BJMG 26 (1), 2023 • 43-50
DOI: 10.2478/bjmg-2023-0010
sciendo

ORIGINAL ARTICLE

DIFFICULTIES IN DIAGNOSING FABRY DISEASE IN PATIENTS WITH UNEXPLAINED LEFT VENTRICULAR HYPERTROPHY (LVH): IS THE NOVEL GLA GENE MUTATION A PATHOGENIC MUTATION OR POLYMORPHISM?

Aladağ N[1,*], Ali Barman H[2], Şipal A[3], Akbulut T[3], Özdemir M[4], Ceylaner S[5]

[*]**Corresponding Author:** Nesim Aladağ, MD, nesimaladag@hotmail.com, Van Yüzüncü yıl University, Faculty of Medicine, Department of Cardiology, Van, Turkey.
Phone: +90 544 961 31 69 / Fax: +90 432 486 54 07

ABSTRACT

Fabry disease (FD) is an X-linked, lysosomal glycosphingolipid storage disorder that occurs very rarely. Cardiac involvement may comprise of left ventricular hypertrophy (LVH), arrhythmias, conduction abnormalities, heart failure and valvular abnormalities. The goal of this study was to conduct gene analysis for FD in patients suffering from unexplained LVH. 120 patients over the age of 30 who were diagnosed by echocardiography with idiopathic LVH were included in the study. Patients with severe hypertension, intermediate valve disease such as moderate aortic stenosis, known FD, and a family history of autosomal dominant hypertrophic cardiomyopathy were excluded from the study. *GLA* gene mutations were studied by Sanger sequence analysis in all patients. Of the 120 total patients included in this study, 69 were female (58%) and 51 were male (42%). The mean age was 60.3 ± 15.7. *GLA* gene mutations were detected in three male patients. The detected mutations are as follows: *NM_000169.2:IVS6-10G>A (c.1000–10G>A), NM_000169.2:c.937G>T (p.D313Y) (p.Asp313Tyr)* and *NM_000169.2:c.941A>T (p.K314M) (p.Lys314Met)*. Early diagnosis is of vital importance in FD, which can be treated with enzyme replacement. Genetic screening in patients diagnosed with idiopathic LVH by echocardiography is important in the early diagnosis and treatment of FD. Patients over 30 years of age with idiopathic LVH should be screened for FD. Various new polymorphisms can be detected in genetic screening. Identifying new polymorphisms is important for knowing the true mutations in FD.

Key Words: Unexplained left ventricular hypertrophy, Fabry Disease, GLA mutation, polymorphism.

INTRODUCTION

The heritable lysosomal storage disorder Fabry disease (FD) occurs because of an α-galactosidase (α-GLA) enzyme deficiency that advances with glycosphingolipid metabolism disorder (1). Being an X-linked disease, FD can be transferred by males as well as females (2). Over 1,000 *GLA* gene variants have been discovered across the world. Fabry disease may be caused by a single nucleotide variation in the *GLA* (3). Due to a defect in the α-GLA enzyme, glycosphingolipids steadily accumulate in lysosomes, particularly globotriaosylceramide (Gb3) and globotriaosylsphingosine (lyso-Gb3), in different cell types within the body. This gives rise to multisystemic issues, such as ocular, neurological, renal, brain, skin, and cardiac symptoms (4).

Brain, renal, and cardiac activity play vital parts in diagnosing FD. Approximately 60% of patients with FD have some form of heart involvement (5). The accumulation of glycosphingolipids in myocytes causes hypertrophy and the eventual fibrosis of the myocardium (6). Although the actual prevalence of FD is unknown, it is estimated to range from 1:40,000 to 1:117,000 people (7). The actual incidence and prevalence are unknown due to the presence of atypical or oligosymptomatic forms (8). Patients with stroke, idiopathic renal failure, or cardiomyopathy are

[1] Van Yüzüncü yıl University, Faculty of Medicine, Department of Cardiology, Van, Turkey

[2] Istanbul University – Cerrahpaşa, Institute of Cardiology, Department of Cardiology, Istanbul, Turkey

[3] University of Health Sciences, Van Training and Research Hospital, Department of Cardiology, Van, Turkey

[4] Bayrampaşa Kolan Hospital, Department of Cardiology, İstanbul, Turkey

[5] Intergen Genetic Centre, Department of Medical Genetics, Ankara, Turkey

frequently screened for FD in screening studies (9). Cardiac involvement is the leading cause of death in patients with FD. For this reason, it is important to diagnose these patients early because the available treatments are more efficient when they are started before the progression of the disease (10, 11).

Enzyme replacement therapy (ERT) and pharmacological chaperones can be used in the treatment of FD. Since the most common finding in patients with cardiac involvement is left ventricular hypertrophy (LVH), LVH has been evaluated in terms of FD in patients with unexplained LVH in screening studies (12). In these studies, the prevalence of FD varies according to the subject country and the screening methods used. In addition to known genetic mutations associated with FD, new genetic mutations or polymorphisms associated with the disease can be detected in screening studies.

This study aimed to present screening results for FD in patients with unexplained LVH diagnosed by two-dimensional echocardiography.

MATERIALS AND METHODS

Over 120 patients older than 30 years who were diagnosed with unexplained left ventricular (LV) wall thickness of ≥ 13 mm through echocardiography were screened for FD between March 2020 and March 2021. Patients were excluded who were suffering from major heart valve disease, significant hypertension, coarctation, strain conditions like aortic stenosis, earlier diagnosis of FD, previous history of any disease that was linked to LVH, or a family history of autosomal dominant hypertrophic cardiomyopathy (HCM) or FD. All participants provided their written informed consent. The study was conducted in conformance to the Helsinki Protocol and obtained the approval of the local ethics committee.

The collection of peripheral venous blood samples from all the participants of the study was carried out in the EDTA tubes (2 ml) for performing mutation analysis. These samples were sent to a laboratory specialized in diagnosing genetic disorders. In all patients, GLA gene sequence analysis was carried out. If a GLA gene mutation was identified, measurements for α-Gal A enzyme activity and lyso-Gb3 levels were obtained. In patients with gene mutations, a family screening was also carried out.

Trans-Thoracic Echocardiography

Trans-thoracic echocardiography (TTE) on the left lateral decubitus position was performed in all patients after they had rested for at least 15 minutes (Philips iE33 Healthcare, Andover, Massachusetts, USA). Echocardiography images were obtained from four standard views (two-chamber apical, long-axis parasternal, short-axis parasternal and four-chamber apical). M-mode recording was used in 2D echocardiography to achieve the standard value of LV diameter and function. According to the recommendations of the American Society of Echocardiography, an average of at least three cardiac cycles was obtained to evaluate the M-mode echocardiogram, (13). The M-mode echocardiography was used to compute the left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic diameter (LVESD), left ventricular posterior wall thickness (LVPW) and interventricular septum (IVS) from the long-axis parasternal view in millimeters. The biplanar disc technique (modified Simpson's rule) was used to compute the LV ejection fraction (EF).

α-Gal A enzyme activity and lyso-Gb3 measurements

α-Gal A DBS card study is performed by fluorimetric method. 4-Methylumbelliferyl-α-D-galactopyranoside (TRC, M334475) was used as substrate and N-Acetyl-D-galactosamine (Sigma, A2795) was used as inhibitor. The reaction is stopped after a 3 mm dried blood spot (DBS) punch, inhibitor, and substrate incubation for 17 hours at 37°C. Fluorescence is recorded at Ex: 366 nm, Em: 442 nm in the fluorimeter. The calibration curve is created with 4-Methylumbelliferone (Sigma M1381) and the results are evaluated. The LC-MS/MS system is used to measure the lyso-Gb3 level. A 5 mm DBS punch is taken from standards, controls and samples, internal standard N-Gly-Lyso-Gb3 is added. After extraction, it is taken into vials and analyzed in 10 µL LC-MS/MS system.

Mutation analysis - Polymerase Chain Reaction - Sequencing

Peripheral blood samples of 200 µl were obtained from the study participants. These samples were stored at a temperature of -200 °C till the polymerase chain reaction (PCR) step was carried out. The design of the in-house PCR primers was done for the coding region and the exone-intron borders of the GLA gene. The Sanger technique was used to perform sequencing on a genetic analyser (Applied Biosystems Inc.). SeqScape 2.5.0 was used to evaluate the data (Applied Biosystems Inc.).

Statistical Analysis

Analysis was performed on the demographic properties and echocardiographic parameters of all the screened patients. The Statistical Package for the Social Sciences (SPSS) program, version 19.0 (SPPS Statistics IBM®) was used to perform the descriptive analysis of the data, and the outcomes were presented as percentages, numbers, or mean values ± a standard deviation.

RESULTS

The clinical and demographic characteristics and the echocardiographic measurements of 120 patients with idiopathic LVH who underwent genetic examination are shown in **Table 1**. The majority of the patients were female (*n* = 58, 69%), and the mean age was 60.3 ± 15.7 years. The mean echocardiographic parameters were as follows: LVEF: 61.4% ± 4.8%, IVS: thickness 15.2 ± 5.4 mm, LVPWD: 13.5 ± 2.1 mm, LVEDD: 50.4 ± 5.2 mm, LVESD: 30.8 ± 8.5 mm, and left atrial diameter: 44.5 ± 6.5 mm. *GLA* gene mutations were detected in three male patients.

Table 1. Demographic, clinical, and echocardiographic characteristics in patients with unexplained left ventricular hypertrophy

	HCM (n=120)
Male / Female, n (%)	51 (42%) / 69 (58%)
Age (years)	60.3±15.7
BMI (kg/m^2)	26.3 ± 4.7
HT, n (%)	19 (16%)
DM, n (%)	7 (6%)
HPL, n (%)	16 (13%)
CAD, n (%)	5 (4%)
Ejection fraction (%)	61.4 ± 4.8
Interventricular septal wall thickness (mm)	15.2 ± 5.4
Posterior wall thickness (mm)	13.5 ± 2.1
LV end-diastolic diameter (mm)	50.4 ± 5.2
LV end-systolic diameter (mm)	30.8 ± 8.5
Left atrial diameter (mm)	44.5 ± 6.5

Abbreviations: HCM, Hypertrophic cardiomyopathy; BMI, body mass index; HT, hypertension; DM, diabetes mellitus; HPL, hyperlipidemia; CAD, coronary artery disease; LV, left ventricular.

One of these mutations (*NM_000169.2:IVS6-10G>A [c.1000-10G>A]*) was previously associated with FD. Another mutation's association with FD was considered a benign GLA variant (*NM_000169.2:c.937G>T [p.D313Y] [p.Asp313Tyr]*). A third was a mutation that had not been previously associated with FD (*NM_000169.2:c.941A>T [p.K314M] [p.Lys314Met]*). The three mutations mentioned are discussed below with case examples.

Case 1.

This 56-year-old male patient presented to cardiology with dyspnea on exertion and palpitations. His systemic arterial pressure was 135/82 mmHg, and his heart rate was arrhythmic at 110 beats per minute. His electrocardiogram demonstrated atrial fibrillation and LVH, and his TTE showed concentric LVH, with an EF of 65%. Cerebral white matter lesions were detected on cranial magnetic resonance imaging, and microalbuminuria was detected in a urine analysis. The patient had low α-Gal A activity at 2.80 nmol/mg/h (normal range: >23.10 nmol/mg/h), and his lyso-Gb3 level was high at 12.80 ng/mL (normal range: <1.30). A genetic analysis revealed the *NM_000169.2:IVS6-10G>A (c.1000-10G>A)* mutation, which was consistent with the diagnosis of FD (14) **(Figure 1**). The patient's family screening showed a similar mutation in seven relatives. For the index patient, ERT was started.

Case 2:

This 58-year-old male patient, who was experiencing chest pain and shortness of breath, presented to the cardiology department. The patient was suffering from coro-

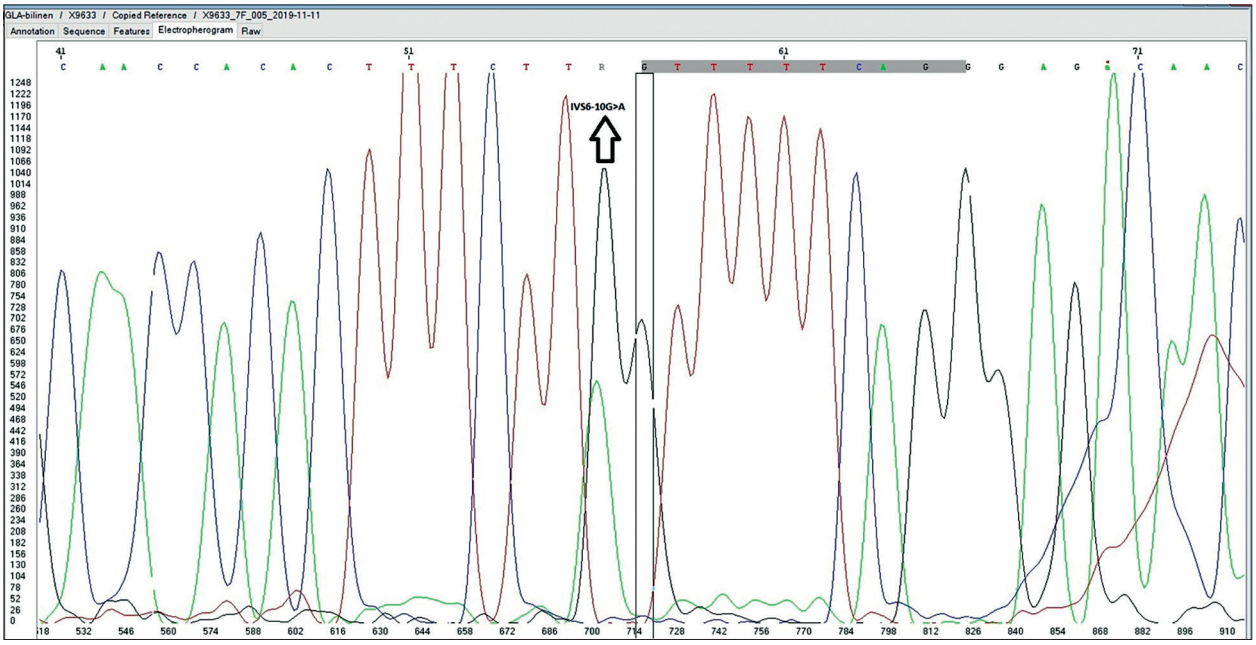

Figure 1. Genetic analysis showed the NM_000169.2:IVS6-10G>A (c.1000-10G>A) mutation consistent with the diagnosis of Fabry Disease

nary artery disease and hypertension and had undergone stunting in the right coronary artery and the left anterior descending coronary artery. A genetic assessment was performed because of unexplained concentric LVH on echocardiography, and the *NM_000169.2:c.937G>T (p.D313Y)* (p.Asp313Tyr) mutation was found (15) (**Figure 2**). The patient had normal α-Gal A activity and normal lyso-Gb3 levels. His nephrological, neurological, and ocular assessments were also found to be normal.

Case 3.

This 58-year-old male patient presented to cardiology with palpitations. He had no known cardiac history. His electrocardiography results showed a normal sinus rhythm and concentric LVH, and his neurological, nephrological, ocular, and other systemic examinations were normal. A genetic analysis revealed the *NM_000169.2:c.941A>T (p.K314M) (p.Lys314Met)* mutation (14) (**Figure 3**). The patient had normal α-Gal A activity and normal lyso-Gb3

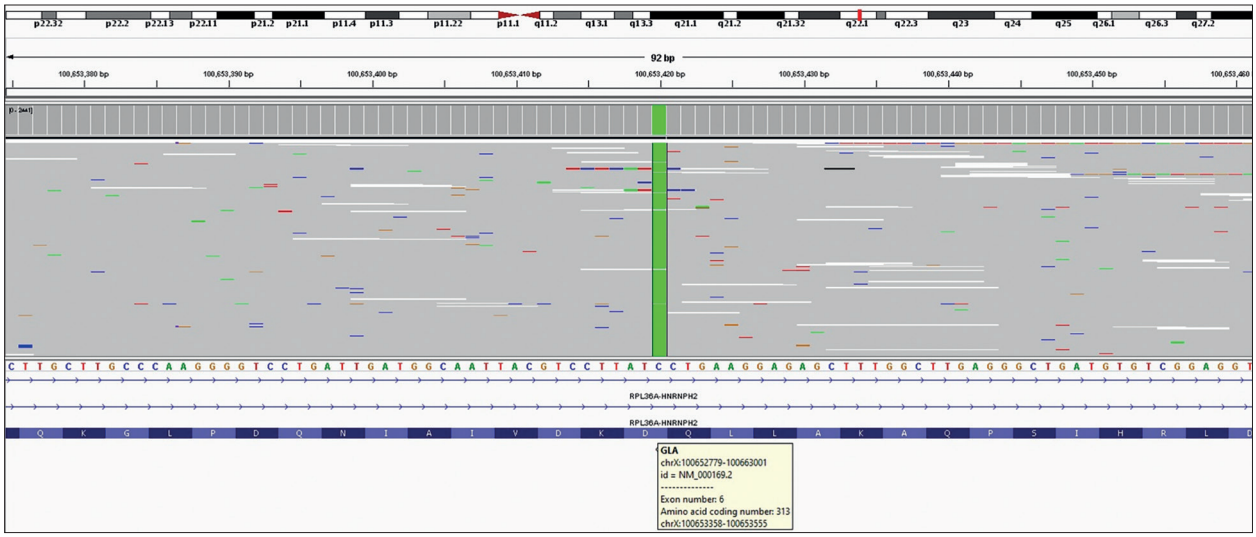

Figure 2. Genetic analysis showed the NM_000169.2:c.937G>T (p.D313Y) (p.Asp313Tyr) mutation

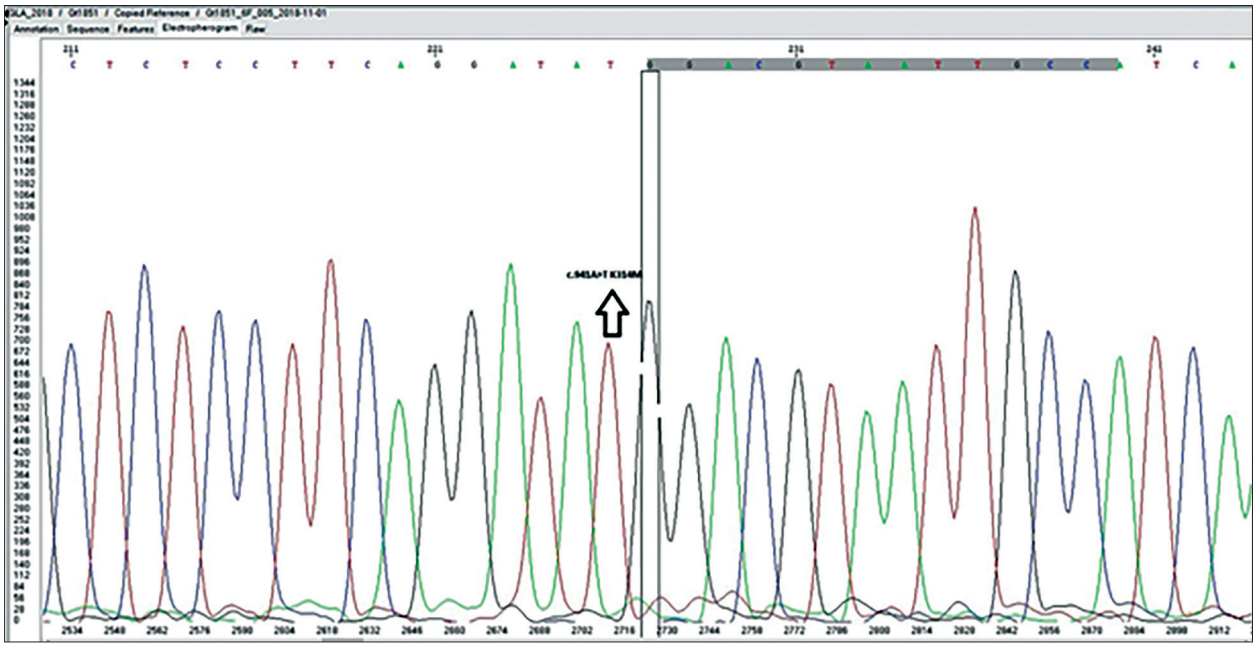

Figure 3. Genetic analysis revealed the NM_000169.2:c.941A>T (p.K314M) (p.Lys314Met) mutation

levels. The same genetic mutation was discovered in two siblings during family screening. However, the patient's echocardiographic examinations were normal.

DISCUSSION

In this study, 120 patients with idiopathic LVH on echocardiography were genetically screened for FD. A genetic mutation was detected that was previously associated with FD in one of the participants (Case 1). The D313Y mutation was detected in another participant (Case 2). This mutation was previously described as a benign *GLA* gene mutation. In the third participant (Case 3), a result was obtained that is considered a polymorphism in the foreground.

Variants in the *α-GLA A* gene present on the X chromosome cause FD (1). Due to a deficiency in the α-Gal A enzyme, the glycolipid Gb3 steadily accumulates within lysosomes. The degree of X inactivation determines the intensity of FD in females (4). Multisystemic involvement is exhibited in FD (16). It is evident from the recurring observation of cardiac involvement in FD that the heart is extremely sensitive to α-GLA A (17, 18) and that cardiac involvement is the main cause of morbidity and mortality in patients with FD (10).

Differences in the incidence and occurrence of FD signify variations in study design and population. There is a low prevalence of the disease in the general population; however, it is increasing in different at-risk patient populations, for example, those with kidney failure, stroke, or HCM (12). Nakao et al. determined seven patients to be suffering from FD out of a group of 230 Japanese males with LVH (12). Sachdev et al. identified six patients as having FD out of 153 males with HCM (4%) in the United Kingdom, with a prevalence of 6% for males diagnosed at over 40 years of age (19). Ommen et al. determined that out of 100 patients with HCM in the United States who had undergone septal ablation, none had FD (20). Chimenti et al. determined four Italian patients to have FD out of a total of 34 females (12%) with HCM who had undergone endomyocardial biopsy (17). Monserrat et al. screened plasma for α-Gal A activity in a Spanish population of patients with HCM and determined an FD prevalence of almost 2% (0.9% in males and 1.1% in females). However, this study included many variants of unknown significance (21). Havndrup et al. identified three patients with FD from a group of 90 patients with LVH in Denmark (18). Elliot et al. identified seven patients with FD from a group of 1,386 European patients (3.4%) with HCM or unexplained LVH (22). Hagege et al. performed systematic screening for FD (an α-Gal A assay on dried blood spots via a filter paper test) in a French population of patients diagnosed with HCM and found four patients with FD from a total of 278 males (23). However, Mawatari found no FD in a Japanese population of 738 patients with unexplained LVH (24). Five patients with FD were identified by Terryn et al. from a group of 560 Belgian patients with unexplained LVH (25). Out of a group of 100 Czech patients, Palecek et al. found four patients with FD (26). Baptista et al. performed screening for FD in Portuguese patients with LVH, and a single case of FD was identified from a total of 47 patients (27). A Spanish group of 805 patients with clinical symptoms related to FD was examined by Vieitez et al., and 21 patients were found to have FD (28). Maron et al. performed screening for FD among patients from North America with diagnosed HCM. The study found two patients with FD from a total of 585 patients (29). From a Korean group of 988 patients with LVH, Kim et al. found five patients with FD (30). Similarly, in our study, two patients out of 120 with unexplained LVH were diagnosed with FD by genetic analysis (1.6%) (**Figure 4**). In these screening studies, different LVH cut-off values were used. Similarly, the diagnostic methods for FD varied in these studies.

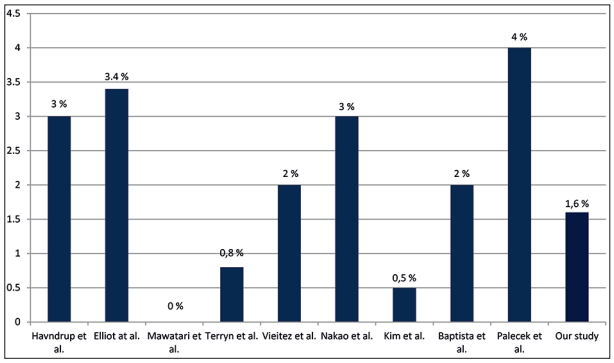

Figure 4. Prevalence of Fabry disease in various populations in patients with unexplained LVH

The *D313Y* genotype, previously described as a benign mutation not clinically relevant to FD (15), was reported in 2016 to have a significant impact on health-related quality of life of respective individual patients. This mutation might represent a confounding risk factor for certain isolated symptoms, triggering a specific mild clinical variant of FD (31).

During these screening studies, some of the genetic mutations detected by genetic analyses were pathogenic, while some were polymorphisms. For instance, Montserrat et al. reported an incidence of 1% in the Spanish population but includes many variants of unknown significance (21). The difficulty lies in identifying *GLA* variants that may give rise to the clinical symptoms of FD. There is a lack of en-

zyme activity in patients with classic FD who have clinically significant α-GLA A enzyme deficiency (32). It was found that the residual activity of 30% to 35% of the mean normal α-GLA A activity was the threshold for diagnosing FD (33).

There are three categories of α-GLA A residual activity into which GLA variants are grouped. In the first category, patients have benign *GLA* gene mutations and a 10% change in enzyme levels (34). In the second category, enzyme activity is between 15% to 30% of the standard activity in males (35). The clinical expression of the second group is highly dependent on genetic and epigenetic modifiers. In the third category, there is considerably reduced enzyme activity (GLA variants less than 35% to 40% of the average normal male controls). The true pathogenicity threshold for FD in terms of enzyme levels is unknown.

Normal enzyme levels in male patients indicate benign GLA variants and polymorphisms in these patients. Since the enzyme levels of the patients in Case 2 and Case 3 were normal, the genetic result was evaluated as a predominantly benign GLA variant and polymorphism. Their cardiomyopathy likely has another etiology, and the variant found was a benign polymorphism. These results demonstrate that enzyme activity levels alone are insufficient to determine whether a particular GLA variant is pathogenic. In addition to enzyme levels, the accumulation of substrate in plasma or urine or altered sphingolipid levels (Gb3 or lyso-Gb3) may be helpful in the diagnosis of FD (14, 15). However, recognizing typical lysosomal inclusions in tissue biopsy specimens is suggested as the best method of diagnosing FD (36).

Fabry disorder is rare in that it is one of the metabolic diseases that can be treated. As it is a progressive disease, the early diagnosis of FD is vital, and it can be treated with ERT and pharmacological chaperones (37). If FD is diagnosed via screening, it is extremely important to screen family members. Some individuals may be identified via family screening who have not yet developed organ damage (with the exception of the index case).

CONCLUSION

In conclusion, early diagnosis is of vital importance in FD, which can be treated with enzyme replacement. Patients over 30 years of age with idiopathic LVH should be screened for FD because a patient with unexplained LVH is part of a high-risk group that has a high incidence of FD. In screening studies, clinical evaluation of organ involvement as well as enzyme and lyso-Gb3 levels are required to show whether the newly detected mutations are true pathogenetic mutations or polymorphisms. Identifying new polymorphisms is important to know the true mutations in FD.

Limitations

A key limitation of this study is the comparatively small number of study participants. Another limitation of this study is the fact that cardiac MRI and biopsy were not performed on the patients with gene mutations. As such, the diagnoses of FD in these patients are not definitively established.

REFERENCES

1. Desnick RJ, Brady R, Barranger J, Collins AJ, Germain DP, Goldman M, et al. Fabry disease, an under-recognized multisystemic disorder: expert recommendations for diagnosis, management, and enzyme replacement therapy. Ann Intern Med. 2003 Feb 18;138(4):338-46. doi: 10.7326/0003-4819-138-4-200302180-00014.
2. Pinto LL, Vieira TA, Giugliani R, Schwartz IV. Expression of the disease on female carriers of X-linked lysosomal disorders: a brief review. Orphanet J Rare Dis. 2010 May 28;5:14. doi: 10.1186/1750-1172-5-14.
3. Garman SC, Garboczi DN. The molecular defect leading to Fabry disease: structure of human alpha-galactosidase. J Mol Biol. 2004 Mar 19;337(2):319-35. doi: 10.1016/j.jmb.2004.01.035.
4. Kampmann C, Baehner F, Whybra C, Martin C, Wiethoff CM, Ries M, et al. Cardiac manifestations of Anderson-Fabry disease in heterozygous females. J Am Coll Cardiol. 2002 Nov 6;40(9):1668-74. doi: 10.1016/s0735-1097(02)02380-x.
5. Mehta A, Ricci R, Widmer U, Dehout F, Garcia de Lorenzo A, Kampmann C, et al. Fabry disease defined: baseline clinical manifestations of 366 patients in the Fabry Outcome Survey. Eur J Clin Invest. 2004 Mar;34(3):236-42. doi: 10.1111/j.1365-2362.2004.01309.x.
6. Morrissey RP, Philip KJ, Schwarz ER. Cardiac abnormalities in Anderson-Fabry disease and Fabry's cardiomyopathy. Cardiovasc J Afr. 2011 Jan-Feb;22(1):38-44.
7. Meikle PJ, Hopwood JJ, Clague AE, Carey WF. Prevalence of lysosomal storage disorders. JAMA. 1999 Jan 20;281(3):249-54. doi: 10.1001/jama.281.3.249.
8. Barba-Romero MÁ, Rivera-Gallego A, Pintos-Morell G; Spanish FOS-Study Group. Fabry disease in Spain: description of Spanish patients and a comparison with other European countries using data from the Fabry Outcome Survey (FOS). Int J Clin Pract. 2011 Aug;65(8):903-10. doi: 10.1111/j.1742-1241.2011.02695.x.

9. Linthorst GE, Bouwman MG, Wijburg FA, Aerts JM, Poorthuis BJ, Hollak CE. Screening for Fabry disease in high-risk populations: a systematic review. J Med Genet. 2010 Apr;47(4):217-22. doi: 10.1136/jmg.2009.072116.
10. Mehta A, Clarke JT, Giugliani R, Elliott P, Linhart A, Beck M, et al; FOS Investigators. Natural course of Fabry disease: changing pattern of causes of death in FOS - Fabry Outcome Survey. J Med Genet. 2009 Aug;46(8):548-52. doi: 10.1136/jmg.2008.065904.
11. Waldek S, Patel MR, Banikazemi M, Lemay R, Lee P. Life expectancy and cause of death in males and females with Fabry disease: findings from the Fabry Registry. Genet Med. 2009 Nov;11(11):790-6. doi: 10.1097/GIM.0b013e3181bb05bb.
12. Nakao S, Takenaka T, Maeda M, Kodama C, Tanaka A, Tahara M, Yoshida A, Kuriyama M, Hayashibe H, Sakuraba H, et al. An atypical variant of Fabry's disease in men with left ventricular hypertrophy. N Engl J Med. 1995 Aug 3;333(5):288-93. doi: 10.1056/NEJM199508033330504.
13. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. Eur Heart J Cardiovasc Imaging. 2015 Mar;16(3):233-70. doi: 10.1093/ehjci/jev014.
14. Niemann M, Rolfs A, Störk S, Bijnens B, Breunig F, Beer M, et al. Gene mutations versus clinically relevant phenotypes: lyso-Gb3 defines Fabry disease. Circ Cardiovasc Genet. 2014 Feb;7(1):8-16. doi: 10.1161/CIRCGENETICS.113.000249.
15. Niemann M, Rolfs A, Giese A, Mascher H, Breunig F, Ertl G, Wanner C, Weidemann F. Lyso-Gb3 Indicates that the Alpha-Galactosidase A Mutation D313Y is not Clinically Relevant for Fabry Disease. JIMD Rep. 2013;7:99-102. doi: 10.1007/8904_2012_154.
16. Germain DP. Fabry disease. Orphanet J Rare Dis. 2010 Nov 22;5:30. doi: 10.1186/1750-1172-5-30
17. Chimenti C, Pieroni M, Morgante E, Antuzzi D, Russo A, Russo MA, et al. Prevalence of Fabry disease in female patients with late-onset hypertrophic cardiomyopathy. Circulation. 2004 Aug 31;110(9):1047-53. doi: 10.1161/01.CIR.0000139847.74101.03.
18. Havndrup O, Christiansen M, Stoevring B, Jensen M, Hoffman-Bang J, Andersen PS, et al. Fabry disease mimicking hypertrophic cardiomyopathy: genetic screening needed for establishing the diagnosis in women. Eur J Heart Fail. 2010 Jun;12(6):535-40. doi: 10.1093/eurjhf/hfq073.
19. Sachdev B, Takenaka T, Teraguchi H, Tei C, Lee P, McKenna WJ, et al. Prevalence of Anderson-Fabry disease in male patients with late onset hypertrophic cardiomyopathy. Circulation. 2002 Mar 26;105(12):1407-11. doi: 10.1161/01.cir.0000012626.81324.38.
20. Ommen SR, Nishimura RA, Edwards WD. Fabry disease: a mimic for obstructive hypertrophic cardiomyopathy? Heart. 2003 Aug;89(8):929-30. doi: 10.1136/heart.89.8.929.
21. Monserrat L, Gimeno-Blanes JR, Marín F, Hermida-Prieto M, García-Honrubia A, Pérez I, et al. Prevalence of fabry disease in a cohort of 508 unrelated patients with hypertrophic cardiomyopathy. J Am Coll Cardiol. 2007 Dec 18;50(25):2399-403. doi: 10.1016/j.jacc.2007.06.062.
22. Elliott P, Baker R, Pasquale F, Quarta G, Ebrahim H, Mehta AB, et al; ACES study group. Prevalence of Anderson-Fabry disease in patients with hypertrophic cardiomyopathy: the European Anderson-Fabry Disease survey. Heart. 2011 Dec;97(23):1957-60. doi: 10.1136/heartjnl-2011-300364.
23. Hagège AA, Caudron E, Damy T, Roudaut R, Millaire A, Etchecopar-Chevreuil C, et al; FOCUS study investigators. Screening patients with hypertrophic cardiomyopathy for Fabry disease using a filter-paper test: the FOCUS study. Heart. 2011 Jan;97(2):131-6. doi: 10.1136/hrt.2010.200188.
24. Mawatari K, Yasukawa H, Oba T, Nagata T, Togawa T, Tsukimura T, et al. Screening for Fabry disease in patients with left ventricular hypertrophy. Int J Cardiol. 2013 Aug 10;167(3):1059-61. doi: 10.1016/j.ijcard.2012.10.076.
25. Terryn W, Deschoenmakere G, De Keyser J, Meersseman W, Van Biesen W, Wuyts B, et al. Prevalence of Fabry disease in a predominantly hypertensive population with left ventricular hypertrophy. Int J Cardiol. 2013 Sep 10;167(6):2555-60. doi: 10.1016/j.ijcard.2012.06.069.
26. Palecek T, Honzikova J, Poupetova H, Vlaskova H, Kuchynka P, Golan L, et al. Prevalence of Fabry disease in male patients with unexplained left ventricular hypertrophy in primary cardiology practice: prospective Fabry cardiomyopathy screening study (FACSS). J Inherit Metab Dis. 2014 May;37(3):455-60. doi: 10.1007/s10545-013-9659-2.
27. Baptista A, Magalhães P, Leão S, Carvalho S, Mateus P, Moreira I. Screening for Fabry disease in left

ventricular hypertrophy: documentation of a novel mutation. Arq Bras Cardiol. 2015 Aug;105(2):139-44. doi: 10.5935/abc.20150090.

28. Vieitez I, Souto-Rodriguez O, Fernandez-Mosquera L, San Millan B, Teijeira S, Fernandez-Martin J, et al. Fabry disease in the Spanish population: observational study with detection of 77 patients. Orphanet J Rare Dis. 2018 Apr 10;13(1):52. doi: 10.1186/s13023-018-0792-8.
29. Maron MS, Xin W, Sims KB, Butler R, Haas TS, Rowin EJ, et al. Identification of Fabry Disease in a Tertiary Referral Cohort of Patients with Hypertrophic Cardiomyopathy. Am J Med. 2018 Feb;131(2):200.e1-200.e8. doi: 10.1016/j.amjmed.2017.09.010.
30. Kim WS, Kim HS, Shin J, Park JC, Yoo HW, Takenaka T, et al. Prevalence of Fabry Disease in Korean Men with Left Ventricular Hypertrophy. J Korean Med Sci. 2019 Feb 15;34(7):e63. doi: 10.3346/jkms.2019.34.e63.
31. Oder D, Üçeyler N, Liu D, Hu K, Petritsch B, Sommer C, et al. Organ manifestations and long-term outcome of Fabry disease in patients with the GLA haplotype D313Y. BMJ Open. 2016 Apr 8;6(4):e010422. doi: 10.1136/bmjopen-2015-010422.
32. Branton MH, Schiffmann R, Sabnis SG, Murray GJ, Quirk JM, Altarescu G, et al. Natural history of Fabry renal disease: influence of alpha-galactosidase A activity and genetic mutations on clinical course. Medicine (Baltimore). 2002 Mar;81(2):122-38. doi: 10.1097/00005792-200203000-00003.
33. Ferreira S, Ortiz A, Germain DP, Viana-Baptista M, Caldeira-Gomes A, Camprecios M, et al. The alpha-galactosidase A p.Arg118Cys variant does not cause a Fabry disease phenotype: data from individual patients and family studies. Mol Genet Metab. 2015 Feb;114(2):248-58. doi: 10.1016/j.ymgme.2014.11.004.
34. Eng CM, Desnick RJ. Molecular basis of Fabry disease: mutations and polymorphisms in the human alpha-galactosidase A gene. Hum Mutat. 1994;3(2):103-11. doi: 10.1002/humu.1380030204.
35. Eng CM, Ashley GA, Burgert TS, Enriquez AL, D'Souza M, Desnick RJ. Fabry disease: thirty-five mutations in the alpha-galactosidase A gene in patients with classic and variant phenotypes. Mol Med. 1997 Mar;3(3):174-82.
36. Van der Tol L, Smid BE, Poorthuis BJ, Biegstraaten M, Deprez RH, Linthorst GE, et al. A systematic review on screening for Fabry disease: prevalence of individuals with genetic variants of unknown significance. J Med Genet. 2014 Jan;51(1):1-9. doi: 10.1136/jmedgenet-2013-101857.
37. Hoffmann B, Beck M, Sunder-Plassmann G, Borsini W, Ricci R, Mehta A; FOS European Investigators. Nature and prevalence of pain in Fabry disease and its response to enzyme replacement therapy--a retrospective analysis from the Fabry Outcome Survey. Clin J Pain. 2007 Jul-Aug;23(6):535-42. doi: 10.1097/AJP.0b013e318074c986.