Taylor & Francis
Taylor & Francis Group

Disease manifestations and X inactivation in heterozygous females with Fabry disease

ESTHER M. MAIER[1], STEPHANIE OSTERRIEDER[1], CATHARINA WHYBRA[2], MARKUS RIES[3], ANDREAS GAL[4], MICHAEL BECK[2], ADELBERT A. ROSCHER[1] & ANIA C. MUNTAU[1]

[1]*Research Centre, Department of Biochemical Genetics and Molecular Biology, Dr von Hauner Children's Hospital, Ludwig-Maximilian University, Munich, Germany;* [2]*Department of Paediatrics, University of Mainz, Mainz, Germany* [3]*Developmental and Metabolic Neurology Branch, National Institutes of Health, Bethesda, Maryland, USA, and* [4]*Institute of Human Genetics, University Medical Centre Eppendorf, Hamburg, Germany*

Abstract

Aim: Fabry disease is an X-linked lysosomal storage disorder characterized by an accumulation of neutral glycosphingolipids in multiple organ systems caused by α-galactosidase A deficiency due to mutations in the *GLA* gene. The majority of heterozygous females show the characteristic signs and symptoms of the disease, and some of them are severely affected. The current hypothesis for the occurrence of disease manifestations in females is skewed X inactivation favouring the mutant *GLA* allele. *Method:* We analyzed the patterns of X inactivation in the leukocytes of 28 biochemically and genetically characterized symptomatic Fabry disease heterozygotes and their correlation with clinical and biochemical disease expression. *Results:* X inactivation patterns in symptomatic females who are heterozygous for Fabry disease did not differ from those of female controls of the same age ($p = 0.669$). Thirteen (46%) of the 28 females with Fabry disease showed random X inactivation, ten (36%) moderate skewing, and five (18%) highly skewed X inactivation. Segregation analysis was performed in the families of six females who had highly or moderately skewed X inactivation. In four of these females, skewing favoured the wild-type *GLA* allele and in the other two skewing favoured the mutant allele. Patterns of X inactivation or the extent of skewing were not related to the severity of clinical manifestations or to residual enzyme activity.

Conclusion: In this study we provide evidence that heterozygous females with Fabry disease show random X inactivation. Our data do not support the hypothesis that the occurrence and severity of disease manifestations in the majority of Fabry heterozygotes are related to skewed X inactivation.

Key Words: *Fabry disease, lysosomal storage disorder, heterozygotes, X inactivation, HUMARA assay*

Fabry disease (MIM# 301500) is an X-linked inborn error of glycosphingolipid catabolism resulting from a deficiency of the lysosomal enzyme α-galactosidase A (α-Gal A, EC 3.2.1.22). The enzyme defect leads to a progressive accumulation of neutral glycosphingolipids, mainly globotriaosylceramide, in visceral tissues, body fluids and endothelial cells [1]. The clinical hallmarks of Fabry disease in males comprise characteristic skin lesions (angiokeratoma), acroparaesthesias, recurrent crises of excruciating pain, corneal and lenticular opacities, hypohidrosis, cerebral ischaemia and infarction, and cardiac and renal injury. Fabry disease is caused by mutations in the *GLA* gene, and more than 300 have been reported to date (Human Gene Mutation Database [2]). The *GLA* gene was mapped to Xq22 and shown to be subject to X inactivation [3].

In the majority of X-linked disorders, heterozygous females are asymptomatic [4,5]. In Fabry disease, however, recent studies revealed that the majority of heterozygous females show the characteristic signs and symptoms of the disease, and many of them are severely affected. Heterozygotes may present with the full spectrum of disease manifestations, but do show a later onset of symptoms, a slower rate of progression and a higher phenotypic variability than male patients [6]. Life expectancy is reduced in both female and male patients, by about 20 and 30 years, respectively [6,7]. α-Gal A activity can be normal, decreased, or undetectable in females [1,6].

Why do females who are heterozygous for an X-linked trait display clinical symptoms? Skewed X inactivation has been shown to correlate with the occurrence and severity of neurological symptoms in females heterozygous for X-linked adrenoleukodystrophy [8]. Similarly, the current hypothesis, which has been extensively referred to in the literature, is that the biochemical and clinical disease manifestations in females with Fabry disease are also related to skewed X inactivation [1,6,9–16]. This assumption appears to be supported by two case reports, on female monozygotic twins and on four females with Fabry disease from one family [17,18], and by a very recent study of 38 symptomatic females with Fabry disease [19]. In the latter study, ten heterozygous females with a preferential inactivation of the wild-type *GLA* allele showed a more rapid disease progression compared with 27 heterozygous females with balanced X inactivation [19]. In view of these first data, it has been suggested that X inactivation studies could be helpful in predicting the female phenotype and give useful indications for therapeutic management [17,19].

To address this issue, we analysed the patterns of X inactivation in a cohort of 28 clinically, biochemically and genetically characterized heterozygous females with Fabry disease and 56 female controls asking the following three questions: (i) Are females who are heterozygous for Fabry disease prone to skewed X inactivation? (ii) Is the pattern of X inactivation related to the severity of clinical symptoms or the residual enzyme activity? (iii) Is there any evidence for cell selection occurring in favour of the mutant Fabry disease allele?

Subjects and Methods

Subjects

Twenty-eight symptomatic heterozygous females with Fabry disease from a total of 19 families were analysed. Two patients were monozygotic twins (H22 and H25). All patients were seen in a tertiary referral centre. Blood samples for genetic analyses were donated in accordance with consent principles. Patients with Fabry disease were aged between 3 and 71 years (mean age, 36.3 years). In order to minimize bias, all consenting females were included in the study. Heterozygote status was confirmed by mutation analysis of the *GLA* gene [16]. All but two families sharing the mutation R220X had 'private' mutations. In view of identical haplotypes defined by three intragenic DNA polymorphisms (data not shown), these two kindreds may be remotely related.

Controls were matched according to age-groups of 10 years and consisted of 56 females aged between 2 and 73 years (mean age, 36.7 years). Blood samples

from these subjects were obtained anonymously from the central laboratory of the Ludwig-Maximilian University Hospital 'Klinikum Innenstadt'.

X inactivation analysis in leukocytes

DNA was extracted from peripheral blood leukocytes using a standard salting out procedure [20]. Patterns of X inactivation were determined by polymerase chain reaction (PCR) analysis of a polymorphic CAG repeat in the first exon of the human androgen receptor gene (*AR*) as previously described (HUMARA assay) [8].

X inactivation patterns were divided into three groups: random X inactivation (a ratio of between 50:50 and 64:36), moderate skewing (a ratio of between 65:35 and 80:20), and highly skewed X inactivation (a ratio of greater than 80:20) [21].

Parental origin of *AR* alleles

The parental origin of *AR* alleles was analysed in heterozygous females with highly or moderately skewed X inactivation [22]. In order to determine the correct phase, this analysis was performed only if a DNA sample from the father of the heterozygote was available.

Biochemical and clinical evaluation

α-Gal A activity in leukocytes was quantified in all Fabry heterozygotes by standard procedures [23]. None of the heterozygotes had been treated with agalsidase alfa enzyme replacement therapy before, or at the time of blood sampling. All heterozygous females underwent comprehensive diagnostic evaluations performed by physicians or nurse specialists experienced in Fabry disease. The individual clinical phenotypes were assessed using the Mainz Severity Score Index (MSSI) [24]. The MSSI was specifically developed for Fabry disease and consists of four components comprising the specific general, neurological, cardiovascular and renal signs and symptoms of the disease. General and renal components have a maximum score of 18, whereas neurological and cardiovascular components have a maximum score of 20. The four components are summed to give the total MSSI score ranging from 0 to 76. The severity of Fabry disease is classified as mild (total MSSI score <20), moderate (total MSSI score 20–40) and severe (total MSSI score >40) [24].

Statistics

Differences between the distribution of patterns of X inactivation in heterozygous females with Fabry disease and controls were analysed using a non-para-

metric Mann–Whitney U test. To establish a correlation between patterns of X inactivation and α-Gal A activity and clinical findings (MSSI scores), respectively, a non-parametric Spearman's rho correlation was applied. Statistical significance was set at $p < 0.05$. Statistical analyses were performed using SPSS version 6.1.3 for Windows.

Results

Pattern of X inactivation in heterozygous females with Fabry disease and controls

We addressed the issue of whether symptomatic Fabry heterozygotes are prone to skewed X inactivation in order to explain the high incidence of clinical signs and symptoms in these individuals. Thirteen (46%) of the 28 symptomatic females showed random X inactivation, ten (36%) moderate skewing, and five (18%) highly skewed X inactivation (Figure 1A). The distribution of X inactivation patterns was of a bell-shaped Gaussian type and did not differ from the control group. Random X inactivation was found in 29 (52%) of the 56 controls, moderate skewing in 16 (29%), and highly skewed patterns of X inactivation in 11 (20%) ($p = 0.649$; Mann–Whitney U test) (Figure 1B).
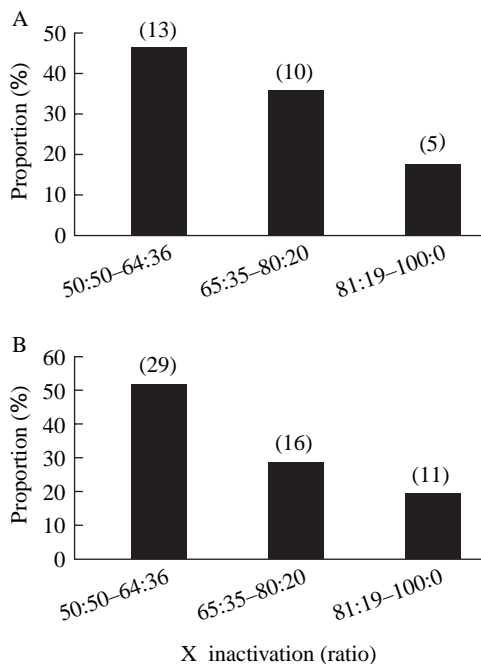

Figure 1. Distribution of patterns of X inactivation in leukocytes of (A) 28 heterozygous females with Fabry disease and (B) 56 female controls. Number of females is given in parentheses. X inactivation was divided into random (a ratio of between 50:50 and 64:36), moderately skewed (a ratio of between 65:35 and 80:20) or highly skewed (a ratio greater than 80:20) [21].

Parental origin of X-chromosomes

We aimed to determine whether the wild-type or the mutant *GLA* allele was preferentially inactivated in the 15 heterozygotes displaying highly or moderately skewed X inactivation. As samples were not available for analysis from nine of the fathers (in six out of nine cases because of the advanced age of the female patient), the parental origin of the *AR* alleles was analysed in the remaining six heterozygotes with skewed X inactivation (Figure 2). In heterozygotes H4, H6 and H13, the mutant *GLA* allele was inherited from the father and was preferentially inactivated in H4 and H6. Heterozygotes H2, H3 and H9 inherited the wild-type *GLA* allele from their fathers and it was preferentially inactivated in H2. In summary, the mutant *GLA* allele was preferentially inactivated in four of the six heterozygotes, namely H3, H4, H6 and H9.

Clinical scores, α-Gal A activity and X inactivation in heterozygote females with Fabry disease

The clinical findings in Fabry heterozygotes included in this study have previously been reported [16,25–27]. Median clinical scores were 21 for total MSSI (5.5 for the general, 7.0 for the neurological, 1.5 for the cardiovascular and 4.0 for the renal components). Two of the females were severely affected, 16 moderately affected and ten mildly affected (Table I).

Two of the patients (H8 and H10) were severely affected and showed moderate skewing, whereas carriers displaying highly skewed patterns of X inactivation showed only mild to moderate clinical symptoms (H1 to H5), although patient H2 had the wild-type *GLA* allele preferentially inactivated. Likewise, mild and moderate phenotypic expressions were found in patients with random X inactivation patterns. Both members of the monozygotic twin pair showed random X inactivation despite discordant phenotypes, with only mild symptoms in patient H22 and moderate symptoms in patient H25. No significant correlation between the extent of skewing of X inactivation and the severity of clinical symptoms could be established in a Spearman's rho correlation; either for the total MSSI score ($p = 0.413$), or for the four separate components (general MSSI [$p = 0.627$], neurological MSSI [$p = 0.563$], cardiovascular MSSI [$p = 0.838$] and renal MSSI score [$p = 0.432$]) (Table I).

As both skewing of X-inactivation and severity of Fabry disease manifestations have been shown to increase with age, heterozygotes older than 40 years of age with more severe symptoms (total MSSI > 30) might be expected to show skewed X inactivation. In our cohort, however, the X inactivation patterns of the six females who fell into this group were variable, with
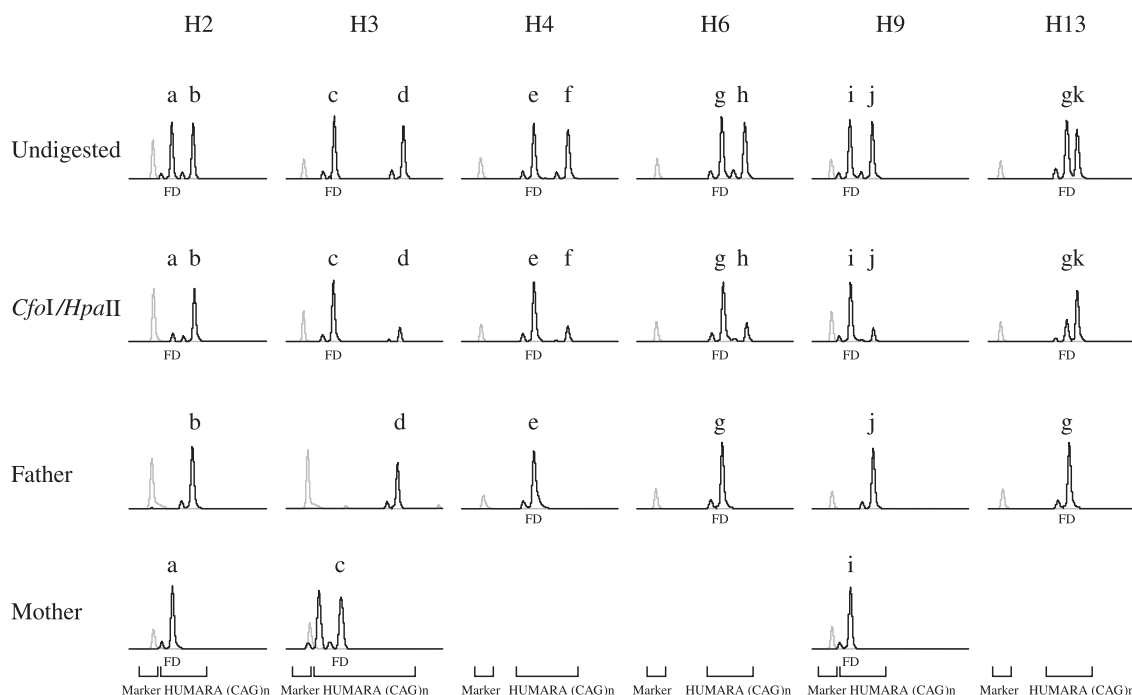
Figure 2. Parental origin of androgen receptor gene (*AR*) alleles in six Fabry heterozygotes displaying a highly or moderately skewed pattern of X inactivation. Patterns of X inactivation were determined by the HUMARA assay based on amplification of a polymorphic CAG repeat in the first exon of the human *AR* gene. Fragment analyses of the parental *AR* alleles were performed and the results compared with the alleles of each heterozygote to determine whether the wild type or the mutant *GLA* allele had been preferentially inactivated. Heterozygote 2 (H2) shows two different *AR* alleles (a and b) amplified from undigested DNA, indicating heterozygosity for the *AR* repeat (first row, Undigested). Predigestion with the methylation-sensitive restriction enzymes *Hpa*II and *Cfo*I revealed skewing of X inactivation with a preferential inactivation of allele b (second row, *Cfo*I/*Hpa*II) which was inherited from the healthy father (third row, Father). The mutant *GLA* allele associated with *AR* allele a, was transmitted by the mother (bottom row, Mother). Thus, skewing of X inactivation occurred favouring the mutant allele in this female. Heterozygote 3 (H3) was found to be heterozygous for the *AR* alleles c and d. She showed skewing of X inactivation, with the paternally derived *AR* allele d, being active in most of her cells. The maternally derived *AR* allele c, which was associated with the mutant *GLA* allele, was found to be preferentially inactivated. Heterozygote 4 (H4) was heterozygous for the *AR* alleles e and f. Skewing of X inactivation was found with a preferential inactivation of *AR* allele e. This allele was transmitted together with the mutant *GLA* allele by the father, indicating that skewing of X inactivation occurred favouring activation of the wild-type allele. Heterozygote 6 (H6) was found to be heterozygous for the *AR* alleles g and h. She showed skewing of X inactivation with a preferential inactivation of the paternally derived *AR* allele g, which is associated with the mutant *GLA* allele. Heterozygote 9 (H9) was found to be heterozygous for the *AR* alleles i and j. She showed skewing of X inactivation, with the paternally derived *AR* allele j, being active in most of her cells. The maternally derived *AR* allele i, which was associated with the mutant *GLA* allele, was found to be preferentially inactivated. Heterozygote 13 (H13, sister of H6) was found to be heterozygous for the *AR* alleles g and k. She showed skewing of X inactivation with the paternally derived *AR* allele g, which is associated with the mutant *GLA* allele, being preferentially active.

random inactivation in two (H18 and H28), moderately skewed inactivation in three (H8, H10, and H14) and highly skewed inactivation in one female (H5). The age of females with Fabry disease showed no correlation with the patterns of X inactivation ($p = 0.634$), whereas age correlated strongly with the severity of clinical symptoms as assessed by total MSSI score ($p < 0.001$).

α-Gal A activity was determined in leukocytes of all Fabry disease heterozygotes. Only 6 of the 28 patients (H2, H5, H7, H17, H20, and H21) showed decreased activity (Table I). There was no significant correlation between X inactivation in leukocytes and α-Gal A activity ($p = 0.768$; Spearman's rho correlation).

Discussion

Females who are heterozygous for X-linked traits are usually asymptomatic due to random inactivation of one of the two X chromosomes in somatic cells leading to a mosaic of cell populations, which express either the wild-type or the mutant allele. Thus, in females who are heterozygous for X-linked diseases, the wild-type allele is active in approximately 50% of cells, and the gene product provided by these cells is usually sufficient to spare heterozygous females from the clinical effects of the disease [4]. The manifestation of clinical signs and symptoms in the majority of females who are heterozygous for Fabry disease [6] indicates a disease penetrance of close to 100%, an

Table I. Genotype, X inactivation, enzyme activity and clinical scores of females who are heterozygous for Fabry disease.

		Genotype					Phenotype MSSI score[c]			
Patient	Age (years)	Mutation	X inactivation[a]	α-Gal A activity (mU/mg)[b]	Total (0−76)	General (0−18)	Neurological (0−20)	Cardiovascular (0−20)	Renal (0−18)
1	31	IVS2+1G >A	92:08	1.008	17	5	12	0	0
2	23	Q321X	84:16	0.176	11	5	6	0	0
3 [(D12)]	14	R220X	84:16	0.845	4	2	2	0	0
4	3	c.744delT	84:16	0.736	1	1	0	0	0
5	67	R220X	83:17	0.105	38	7	13	10	8
6 [(S13)]	18	c.34del24	80:20	0.877	21	5	9	1	6
7	63	c.912delC	77:23	0.110	29	10	6	5	8
8 [(M11)]	68	W236C	76:24	0.830	44	11	9	16	8
9	17	R301X	76:24	1.031	8	1	5	2	0
10 [(M24)]	71	W340X	75:25	1.297	41	9	14	14	4
11 [(D8)]	37	W236C	74:26	0.500	18	7	7	0	4
12 [(M3)]	54	R220X	70:30	1.237	20	5	7	8	0
13 [(S6)]	17	c.34del24	69:31	0.361	21	7	10	0	4
14	62	A350P	67:33	0.498	36	9	5	14	8
15	20	H46R	67:33	0.355	16	2	8	2	4
16	38	A350P	63:37	0.732	26	3	7	12	4
17	34	N320I	63:37	0.190	22	6	12	0	4
18 [(S20)]	45	IVS5-2delCA	61:39	0.582	37	10	14	1	12
19	36	L129P	60:40	0.460	29	4	11	14	0
20 [(S18)]	31	IVS5-2delCA	60:40	0.190	29	10	15	0	4
21	8	c.1187delT	60:40	0.180	2	1	1	0	0
22 [(MZT25)]	20	R112H	57:43	1.171	1	1	0	0	0
23	33	c.718delAA	56:44	0.725	17	5	7	1	4
24 [(D10)]	47	W340X	53:47	0.703	26	3	7	8	8
25 [(MZT22)]	20	R112H	53:47	0.938	25	6	11	0	8
26	50	C52S	53:47	0.766	20	6	6	8	0
27	28	C63Y	51:49	0.338	21	8	13	0	0
28	62	L131P	51:49	0.663	32	9	7	8	8
p [d]				0.768	0.413	0.627	0.563	0.838	0.432

[a]Random X inactivation: 50:50−64:34; moderately skewed X inactivation: 65:35−80:20; highly skewed X inactivation: >80:20 [21].
[b]α-Gal A reference range: 0.23−1.14 mU/mg protein.
[c]Mild disease: Total MSSI <20; moderate disease: Total MSSI 20−40; severe disease: Total MSSI >40 [24].
[d]Nonparametric Spearman's rho correlation.
[D], Daughter; α-Gal A, α-Galactosidase A; [M], Mother; MSSI, Mainz Severity Score Index; [MZT], Monozygotic twins; [S], Sister.

uncommon feature for females who are heterozygous for an X-linked trait [28].

Significant disease manifestation in females who are heterozygous for X-linked diseases has often been attributed to an unfavourable skewing of X inactivation, with the mutant allele being active in most cells [29]. At least three major mechanisms leading to unbalanced X inactivation are known [30–32]. (i) Skewing of X inactivation may occur as stochastic variation of a random process. (ii) Skewing may be due to genetic factors influencing the X inactivation process itself. (iii) Skewed X inactivation may be the result of a post-inactivation selection mechanism. The first two mechanisms are rare and occur independently of the underlying defective *GLA* allele. Therefore, these approaches would explain the manifestation of clinical signs and symptoms in only a small proportion of Fabry heterozygotes. To explain the occurrence of clinical signs and symptoms in the majority of heterozygotes, one would expect that skewed X inactivation is a frequent finding in females with Fabry disease and is related to post-inactivation selection mechanisms favouring the mutant *GLA* allele. In our study, however, the incidence of skewed X inactivation was not higher in symptomatic females who are heterozygous for Fabry disease than in healthy controls of the same age. Highly skewed X inactivation, i.e., X inactivation ratios greater than 80:20, that are considered to be pathogenic [21, 33,34], were identified in only 18% of symptomatic females with Fabry disease.

Moreover, if there was a relationship between the clinical phenotype and X inactivation, pronounced skewing of X inactivation would be expected to occur particularly in the more severely affected heterozygotes [35]. In our cohort, however, severely affected heterozygous females (H8 and H10) showed only moderate skewing, whereas females with highly skewed patterns of X inactivation were only mildly (H1 and H2) or moderately (H5) affected. We found no significant correlation between the X inactivation pattern and the clinical phenotype, either in terms of severity, or in terms of affected organ systems. Likewise, there was no correlation between X inactivation and α-Gal A enzyme activity in leukocytes. It is important to consider, however, that there are certain limitations in the instrument used to assess the severity of symptoms in heterozygotes. It is known that the MSSI score increases progressively with age and that this system has not been independently validated. However, taking into account these limitations, this scoring system is still useful for reflecting the severity of disease.

Our data indicate that symptomatic heterozygous females with Fabry disease are not more likely to have skewed X inactivation, but show random X inactivation, as has been suggested for heterozygotes for most X-linked diseases [29,33]. Consistent with these findings, among females with skewed X inactivation, equal numbers showed preferential inactivation of the wild-type and the mutant alleles, indicating no evidence for selection mechanisms favouring the mutant allele (Figure 2). Thus, the occurrence and severity of clinical signs and symptoms in heterozygous females with Fabry disease is not attributable to a high frequency of skewed X inactivation. X inactivation studies will therefore not, as previously proposed [17,19], be helpful in predicting the female phenotype and cannot give useful indications for therapeutic management.

These data are in agreement with that of a very recent study on symptomatic Fabry heterozygotes from the Czech and Slovak Republics [19]. In both cohorts, symptomatic females were not more likely to have unbalanced X inactivation, with skewing (ratio of greater than 75:25) being observed in 29% in the Czech/Slovak and in 32% in the German study. These figures correspond to the incidence of skewed X inactivation found in the normal female population [31] and to that of our control group (21%). Nonetheless, the observation that ten of the 11 females with skewed X inactivation showed preferential inactivation of the wild-type *GLA* allele in the Czech/Slovak cohort appears to be in contrast with our finding of only two of six females showing preferential inactivation of the wild-type *GLA* allele. This difference, however, might be due to the small numbers of individuals with skewed X inactivation studied in both cohorts. Dobrovolny et al. [19] reported that the ten females with skewed X inactivation favouring the mutant *GLA* allele showed a more rapid clinical deterioration compared with the symptomatic females with balanced X inactivation and a symptomatic female with skewed X inactivation favouring the wild-type *GLA* allele. In spite of the fact that skewing (>75:25) occurred only in 29% of Fabry disease heterozygotes analysed, the authors concluded that X inactivation was a major factor determining the morbidity of Fabry disease in females.

Our data contrast with the reports by Redonnet-Vernhet et al. [18] and Morrone et al. [17]. These groups described an association between X inactivation and clinical phenotype in a pair of female monozygotic twins and in four females with Fabry disease from one family, respectively. However, the observed skewing was presumably not related to the mutant *GLA* allele in these females. Variable disease expression with divergent skewing of X inactivation is an apparently common phenomenon in monozygotic female twins heterozygous for X-linked disorders [31] and has been observed in various X-linked traits [36–39]. In addition, skewed patterns of X inactivation have been shown to run within families [30,40–42].

We and others [17–19] have studied X inactivation patterns in leukocytes of females who are heterozygous for Fabry disease. Beside the fact that leukocytes are easily accessible, they are suitable for use to study the hypothesis that selection mechanisms may occur in Fabry disease. These mechanisms take place in tissues that express the mutant gene, and are most pronounced in tissues such as rapidly dividing blood cells, which are exposed to high selective pressure [29,30,33]. In females who are heterozygous for Fabry disease, no significant difference has been found so far between the individual X inactivation patterns seen in peripheral blood cells, and urinary and buccal epithelia [19]. Similarly, ultrastructural examination of cardiac and renal tissue from symptomatic women with Fabry disease showed two cell populations, normal cells and cells with lipid accumulation [7,43,44]. Nonetheless, patterns of X inactivation have been described to vary between different tissues [45,46]. Therefore, it cannot be excluded that a correlation between X inactivation and disease phenotype may be revealed in some tissues or organs affected by Fabry disease such as the kidneys or nervous system.

In the light of these considerations, how do random patterns of X inactivation correlate with clinical signs and symptoms in females who are heterozygous for Fabry disease? Females heterozygous for X-linked traits are usually spared from symptoms due to their proportion of functionally normal cells. Alternatively, growth differences between two cell populations may lead to a loss of the deficient cell line due to a selective disadvantage [4,29]. Pronounced differences result in an early loss of the disadvantaged cell population and in marked skewing of X inactivation, whereas more subtle differences lead to a gradual loss of the disadvantaged cell population throughout life and to skewing of X inactivation in older heterozygotes [29]. Indeed, subtle differences between the maternal and the paternal X chromosomes lead to an increase of skewing of X inactivation with age in the healthy female population [46,47]. In the symptomatic female patients in our study, skewing was not a frequent finding compared with females of the same age. A significant correlation between age and X inactivation was not established in the Fabry disease cohort, most likely due to the small sample size. However, we found a strong correlation between age and severity of disease manifestation as seen in hemizygous male patients [6]. Remarkably, two of the elderly female patients (H18 and H28) showed severe manifestations but random patterns of X inactivation. Thus, it seems that disease progression with age reflects the natural course of the disease and is due to the detrimental effect of the mutant *GLA* allele itself rather than being a result of skewing of X inactivation with age arising from a selective advantage of the mutant *GLA* allele.

After random inactivation, cells expressing the mutant *GLA* allele proliferate unhindered and the negative effects of lysosomal lipid accumulation increase with age. Although cells with an active wild-type *GLA* allele produce fully functional enzyme, part of which is secreted and can be taken up through endocytosis by α-Gal A-deficient cells, this cross-correction is of limited efficiency and can only postpone or ameliorate the pathology – and the manifestation of the signs and symptoms of Fabry disease in females. Consistent with this hypothesis, signs and symptoms in females can be as severe as in males but occur at a later age [6,7].

In conclusion, we provide evidence that females with Fabry disease show random X inactivation. Our data do not support the hypothesis that the occurrence and severity of disease manifestations in the majority of females with Fabry disease are related to unfavourable skewing of X inactivation.

Acknowledgements

References

[1] Desnick RJ, Ioannou YA, Eng CM. α-Galactosidase A deficiency: Fabry disease. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. The metabolic and molecular bases of inherited disease. 8th edn. New York: McGraw-Hill; 2001. p. 3733–74.

[2] www.uwcm.ac.uk/uwcm/mg/hgmd0.html.

[3] Carrel L, Cottle AA, Goglin KC, Willard HF. A first-generation X-inactivation profile of the human X chromosome. Proc Natl Acad Sci USA 1999;96:14440–4.

[4] Puck JM, Willard HF. X inactivation in females with X-linked disease. N Engl J Med 1998;338:325–8.

[5] Dobyns WB. The pattern of inheritance of X-linked traits is not dominant or recessive, just X-linked. Acta Paediatr 2006; 95(Suppl 451):11–15.

[6] 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;34:236–42.

[7] MacDermot KD, Holmes A, Miners AH. Anderson-Fabry disease: clinical manifestations and impact of disease in a

cohort of 60 obligate carrier females. J Med Genet 2001;38: 769–75.

[8] Maier EM, Kammerer S, Muntau AC, Wichers M, Braun A, Roscher AA. Symptoms in carriers of adrenoleukodystrophy relate to skewed X inactivation. Ann Neurol 2002;52:683–8.

[9] Ashton-Prolla P, Ashley GA, Giugliani R, Pires RF, Desnick RJ, Eng CM. Fabry disease: comparison of enzymatic, linkage, and mutation analysis for carrier detection in a family with a novel mutation (30delG). Am J Med Genet 1999;84:420–4.

[10] Davies JP, Winchester BG, Malcolm S. Mutation analysis in patients with the typical form of Anderson-Fabry disease. Hum Mol Genet 1993;2:1051–3.

[11] Eng CM, Desnick RJ. Molecular basis of Fabry disease: mutations and polymorphisms in the human alpha-galactosidase A gene. Hum Mutat 1994;3:103–11.

[12] Hauser AC, Lorenz M, Sunder-Plassmann G. The expanding clinical spectrum of Anderson-Fabry disease: a challenge to diagnosis in the novel era of enzyme replacement therapy. J Intern Med 2004;255:629–36.

[13] Masson C, Cisse I, Simon V, Insalaco P, Audran M. Fabry disease: a review. Joint Bone Spine 2004;71:381–3.

[14] Rodriguez-Mari A, Coll MJ, Chabas A. Molecular analysis in Fabry disease in Spain: fifteen novel GLA mutations and identification of a homozygous female. Hum Mutat 2003;22:258.

[15] Stromberger C, Bodamer OA. Fabry disease defined. Eur J Clin Invest 2004;34:449.

[16] Whybra C, Kampmann C, Willers I, Davies J, Winchester B, Kriegsmann J, et al. Anderson-Fabry disease: clinical manifestations of disease in female heterozygotes. J Inherit Metab Dis 2001;24:715–24.

[17] Morrone A, Cavicchi C, Bardelli T, Antuzzi D, Parini R, Di Rocco M, et al. Fabry disease: molecular studies in Italian patients and X inactivation analysis in manifesting carriers. J Med Genet 2003;40:e103.

[18] Redonnet-Vernhet I, Ploos van Amstel JK, Jansen RP, Wevers RA, Salvayre R, Levade T. Uneven X inactivation in a female monozygotic twin pair with Fabry disease and discordant expression of a novel mutation in the alpha-galactosidase A gene. J Med Genet 1996;33:682–8.

[19] Dobrovolny R, Dvorakova L, Ledvinova J, Magage S, Bultas J, Lubanda JC, et al. Relationship between X-inactivation and clinical involvement in Fabry heterozygotes. Eleven novel mutations in the alpha-galactosidase A gene in the Czech and Slovak population. J Mol Med 2005;83:647–54.

[20] Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215.

[21] Harris A, Collins J, Vetrie D, Cole C, Bobrow M. X inactivation as a mechanism of selection against lethal alleles: further investigation of incontinentia pigmenti and X linked lymphoproliferative disease. J Med Genet 1992;29:608–14.

[22] Pegoraro E, Schimke RN, Arahata K, Hayashi Y, Stern H, Marks H, et al. Detection of new paternal dystrophin gene mutations in isolated cases of dystrophinopathy in females. Am J Hum Genet 1994;54:989–1003.

[23] Mayes JS, Scheerer JB, Sifers RN, Donaldson ML. Differential assay for lysosomal alpha-galactosidases in human tissues and its application to Fabry's disease. Clin Chim Acta 1981;112:247–51.

[24] Whybra C, Kampmann C, Krummenauer F, Ries M, Mengel E, Miebach E, et al. The Mainz Severity Score Index: a new instrument for quantifying the Anderson-Fabry disease phenotype, and the response of patients to enzyme replacement therapy. Clin Genet 2004;65:299–307.

[25] Baehner F, Kampmann C, Whybra C, Miebach E, Wiethoff CM, Beck M. Enzyme replacement therapy in heterozygous females with Fabry disease: results of a phase IIIB study. J Inherit Metab Dis 2003;26:617–27.

[26] 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; 40:1668–74.

[27] Wendrich K, Whybra C, Ries M, Gal A, Beck M. Neurological manifestation of Fabry disease in females. Contrib Nephrol 2001:241–4.

[28] Dobyns WB, Filauro A, Tomson BN, Chan AS, Ho AW, Ting NT, et al. Inheritance of most X-linked traits is not dominant or recessive, just X-linked. Am J Med Genet A 2004;129:136–43.

[29] Migeon BR. Non-random X chromosome inactivation in mammalian cells. Cytogenet Cell Genet 1998;80:142–8.

[30] Belmont JW. Genetic control of X inactivation and processes leading to X-inactivation skewing. Am J Hum Genet 1996; 58:1101–8.

[31] Willard HF. The sex chromosomes and X chromosome inactivation. In: Scriver CR, Beaudet AL, Valle D, Sly WS, editors. The metabolic and molecular bases of inherited disease, 8th edn. New York: McGraw-Hill; 2001. p. 1191–211.

[32] Ørstavik KH. Skewed X inactivation in healthy individuals and in different diseases. Acta Paediatr 2006;95(Suppl 451): 24–29.

[33] Lyon MF. X-chromosome inactivation and human genetic disease. Acta Paediatr Suppl 2002;91:107–12.

[34] Watkiss E, Webb T, Bundey S. Is skewed X inactivation responsible for symptoms in female carriers for adrenoleucodystrophy? J Med Genet 1993;30:651–4.

[35] Lyon MF. Gene action in the X-chromosome of the mouse (Mus musculus L.). Nature 1961;190:372–3.

[36] Jorgensen AL, Philip J, Raskind WH, Matsushita M, Christensen B, Dreyer V, et al. Different patterns of X inactivation in MZ twins discordant for red-green color-vision deficiency. Am J Hum Genet 1992;51:291–8.

[37] Richards CS, Watkins SC, Hoffman EP, Schneider NR, Milsark IW, Katz KS, et al. Skewed X inactivation in a female MZ twin results in Duchenne muscular dystrophy. Am J Hum Genet 1990;46:672–81.

[38] Valleix S, Vinciguerra C, Lavergne JM, Leuer M, Delpech M, Negrier C. Skewed X-chromosome inactivation in monochorionic diamniotic twin sisters results in severe and mild hemophilia A. Blood 2002;100:3034–6.

[39] Willemsen R, Olmer R, De Diego Otero Y, Oostra BA. Twin sisters, monozygotic with the fragile X mutation, but with a different phenotype. J Med Genet 2000;37:603–4.

[40] Naumova AK, Olien L, Bird LM, Smith M, Verner AE, Leppert M, et al. Genetic mapping of X-linked loci involved in skewing of X chromosome inactivation in the human. Eur J Hum Genet 1998;6:552–62.

[41] Naumova AK, Plenge RM, Bird LM, Leppert M, Morgan K, Willard HF, et al. Heritability of X chromosome-inactivation phenotype in a large family. Am J Hum Genet 1996;58: 1111–9.

[42] Pegoraro E, Whitaker J, Mowery-Rushton P, Surti U, Lanasa M, Hoffman EP. Familial skewed X inactivation: a molecular trait associated with high spontaneous-abortion rate maps to Xq28. Am J Hum Genet 1997;61:160–70.

[43] Gubler MC, Lenoir G, Grunfeld JP, Ulmann A, Droz D, Habib R. Early renal changes in hemizygous and heterozygous patients with Fabry's disease. Kidney Int 1978;13:223–35.

[44] Itoh K, Takenaka T, Nakao S, Setoguchi M, Tanaka H, Suzuki T, et al. Immunofluorescence analysis of trihexosylceramide accumulated in the hearts of variant hemizygotes and heterozygotes with Fabry disease. Am J Cardiol 1996;78:116–7.

[45] Gale RE, Wheadon H, Boulos P, Linch DC. Tissue specificity of X-chromosome inactivation patterns. Blood 1994;83: 2899–905.

[46] Sharp A, Robinson D, Jacobs P. Age- and tissue-specific variation of X chromosome inactivation ratios in normal women. Hum Genet 2000;107:343–9.

[47] Kristiansen M, Knudsen GP, Bathum L, Naumova AK, Sorensen TI, Brix TH, et al. Twin study of genetic and aging effects on X chromosome inactivation. Eur J Hum Genet 2005;13:599–606.