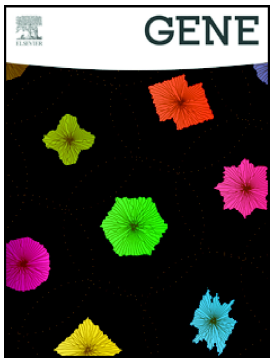

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PII: S0378-1119(17)30909-5
DOI: doi:10.1016/j.gene.2017.10.064
Reference: GENE 42285

To appear in: *Gene*

Received date: 3 August 2017
Revised date: 29 September 2017
Accepted date: 20 October 2017

Please cite this article as: Patrycja Juchniewicz, Anna Kloska, Anna Tylki-Szymańska, Joanna Jakóbkiewicz-Banecka, Grzegorz Węgrzyn, Marta Moskot, Magdalena Gabig-Cimińska, Ewa Piotrowska , Female Fabry disease patients and X-chromosome inactivation. The address for the corresponding author was captured as affiliation for all authors. Please check if appropriate. Gene(2017), doi:10.1016/j.gene.2017.10.064

Female Fabry disease patients and X-chromosome inactivation

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Abstract

Fabry disease is an X-linked inherited lysosomal storage disorder caused by mutations in the gene encoding α-galactosidase A (GLA). Once it was thought to affect only hemizygous males. Over the last fifteen years, research has shown that most females carrying mutated allele also develop symptoms, demonstrating a wide range of disease severity, from a virtually asymptomatic to more classical profile, with cardiac, renal, and cerebrovascular manifestations. This variable expression in females is thought to be influenced by the process of X-chromosome inactivation (XCI). The aim of this study was to assess severity of the clinical phenotype, to analyze XCI patterns, and to estimate their effect on disease manifestation in twelve female Fabry disease patients from five unrelated Polish families. Our analyses revealed that patients presented with the broad range of disease expression - from mild to severe, and their clinical involvement did not correlate with XCI profiles. Female carriers of the mutation in the *GLA* gene with the random XCI may present with the wide range of disease signs and symptoms. Thus, XCI is not a main factor in the phenotype variability of Fabry disease manifestation in heterozygous females.

Keywords: FOS-MSSI; heterozygotes; MSSI; severity score; XCI.

Abbreviations list:

AR - androgen receptor

DS3 - Fabry disease severity scoring system

FOS - Fabry Outcome Survey

FOS-MSSI - Fabry Outcome Survey Mainz Severity Score Index

α-GAL A - α-galactosidase A

Gb3 – globotriaosylceramide

GL3 - globotriaosylceramide

GLA - α-galactosidase A

lyso-Gb3 - globotriaosylsphingosine

MSSI - Mainz Severity Score Index

XCI - X-chromosome inactivation

1. Introduction

Fabry disease (OMIM #301500), also known as Anderson-Fabry disease or angiokeratoma corporis diffusum, is a lysosomal storage disorder caused by a deficiency of α-galactosidase A (α-GAL A, GLA), an enzyme required for the degradation of globotriaosylceramide (Gb3, GL3). The *GLA* gene coding for α-GAL A maps to Xq22.1 (locus MIM number: *300644). The earliest symptoms observed in patients with the classic form of the disorder in childhood and adolescence (in males earlier than in females) include neuropathic pain, vascular skin lesions, sweating abnormalities, characteristic ocular changes, gastrointestinal problems, temperature intolerance, and proteinuria (Mehta et al., 2010; Mehta and Hughes, 2002). These symptoms tend to worsen with age and are often followed by progressive renal failure, as well as cardiac disease, transient ischemic attacks, and stroke. Many male patients are additionally characterized by typical facial features (MacDermot et al., 2001). Compared with the general population of the United States in 2008, the life expectancy of patients with Fabry disease was reduced by 22% in males and 5.75% in females, with cardiovascular disease being the most common cause of death among both genders (Waldek et al., 2009).

Historically, Fabry disease was considered to be inherited in an X-linked recessive manner, and affect primarily males. In the majority of X-linked recessive disorders, heterozygous females are usually asymptomatic carriers of the condition. Symptomatic carriers occur

occasionally, usually due to chromosomal rearrangements or nonrandom X-chromosome inactivation (XCI), as it was also described for two other X-linked lysosomal storage disorders, mucopolysaccharidosis type II (Kloska et al., 2011) and Danon disease (Hedberg Oldfors et al., 2015). However, in case of Fabry disease, most of heterozygous females are clinically affected, presenting with a wide spectrum of signs and symptoms. The phenotype variability of disease manifestation in heterozygous females is much higher than in males, symptoms usually occur later in life and progress slower. Disease manifestation in heterozygous females is hypothesized to be related to skewed X-inactivation favoring the mutant *GLA* allele. Nonetheless, previous studies evaluating the existence of skewed XCI in females with Fabry disease have led to ambiguous results. Some of them suggest a correlation between the severity of clinical involvement and XCI (Dobrovolny et al., 2005; Echevarria et al., 2016; Morrone et al., 2003; Redonnet-Vernhet et al., 1996), while others do not support it (Elstein et al., 2012; Maier et al., 2006). In order to further investigate the relationship between XCI patterns and severity of Fabry disease signs and symptoms in carriers, we examined the contribution of X-inactivation patterns to disease manifestations in a cohort of Polish Fabry heterozygous females.

2. Materials and Methods

2.1. Patients

Twelve symptomatic heterozygous females with family history of Fabry disease from five unrelated Polish families were recruited for the study. At the time of recruitment in 2014-2015 they were aged between 11 and 67 years (mean age, 40.7 years) and none of them received enzyme replacement therapy (ERT). Saliva samples were collected from all subjects using the Oragene® DNA Self-Collection Kit (OG-500) and DNA was isolated according to the prepIT®•L2P protocol (DNA Genotek Inc., Ontario, Canada). The study protocol was

approved by the Ethical Committee of the Regional Chamber of Physicians and written informed consent was obtained from the participants or parents before enrollment.

2.2. Clinical analysis

Clinical phenotypes of the patients were evaluated by physical examination according to four severity scores: a) Mainz Severity Score Index (MSSI) developed to assess the severity of Fabry disease and monitor its clinical course in response to enzyme replacement therapy (Whybra et al., 2004), b) its derivative considering data collected in the Fabry Outcome Survey (FOS) database, the FOS-MSSI (Whybra et al., 2006), c) the age-adjusted score that allows comparison of Fabry disease severity in different subgroups without age or sex confounding factors (Hughes et al., 2010), and d) Fabry disease severity scoring system (DS3) developed for easy use in the general Fabry patient population (Giannini et al., 2010). The analysis included general, neurological, cardiovascular and renal signs and symptoms that are associated with Fabry disease (Giannini et al., 2010; Whybra et al., 2006, 2004).

2.3. GLA sequencing

Data on *GLA* gene mutations identified in males with Fabry disease from families 1, 2, 4, and 5 were available after genetic confirmation of the diagnosis. Heterozygous status in *GLA* gene in females from these families was confirmed by sequencing exons 1, 6 or 7. Fragments of the *GLA* gene were amplified using following M13-tailed primer pairs:

exon 1: 5’-TGTAAAACGACGGCCAGTGTTCCCGTTGAGACTCTCCAGTTC-3’,
5’-CAGGAAACAGCTATGACCGGAAATAGGGCGGGTCAATATCAAG-3’,

exon 6: 5’-TGTAAAACGACGGCCAGTTGATAGTAACATCAAGAGCAAGGGAAA-3’,
5’-CAGGAAACAGCTATGACCCCTTGTTCAAGACCCTGCGG-3’,

exon 7: 5’-TGTAAAACGACGGCCAGTTGAATGGAGAAAAAGGTGGACAGGA-3’,
5’-CAGGAAACAGCTATGACCCCCTTGCTCTTGATGTTACTATCAG-3’.

These three pairs of primers were also used to screen for mutations in exons 1, 6 or 7 in females II-1 and III-2 from the family 3.

Genomic DNA (75 ng) was amplified with 1X High Fidelity PCR buffer (Invitrogen), 2 mM $MgSO_4$, 200 µM of each dNTP, 0.2 µM of each primer, and 1U Platinum® Taq DNA Polymerase High Fidelity (Invitrogen) in a total volume of 10 µl. The PCR conditions were 94°C for 2 min (initial denaturation), followed by 35 cycles of 94°C for 15 s, 58°C for 30 s, and 68°C for 45 s. PCR products were purified using ExoSAP (USB Corporation) and sequenced by Genomed S.A., Poland. Chromatograms were analyzed using Chromas Lite 2.1.1 (Technelysium Pty Ltd).

2.4. X-inactivation analysis

Patterns of X-chromosome inactivation were determined by the analysis of methylation status at polymorphic region of the human androgen receptor gene (*AR* or HUMARA) as described previously (Allen et al., 1992), with modifications. Genomic DNA (100 ng) was digested for 16 hours at 37°C with 10 U of HpaII in a total volume of 10 µl. For each sample, an undigested control was prepared with the same amount of genomic DNA, enzyme digestion buffer, but without restriction enzyme. To determine the XCI pattern in the *AR* assay, both, digested and undigested samples (5 µl) were amplified with 1X Roche PCR reaction buffer with 1.5 mM $MgCl_2$, 200 µM of each dNTP, 0.2 µM of each primer, and 0.75 U of Taq DNA Polymerase (Roche) in a total volume of 15 µl. The forward PCR primer was labeled with the 6-FAM. The PCR conditions were 95°C for 5 min (initial denaturation) followed by 35 cycles of 95°C for 40 s, 62°C for 40 s, and 72°C for 40 s, with a final extension at 72°C for 7 min. All samples were analyzed in duplicate, and the ratios obtained were averaged. DNA of a female with 80:20 skewed XCI was used as a control in each assay run to assess inter-assay variability. PCR products were separated by capillary electrophoresis on an ABI Prism 310

Genetic Analyzer in denaturing conditions. Product length and peak areas were analyzed using Peak Scanner Software v1.0 (Applied Biosystems). The XCI pattern was classified as random (50:50 to 75:25) or skewed (75:25 and more).

Complete HpaII digestion of genomic DNA prior to *AR* gene assay was confirmed by amplification of the 5' region of the *MIC2* gene (an X-linked gene that escapes X-inactivation and should be completely digested by methylation-sensitive HpaII enzyme) (Anderson and Brown, 2002; Goodfellow et al., 1988) according to a modified protocol of Wu et al. (Wu et al., 2014). Both, HpaII-digested and undigested samples (4 µl) were amplified with 1X reaction buffer with 1.5 mM $MgCl_2$, 200 µM of each dNTP, 0.2 µM of each primer, and 0.75 U of Taq DNA Polymerase (Roche) in a total volume of 15 µl. The PCR conditions were 95°C for 5 min (initial denaturation), followed by 35 cycles of 95°C for 40 s, 63°C for 40 s, and 72°C for 40 s, with a final extension at 72°C for 7 min. PCR products were separated by standard agarose gel electrophoresis.

2.5. Statistical analysis

Pearson correlation coefficients were calculated to assess the linear relation between XCI pattern and clinical phenotype severity scores (MSSI, FOS-MSSI, DS3, and age-adjusted score), age and these scores, and age and XCI pattern. A p value of <0.05 was considered statistically significant. Analyses were performed using Statistica 13 (Dell Inc.).

3. Results

3.1. Clinical characteristics

Familial relationships of the female Fabry disease participants in this study are outlined in Figure 1. Although the grandmother from the family 1 was not tested by us, as the mother of

three affected daughters, she is an obligate heterozygote. Moreover, her medical history shows that she was also symptomatic - she developed cerebrovascular disease and died of stroke at the age of 42. Heterozygous status of the woman III-1 from family 3 (not included in this analysis due to her moving abroad) was confirmed by independent commercial laboratories.

Table 1 provides the age at diagnosis and first manifestations of Fabry disease in the analyzed group of females. The mean age at symptom onset was 14 years (range: 5-35 years). Except for the two youngest patients, who were diagnosed pre-symptomatically, all experienced delay in diagnosis (mean: 21, range: 8-37 years). Eight of eleven females (one did not specify early symptoms of the disease) reported severe pain in the extremities (acroparesthesia) as the first manifestation of the disease.

Analyzed females with Fabry disease had varied symptoms without any organ- or tissue-specific expression. The severity of disease manifestations was assessed by MSSI and FOS-MSSI scores and their sub-scores: general, neurological, cardiovascular, and renal, as well as DS3 (Table 2). Using MSSI scores, all three levels of severity were observed, with 3 patients expressing mild, 7 moderate, and 2 severe phenotype. The total FOS-MSSI scores obtained for our patients shifted the severity classification of 2 patients with severe phenotype into moderate, and 3 moderate into mild. This inconsistency in severity classification results from the different weighting of Fabry disease signs and symptoms applied in calculation of both severity indices (according to the contribution to the morbidity of the disease in MSSI and according to the data collected in FOS in FOS-MSSI) and the fact that FOS-MSSI, unlike MSSI, can only be used to give a description of the accumulated disease burden (Whybra et al., 2006). MSSI and FOS-MSSI median sub-scores were respectively 7.5 and 7.5 (general), 10.0 and 6.0 (neurological), 2.5 and 2.0 (cardiovascular), and 4.0 and 4.0 (renal). Among our

cohort, 5/12 patients (III-6, family 1, II-3 and II-5, family 2, III-2, family 3, and I-1, family 5) had normal cardiovascular functions (cardiovascular sub-score = 0), while patient I-1 from family 2 scored the maximum values for the cardiovascular sub-scores (20 in MSSI and 18 in FOS-MSSI). In the other 6 patients cardiovascular sub-sores were in the range 2-11 (MSSI) and 1-13 (FOS-MSSI). In all patients renal sub-scores contributed the least to the overall scores, with the highest value of 8/18 only in patient II-3, family 1. Six patients had mild (4/18) and five had no (0/18) renal involvement. DS3 scores ranged from 1.6 to 15.3.

As all three severity scores used in our analysis positively correlate with age (here, correlation coefficients were 0.885, 0.882, and 0.835 for MSSI, FOS-MSSI, and DS3, respectively), an equation based on age and gender was applied to calculate the age-adjusted severity score (Hughes et al., 2010) (Table 2). Only the youngest patient in the studied cohort (III-6, family 1) had its value of 0, indicating disease expression convergent with her age. In the other eleven patients, age-adjusted severity scores took positive values (from 2.2 to 25.5), implying that their phenotypes were more severe than expected for their age. Significant correlation of age-adjusted severity score with age was noticed (r=0.742).

3.2. Genotype analysis

All mutations identified in this study are characterized in Table 3. Our genetic analysis confirmed heterozygous change in the *GLA* gene in all females from families 1 and 2, in one female from family 4, and one from family 5. Screening for mutations in females from family 3 revealed a novel mutation c.1055_1056delCT in exon 7 (not registered in http://fabry-database.org, 2017/04/10 ver.3.0.7) present in patient II-1 and III-2. Concurrently it was also identified by independent commercial laboratories in hemizygous male patients from this family.

Mutations found in families 1-4 create early stop codons resulting in a truncated protein, while a mutation found in family 5 eliminates a stop codon resulting in an abnormally long protein (448 amino acids instead of 429).

3.3. X-chromosome inactivation

Eleven of twelve analyzed Fabry females were informative for the HUMARA locus polymorphism. In ten of them, random XCI pattern (ranging from 50:50 to 65:35) was found, while one, second youngest female in our cohort (III-2, family 3) was identified with slightly skewed XCI favoring expression of maternal chromosome bearing the mutated *GLA* allele (Table 2). XCI pattern did neither correlate with Fabry disease severity scores and sub-scores nor with patient's age.

4. Discussion

Fabry disease female patients may display much variability in disease onset, severity, and progression (Lenders et al., 2016), which was also observed in this analysis. Our patients presented with all three forms of severity (mild to severe) and suffered a range of Fabry-related manifestations including acroparasthesia, which was the most common and the earliest presenting symptom. Despite a small number of patients in our study, distributions of ages at onset of first Fabry symptoms and diagnosis were consistent with larger study encompassing patients enrolled in the Fabry Registry (Eng et al., 2007; Germain, 2010). It is worth mentioning that all female patients presented in this study were diagnosed after Fabry disease was recognized in the male members of their families, what highlights the importance of pedigree analysis as a useful tool to detect relatives (particularly females) at risk for Fabry disease (Gutiérrez-Amavizca et al., 2014).

In the past, Fabry disease was considered to be transmitted as an X-linked recessive trait. When it was found that heterozygous females may be affected in the same manner as hemizygous males, the disease was postulated to be classified as X-linked dominant (Whybra et al., 2001) or X-linked semi-dominant (Elstein et al., 2012), however following the recommendations of Dobyns (Dobyns et al., 2004), we prefer to simply describe it as X-linked.

The severity and progression of Fabry disease female patients in this study was evaluated by the MSSI, FOS-MSSI, and DS3 scoring systems. As they all correlate with age, an age-adjusted scoring system was applied to calculate age-adjusted severity scores. It appeared that scores predicted by this model in our cohort also correlated with age, making comparison of patients severity regardless of age impossible. This may be explained by the fact that the system of severity score correction does not take mutation type into account. In our patients, cells with the mutated X chromosome (carrying either nonsense or frameshift mutation) were deprived of the mitigating effect of residual enzyme activity present in cells of patients with missense mutation. It suggests that not only age and gender but also mutation type or residual enzyme activity be considered in adjusting severity score in Fabry disease patients.

The selection pressure for or against cells carrying an X-linked mutation was reported for several X-linked disorders (Gribnau and Barakat 2017, submitted). Skewed X-inactivation favoring the mutant *GLA* allele was also postulated to elucidate the frequent expression of Fabry disease manifestations in females. In the present study, all but one patient showed random X-chromosome inactivation, irrespective of the disease severity. Moreover, in three females with the highest MSSI, FOS-MSSI, and DS3 scores of 40-47, 36.5-37.5, and 14.3-15.3, respectively (II-1, II-3, family 1, and I-1, family 2), XCI patterns were in the range of 51:49 to 59:41, while the only patient with a slightly skewed XCI pattern of 75:25 with

preferential inactivation of the chromosome with the wild-type *GLA* allele (III-2, family3) had a relatively low disease severity scores (6, 5, and 2). This is at odds with the expectation of pronounced skewing of X-inactivation in more severely affected heterozygotes and any selection of cells carrying mutation.

Several studies have undertaken XCI analysis in Fabry disease heterozygotes. Redonnet-Vernhet et al. (Redonnet-Vernhet et al., 1996) described 26-year-old female monozygotic twins heterozygous for Fabry disease, one clinically affected and the other asymptomatic. Completely opposite skewed XCI patterns (0:100 and 97:3) were found in their fibroblasts which seemed to explain the observed phenotypic discordance between the twin sisters. Similarly, in 4 Fabry disease carriers from the Italian family skewed XCI pattern in favor of the mutant allele was detected in two manifesting females while the opposite XCI pattern was found in two asymptomatic ones, suggesting a correlation between the clinical phenotype and X-inactivation (Morrone et al., 2003). Another study supporting XCI contribution to the morbidity of Fabry disease heterozygotes was conducted in 38 Czech and Slovak female patients (Dobrovolny et al., 2005). Skewed XCI ratio was detected in 11 of them (in 10 the wild type allele was preferentially inactivated), including six females under 30 with only minor clinical symptoms, four aged 42-57 with MSSI scores of 38-54, indicating moderate and severe phenotype, and one 59-year-old woman with preferential inactivation of the mutated allele and MSSI score of 16 (mild phenotype). The authors observed that heterozygotes with the inactivated wild type allele tended to deteriorate faster than heterozygotes with random XCI and suggested a strong influence of X-inactivation on the clinical phenotypes of Fabry female patients (Dobrovolny et al., 2005), although 27 heterozygotes (71%) without XCI skewing presented with the full spectrum of Fabry disease severity. Interestingly, the same proportion of Fabry heterozygotes with random XCI was reported in other studies: 55/77 (Elstein et al., 2012) and 39/55 (Echevarria et al., 2016).

Maier and coworkers (Maier et al., 2006), who defined random XCI as a ratio of between 50:50 and 64:36, found 13/28 (46%) symptomatic Fabry disease females with random XCI, however using the cut-off ratio of 75:25, which was applied in the other studies, this number would increase to 18/28 (64%). An analogous distribution of X-inactivation patterns was observed in the age-matched controls (Maier et al., 2006) as well as general female population (Amos-Landgraf et al., 2006). In our cohort 10/11 (91%) patients had a random XCI pattern, meaning that the incidence of XCI skewing (1/11) did not exceed the average for either healthy nor Fabry disease females.

In the presented analysis clinical involvement of female Fabry disease patients did not correlate with XCI profiles. This is consistent with the results presented by Maier et al. (Maier et al., 2006) and Elstein et al. (Elstein et al., 2012). Yet, most recent research showed that in females with skewed XCI, Fabry disease progression correlated with the direction of skewing (Echevarria et al., 2016). We have not noted that probably due to the limited number of patients and only one patient with the skewed XCI. Our female Fabry disease heterozygotes presented with the wide range of clinical severity. This phenotypic variability cannot be explained by the phenomenon of XCI, as in most of our patients the random XCI pattern was detected.

The limitation of our study is that the X-inactivation assay was carried out on genomic DNA extracted from saliva samples only. DNA isolated from saliva comes admittedly from two sources: buccal epithelial and white blood cells, with the later contributing up to 74% (Thiede et al., 2000), nonetheless its analysis does not detect intertissue variation. In most females there is a significant association of the XCI ratios between easily obtainable tissues (Echevarria et al., 2016; Sharp et al., 2000). Fabry disease is a multiorgan disorder with serious renal, cardiac, and cerebrovascular involvement so a survey of tissue-specific XCI

patterns in the affected organs would give the best perspective on the contribution of XCI phenomenon to the clinical variability in female patients. Though such analysis would require invasive biopsies. On the basis of our findings, skewed X-inactivation patterns in saliva samples do not explain different rates of penetrance and expressivity in females with Fabry disease.

Several other factors have been discussed to influence the phenotypic expression of Fabry disease in females but none was crucial. The nature of the mutation seems important in disease progression in males but in females the presence of a nonsense or missense mutation does not appear to affect the heterogeneous clinical picture (Lenders et al., 2016). Our results confirm this observation, as patients analyzed here carried either nonsense or frameshift mutations. Measurement of α-GAL A enzyme activity is used to distinguish classic from atypical Fabry disease in males but in females this parameter is unreliable even for identification of heterozygotes (Mehta and Hughes, 2002; Pasqualim et al., 2014), not to mention prediction of the disease severity. Since deacylated Gb3, globotriaosylsphingosine (lyso-Gb3), was reported to be dramatically increased in plasma of classically affected male Fabry patients (Aerts et al., 2008), there have been some successful attempts to use it as a prognostic marker to elucidate disease severity and progression in males. It is considered a potential biomarker that may improve initial diagnose of clinically relevant Fabry disease, particularly in females (Nowak et al., 2017). Nonetheless lyso-Gb3 levels are difficult to correlate with disease burden and progression in female patients (Lenders et al., 2016), especially that in the later-onset heterozygotes, the lysoGb3 levels may be very low or normal (Nowak et al., 2017). Further investigations are needed to elucidate the reason for variable clinical expression of Fabry disease in females.

Conflict of interest

The authors do not have a commercial or other association that might pose a conflict of interest. Prof. Anna Tylki-Szymańska has received speaker honoraria from Sanofi Genzyme and Shire HGT; member of the European Advisory Board of the Fabry Registry.

Acknowledgments

This work was supported by the National Science Centre, Poland [project grant: 2011/01/D/NZ2/03633]. PJ was supported by the European Social Fund.

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Table 1. The age at diagnosis and first symptoms.

Family	Patient symbol	Age at first symptoms (years)	Age at diagnosis (years)	First signs and symptoms reported by patients
1	II-1	10	47	severe pain in the extremities, increased body temperature, no weight gain for several years, abdominal pain, fatigue, arrhythmia
1	II-3	17	45	pain in the feet and toes, chronic proteinuria
1	II-5	9	36	episodes of fainting, arrhythmia, supraventricular tachycardia, pain in the hands and feet
1	III-6	9	7	hypohidrosis, poor tolerance of high temperature, headache
2	I-1	24	53	cardiac disease, hypertension, endocrine dysfunction
2	II-1	7	22	burning pain in the distal extremities triggered by increased temperature or fatigue
2	II-3	10	27	burning pain in the distal extremities triggered by increased temperature or fatigue
2	II-5	5	13	pain in the fingers and toes associated with fatigue or fever
3	II-1	7	17	burning pain in the hands and feet
3	III-2	8	1	burning pain in the hands and feet
4	I-1	26	51	not specified
5	I-1	35	45	swelling of the legs, fatigue, dyspnea, arrhythmia

Table 2. Severity scores and X-chromosome inactivation patterns.

Family	Patient symbol	MSSI score[a]						FOS-MSSI score[b]						Age- and sex- adjusted score[c]	DS3[d]	XCI (saliva)
		G	N	C/V	R	Total	severity	G	N	C/V	R	Total	severity			
1	II-1	14	16	10	0	40	moderate	13,5	12	12	0	37.5	moderate	25.5	15.3	51:49
1	II-3	11	15	9	8	43	severe	10,5	9	9	8	36.5	moderate	25.2	14.3	59:41
1	II-5	6	9	8	4	27	moderate	6	6	8	4	24.0	moderate	15.5	8.7	50:50
1	III-6	2	1	0	0	3	mild	2	0	0	0	2.0	mild	0.0	1.6	56:44
2	I-1	13	10	20	4	47	severe	11,5	3	18	4	36.5	moderate	19.6	14.6	57:43
2	II-1	13	10	2	4	29	moderate	13,5	6	1	4	24.5	moderate	14.8	8.0	63:37
2	II-3	4	13	0	4	21	moderate	4,5	8	0	4	16.5	mild	8.9	4.3	65:35
2	II-5	8	11	0	4	23	moderate	8	6	0	4	18.0	mild	13.1	6.7	54:46
3	II-1	4	10	3	4	21	moderate	4	7	3	4	18.0	mild	9.2	5.0	54:46
3	III-2	1	5	0	0	6	mild	1	4	0	0	5.0	mild	2.2	2.0	75:25
4	I-1	7	10	11	0	28	moderate	7	9	13	0	29.0	moderate	16.7	7.3	62:38
5	I-1	11	7	0	0	18	mild	9	6	0	0	15.0	mild	4.4	9.6	nd

[a] The total MSSI score consists of general (G), neurological (N), cardiovascular (C/V), and renal (R) sub-scores with the maximum values of 18, 20, 20, and 18 respectively; based on MSSI scores, clinical phenotypes are assessed as mild (<20), moderate (20-40) or severe (>40) (Whybra et al., 2004).

[b] The total FOS-MSSI score consists of general (G), neurological (N), cardiovascular (C/V), and renal (R) sub-scores with the maximum values of 14.5, 15, 18, and 18 respectively; based on FOS-MSSI scores, clinical phenotypes are assessed as mild (≤ 18), moderate (19–38) or severe (> 38) (Whybra et al., 2006).

[c] Age-adjusted score is calculated by subtraction of predicted FOS-MSSI score (model equation for females: $[0.96+(0.05*Age)^2]$) from the actual observed FOS-MSSI score; positive values indicate more severe phenotype and negative values - milder phenotype as average phenotype in age-matched Fabry disease population (Hughes et al., 2010).

[d] Fabry disease severity scoring system (DS3) consists of four clinical domains: peripheral nervous system, renal, cardiac and central nervous system plus patient reported domain; the score may range from “minimal severity=0” to “maximal severity=32” (Giannini et al., 2010).

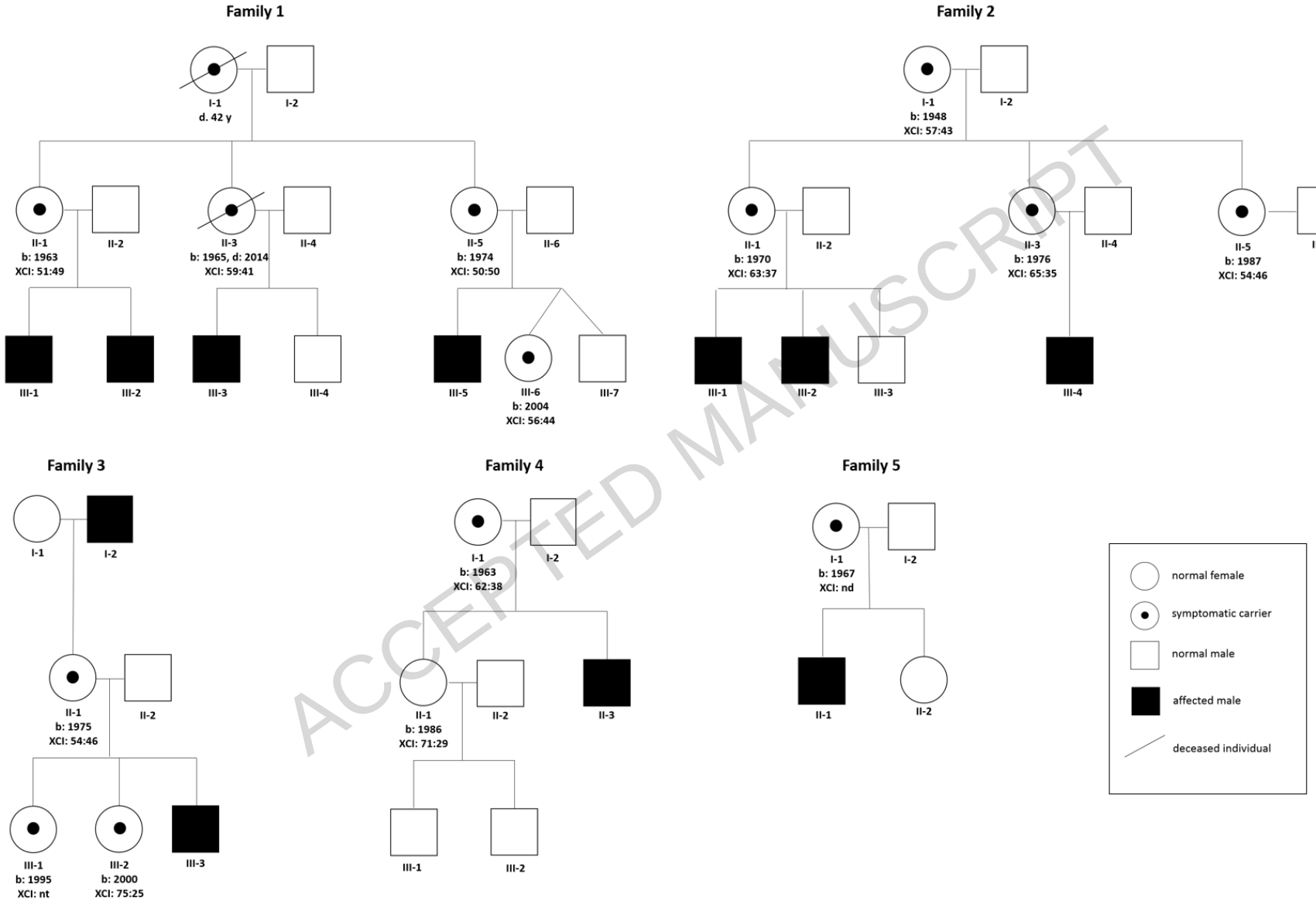
nd – XCI ration not determined due to homozygosity in HUMARA locus

Table 3. Characteristics of *GLA* gene mutations detected in five analyzed families with history of Fabry disease.

Family	Gene mutation	Location	Protein change	Reference
1	c.71G>A	exon 1	p.Trp24Ter	(Buda et al., 2012)
2	c.803_806delTAGT	exon 6	p.Leu268Ter	(Lee et al., 2000)
3	c.1055_1056delCT	exon 7	p.Ala352AspfsTer22	this work
4	c.1057_1058delAT	exon 7	p.Met353AspfsTer21	(Eng et al., 1997)
5	c.1235_1236delCT	exon 7	p.Thr412SerfsTer38	(Blaydon et al., 2001)

Fig. 1.

Pedigrees of the families reported in this study; b- year of birth, d- year of death (II-3, family 1) or age at death (I-1, family 1), XCI- X-chromosome inactivation pattern, nt- not tested, nd- not determined.



Highlights

- Fabry disease severity in Polish female patients was assessed
- Effect of X-chromosome inactivation (XCI) pattern on disease severity was estimated
- Patients presented with the broad range of disease expression - from mild to severe
- Clinical involvement did not correlate with XCI profiles
- XCI is not a main factor in the phenotype variability of Fabry disease in females