

# Identification of a Novel *GLA* Gene Mutation, p.Ile239Met, in Fabry Disease With a Predominant Cardiac Phenotype

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## SUMMARY

Fabry disease (FD) is an X-linked inherited lysosomal storage disorder caused by mutations in the *GLA* gene, encoding for the enzyme  $\alpha$ -galactosidase A. Although hundreds of mutations in the *GLA* gene have been described, many of them are variants of unknown significance. Here we report a novel *GLA* mutation, p.Ile239Met, identified in a large Hungarian three-generation family with FD. A 69 year-old female index patient with a clinical history of renal failure, hypertrophic cardiomyopathy, and 2nd degree AV block was screened for mutation in the *GLA* gene. Genetic screening identified a previously unreported heterozygous mutation in exon 5 of the *GLA* gene (c.717A>G; p.Ile239Met). Family screening indicated that altogether 6 family members carried the mutation (5 females, 1 male, average age: 55  $\pm$  16 years). Three family members, including the index patient, manifested the cardiac phenotype of hypertrophic cardiomyopathy, while two other family members were diagnosed with left ventricular hypertrophy. Taking affection status as the presence of hypertrophic cardiomyopathy, left ventricular hypertrophy or elevated lyso-Gb3 levels, all affected family members carried the mutation. Linkage analysis of the family gave a two-point LOD score of 2.01 between the affection status and the p.Ile239Met *GLA* mutation. Lyso-Gb3 levels were elevated in all carrier family members (range: 2.4-13.8 ng/mL; upper limit of normal +2STD:  $\leq$  1.8 ng/mL). The *GLA* enzyme level was markedly reduced in the affected male family member ( $<$  0.2  $\mu$ mol/L/hour; upper limit of normal  $\pm$  2STD:  $\geq$  2.6  $\mu$ mol/L/hour). We conclude that the p. Ile239Met *GLA* mutation is a pathogenic mutation for FD associated with predominant cardiac phenotype. (Int Heart J 2017; 58: 454-458)

**Key words:**  $\alpha$ -galactosidase A, Hypertrophic cardiomyopathy, Left ventricular hypertrophy, Renal failure

**F**abry disease (FD, OMIM: 301500) is a rare X-linked disorder caused by mutations in the *GLA* gene (OMIM: 300644), encoding a lysosomal hydrolase enzyme,  $\alpha$ -galactosidase A (*GLA*; EC 3.2.1.22) (reviewed in detail in Germain DP, 2010<sup>1</sup>). Mutations affecting the *GLA* gene and enzyme will result in the accumulation of complex sphingolipids, mainly globotriaosyl-ceramide (Gb3) in the lysosome, which subsequently will lead to Fabry disease, a systemic disorder with multiple organ involvement. In hemizygous male patients, symptoms of acroparesthesia, abdominal pain, and fever are typically first experienced in early childhood. During adolescence, the affected subjects may exhibit angiokeratomas, hypohidrosis, proteinuria, progressive renal insufficiency, and cornea verticillata. In the fourth decade patients may manifest cardiomyopathy, arrhythmia, and cerebrovascular complications. Cardiac involvement as left ventricular (LV) hypertrophy, hypertrophic cardiomyopathy, and conduction disturbances are detected in 60% of Fabry patients.<sup>2</sup> The most common causes of death are renal failure,

heart failure, myocardial infarction, and stroke.

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## Editorial p.305

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Over 670 mutations in the *GLA* gene have been described, mostly appearing in single families. While the pathogenicity of some *GLA* mutations is well described, many subjects often have a *GLA* genetic variant/mutation of unknown significance (VUS).<sup>3,4</sup> In a recent recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP), a process is described for classifying variants based on criteria using typical types of variant evidence.<sup>5</sup> These types of evidence include population data, computational data, functional data, and most importantly, segregation data in a given family. For the majority of the metabolic diseases there is the advantage of having the data of the concentration of the effected enzyme in clinical materials and for some few ones also information about the biomarker, like in Fabry disease.<sup>6</sup> As a specific enzyme replacement therapy (ERT) for Fabry disease is availa-

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The work was supported by the University of Szeged.

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Received for publication August 3, 2016. Revised and accepted September 13, 2016.

Released in advance online on J-STAGE May 12, 2017.

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ble,<sup>7)</sup> early diagnosis of a real FD patient is of great importance to initiate the otherwise invasive and expensive ERT.

Here we describe a novel *GLA* mutation, p.Ile239Met, identified in a large Hungarian family with Fabry disease. The 6 affected family members in 3 generations allowed us to delineate the dominant cardiac phenotype of the disease and also presented multiple lines of evidence, including linkage analysis, to suggest the pathogenic nature of the p.Ile239Met *GLA* mutation for Fabry disease.

### CASE REPORT

**Index patient:** Past medical history of the Hungarian female index patient (subject H 332.0, see family tree in Figure 1 and the Table) included nephrology assessment at the age of 43 years because of proteinuria which was interpreted as being due to mesangioproliferative glomerulonephritis. Angina pectoris, ischemic heart disease, and left ventricular hypertrophy diagnosed at 63 years of age, were also known. At that time, renal insufficiency had worsened and necessitated continuous ambulatory peritoneal dialysis, and later hemodialysis. At 67 she received a successful renal transplant but rejection occurred 2 years later, and hemodialysis continued.

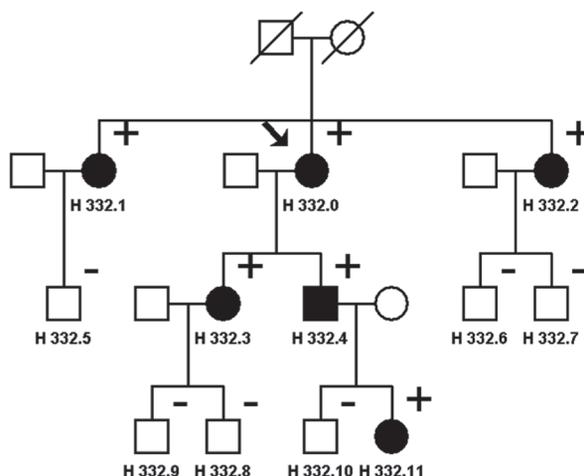
At age 69 the patient exhibited syncopal episodes which proved to be caused by intermittent 2nd degree, 2:1 atrioventricular (AV) block. Echocardiography revealed marked LV hypertrophy in the form of obstructive hypertrophic cardiomyopathy with a left ventricular outflow tract gradient of 120 mmHg measured at a heart rate of 40/minute due to the 2:1 AV block. The morphology of the heart was that of hypertrophic cardiomyopathy with marked left ventricular hypertrophy (maximal LV wall thickness, 27 mm), papillary muscle hypertrophy, and right ventricular (RV) hypertrophy (maximal RV wall thickness, 14 mm) (Figure 2). NT-pro-BNP levels were extremely high (> 35,000 pg/mL, upper limit of normal: < 200 pg/mL). Because of the high degree AV block a dual-chamber pacemaker implantation was performed, and the outflow tract

gradient decreased to 20 mmHg with optimization of the AV delay.

Further examinations revealed neither angiokeratomas nor cornea verticillata on dermatology and ophthalmology assessments. Ear, nose, and throat examinations revealed perception hypoacusis. Neurological assessment indicated a left side dominant paraparesis which was more prominent distally, with a muscle strength of 3-4/5. Electromyography and electroneurography indicated primarily motor neuropathy with axonal loss, without myopathy.

Current laboratory findings include elevated renal function (UN: 16.1 mmol/L; creatinine: 294 μmol/L; eGFR: 13.7 mL/minute/m<sup>2</sup>) and lyso-Gb3 level (10.6 ng/mL, upper limit of normal + 2SD: < 1.8 ng/mL).

**Family members:** The index patient belongs to a 3-generation

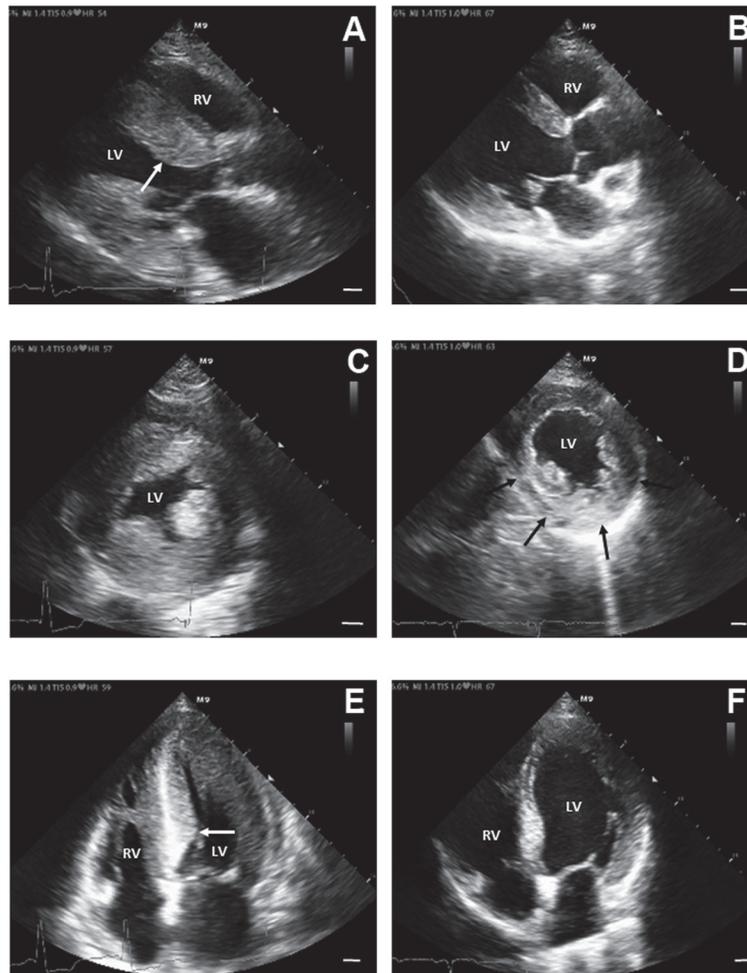


**Figure 1.** Pedigree of the p.Ile239Met *GLA* gene mutation carrier family. Squares and circles denote males and females, respectively; filled symbols indicate clinically affected family members. The arrow points to the index patient. Deceased individuals are slashed. Mutation carriers are labelled with a plus (+) sign, and non-carriers with a minus (-) sign.

**Table.** Demographic and Clinical Characteristics of Genetically Affected Family Members Carrying the *GLA* p.Ile239Met Mutation

Subject	Sex	Age (years)	Cardiac morphology	ECG changes	LVmax (mm)	LV mass (g)	GLA enzyme level (μmol/L/hour)*	lyso-Gb3 level (ng/mL)**	Other organ involvement
H 332.0	female	69	HCM	2nd degree AV block, LVH, intraventricular conduction delay	27	ND	ND	10.6	renal failure
H 332.1	female	73	LVH	negative T waves in leads I-aVL, V2-6	13	107	ND	2.4	non-significant proteinuria
H 332.2	female	62	HCM	LVH, negative T waves in leads I-aVL, V4-6	16	131	ND	4.2	non-significant proteinuria
H 332.3	female	52	LVH	negative T waves in leads II-III-aVF, V4-6	14	144	ND	2.9	-
H 332.4	male	49	HCM	LVH, negative T waves in leads II-III-aVF, V4-6	20	268	< 0.2	13.8	-
H 332.11	female	26	none	none	8	87	ND	3.2	-

ECG indicates electrocardiogram; LVmax, maximal left ventricular wall thickness; GLA, α-galactosidase A; lyso-Gb3, lysosomal globotriaosyl-ceramide; ND, not done; HCM, hypertrophic cardiomyopathy; and LVH, left ventricular hypertrophy. \* upper limit of normal ± 2SD: ≥ 2.6 μmol/L/hour. \*\* upper limit of normal + 2SD: ≤ 1.8 ng/mL.



**Figure 2.** Transthoracic echocardiography images of hypertrophic cardiomyopathy in the index patient (subject H 332.0) and her son (subject H 332.4) illustrating heterogeneity in the extent and distribution of left ventricular hypertrophy. In the index patient, marked left ventricular hypertrophy (maximal LV wall thickness 27 mm, white arrows) is seen, predominating at the interventricular septum, with papillary muscle hypertrophy and right ventricular hypertrophy (A, C and E). In the affected son, the hypertrophy affects mostly the inferior septum and the infero-postero-lateral wall of the left ventricle (black arrows, B, D and F). Parasternal long axis view (A and B), parasternal short axis view (C and D), and apical 4-chamber view (E and F) of transthoracic echocardiography. Scale bars at the lower right hand corner in each panel represent 1 cm. LV indicates left ventricle; and RV, right ventricle.

family (see family tree in Figure 1). The collection of case history data, physical examinations, overview of available clinical documentation, 12-lead ECG, and transthoracic echocardiography were carried out in all available family members.<sup>8,9</sup> In selected cases, patients were hospitalized for detailed in-hospital assessment including nephrology, dermatology, ophthalmology, neurology, and ear, nose and throat examinations.

Altogether, 12 family members (5 females, 7 males, average age:  $45 \pm 17$  years, see family tree in Figure 1 and the Table) were available for clinical and genetic screening. Three family members, including the index patient (subject H 332.0, H 332.2, and H 332.4), manifested the cardiac phenotype of hypertrophic cardiomyopathy (defined as maximal LV wall thickness  $\geq 15$  mm). The degree and distribution of cardiac hypertrophy were highly variable; while it was most marked at the interventricular septum in the index patient, it affected mostly the inferior septum and the infero-postero-lateral wall of the left ventricle in the son of the index patient (Figure 2). Two other family members (subject H 332.1 and H 332.3)

were diagnosed with LV hypertrophy (defined as maximal LV wall thickness  $\geq 12$  mm). ECG changes indicating LV hypertrophy and repolarization changes were present in all patients with echocardiographic evidence of LV hypertrophy. A further family member, the 26 year-old niece of the index patient (subject H 332.11) did not exhibit cardiac phenotype, but showed increased lyso-Gb3 levels, indicating subclinical disease. Non-significant proteinuria was present in two family members. No other family member showed other extra-cardiac (renal, central and peripheral nervous system, skin, eye, etc.) manifestation of the disease.

**Molecular genetic analysis:** The investigations were approved by the Institutional Ethics Committee of the University of Szeged, and were conducted according to the Helsinki Declaration. Family members gave written informed consent to the molecular genetic analysis. Molecular genetic analysis was conducted by standard direct sequencing on an ABI Prism 310 Genetic Analyzer (Applied Biosystems). The levels of alpha-galactosidase A enzyme and lyso-Gb3<sup>10</sup> were measured at a

specialized laboratory (CentoGene AG, Rostock, Germany). Nucleotide and amino acid changes are reported according to the Ensembl database (release 84 - March 2016) using *GLA*-001 (ENST00000218516) as a reference sequence. The size of the family and the presence of affected family members in all 3 generations of the family allowed us to conduct linkage analysis in the family. Linkage analysis was performed with the FASTLINK program. Linkage between the affection status and the mutation was modeled with the following parameters: disease allele frequency: 1:10,000, disease penetrance: 90%.

Molecular genetic analysis identified a previously unreported heterozygous mutation in exon 5 of the *GLA* gene in the index patient (c.717A>G; Figure 3). The mutation changes the ATA triplet at codon 239, encoding for isoleucine, to ATG, encoding for methionine (p.Ile239Met, missense mutation). This mutation is located in a weakly conserved nucleotide and moderately conserved amino acid position, with small physicochemical differences between the amino acids isoleucine and methionine (Alamut v.2.7.1). Software analyses by PolyPhen-2, SIFT, and Mutation Taster indicate this mutation is probably damaging. Corresponding base changes were present in the same position in the reverse strand. To date, this mutation is not described in the Exome Aggregation Consortium, Exome Sequencing Project, or the 1000 Genomes Browser.

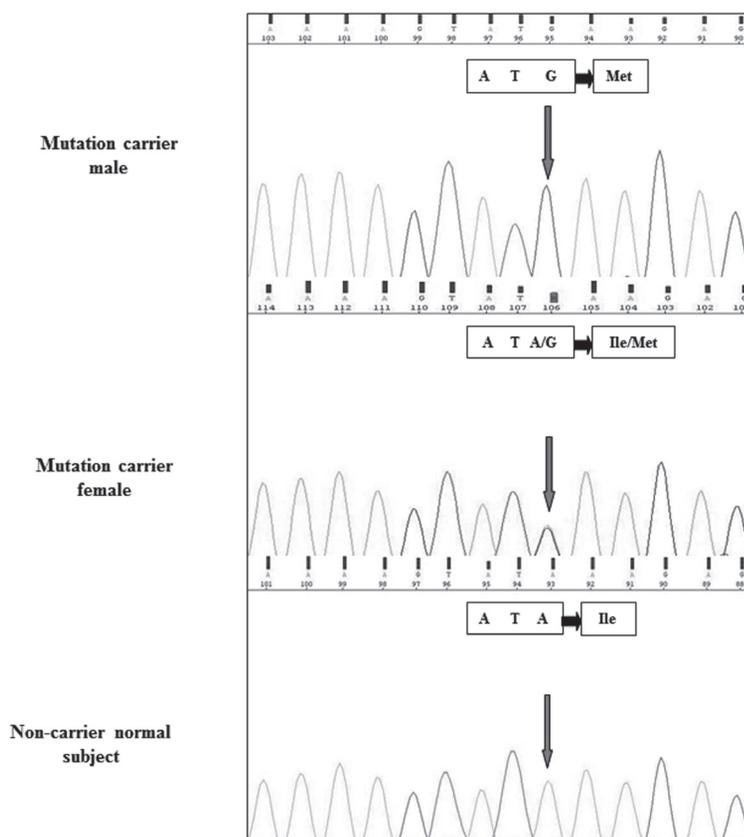
All of the available 12 family members were genotyped for the *GLA* p.Ile239Met mutation. Six family members carried the mutation (5 females, 1 male, average age: 55 ± 16

years, see family tree in Figure 1 and the Table). Taking affection status as the presence of hypertrophic cardiomyopathy, LV hypertrophy, or elevated lyso-Gb3 levels, all affected family members carried the mutation while all non-affected family members were non-carriers. Linkage analysis of the family gave a two-point LOD score of 2.01 between the affection status and the presence of the p.Ile239Met *GLA* mutation, favoring linkage. Lyso-Gb3 levels were elevated in all carrier family members (range: 2.4-13.8 ng/mL; upper limit of normal + 2 STD: ≤ 1.8 ng/mL). The *GLA* enzyme level was markedly reduced in the affected male family member (< 0.2 μmol/L/hour; upper limit of normal ± 2 STD): ≥ 2.6 μmol/L/hour).

## DISCUSSION

Here we report the identification of a novel *GLA* gene mutation, p.Ile239Met, in a large family with Fabry disease. The disease phenotype showed primarily cardiac involvement in the form of hypertrophic cardiomyopathy, left ventricular hypertrophy, and ECG changes in all of the adult mutation carriers, while progressive renal failure, necessitating renal transplantation, developed only in the index patient. Lyso-Gb3 levels were increased in all carrier family members and the *GLA* enzyme level was markedly reduced in the affected male family member.

The reported p.Ile239Met *GLA* mutation is a novel muta-



**Figure 3.** Sequence analysis of exon 5 of the *GLA* gene illustrating a c.717A>G nucleotide transition. The mutation changes the ATA triplet at codon 239, encoding for isoleucine, to ATG, encoding for methionine (p.Ile239Met). The sequences of a mutation carrier, hemizygous male; of a mutation carrier heterozygous female, and of a non-carrier normal subject are shown.

tion, although an amino acid change at this position has already been described as disease-causing for Fabry disease by Kotanko in 2004 (c.716T>C, p.Ile239Thr, HGMD ID: CM044637).<sup>11)</sup> The mutation was identified in a male patient, who experienced recurrent fever, pain, lymphadenopathy, and acroparesthesia in early childhood. Later on a shrunken right kidney and stage 4 chronic kidney disease were diagnosed, and acute hearing loss, dizziness, headache, dysphasia, and dysarthria developed. Multiple cerebral lesions detected by magnetic resonance imaging were related to stage 3 hypertension. Fabry disease was diagnosed during the course of the screening study. Reexamination of the kidney biopsy material by electron microscopy showed typical signs of Fabry disease that were previously not identified by light microscopy. The patient also had cornea verticillata and mild left ventricular hypertrophy. His 48-year-old mother (normal GLA activity) and his two daughters (aged 3 and 8 years; decreased GLA activity in both) exhibited the same mutation. These 3 female relatives do not show clinical signs and symptoms of Fabry disease.

On the basis of the current ACMG/AMP recommendations<sup>5)</sup> there are multiple lines of evidence to characterize the p.Ile239Met mutation as 'pathogenic' for Fabry disease. First and most important, the observation of the markedly decreased GLA enzyme level in the affected male carrier and the increased lyso-Gb3 levels in all the mutation carriers provides evidence for the damaging consequences of the mutation on the gene product (PS3, strong evidence of pathogenicity). Second, the mutation is absent from controls in the Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium (PM2, moderately strong evidence of pathogenicity). Third, the mutation is a novel missense change at an amino acid residue where a different missense change (p.Ile239Thr, see above) determined to be pathogenic has been seen before (PM5, moderately strong evidence of pathogenicity). Fourth, cosegregation with disease in multiple affected family members in the *GLA* gene, definitively known to cause the disease, has been shown (PP1, supporting evidence of pathogenicity). It is worth noting that the > 2 LOD score we obtained by linkage analysis in the family is suggestive, although not conclusive in itself, for the causative role of the variant, as it indicates cosegregation of the mutation with the disease phenotype. Fifth, multiple lines of computational evidence support a deleterious effect on the gene or gene product (PP3, supporting evidence of pathogenicity). By applying the rules of the ACMG/AMP guideline for combining criteria to classify sequence variants as 'pathogenic' (1 Strong AND 2 Moderate AND  $\geq 2$  Supporting), the p.Ile239Met *GLA* variant that we have identified completely satisfies the criteria of being 'pathogenic'.

In conclusion, we have described a family with a novel p.Ile239Met *GLA* gene mutation in Fabry disease. Cardiac involvement in the form of hypertrophic cardiomyopathy, LV hypertrophy, and ECG changes was the most common manifestation of the disease and severe renal failure occurred in one family member. We conclude that the p.Ile239Met *GLA* mutation is a pathogenic mutation for Fabry disease and obviously

associated with a late onset and predominantly a cardiac variant of the disease.

## ACKNOWLEDGMENTS

The skillful assistance of Ms. Molnár Jánosné Klári is gratefully acknowledged.

## DISCLOSURE

**Conflict of interest:** The authors have no conflict of interests to declare.

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