our knowledge, this was the first study to determine the prevalence of fabry disease in Chinese patient diagnosed as HCM. In present study, pathogenic mutations of GLA gee were identified in 2 patients, with a prevalence of 0.45%. Our data suggest the frequency of fabry disease was relatively low in Chinese HCM patients.

GLA mutations are responsible for the deficiency of activity of the enzyme α-galactosidase A in patients with fabry disease. The deficiency of enzyme activity lead

to the abnormol accumulation of neutral glycosphingolipids, mainly globotriaosylceramide , in various organ systems[3]. Patients with fabry disease show a variety of progressive clinical manifestations, included skin, renal, cardiac, cerebrovascular disease [4]. There are also atypical forms with late-onset isolated renal or cardiac manifestations, 4especially cardiac involvement often express LVH resemble HCM[5,6].

Various screening methods have been used to evaluate the prevalence of fabry disease in American and European with the presence of LVH. The measurement of plasma α-galactosidase activity was used to diagnose fabry disease in the majority of study. In several early study, a high prevalence was determined in a small number of males patients. By the measurement of plasma α-galactosidase activity, Nakao et al found 7 patients present with low plasma α-galactosidase activity in 230 consecutive male patients with left ventricular [9], and Sachdev et al found 5 low plasma α-galactosidase activity patients in 153 male patients [4],A similar 3% prevalence was determined in two study. But genetic analysis of GLA only found 2 mutations was harbored in 2 patients in for-

mer study. Of these 5 patients, 4 patients diagnosed at ⩾40 y in the latter study. Of course, a high prevalence was also determined in a small number of female patients.4 patients was diagnosed with fabry disease in 34 women HCM patients underwent endomyocardial biopsy. Genetic analysis showed the presence of -Gal A gene mutation in all 4 cases. 12% prevalence was determined in female HCM patients[10]. To the contrary, two studies showed none of fabry disease patients was found in HCM patients. Electron microscopy examination of myectomy specimens was performed in 100 hypertrophic obstructive cardiomyopathy underwent subaortic ventricular septal myectomy. But Ommen et al failed to found nobody with myeloid figures characteristic of fabry disease. Similarly [11], though Arad et al analyses the sequence of GLA gene of 75 consecutive unrelated patients with hypertrophic cardiomyopathy, no variant in GLA gene was found[12]. Recent year, the evaluation of prevalence of fabry disease with LVH in a large number of patients was carried out 1 % prevalence was determined by the measurement of Plasma α-galactosidase activity or sequencing analysis in both genders. Monserrat et al found low plasma activity in 15 of 508 unrelated patients with HCM patients. Of these 15 patients , genetic analysis showed the presence of mutation in 5patients included 3men and 2 women [13]. Similar prevalence was determined by dried blood spot using a filter paper test in 392 patients with hypertrophic cardiomyopathy, and 9 male patients had a low plasma activity and no fabry disease was diagnosed among women. Of these 9 male patients, only 4 patients harbored missense mutation[14].

A lower 0.5% prevalence was determined only by direct sequencing of GLA. Elliott et al found 7 patient harbored pathogenic α-galactosidase A mutations in 1386 European patients with HCM[3].

0% to 12% prevalence was showed in those studies. The difference of both diagnosis criteria and study population lead to the controversial. In our study 0.45% prevalence was determined, resembled the screening result of the large number of patients. In this study, the age distribution of study population was wider, and both genders was included. Though fabry disease was not found in children in this study, Havranek et al found 3 younger patients with LVH in 22 children with fabry disease [15]. So children with HCM should not excluded.

LVH caused by fabry disease was mimic the phenotypic expression of HCM. It was difficult to differentiate between fabry disease and HCM by physical examination, imaging examination or ECG [16]. Patients are often misdiagnosed as HCM and receive inappropriate treatment. Study showed that the treatment of ERT may alter the natural course of the disease and reduced morbidity and mortality [17,18]. It is of great important to make the differential diagnoses between HCM and fabry disease. Genetic testing is helpful in differentiation of the two diseases.

The deficiency was the lack of the measurement of plasma α-galactosidase activity. It is generally believed that the lever of plasma α-galactosidase activity was normal in the most female patients with fabry disease, and a low GLA activity in male with fabry disease[19]. So plasma α-galactosidase activity should be measured in male patients, though some of study shown screening using plasma will fail to detect some patients with fabry disease[20].

4 Conclusions

The prevalence of fabry disease in Chinese patients with HCM was 0.45%. Genetic testing is helpful in differentiation of the two diseases.

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Fabry 病在中国汉族肥厚型心肌病人群的患病率 *

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摘要 目的：研究中国汉族肥厚型心肌病人群中α-Galactosidase A 突变的患病率及其临床表现。**方法：**对 439 名肥厚型心肌病患者及 156 名健康对照 GLA 基因进行全外显子测序，及基因型及临床表型进行关联分析。**结果：**确定了 2 个致病性突变，包括 1 个错义突变 E66Q 和 1 个剪接位点的突变 c.547+1G>C。2 个突变在 156 名健康人群未发现，在 1000 人基因组计划中未报道。确定中国汉族肥厚型心肌病人群中 α-Galactosidase A 突变 0.45%的患病率。**结论：**Fabry 病在中国汉族肥厚型心肌病人群中 α-Galactosidase A 突变的患病率较低。基因检测有助于 Fabry 病与肥厚型心肌病的鉴别诊断。

关键词：肥厚型心肌病；Fabry 病；患病率；突变

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