BMC Medical Genomics

RESEARCH

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Successful experience with high-risk and family screening for Fabry disease in Ninghai County, Zhejiang Province, Eastern China: genotype–phenotype analysis of the *GLA* IVS4 + 919G > A variant

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Abstract

Background Fabry disease is a rare and non-specific disease that is difficult and expensive to diagnose. This study aimed to investigate the clinical phenotypes and genetic characteristics of patients with Fabry disease characterised by the *GLA* IVS4 + 919G > A variant in China through a proposed pilot program integrating high-risk and family screening.

Methods The 31-months-long pilot program assessed high-risk screening for Fabry disease in 388 patients by integrating previous screening methods that measure their dry blood spot (DBS) α-galactosidase A (α-GAL) activity, globotriaosylsphingosine (Lyso-GL-3), and *GLA* gene sequence at Ninghai First Hospital. Patients whose dried blood spot (DBS) α-GAL enzyme activity was low (< 2.40 μmol·L^{-1}·h^{-1}) or whose Lyso-GL-3 level was high (> 1.10 ng/mL) underwent GLA genetic testing for diagnostic confirmation. Gender-specific family screening and evaluation was carried out on the proband, and the clinical and genetic characteristics of Fabry disease characterised by the *GLA* IVS4 + 919G > A variant were summarised.

Results A yield of Fabry disease diagnosis of 1.80% (7/388) was achieved, which is much larger than have been previously reported. These diagnoses include a 9.8-year-old girl, who was screened because of a high-risk profile of severe pain in the extremities, as well as 6 males, who were diagnosed with Fabry disease and who were screened because of unexplained left ventricular hypertrophy. All 7 diagnosed patients were carriers of the *GLA* IVS4 + 919G > A variant. Family screening of 7 probands revealed that 18 family members carried pathogenic variants, resulting in a diagnosis rate of 6.44% (25/388); 13 were clinically affected, 2 were asymptomatic carriers, and 3 declined further clinical assessment. The 25 patients had multiple affected organs and systems included the heart (60.00%), peripheral nerves (16.00%), kidney (36.00%), eye (20.00%), brain (12.00%), and gastrointestinal tract (24.00%).

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Conclusions Screening high-risk populations and family screening is critical for early diagnosis and timely intervention in patients with Fabry disease. The *GLA* IVS4 + 919G > A variant is associated with diverse phenotypes of Fabry disease and is highly prevalent in late-onset cases in Ninghai County, Zhejiang Province, Eastern China.

Keywords Fabry disease, High-risk screening, Family screening, Early diagnosis, *GLA* gene variant, IVS4 + 919G > A

Introduction

Fabry disease (OMIM #301500) is a rare, X-linked lysosomal storage disorder caused by pathogenic variants in the *GLA* gene, resulting in a deficiency in α-Gal A activity. This deficiency leads to the progressive accumulation of globotriaosylceramide (GL-3) and globotriaosylsphingosine (Lyso-GL-3) in cells and multiple organ systems throughout the body [1, 2], causing multiorgan pathology resulting in substantial mortality and a reduced life expectancy [3]. The clinical spectrum ranges from classic early-onset to atypical late-onset phenotypes. The clinical manifestations include renal insufficiency of unknown etiology, acroparesthesia, unexplained left ventricular hypertrophy (LVH), cardiac arrhythmia, unexplained stroke, angiokeratomas, sweating abnormalities, cornea verticillata, lenticular opacities, and unexplained gastrointestinal symptoms [1]. Owing to skewed X chromosome inactivation, heterozygous females generally present with milder clinical manifestations and a delayed age of onset relative to those of hemizygous males [1, 2].

The early symptoms of patients with Fabry disease are nonspecific. Therefore, early diagnosis remains a clinical challenge. To enhance the early diagnosis of Fabry disease, some countries have systematically conducted screening used for high-risk, newborn, and genetic screening procedures [2, 4]. This study was designed to combine high-risk and family screening to increase the yield of diagnoses of Fabry disease presenting with different but relevant phenotypic traits (isolated or combined).

Methods

Patient characteristics

This study involved collaboration across thirteen hospitals in Zhejiang province. From October 2021 to May 2024, a total of 388 individuals (233 males and 155 females; median age, 46.5 years) at Ninghai First Hospital were enrolled. The patient group included 165 patients with unexplained chronic kidney disease, 58 patients with unexplained stroke, 114 patients with unexplained cardiovascular diseases, 38 patients with acroparesthesia, 4 patients with clustered angiokeratoma, 5 patients with hypo-anhidrosis, 1 patient with cornea verticillata and lenticular opacities, and 3 patients with abdominal pain or diarrhea of unknown etiology.

The inclusion criteria for the patient group were: (a) unexplained chronic kidney disease (including unexplained proteinuria or haematuria); (b) unexplained stroke; (c) unexplained cardiovascular diseases (unexplained LVH or cardiac failure); (d) acroparesthesias (periodic crises of severe pain in the extremities); (e) clustered angiokeratoma; (f) hypo-anhidrosis; (g) cornea verticillata and lenticular opacities; and (h) abdominal pain or diarrhoea of unknown aetiology [2].

Experimental design

DBS samples of all participants were analysed at Ninghai First Hospital. We carried out gender-specific screening, as suggested by previous studies [2, 3]. For males, the level of α-Gal A determined below normal (< 2.40 $\mu mol \cdot L^{-1} \cdot h^{-1}$) was followed by long-range PCR and sequencing of the *GLA* gene, and the level of lyso-GL-3 was tested. For female adults, a level of lyso-GL-3 above normal (> 1.10 ng/mL) was tested via long-range PCR, the *GLA* of the gene was sequenced, and the level of α-Gal A was determined. For female children, a level of α-Gal A determined below normal or a level of lyso-GL-3 tested above normal was followed by long-range PCR and sequencing of the *GLA* gene. Family screening, including genetic testing, enzymatic activity measurement of the level of α-Gal A and the measurement of Lyso-GL-3, was offered to all the relatives of the probands. Patients who did not agree to undergo measurement of α-Gal A activity, Lyso-GL-3 and genetic analysis were excluded. This study was approved by the Ethical Committee of Ninghai First Hospital, Zhejiang Province, China (No. Nhyy-IEC-KY202304).

Enzymatic activity measurement of α-Gal A

The NeoLSD MSMS kit was used for the quantitative measurement of the activity of the enzyme GLA in dried blood spots (DSSs). A 3.2 mm (1/8 in) DBS was incubated in the buffer solution containing the internal standard and substrate provided by the kit at 37 °C for 18.5 h. Then, water and NeoLSD Extraction Solution were added for extraction. Fifty microliters of the supernatant was transferred to a new well and dried with nitrogen. One hundred microliters of flow solvent was added to the well before it was run on a Waters UPLC/Xevo TQD MS/MS system. Data were obtained by using the multiple reaction monitoring (MRM) mode of the tandem mass spectrometry (MSMS) system. The enzymatic activity could be measured according to the signal response of the target analyte relative to the internal standard.

Measurement of Lyso-GL-3

An HPLC–MS/MS instrument was used for the quantitative measurement of Lyso-GL-3 in DBSs. Two 3.2 mm (1/8 in) DBSs were incubated in buffer solution containing an internal standard. The supernatant was transferred to a new well before being run on the HPLC–MS/MS system. Data were obtained by using the multiple reaction monitoring (MRM) mode of the tandem mass spectrometry (MSMS) system. The standard curve was generated via data fitting of the standard. The x-axis represents the ratio of the peak area of the target analyte to that of the internal standard, and the y-axis represents the concentration. The concentration of the target analyte in the sample can be obtained by inputting the ratio of the peak area into the regression equation of the standard curve.

Genetic test of *GLA*

A sufficient amount of genomic DNA was extracted from dry blood through lysis solution and ground. The whole-genome sequence of the *GLA* gene was amplified via long-range PCR. The purified amplicons were processed to generate paired-end libraries and then sequenced on an Illumina NovaSeq platform. The data were processed and analysed to identify related variants.

Data analysis and statistics

SPSS 22.0 software was used for statistical processing and descriptive analysis, including the mean ± standard deviation (SD), median and interquartile range (IQR). We compared changes in plasma α-Gal A activity and Lyso-GL-3 between patients with Fabry disease with normal controls, respectively for each gender. Mann-Whitney U tests were used for comparison between two unpaired groups, as the data were not normally distributed. Differences were considered to be statistically significant when the p value was < 0.05.

Results

Measurement of α-Gal A activity and Lyso-GL-3

We measured α-Gal A activity and Lyso-GL-3 via a DBS test. α-Gal A activity values < 2.40 μmol/(L·h) were observed in 33 patients (25 males and 8 females), Lyso-GL-3 values > 1.10 ng/mL were observed in 3 patients (3 females), α-Gal A activity values were low, and Lyso-GL-3 values increased in 17 patients (14 males and 3 females).

Genetic tests and assays

By sequencing the full-length sequence of the *GLA* gene, including the exon coding region and intron region, the *GLA* variant IVS4 + 919G > A was detected in 13 hemizygous males and 12 heterozygous females, including 7 probands, who were diagnosed with Fabry disease. According to the interpretation guidelines of the American Society of Medical Genetics and Genomics, this variant was rated as the pathogenic variant [5–7].

Clinical characteristics of patients with the pathogenic *GLA* variant

Through high-risk screening, we identified 7 probands with Fabry disease. Subsequent family screening of these probands revealed 18 family members carrying pathogenic variants; 13 were clinically affected, 2 were asymptomatic carriers, and 3 declined further clinical assessment. The clinical characteristics of the 25 patients (13 males and 12 females) carrying the pathogenic *GLA* variant IVS4 + 919G > A are summarised in Tables 1 and 2; the affected organs and systems included the kidney (36.00%), brain (12.00%), heart (60.00%), peripheral nerves (16.00%), eye (20.00%), gastrointestinal tract (24.0%), and hypertension (8.00%).

Among 25 patients (average age, 51.16 ± 21.52 years), α-Gal A activity was within the normal reference range (2 females), α-Gal A activity was significantly reduced in 5 patients, falling below the standard reference (1 male and 4 females), Lyso-GL-3 levels were abnormally elevated in one patient (1 female), and both α-Gal A activity and Lyso-GL-3 were abnormally altered in 17 patients (11 males and 6 females). In our cohort, there were extremely significant differences in α-Gal A activity and Lyso-GL-3 between patients with Fabry disease and patients without Fabry disease of the same gender ($P < 0.05$) in Tables 3.

Proband 1 was a 9-year-old girl (A-III-10) with pain in both lower limbs, echocardiogram indicated that the left cardiac mass index was 27 ($g/m^{2.7}$) and cardiovascular magnetic resonance imaging revealed that the ventricular septum was thick. The α-Gal A activity was below the normal range, Lyso-GL-3 was within the normal range, and genetic analysis revealed a heterozygous pathogenic variant in *GLA* IVS4 + 919G > A, which has been reported previously [1]. Through family screening, 4 family members (proband's father, elder sister, elder male cousin and elder female cousin) were diagnosed with Fabry disease, the proband′s father complained of the knee joint pain, echocardiogram indicated that the left cardiac mass index is above the upper limit of normal, suggesting left ventricular hypertrophy, the male cousin of proband 1, a boy aged 13-year-old had a history of intermittent pain in both lower extremities, Echocardiogram showed no obvious abnormalities and he refused to undergo cardiovascular magnetic resonance imaging, the proband′s elder sister had decreased vision, and a family member (proband's fifth aunt) carried the *GLA* variant with no symptoms. The age at diagnosis ranged from 9.0 years old to 50.0 years old in this Fabry disease family (Fig. 1A).

Proband 2 was a 55-year-old male (B-II-11) with LVH, acroparesthesia, and gastrointestinal disorders,

Table 1 Clinical characteristics of patients with the GLA IVS4+919G>A variant

Pt	Family tree No	Gender	Age years	α-Gal A (μmol·L^{-1}·h^{-1})	Lyso-GL-3 (ng/mL)	Comorbidity
1	A-III-10	F	9	1.09	0.47	LVH, Acroparesthesias, Bile reflux gastritis
2	A-III-9	F	19	2.33	0.90	Decreased vision
3	A-Ⅱ-11	M	50	0.71	3.06	LVH, Acroparesthesias
4	A-Ⅱ-10	F	47	3.84	0.74	Decreased vision
5	A-III-7	M	13	0.87	0.78	Acroparesthesias
6	A-III-8	F	22	1.60	0.66	No symptoms
7	B-Ⅱ-11	M	55	0.74	3.98	LVH, CAD, HUA, Chronic gastritis, Acroparesthesias, CKD
8	B-Ⅱ-1	M	61	0.93	3.65	LVH, HUA, Conjunctival vessel tortuosity, CKD
9	B-Ⅱ-9	M	72	0.72	3.79	LVH, Cerebral ischemic stroke, CKD
10	B-Ⅱ-5	M	72	0.57	4.99	LVH, CKD
11	B-Ⅱ-13	F	66	1.28	1.58-	No symptoms
12	B-Ⅲ-14	F	40	1.69	1.13	No symptoms
13	B-Ⅲ-2	F	33	1.45	1.13	No symptoms
14	B-Ⅳ-4	F	18	2.11	1.13	No symptoms
15	C-Ⅰ-1	M	78	0.77	4.31	LVH, CAD, COPD, Strock, Arrhythmia（PVC）, Hypertension, Gastroduodenal ulcer, CKD
16	C-Ⅱ-9	F	56	2.05	1.12	Conjunctival vessel tortuosity, Arrhythmia (Sinus bradycardia)
17	C-Ⅱ-11	F	60	3.52	0.40	Conjunctival vessel tortuosity, Arrhythmia (Sinus bradycardia)
18	D-Ⅱ-3	M	66	0.95	13.9	LVH, Pacemaker（Third-degree atrioventricular block）, Hypertension, Duodenal bulb ulcer, CKD
19	E-Ⅱ-3	M	68	0.49	8.25	LVH, Pacemaker（Third-degree atrioventricular block）, CKD
20	E-III-1	M	45	0.69	4.79	CAD, Arrhythmia (Complete right bundle branch block)
21	E-Ⅱ-4	F	70	0.67	0.36	No symptoms
22	F-Ⅱ-6	M	74	0.42	5.57	LVH, Cerebral ischemic stroke, CAD, HUA, Gastrectomy for gastric cancer, Chronic gastritis, CKD
23	F-Ⅲ-1	F	44	3.15	4.10	Gastroduodenal ulcer
24	G-Ⅱ-1	M	80	1.20	5.30	LVH, Pacemaker（Third-degree atrioventricular block）, CAD, HUA, CKD
25	G-Ⅱ-7	M	61	1.01	6.05	LVH, Arrhythmia (Sinus bradycardia), COPD, stroke

F Female, *M* Male α-Gal A activity, normal range（2.40~17.65）μmol·L-1·h-1, Lyso-GL-3, normal range（<1.10 ng·mL-1), *LVH* Left ventricular hypertrophy, *CAD* Coronary atherosclerotic heart disease, *HUA* hyperuricemia, *CKD* Chronic kidney disease, *COPD* Chronic obstructive pulmonary disease, *PVC* Premature ventricular complex

echocardiogram indicated left ventricular hypertrophy. The level of α-Gal A was markedly low, and genetic analysis revealed a hemizygous pathogenic variant in *GLA* IVS4 + 919G > A. Family screening for Fabry disease led to a diagnosis of 3 elder brothers, one elder sister (B-II-13), 2 nieces and one granddaughter (B-Ⅳ-4). The age at diagnosis ranged from 18.0 years old to 72.0 years old in this Fabry disease family (Fig. 1B).

Proband 3 was a 78-year-old male (C-Ⅰ-1) with LVH, ischemic stroke, acroparesthesia, kidney disease, and gastrointestinal disorders, and he also suffered from hypertensive disease. The level of α-Gal A was markedly low, Lyso-GL-3 was above the normal range, and genetic analysis revealed a hemizygous pathogenic variant in *GLA* IVS4 + 919G > A. Family screening identified the *GLA* pathogenic variant in the proband's elder daughter (diagnosed with Fabry disease) and younger daughter (an asymptomatic carrier). The age at diagnosis ranged from 56.0 years to 78.0 years (Fig. 1C).

Proband 4 was a 66-year-old male (D-Ⅱ-3) with LVH, hypertensive disease, and gastrointestinal disorders; he also experienced acute irreversible visual loss of his left eye due to ophthalmic artery occlusion and underwent pacemaker implantation. The level of α-Gal A was low, Lyso-GL-3 was above normal, and genetic analysis revealed a hemizygous pathogenic variant in *GLA* IVS4 + 919G > A. The age at diagnosis was 66.0 years (Fig. 1D).

Proband 5 was a 68-year-old male (E-Ⅱ-3) with LVH, kidney disease and gastrointestinal disorders, and he also underwent pacemaker implantation. The level of α-Gal A was markedly low, and that of Lyso-GL-3 was above normal. Genetic analysis revealed a hemizygous pathogenic variant in *GLA* IVS4 + 919G > A. Since Fabry disease is inherited in an X-linked manner, the sons of proband 5 are expected to be normal. However, the proband′s son strongly urged family screening because of coronary atherosclerotic heart disease (CAD) and arrhythmia (complete right bundle branch block); subsequently, genetic analysis revealed *GLA* IVS4 + 919G > A in the proband′s son(E-Ⅲ-1)and the proband′s wife (E-Ⅱ-4). Thus, the proband′s son inherited it from his mother (the proband's

Table 2 Clinical evaluation of cardiac status and renal function in patients with the GLA IVS4+919G>A variant

Pt	Family tree No	Gender	Age years	Cardiac biomarkers		Echocardiography				renal function	
				CTnI	BNP	IVST	LVPWT	LVEF	LVMI	Scr	GFR
				(μg/L)	(ng/L)	(mm)	(mm)	(%)	(g/m2.7)	(μmol/L)	(ml/min)
1	A-III-10	F	9	<0.100	141	5.4	4.9	72	27.3	34	187
2	A-III-9	F	19	<0.100	68	8	7	72	29.4	NA	NA
3	A-Ⅱ-11	M	50	<0.100	320	12	11	68	48.5	63	109
4	A-Ⅱ-10	F	47	<0.100	89	10	8	66	44.5	NA	NA
5	A-III-7	M	13	<0.100	56	6	6	70	16.5	NA	NA
6	A-III-8	F	22	<0.100	70	8	7	69	27.9	NA	NA
7	B-Ⅱ-11	M	55	0.459	1290	20	17	56	86.0	99	73.58
8	B-Ⅱ-1	M	61	<0.100	16	12	12	61	43.6	82	88.59
9	B-Ⅱ-9	M	72	<0.100	512	14	13	66	68.1	80	84.48
10	B-Ⅱ-5	M	72	<0.100	173	12	12	54	63.6	84	79.64
11	B-Ⅱ-13	F	66	<0.100	77	10	11	51	62.0	57	92.76
15	C-Ⅰ-1	M	78	0.151	347	13	12	65	68.2	138	41.6
16	C-Ⅱ-9	F	56	<0.100	479	9	9	67	43.7	63	94.8
17	C-Ⅱ-11	F	60	<0.100	21	9	9	68	40.7	62	95.3
18	D-Ⅱ-3	M	66	0.661	506	21	12	59	82.8	83	83.69
19	E-Ⅱ-3	M	68	<0.100	1006	27	18	55	81.7	80	86.89
20	E-Ⅲ-1	M	45	<0.100	NA	10	10	68	52.5	82	99.12
22	F-Ⅱ-6	M	74	<0.1008	3419	24	9	38	98.4	150	38.96
23	F-Ⅲ-1	F	44	NA	NA	9	8	68	30.9	47	115.36
24	G-Ⅱ-1	M	80	0.273	2745	11	11	46	98.6	112	53.00
25	G-Ⅱ-7	M	61	0.353	517	23	18	64	95.6	74	94.64

F Female, *M* Male, *CTnI* Troponin I（normal range 0.000~0.100 μg·L-1), *BNP* B-type natriuretic peptide（normal range 0~100 pg·ml-1), *IVST* Interventricular septal thickness, *LVPWT* Left ventricular posterior wall thickness, *LVEF* Left ventricular ejection fraction, *LVMI* Left ventricular mass index（left ventricular hypertrophy was defined as LVMI>51 g/m2.7 in males and LVMI>48 g/m2.7 in females), *NA* Not available

Table 3 Baseline α-Gal A and Lyso-GL-3 Data of Males and Females with Fabry Disease

Test	Male		Female	
	n	*median (IQR)*	*n*	*median (IQR)*
α-Gal A (Fabry disease) μmol·L-1·h-1	13	0.74 (0.63, 0.94)	12	1.87 (1.32, 2.95)
α-Gal A (without Fabry disease) μmol·L-1·h-1	220	4.05 (2.94, 5.42)	59	3.96 (3.04, 6.95)
P value	—	<0.05	—	<0.05
Lyso-GL-3 (Fabry disease) ng/mL	13	4.79 (3.72, 5.81	12	1.01 (0.52, 1.13)
Lyso-GL-3 (without Fabry disease) ng/mL	28	0.22 (0.02, 0.43)	143	0.30 (0.04, 0.60)
P value	—	<0.05	—	<0.05

*Probability values refer to Mann-Whitney U tests, significance at $p<0.05$

wife). however, the proband's wife refused further examination. The age at diagnosis ranged from 45.0 years old to 70.0 years old (Fig. 1E).

Proband 6 was a 74-year-old male (F-Ⅱ-6) with LVH, kidney disease, ischaemic stroke and gastrointestinal disorders. The level of α-Gal A was markedly low, Lyso-GL-3 was above standard, and genetic analysis revealed a hemizygous pathogenic variant in *GLA* IVS4 + 919G > A. Family screening indicated that his daughter (F-Ⅲ-1) was diagnosed with Fabry disease. The age at diagnosis ranged from 44.0 years to 74.0 years (Fig. 1F).

Proband 7 was an 80-year-old male (G-Ⅱ-1) with LVH, kidney disease, ischaemic stroke and gastrointestinal disorders. The level of α-Gal A was markedly low, Lyso-GL-3 was above normal, and genetic analysis revealed a hemizygous pathogenic variant in *GLA* IVS4 + 919G > A. Family screening indicated that his brother (G-Ⅱ-7) was diagnosed with Fabry disease. The age at diagnosis ranged from 61.0 years to 80.0 years (Fig. 1G).

Among 25 patients with Fabry disease and 2 patients with the *GLA* IVS4 + 919G > A variant, proband 1 and his father were treated regularly via enzyme replacement therapy (ERT); after 4 months of ERT, the pain in both lower limbs of proband 1 improved, and her father received ERT from September 2023. The levels of Lyso-GL-3 decreased from baseline values of 3.06 ng/mL to 2.20 ng/mL after 4 months of treatment. After the diagnosis of Fabry disease, proband 7 and the brother of proband 7 underwent pretreatment evaluation and were treated with ERT regularly. The levels of Lyso-GL-3 decreased from baseline values of 6.05 ng/mL and 5.30 ng/mL to 4.58 ng/mL and 5.07 ng/mL, respectively. Other patients have not initiated ERT and only receive different degrees of symptomatic treatment.

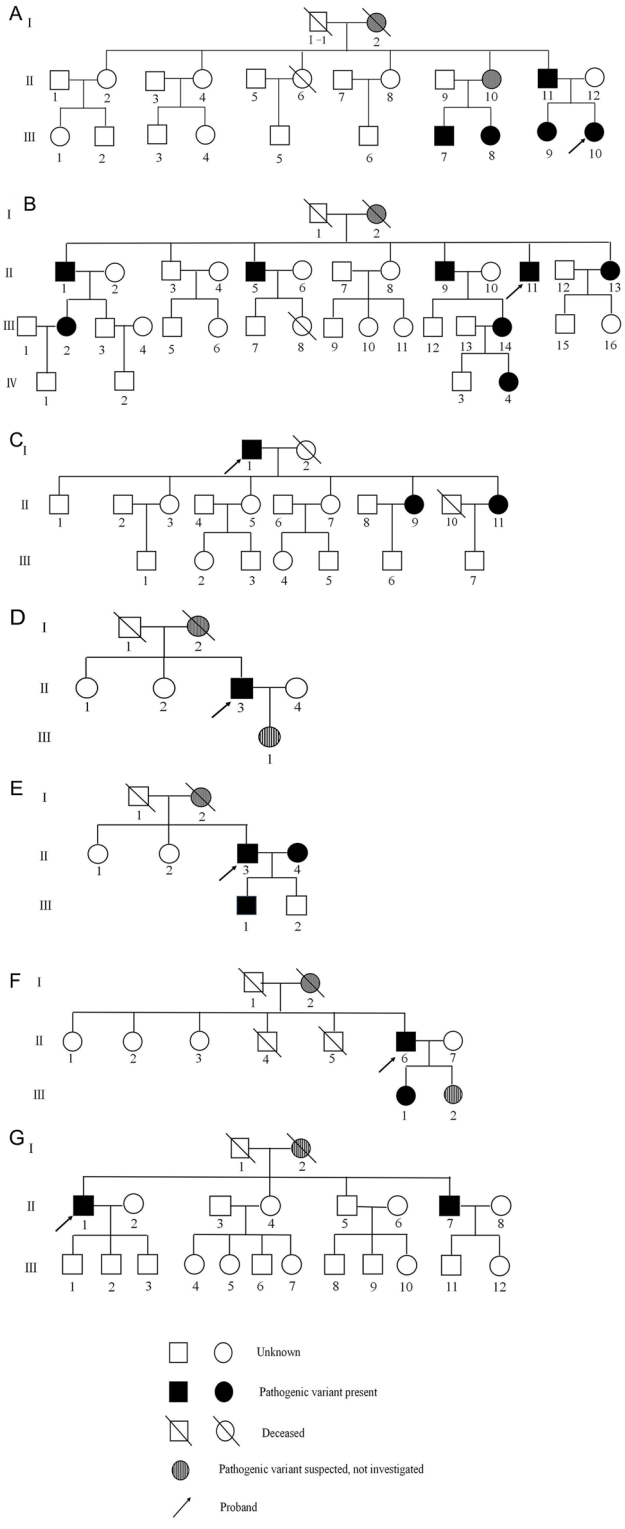

Fig. 1 Pedigrees of families with Fabry disease. **A** Pedigree of proband 1's family. **B** Pedigree of proband 2's family. **C** Pedigree of proband 3's family. **D** Pedigree of proband 4's family. **E** Pedigree of proband 5's family. **F** Pedigree of proband 6's family. **G** Pedigree of proband 7's family

Discussion

Fabry disease patients with rare genetic diseases and nonspecific symptoms often experience substantial diagnostic delays, sometimes more than a decade [4]. If not treated in time, the resulting end-organ damage significantly impairs quality of life and reduces life expectancy [4, 5]. To improve the diagnostic yield of Fabry disease, we integrated high-risk screening, family screening, and newborn screening programs previously adopted in some countries and regions.

Compared with prior studies, the screening strategy implemented in this study demonstrated an enhanced diagnostic yield of Fabry disease, compared to previously reported. For our study 388 cases of high-risk screening combined with family screening for Fabry disease were conducted a total of 13 male hemizygotes and 12 female heterozygotes were detected, resulting in a diagnosis rate of 6.44%. In comparison, some studies have screened for Fabry disease in different populations (i.e., dialysis patients, LVHs, strokes, and newborns), and the prevalence of Fabry disease is 0.41% (0.33% for males and 0.10% for females) in dialysis patients. In addition, the prevalence of Fabry disease is at least 0.94% for both genders in LVH, and the prevalence of Fabry disease in premature stroke patients is 0.20% (0.13% for males and 0.14% for females) [6]. Newborn screening studies have reported that the prevalence of Fabry disease is unexpectedly high, reaching 0.04% for infants in the Chinese Taiwan population (Table 4) [8–19].

This study revealed that genetic testing is superior to Lyso-GL-3 quantification for early diagnosis of late-onset Fabry disease in females, mainly due to the limited sensitivity of Lyso-GL-3 in heterozygous individuals with skewed X inactivation. Our results showed that the level of Lyso-GL-3 did not increase significantly in the majority of females with Fabry disease, except for proband 1; the other female patients were successfully diagnosed through family screening in this study. Importantly, genetic testing allows identification of undiagnosed Fabry patients. According to cased shared by the international Fabry Family Screening Advisory Board, family screening of 365 probands with Fabry disease revealed that 1,744 family members carried pathogenic *GLA* variants; in other words, each proband affected 4.8 family members on average [4].

Although family screening is the most effective strategy for identifying undiagnosed Fabry patients, our study experienced similar family-based patient resistance as reported in previous studies. For our study, some data were missing due to patients' rejection for genetic test and treatment due to cultural reasons. Comparably, previous studies have attributed the resistance to logistics economics and the numerous geographic, societal, and cultural barriers that limit the adoption of family genetic

Table 4 Summary of global studies on Fabry cases with the IVS4+919G>A variant

Pub year	Region	No. of screening	Pop	Inclusion criteria	No. of FD	No. of-Cases with IVS4+919G>A	Ref
2017	Japan	177	LVH	consecutive unrelated male patients with HCM, the diagnosis of HCM was based on echocardiographic demonstration of unexplained LVH, i.e. maximum LV wall thickness ≥ 15 mm	2 (1.13%)	1 (0.56%)	[8]
2020	Canada andChinese Hongkong	266	LVH	Inclusion criteria for the study included the following: 1) ability to provide informed consent, 2) LVH based on echocardiography. Participants were excluded from the study if other known causes of hypertrophic remodeling were present in their clinical history, such as the following: 1) uncontrolled hypertension, 2) hemodynamically stable aortic valvular stenosis, 3) coarctation of the aorta, 4) cardiac amyloidosis, and 5) previously diagnosed hypertrophic cardiomyopathy. Patients that were pregnant or suspected to be pregnant were also excluded	5 (1.88%)	5 (1.88%)	[9]
2021	Hongkong, China	499	LVH	Adult (age ≥18 years) Han Chinese subjects with LVH, defined as a maximal interventricular septal (IVST) and/or posterior wall thickness (PWT) ≥13 mm on echocardiography, were identified from our echo lab and prospectively recruited for Fabry screening in this study. Exclusion criteria were LVH caused by known sarcomere gene mutation, infiltrating cardiomyopathies (e.g., amyloidosis), athletic heart, non-compaction cardiomyopathy, and other non-Fabry metabolic or syndromic conditions associated with LVH.	8 (1.60%)	8 (1.60%)	[10]
2024	Hongkong, China	426	LVH	Adult (aged ≥18 years) consecutive patients with LVH were recruited for Fabry screening if they had maximal left ventricular (septal and/or posterior) wall thickness ≥13 mm, as determined by transthoracic echocardiography. Patients with abnormal loading conditions, namely hypertension and aortic stenosis, were included in the study. Exclusion criteria included those with a diagnosis of HCM (i.e., increased LV wall thickness of ≥13 mm not solely explained by abnormal loading conditions or those with known sarcomere mutations), other infiltrative cardiomyopathies (e.g., cardiac amyloidosis), LV non-compaction, athlete's heart, morbid obesity, and other non- Fabry infiltrative cardiomyopathies associated with LVH.	4 (0.94%)	3 (0.70%)	[11]
2018	Taiwan, China	1000	Stroke	Young stroke patients were aged 18–55 years	2 (0.20%)	2 (0.20%)	[12]
2018	Taiwan, China	1012	CKD	Patients at different CKD stages and not on dialysis with unknown etiology	6 (0.59%)	3 (0.30%)	[13]
2023	Taiwan, China	1812	ESRD	Male patients from Pre-End Stage Renal Disease Program, hemodialysis, peritoneal dialysis, and kidney transplant programs. Patients aged younger than 20 years were excluded.	3 (0.16%)	2 (0.11%)	[14]
2024	Zhejiang, China*	244	ESRD	patients under dialysis	1 (0.41%)	1 (0.41%)	[15]
2009	Taiwan, China	171,977	NBS	NA	74 (1/2324)	63 (1/2730)	[16]
2013	Japan	21170	NBS	NA	3 (1/7057)	1 (1/21170)	[17]
2024	Nanjing, China	17171	NBS	NA	13 (1/1321)	6 (1/2862)	[18]
2024	Shanghai, China	50108	NBS	NA	8 (1/6264)	1 (1/50108)	[19]

*In 2024 Zhejiang Study, Family screening of the index patient (41 family members) yielded 11 FD patients, *Abbreviation*: *FD* Fabry disease, *Pub* Publication, *Pop* Population, *LVH* Left Ventricular Hypertrophy, *CKD* Chronic Kidney Disease, *ESRD* End Stage Renal Disease, *NBS* Newborn Screening, *Ref* Reference, *NA* not available

screening [4]. For example, ineffective communication among relatives and geographical dispersion of families can hinder coordinated genetic testing efforts [4, 20].

GLA IVS4 + 919G > A pathogenic variant is associated with the Fabry disease in China, despite rarity in findings with other ethnic populations. Among the patients included in this study, 25 patients had a previously reported *GLA* IVS4 + 919G > A pathogenic variant located in the Xq22.1 region. This variant was first discovered in Kyushu, Japan [5], has a high frequency in Chinese Taiwan populations and has been linked to late-onset Fabry disease [21]; the incidence of this specific variant is as high as 80% in patients with Fabry disease in Taiwan, China. Patients with the *GLA* IVS4 + 919G >

A variant, including mainland China, Chinese Taiwan, Japan, Singapore, Vietnam and Malaysia, who were of Chinese ancestry, were subsequently investigated [8, 15]. The clustering of cases along coastal regions suggests that this variant may represent a regional founder mutation, potentially linked to historical population migration patterns and originating from the common ancestor, which was traced to Chinese ancestors more than 800 years ago via genetic assessments of the common haplotype [22]. The sequence of the patient's intron 4 contains a single G-to-A transversion at IVS4 + 919G > A, causing an increase in alternatively spliced genes; it has been proven that this sequence is related to cardiac diseases, such as LVH, valve regurgitation, cardiac failure, arrhythmia, and myocardial ischemia, described as a cardiac variant of Fabry disease [5, 8, 23].

Our study has taken into consideration of heterogeneity of the Fabry disease through more inclusive diagnostic threshold for the screening. Recent studies suggest that the phenotypes of Fabry disease with the variant IVS4 + 919G > A are diverse; several studies have shown that insidious and irreversible organ injury may progress silently [24, 25], including renal, cardiovascular, cerebrovascular, gastrointestinal, respiratory, and ocular manifestations (Table 5) [15, 26–30]. In this study, the clinical manifestations of 25 patients with Fabry disease identified through high-risk and family screening included LVH, pain in the lower limbs, stroke, chronic kidney disease, gastrointestinal symptoms, and conjunctival vessel tortuosity. Ten patients had LVH, among whom, proband 4 and proband 5 had implanted pacemakers; the visual loss of proband 4 was due to ophthalmic artery occlusion, some cases of visual loss have been reported in the literature [31–33], proband 1 experienced pain in both lower limbs as the first initial symptom. Cardiac magnetic resonance imaging (MRI) revealed that the ventricular septum was thick, which indicates that patients with Fabry disease may have experienced heart injury in childhood. To the best of our knowledge, there is limited literature on patients with a known intronic variant, IVS4 + 919G > A, combined with peripheral nerve pain. Family genetic screening identified 2 affected relatives, the son of proband 5 and his wife, who were also diagnosed with Fabry disease with the *GLA* IVS4 + 919G > A variant. To date, it has been unexpectedly and rarely reported. The clinical characteristics of Fabry disease are heterogeneous; some studies show that Fabry patients may have one or more general risk factors for cardiovascular disease, such as diabetes, smoking, hypertension, hypercholesterolaemia, Fabry disease may be considered a major risk factor [3], therefore, to reduce the chance of missing a diagnosis of Fabry disease, the proband's relatives should be asked to provide more detailed information, and family genetic screening should be offered to all the relatives of the probands [20, 34].

Table 5 Systematic review of Fabry disease cases with the IVS4+919G>A variant in China

Publication Year	Region	No. of Cases with IVS4+919G>A cases	Extra-cardiac involvement of IVS4+919G>A cases	Ref
2010	Taiwan, China	94	Microalbuminuria in 17 cases；At least one ocularmanifestation consistent with Fabry disease in 41cases	[26]
2013	Taiwan, China	21	Elevated urine ACR in 6 cases; Abnormal eGFR in 1 case；Cerebrovascular disorders in 1 case; cornea verticillata in 2 cases; Fabry cataract in 5 cases	[27]
2014	Taiwan, China	22	ESRD in 1 case; Chronic renal insufficiency in 1 case	[28]
2016	Taiwan, China	26	Infarctions in 9 cases；pulvinar sign in 8 cases	[29]
2024	Zhejiang, China	12	Renal involvement in 9 case（75.00%）, among them, 9 with albuminuria or hematuria, 1 with ESRD; abnormal brain MRI in 4 cases	[15]
2024	Zhejiang, China	6	Pain in both lower limbs in 1 case	[30]

ACR Albumin-to-creatine ratio, *eGFR* estimated glomerular filtration rate, *ESRD* End Stage Renal Disease, *Ref* Reference

Our pilot program was facilitated by patient-friendly government policy to treat the Fabry disease. Fabry disease therapies were included in Zhejiang Province's rare disease reimbursement drug list for timely treatment initiation, significantly reducing the risk of irreversible organ damage through early intervention [35]. In May 2018, Fabry disease was listed as the first batch of rare diseases in China [36]. Agalsidase alfa and agalsidase beta were approved by China's National Medical Products Administration [37]. Proband 1 and his father underwent pretreatment ERT and successfully received an intravenous infusion of agalsidase beta, and the pain in both lower limbs was alleviated. Furthermore, medical cost was greatly reduced due to the more effective evaluation of the screening results based on gender-specific normal range (~ 80% less) as compared to the less efficient evaluation of the screening results collapsing both genders.

This study has several limitations. Firstly, there was a lack of pathological research in our current study; the diagnosis of Fabry patients is mainly confirmed through symptoms, α-Gal A activity, Lyso-GL-3 and genetic testing, as the patients refused to undergo further pathological biopsy. Secondly, there were missing data in our sample due to patients' rejection for genetic test and

treatment. This is due to complex family structures, fear of stigmatization, internal migration of families complicating communication. Thirdly, the late-onset Fabry disease is more common than the classic Fabry disease [1], the presumed reason for the rarity of classic Fabry disease is that patients with classic Fabry disease can not marry or have children due to earlier onset, more severe disease progression, or premature death. For future, a large cohort is required to screen for the potential presence of classic Fabry disease and other genotypes in the local population.

In conclusion, the integration of targeted high-risk population screening with family screening significantly improves the early diagnosis of Fabry disease. Patients harboring the *GLA* IVS4 + 919G > A variant exhibit a heterogeneous phenotype, with multisystem involvement extending beyond classical cardiac manifestations to include renal disease, stroke, respiratory symptoms, gastrointestinal symptoms and ocular symptoms. This study identified the *GLA* IVS4 + 919G > A variant as a cause of Fabry disease in a patient who presented with lower limb pain as the initial symptom, expanding the known phenotypic spectrum. Furthermore, our data confirm the high prevalence of this variant in Ninghai County, Zhejiang Province, Eastern China, suggesting a potential regional founder effect.

Abbreviations

DBS	Dried blood spots
α-Gal A	α-Galactosidase A
Lyso-GL-3	globotriaosylsphingosine
LVH	left ventricular hypertrophy
CAD	coronary atherosclerotic heart disease
ERT	enzyme replacement therapy
HUA	hyperuricemia
CKD	chronic kidney disease
COPD	chronic obstructive pulmonary disease
PVC	premature ventricular complex
cTnI	troponin I
BNP	B-type natriuretic peptide
IVST	interventricular septal thickness
LVPWT	left ventricular posterior wall thickness
LVEF	left ventricular ejection fraction
LVMI	left ventricular mass index

Acknowledgements
The authors sincerely thank all the patients and their families who participated in this study.

Authors' contributions
Each author has participated sufficiently in the work to take public responsibility for the content. JHM and ZHL participated in the conception and design of the study; ZHG, ZQD, XDP, KQL, MJY, TTL, HBZ, HLG, GYG and ZGH conducted the data acquisition, analysis, and interpretation; ZHG drafted the article and provided intellectual content of critical importance to the work described; and JHM revised the article. All the authors approved the final manuscript.

Funding
This study was supported by the Ninghai County Science and Technology Plan Project (No. 202325).

Data availability
The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2021) in National Genomics Data Center (Nucleic Acids Res 2024), China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences (GSA-Human: HRA011136) that are publicly accessible at [https://ngdc.cncb.ac.cn/gsa-human].

Declarations

Ethics approval and consent to participate
All procedures performed in studies involving human participants were performed in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Ethics approval for this study was granted by the Ethics Board of Ninghai First Hospital (project number: Nhyy-IEC-KY202304). Informed consent was obtained from all individual participants included in the study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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Received: 2 April 2025 / Accepted: 16 January 2026
Published online: 05 February 2026

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