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**High risk population screening for Fabry disease in
hemodialysis patients in Vojvodina**

Скрининг високоризичне популације на Фабријеву болест међу
хемодијализним пацијентима у Војводини

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High risk population screening for Fabry disease in hemodialysis patients in Vojvodina

Скрининг високоризичне популације на Фабријеву болест међу хемодијализним пацијентима у Војводини

SUMMARY

Introduction Fabry disease (FD) is an X-linked lysosomal storage disease that develops as a consequence of mutation in the alpha-galactosidase A (*GLA*) gene. There are more than 1080 known variants in the *GLA* gene. Some of them are pathogenic, but most of them are benign or represent the genetic change that can be classified as a genetic variant of unknown significance or simply be a representation of genetic polymorphism. There are two main features of FD, classic form and late-onset variants of disease. The main target organs in patients with FD are kidneys, heart and nervous system. Bearing in mind the fact that FD is a rare disease, the best way for active searching of patients is high-risk population screening, after which family screening for every proband case should be performed.

Methods In this paper, we present results of a multicentric pilot study that represents findings from the screening of hemodialysis patients for FD in six hemodialysis units in Vojvodina.

Results We have found one patient with benign mutation and 16 patients with genetic polymorphisms in *GLA* gene. We have learned that genetic changes in *GLA* gene can be frequent, but very rarely are of clinical significance and lead to manifestations of FD.

Conclusion Results of this screening study will give us important insights into our future work

Keywords: Fabry disease; hemodialysis; high-risk population screening

САЖЕТАК

Увод Фабријева болест (ФД) је Х-везана лизозомна болест складиштења која се развија као последица мутације гена алфа-галактозидазе А (ГЛА). Постоји више од 1080 познатих варијанти ГЛА гена. Неки од њих су патогени, али већина их је бенигна или представљају генетску промену која се може класификовати као генетска варијанта непознатог значаја или једноставно представљати генетски полиморфизам. Постоје две главне карактеристике ФД, класични облик и касније варијанте болести. Главни циљни органи код пацијената са овом болешћу су бубрези, срце и нервни систем. Имајући у виду чињеницу да је ФД ретка болест, најбољи начин за активну претрагу пацијената је скрининг популације високог ризика, након чега би требало извршити породични скрининг за сваки случај пробанда.

Метод У овом раду представљамо резултате мултицентричне пилот студије која представља налазе скрининга пацијената на хемодијализи на ФД у шест хемодијализних центара у Војводини.

Резултати Идентификован је 1 пацијент са бенигном мутацијом и 16 пацијената са генетским полиморфизмом ГЛА гена. Утврдили смо да генетске промене на ГЛА гену могу бити честе, али су веома ретко од клиничког значаја и ретко доводе до манифестација ФД.

Закључак Резултати ове скрининг студије ће нам омогућити увид у преваленцу ФД у хемодијализној популацији и усмерити наш будући рад.

Кључне речи: Фабријева болест; хемодијализа; скрининг популације високог ризика

INTRODUCTION

Fabry disease (FD) (OMIM 301500) is an X-linked lysosomal storage disorder caused by mutations in the *GLA* gene that result in markedly reduced or absent activity of the enzyme α -galactosidase A (α -Gal A, EC 3.2.1.22). This leads to the intracellular accumulation of substrates like globotriaosylceramide (Gb3) and also its deacylated derivative globotriaosylsphingosine (lyso-Gb3). Lyso-Gb3 is a valuable biomarker and speaks mainly

about the burden of FD, since it reflects the severity of disease and also corresponds well with tissue accumulation of Gb3. So, Lyso-Gb3 is of great importance in establishing the diagnosis of FD, but also in assessing the disease severity and therapeutic monitoring. [1-4] Until now, more than 1080 variants in the *GLA* gene have been identified in the Human Gene Mutation Database (HGMD). [5] Some of them are pathogenic but others are benign or represent genetic change that can be classified as a genetic variant of unknown significance. After the findings of genetic change in *GLA* gene in each patient, we can scroll through the HGMD and seek information about the clinical significance of found mutation [1,5,6]

Prevalence of FD is in the range of 1:40.000-1:117.000 live male newborns [7], but the exact prevalence is very hard to establish due to the very heterogeneous nature of the disease (even within a group of patients with the same genetic variant) and lack of awareness about the disease. In contrast to other X-linked diseases, in which females can only be carriers, in FD females may be as severely affected as male patients. [8] There are two basic phenotypes of FD: a classic form of the disease that is mainly associated with deletions, frameshifts and nonsense variants in the *GLA* gene, and late-onset variants of FD that are associated with missense variants in the *GLA* gene.[9] The classic form of FD starts at a younger age with earlier manifestations on target organs (heart, kidneys, nervous system) and usually with some other clinical presentations of the disease, like cornea verticillata, angiokeratomas and acroparesthesia (burning sensations on hands and feet). On the other hand, later-onset variants are becoming clinically evident later in life, with cardiomyopathy and/or nephropathy alone [1] Diagnostic criteria for FD require the confirmation of low enzyme activity and presence of genetic variants in the *GLA* gene for male patients or *GLA* gene variants for female patients together with characteristic clinical features of the disease and/or a family member with the diagnosis of FD and/or elevated plasma lyso-Gb3 level. [10] In some cases of suspected FD

target organ biopsy should be performed, with electron microscopy analysis of the tissues, so typical cellular inclusions, “zebra bodies“, can be found [1,2].

The current mainstay of treatment for FD patients includes the administration of enzyme replacement therapy (ERT). Nowadays, in clinical practice, there are two available forms of the drug: agalsidase alpha and agalsidase beta, with different dosage regimes (0.2mg/kg intravenously (i.v.) every other week (eow) and 1mg/kg i.v. eow, respectively) but similar therapeutic efficacy. In some countries, there is also a possibility for oral therapy with migalastat for some types of amenable mutations. [11]

There are only 6 established cases of FD in Serbia, for now. The exact number of FD patients in our country is hard to establish, but should probably be in the range of 50-100 patients. One way of finding of FD patients is high-risk population screening which represents an active search for FD patients in certain patient populations like hemodialysis patients, patients with hypertrophic cardiomyopathy of unknown/uncertain origin or patients with an early stroke (before the age of 55).

FD often remains undiagnosed according to the prevalence calculated in some studies because of the heterogeneous nature of its clinical manifestations. The clinical diagnosis of both phenotypes (classic form and late-onset variants) is challenging, since many of the main symptoms and signs are common in other diseases, and the time between the first symptoms and the settling of diagnosis can take more than ten years. [1,10] Therefore, the precise establishment of the molecular-genetic diagnosis and the earliest possible treatment is essential to avoid significant disease progression. Molecular analysis of FD is also crucial for segregation studies, enabling early diagnosis of family members with pathogenic variants in the *GLA* gene allowing monitoring before the first symptoms appear, and therefore promoting better disease management.

The aim of this study was to perform molecular-genetic analysis of FD in the group of hemodialysis patients to provide an early application of appropriate therapy as well as the provision of genetic advice to families with a high risk for the birth of a child with FD.

METHODS

After the approval of ethic committees from every institution which participated in the screening program and the signing of full informed consent by every patient who participated in the study, we performed a multicentric pilot study from December 2020. until May 2021. The study was based on a FD screening of patients in six hemodialysis centers from different parts of Vojvodina (Stara Pazova, Pančevo, Kikinda, Subotica, Bačka Palanka and Sremska Mitrovica). At the time of the screening process, these hemodialysis centers had a dialysis population of 529 patients in total (58, 135, 82, 70, 64 and 120, respectively). Patients eligible for screening were male patients under 55 years of age and with an unknown cause of end-stage renal disease (ESRD), as well as female patients of all ages and unknown cause of ESRD. Because of the variable clinical presentations of FD we analyzed the presence of other clinical manifestations, besides ESRD, that can be a part of the clinical spectrum of FD in our patient eligible for screening process (left ventricular hypertrophy (LVH), stroke before the age of 55, existence of white matter lesions, cornea verticillata, angiokeratoma, tinnitus, hearing loss, heat or cold intolerance, postprandial pain, acroparesthesia, hypo-, or hyperhidrosis). The screening protocol for FD was based upon genetic analysis of peripheral blood from 117 selected patients. Genetic analysis was performed at the Institute of Molecular Genetics and Genetic Engineering, University of Belgrade.

In patients with genetic polymorphisms or genetic variants of unknown significance in the *GLA* gene, we performed biomarker (Lyso-Gb3) analysis in Centogene, Rostock, Germany, from the blood of our participants through dried blood spots (DBS, CentoCard,

Centogene A.D) analysis with the liquid chromatography mass spectrometry method (HPLC-MS/MS; Thermo Fischer Scientific)

Detection of genetic variants in the *GLA* gene

Genomic DNA was isolated directly from whole peripheral blood using QIA amp DNA Blood Mini Kit (QIAGEN, Hilden Germany), and quantified with a Qubit® 3.0 Fluorimeter (Thermo Fisher Scientific, MA, USA) and Qubit™ dsDNA HS Assay Kit (Thermo Fisher Scientific, MA, USA). All seven exons and flanking intron regions of the *GLA* gene (GenBank: NM_000169.3) were individually PCR amplified using PCR System (Eppendorf, Master cycler Nexus, Hamburg, Germany). Primers were designed in the way that an average length of fragments was between 400 and 700 bp and that all known SNPs were avoided. We used different online available tools: SNP Check [<http://www.ngl.org.uk>], OligoAnalyzer [<http://www.idtdna.com/pages/tools/oligoanalyzer>], In silico PCR [<http://genome.ucsc.edu>]. A list of all primers is given in Table 1.

Reactions were carried out in a final volume of 25µl containing 100 ng of genomic DNA, 1 x PCR buffer (with Mg²⁺), 1 x Q solution, 1.0 mM MgCl₂, 0.2 mM dNTP each, 1 U of HotStarTaq DNA® Polymerase (QIAGEN, Hilden Germany), 10 pmol of each primer and distilled water. Cycling conditions for PCR were as follows: initial denaturation at 95°C for 15 min; 30 cycles at 94°C for 30 sec, 54°C for 30 sec, and 72°C for 1 min, followed by a final extension at 72°C for 5 min.

All PCR fragments were directly sequenced using the Big Dye terminator cycle sequencing kit and the ABI PRISM 310 automated sequencer (Applied Biosystems Life Technologies, USA). Segregation analysis was performed in families when it was possible to determine the carrier status of detected genetic variants.

RESULTS

Hemodialysis, together with peritoneal dialysis and kidney transplantation, represents methods of renal replacement therapy. There are 16 hemodialysis centers in Vojvodina, with around 1600 patients. From the yearly reports of the Registry of Serbian Society of Nephrology, we have learned that around 8% of patients with end-stage renal disease (ESRD) in Serbia have chronic kidney disease of unknown etiology. [12]

FD is a rare disease that physicians usually don't think about in their everyday clinical practice. Having in mind that hemodialysis patients belong to a high-risk population group for FD, we decided to perform a pilot study of screening for FD patients in hemodialysis centers in Vojvodina. According to the previously mentioned criteria for screening eligibility, we have screened 117 patients (40 females) from the aforementioned hemodialysis units with a total dialysis population of 529 (22%) (Table 2). The majority of screened patients had LVH (63.2%), but stroke before the age of 55 and white matter lesions were rare (4.3% and 2.6% respectively). Around one-fifth of screened patients also had tinnitus, hearing loss, heat or cold intolerance, postprandial pain and acroparesthesia. Complete list of comorbidities is given in Table 3.

In this study, we conducted the genetic analysis of 117 patients suspected of FD, and the *GLA* sequencing revealed only one male patient (with a frequency of 0.8%) with a variant in a coding region (p.Asp313Tyr), 16 patients (13.7%) had only non-coding variants (NCVs), while 100 subjects had no variants in the analyzed regions (85.5%). All detected genetic changes were previously described in the literature. Five different combinations of Number of Cited Variants (NCVs), described as complex intronic haplotypes (CIHs), in the *GLA* gene were identified in our group of subjects (Table 4 and 5). The most frequent haplotype is formed by the four variants c.-10C>T, c.370-81_370-77delCAGCC, c.640-16A>G, c.1000-22C>T, and was detected in 8 of 117 (6.8%) patients. Lyso-Gb3 biomarker levels were within normal range

in each tested patient, so all genetic changes we have found could be accounted for nonpathogenic.

DISCUSSION

Fabry disease is a rare systemic metabolic disorder that leads to the accumulation of lipid substrates in lysosomes in various tissues and organs. Having in mind a fact that most important target organs in FD patients are kidneys, heart and brain, most of the screening programs worldwide are directed at patient populations on renal replacement therapies, patients with left ventricular hypertrophy on unknown origin and patients with early cerebrovascular incidents.

In this paper we have summarized the efforts from our pilot study in the screening of hemodialysis patients from 6 hemodialysis centers in Vojvodina. In different screening studies on patients in hemodialysis units the prevalence of *GLA* mutations was up to 0.87% [13,14], but if only pathogenic mutations were checked for, then the prevalence of FD dropped to around 0.14-0.3%. [15] Some screening programs are oriented just on male patients which is unacceptable, since female patients can be as severely affected as their male counterparts. Inconsistency of genotype-phenotype correlation in females is due to X-chromosome inactivation.

Detection of genetic variants in the *GLA* gene is essential to confirm the clinical diagnosis of FD. [16] In the present study, we sequenced the entire *GLA* gene including coding regions and flanking intron sequences to find alterations that could explain observed FD-like characteristics in our group of subjects. In 13.7% (16 of 117) of subjects, we identified five different combinations of CIHs in the *GLA* gene. The most frequent (6.8%) was the haplotype consisting of four variants (c.-10C>T, c.370-81_370-77delCAGCC, c.640-16A>G, c.1000-

22C>T), which coincides with the results of other studies, where the frequency of this haplotype varied from 8.9% to 13.4% in subjects with clinically suspected FD [17,18].

According to Van der Tol and co-workers [19], the prevalence of pathogenic variants in the *GLA* gene is 0.12%. When variants of uncertain significance (VUS) are included, this number increases to 0.62%. The frequencies reported in our study do not reflect the Van der Tol data since these intronic variants have been reported as polymorphic variants in the general population and, as in most cases, have been observed individually rather than in haplotypes. Furthermore, most studies perform sequencing only through coding regions of the *GLA* gene and thus fail to detect heterozygosity in intron regions. [20] Depending on the sequencing design, CIHs often remain unidentified since these regions are not investigated routinely by gene sequencing; consequently, the prevalence of FD may be underestimated. [21] Previous reports have found that CIHs can be associated with different clinical manifestations reflecting mild renal, neurological, and cardiac disorders [22-25]. However, measuring the level of biomarker Lyso-Gb3 in DBS showed the normal level in all tested patients with detected CIHs in this study. Our results are in agreement with the findings of Ferri and colleagues [18], in which are detected 7 different *GLA* haplotypes in control males, indicating that these CIHs are not involved in the development of FD manifestations. But Gervas-Arruga and colleagues [21] suggest that in patients with CIHs, environmental factors, as a pro-inflammatory state, in addition to the accumulation of Gb3 may influence the symptoms. Moreover, it has been shown that DBS lyso-Gb3 levels are not solely enough for defining diagnosis of FD and that secondary analysis should be made, due to possible false-positive or false-negative results. The estimated sensitivity of the HPLC-MS/MS method used for measuring DBS lyso-Gb3 levels is 67%, which indicates the limits of accurate/precise screening outcomes [26]. Hence, the lack of abnormality in Lyso-Gb3 levels is not a reliable parameter for excluding FD. Therefore, further analysis, such as gene expression analysis, should be performed to confirm

or reject the FD diagnosis in subjects with detected CIHs in the *GLA* gene and doubtful diagnosis of disease.

High-risk population screening program represents the best and the easiest way of finding a new FD patient. A diagnostic algorithm can be proposed for screening of patients with ESRD in hemodialysis centers (Figure 1). It's based on the appropriate usage of enzyme activity testing, genetic testing as well as other types of testing that can help physicians in determining the significance of findings during screening process. This algorithm can also be used for patients with milder degrees of chronic kidney disease. In male patients, first step should be enzyme testing, and if needed (when enzyme levels are below normal values), further genetic testing should ensue, while in suspected female FD patients genetic testing should be the first step due to the well-known process of X-chromosome inactivation.

After appropriate high risk population screening is performed, on every index case one could find 3-5 new cases during family screening that should comprise 3 generations in the family tree.

Having in mind the fact that the prevalence of a pathogenic mutation in the *GLA* gene is very low in hemodialysis population, there is a place for future investigations since the total number of patients on hemodialysis in Vojvodina is around 1600 and in Serbia around 5500-6000 patients. [12]

CONCLUSION

The challenges in the establishment of the precise diagnosis of FD and indications for treatment are part of today's clinical practice. In the literature has been described that the enzymatic assay is diagnostic for male patients, while female patients most often need molecular-genetic analysis to get a definite diagnosis. Moreover, the correlation between DBS enzyme activity and *GLA* variants revealed that this screening method is useful for diagnosing

previously described mutations. However, when the patient presents CIH, although our study indicates a possible non-pathogenicity, the diagnosis may not be conclusive and other tools may be necessary to confirm or discard the disease. Because the genotype-phenotype correlation often shows inconsistency with FD manifestations and the effects of multiple intronic variants are not yet fully understood and seem to have individual variations, future expressional and functional studies or research focused on the discovery of possible modifier genes are necessary to confirm FD in these patients.

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Table 1. List of all primers used for amplification of coding and flanking regions of alpha-galactosidase A gene

Primer name	Sequence 5'-3'	Length (bp)
GLA_ex1_F	5`-AGCGGAACGTCTTACGTGAC-3`	522
GLA_ex1_R	5`-CGGGACAGTTTGCTGGG-3`	
GLA_ex2_F	5`-AGGGCGGGAATATTAACGGG-3`	620
GLA_ex2_R	5`-GTTACAGGCGTTCACCACC-3`	
GLA_ex3_F	5`-CTAGCTCAGCAGAACTGGGG-3`	522
GLA_ex3_R	5`-GAGATGGGAGCTCTGGCAC-3`	
GLA_ex4_F	5`-GGGAAGCTGAGACAGAAGAGTC-3`	477
GLA_ex4_R	5`-CCAGGTGATGGTAGCTTAGGC-3`	
GLA_ex5_F	5`-GGTTTAGACCTCCTTATGGAGACG-3`	420
GLA_ex5_R	5`-GCATCCTGCTCTAAGTACTCTCAC-3`	
GLA_ex6_F	5`-GTGAGAGTACTTAGAGCAGGATGC-3`	555
GLA_ex6_R	5`-AGCAACTAGTGATAAGTGGCCC-3`	
GLA_ex7_F	5`-CAATACCAACTTTGTCTTGGGCC-3`	675
GLA_ex7_R	5`-AGGCGGGTCTCAAAGTCC-3`	

Table 2. Patients involved in Fabry disease screening per hemodialysis center

HD center	Patients on HD (N)	Tested female patients (n)	Tested male patients (n)	Tested - both genders (n)	Age of patients
Bačka Palanka	64	4	18	22	55.4 ± 6.4
Stara Pazova	58	3	2	5	36.2 ± 7.5
Kikinda	82	5	3	8	45.4 ± 7.1
Subotica	70	5	9	14	51.2 ± 6.3
Sremska Mitrovica	120	13	29	42	55.7 ± 4.4
Pančevo	135	10	16	26	47.7 ± 7.5
In total	529	40	77	117	48.6 ± 6.3

Table 3. Comorbidities in patients involved in Fabry disease screening

Comorbidities	Bačka Palanka (N = 22)	Stara Pazova (N = 5)	Kikinda (N = 8)	Subotica (N = 14)	Sremska Mitrovica (N = 42)	Pančevo (N = 26)	In total (N = 117)
Left ventricular hypertrophy	12 (54.5%)	2 (40%)	3 (37.5%)	6 (42.8%)	28 (66.7%)	25 (96%)	74 (63.2%)
Cerebrovascular incidents < 55 years	2 (9%)	0	1 (12.5%)	1 (7.2%)	1 (2.4%)	0	5 (4.3%)
White matter lesions	1 (4.5%)	0	1 (12.5%)	0	0	1 (4%)	3 (2.6%)
Cornea verticillata	0	1 (20%)	0	0	1 (2.4%)	1 (4%)	3 (2.6%)
Angiokeratoma	0	0	0	2 (14.4%)	1 (2.4%)	0	3 (2.6%)
Tinnitus	3 (13.5%)	1 (20%)	1 (12.5%)	9 (64.3%)	2 (4.8%)	7 (27%)	23 (19.7%)
Hearing loss	8 (36.4%)	0	1 (12.5%)	2 (14.4%)	6 (14%)	4 (15%)	21 (18%)
Heat/cold intolerance	1 (4.5%)		2 (25%)	8 (57.2%)	1 (2.4%)	8 (31%)	20 (17%)
Acroparesthesia	0	2 (40%)	2 (25%)	2 (14.4%)	6 (14.3%)	13 (50%)	25 (21.4%)
Hypo-/hyperhidrosis	0	1 (20%)	0	3 (21.4%)	7 (16.4%)	0	11 (9.4%)
Post prandial pain	2 (9%)	3 (60%)	1 (12.5%)	2 (14.4%)	3 (7%)	16 (62%)	27 (23%)

Table 4. The summary of complex intron haplotypes found in patients with suspicion of Fabry disease

	Alpha-galactosidase A region	Variant/Haplotype	Patients	
			N = 117	%
Variant 1	5'UTR	c.-10C>T	2	1.7
CIH 1	5'UTR, intron 2, intron 4, intron 6	c.-10C>T, c.370-77_-81delCAGCC, c.640-16A>G, c.1000-22C>T	8	6.8
CIH 2	intron 2, intron 4, intron 6	c.370-77_370-81delCAGCC, c.640-16A>G, c.1000-22C>T	4	3.4
CIH 3	5'UTR, intron4	c.-12G>A, 639+68A>G	1	0.8
CIH 4	5'UTR, intron 2, intron 4, intron 6	c.-12G>A, c.370-77_-81delCAGCC, c.640-16A>G, c.1000-22C>T	1	0.8

Table 5. Genetic changes in alpha-galactosidase A gene in our patients

Patient	Gender	Age	Genetic change	Importance
1.	♀	44	Incomplete CIH (c.-10C>T (g.1170 C>T))	Polymorphism
2.	♂	51	p.Asp313Tyr (D313Y)	Benign
3.	♀	61	CIH (c.- 10C>T (g.1170 C>T), c.370-77_-81delCAGCC (g.7188-7192 del5), c.640-16A>G (g.10115A>G), c.1000-22 C>T (g.10956 C>T))	Polymorphism
4.	♀	32	CIH (c.- 10C>T (g.1170 C>T), c.370-77_-81delCAGCC (g.7188-7192 del5), c.640-16A>G (g.10115A>G), c.1000-22 C>T (g.10956 C>T))	Polymorphism
5.	♀	52	CIH (c.- 10C>T (g.1170 C>T), c.370-77_-81delCAGCC (g.7188-7192 del5), c.640-16A>G (g.10115A>G), c.1000-22 C>T (g.10956 C>T))	Polymorphism
6.	♀	47	CIH (c.- 10C>T (g.1170 C>T), c.370-77_-81delCAGCC (g.7188-7192 del5), c.640-16A>G (g.10115A>G), c.1000-22 C>T (g.10956 C>T))	Polymorphism
7.	♀	44	CIH (c.- 10C>T (g.1170 C>T), c.370-77_-81delCAGCC (g.7188-7192 del5), c.640-16A>G (g.10115A>G), c.1000-22 C>T (g.10956 C>T))	Polymorphism
8.	♀	53	Incomplete CIH (c.-10C>T (g.1170 C>T))	Polymorphism
9.	♂	51	CIH (c.- 10C>T (g.1170 C>T), c.370-77_-81delCAGCC (g.7188-7192 del5), c.640-16A>G (g.10115A>G), c.1000-22 C>T (g.10956 C>T))	Polymorphism
10.	♂	46	CIH (c.- 10C>T (g.1170 C>T), c.370-77_-81delCAGCC (g.7188-7192 del5), c.640-16A>G (g.10115A>G), c.1000-22 C>T (g.10956 C>T))	Polymorphism
11.	♀	35	Incomplete CIH (c.-12G>A (g.1168 G>A), c.639+68A>G (g.8479>G))	Polymorphism
12.	♀	63	Incomplete CIH (c.370-77_-81delCAGCC (g.7188-7192 del5), c.640-16A>G (g.10115A>G), c.1000-22 C>T (g.10956 C>T))	Polymorphism
13.	♂	44	Incomplete CIH (c.370-77_-81delCAGCC (g.7188-7192 del5), c.640-16A>G (g.10115A>G), c.1000-22 C>T (g.10956 C>T))	Polymorphism
14.	♂	29	Incomplete CIH (c.370-77_-81delCAGCC (g.7188-7192 del5), c.640-16A>G (g.10115A>G), c.1000-22 C>T (g.10956 C>T))	Polymorphism
15.	♂	33	CIH (c.-12 G>A (g.1168 G>A), c.639+68A>G (g.8479>G), c.1000-22 C>T (g.10956 C>T))	Polymorphism
16.	♀	66	Incomplete CIH (c.370-81_-77delCAGCC (g.7188-7192del5), c.1000-22C>T (g.10956C>T))	Polymorphism
17.	♀	53	Incomplete CIH (c.-12G>A (g.1168 G>A), c.370-81_-77delCAGCC (g.7188-7192del5), c.639+68A>G (g.8479>G), c.1000-22 C>T (g.10956 C>T))	Polymorphism

Figure 1. Diagnostic algorithm for Fabry disease patients with end-stage renal disease

