Fabry disease: studies on diagnosis, screening and patients' perspectives
Bouwman, M.G.

Citation for published version (APA):
Bouwman, M. G. (2012). Fabry disease: studies on diagnosis, screening and patients' perspectives
EARLY CEREBRAL MANIFESTATIONS IN A YOUNG FEMALE WITH FABRY DISEASE WITH SKewed X-INACTIVATION

Machtelt G. Bouwman¹, Saskia M. Rombach², Gabor E. Linthorst²,
Ben J.H.M. Poorthuis³, Ronald H. Lekanne Deprez⁴,
Johannes M.F.G. Aerts³, Frits A. Wijburg¹

¹Department of Pediatrics, ²Department of Endocrinology and Metabolism, ³Department of Medical Biochemistry, ⁴Department of Clinical Genetics, Academic Medical Center and Amsterdam Lysosome Center, University of Amsterdam, Amsterdam, The Netherlands

Published in part in Clinical Genetics 2011;80:500–502s
ABSTRACT

Fabry disease is an X-linked disorder caused by a deficiency of the enzyme $\alpha$-galactosidase A. As a result its main substrate globotriaosylceramide (Gb3) accumulates in endothelial cells and other cell types. In females, the disease phenotype is highly variable. This variability is not fully understood, but might at least in part be due to skewed inactivation of the X-chromosome during early embryogenesis. Here we report the biochemical, clinical and molecular characteristics of a young female with Fabry disease with CNS involvement from the early age of thirteen years and a silent hemorrhagic infarction in the globus pallidus at the age of sixteen. The activity of $\alpha$-galactosidase A in leucocytes was nearly absent and comparable to activities detected in hemizygote Fabry males. Moreover, the plasma concentration of globotriaosylsphingosine (lysoGb3), which is the deacylated form of Gb3 and is related to a high risk for cerebrovascular white matter lesions in Fabry males, was exceptionally high. Evaluation of X-inactivation in leukocytes showed 100% skewed inactivation of the paternal wild-type allele, potentially explaining the remarkable phenotype in this girl.
INTRODUCTION

Fabry disease (OMIM 301500) is an X-linked lysosomal storage disorder caused by a deficiency of the enzyme α-galactosidase A (αGal A). As a result its main substrate globotriaosylceramide (Gb3) accumulates in endothelial cells and other cell types. In males, the first symptoms of Fabry disease typically occur during childhood and include acroparesthesia, angiokeratoma and hypohidrosis 1. Disease progression may lead to renal insufficiency, cardiomyopathy and neurological complications in adults 2, resulting in a decreased life expectancy 3. It is currently well established that severe disease manifestations may also occur in females with Fabry disease 4-7, even though they generally have a milder course of the disease. Development of symptoms in females has been attributed at least in part to skewed inactivation of the X chromosome 8-11.

Central nervous system (CNS) involvement is mainly characterized by white matter lesions (WML), transient ischemic attacks (TIAs) and strokes 13,14. The pathogenesis of CNS involvement is poorly understood. Fabry patients show alterations in cerebral blood flow and exhibit tortuosity and dilatation of large vessels 15,16. Besides the appearance of WMLs, stroke can be an early and first clinical symptom in Fabry disease 17-19. In the observational international Fabry Registry, of the patients who had a stroke, 50% of the male and 38% of the female patients of all patients who had a stroke, had their first stroke before the diagnosis of Fabry disease was established 17. CNS involvement is uncommon in children and adolescents with Fabry disease, but both WMLs and stroke have been reported in young boys 20-22.

Here, we report the enzymatic, biochemical and molecular characteristics, including a 100% skewed X-inactivation pattern, in a young female patient with Fabry disease who had CNS involvement already at the age of thirteen years.

Case history

At the age of thirteen years, the girl (Figure 1, patient III.1) was evaluated following the diagnosis of Fabry disease in her mother. The girl reported minor acroparesthesia during exercise and on examination a few angiokeratoma were seen on her back.

Clinical evaluation including echocardiography and renal assessment revealed no abnormalities. Magnetic resonance imaging (MRI) of the brain was considered normal at that time. No proteinuria (defined as > 0.3 mg/24hr) or (micro-) albuminuria (defined as albumin-creatinine ratio (ACR) >2.5 mg/mmol in two out of three consecutive early morning voids) was present. Ophthalmologic evaluation revealed cornea verticillata. Audiological studies showed mild bilateral high-frequency hearing loss. MRI of the brain was repeated two years later, at the age of 15 years. A 4 mm periventricular WML on T2 and FLAIR-weighted images was detected near the posterior horn in the right hemisphere (Figure
2a). In retrospect, this lesion was already present on the initial MRI. Treatment with intravenous enzyme replacement therapy (ERT, agalsidase alfa, 0.2 mg/kg every 2 weeks) and anti-platelet therapy was started. MRI of the brain one year later, at the age of sixteen, demonstrated an additional lesion of 7 mm in the left hemisphere in the globus pallidus (Figure 2b) fitting a hemorrhagic infarction, however without clinical symptoms. An MRI one year later showed residual lesions. Clinical risk factors for cardiovascular disease only revealed a history of cigarette smoking. Blood pressure, cholesterol and fasting glucose levels were normal. Laboratory studies on other risk factors for cerebral infarction at a young age, including fibrinogen, clotting factor VIII, antithrombin deficiency, lupus anticoagulant, factor II mutation, factor V Leiden mutation, protein C and S deficiency, elevated lipoprotein, anti-cardiolipin and homocysteine did not reveal any abnormalities.

**Family history**

The girl’s grandmother was the index case of the family (Figure 1: patient I.2) and was diagnosed with Fabry disease when she developed renal insufficiency. Previously she had developed persistent blindness of unknown origin in one eye at the age of 50. She died of renal insufficiency at the age of 57. Her daughter, the mother of the girl (Figure 1, patient II.1) had amaurosis fugax of the left eye at the age of 22 years. At the age of 40 she had a short episode of acute dizziness, loss of muscle control in one leg and nausea, which resolved spontaneously. Later, aged 45, an MRI of the brain revealed a hyperintense lesion on FLAIR imaging of the right cerebellar hemisphere, interpreted as a lacunar infarction (Figure 2c). In addition sensorineural hearing loss and microalbuminuria were found. The aunt of the girl (figure 1: patient II:3), who was asymptomatic, was also diagnosed with Fabry disease. Periventricular WMLs were seen on MRI (Figure 2d) and mild left ventricular hypertrophy was found on echocardiography at the age of 48 years. The girl’s seven years older sister (figure 1: patient III.2) was also diagnosed with Fabry disease. She had no clinical signs or symptoms of the disease and no abnormalities were detected on MRI of the brain, echocardiography, audiological studies or studies on renal function.
METHODS

Enzyme activity, plasma Gb3 and lysoGb3 analysis

Alpha-Galactosidase A activity was measured in leukocytes according to Mayes et al.\textsuperscript{23} Plasma Gb3 and lysoGb3 was measured as described previously\textsuperscript{24,25}.

DNA mutation analysis and analysis of X inactivation patterns

Genomic DNA was isolated from EDTA blood using PUREGENE chemistry (Gentra Systems, Minnesota, Minneapolis, USA). GLA coding region and splice junction variation were analyzed by Sanger sequencing using the Big Dye Terminator\textsuperscript{3.1} cycle sequencing kit (Applied Biosystems, Foster City, California, USA). A precise description of the methods is available on request.

Nomenclature for the description of the sequence variation is according to http://www.hgvs.org/mutnomen/ using with NM\_000169.2 as the reference sequence.

Inactivation status of the X-chromosome was studied in genomic DNA from EDTA blood using polymorphic markers at the Androgen Receptor locus and two methylation sensitive restriction enzymes (Hpall and CfoI) as earlier described\textsuperscript{26}.
As a reference we analyzed skewing in 10 healthy young women (average age 16 years) and found an average skewing of 61% with a standard deviation (SD) of 15%.

RESULTS

Enzyme activity and analysis of plasma Gb3 and lysoGb3
Alpha-Galactosidase A in leucocytes was strongly reduced (1.8 ng/mmol/hr) and plasma concentration of lysoGb3 was markedly elevated (99 nmol/ml) in this young female patient (patient III.1) at the age of 13 years also as compared to the values found in her mother and sister (Table 1).

DNA mutation analysis and analysis of X inactivation patterns
Mutation analysis of the GLA gene revealed a base substitution (c.1025G>A) leading to an amino-acid change p.R342Q. This mutation is often found in Fabry patients with a classic phenotype and is one of the most prevalent mutations in the Fabry registry. This mutation was also detected in the other affected relatives. Sequencing all exons and flanking intron/exon boundaries revealed no other mutations in the GLA gene. Karyotyping revealed a normal 46 XX karyogram, excluding Turner syndrome and an X autosome translocation. Evaluation of X-inactivation in leukocytes of the girl showed 100% skewed inactivation of the paternal wild-type allele (Table 1). In contrast, in both her sister and her mother, the X-chromosomes were randomly inactivated (Table 1).

DISCUSSION

Here we report on a young female Fabry patient with cerebral manifestations and 100% skewed X-inactivation of the wild type X-chromosome. She already had a WML on MRI studies at the age of thirteen and a silent hemorrhagic infarction in the globus pallidus at the age of sixteen. To our knowledge, this is the first report of extensive CNS involvement in such a young female with Fabry disease.

| Table 1. Biochemical and molecular characteristics of the family. |
|--------------|------------|-------------|----------------|----------------|----------------|
|             | Leukocytes αGal activity | Plasma Gb3 | LysoGb3 | X-inactivation | % expression of the mutated allele |
|             | Normal 32-60 nmol/mg/h | Normal < 3μmol/l | Normal<3nM |               |                              |
| II.1        | 27         | 0.77        | 16       | 51% (SD=7)     |                              |
| II.3        | 4.1        | 2.93        | 36       | not done       |                              |
| III.1       | 1.8        | 2.21        | 99       | 100%           |                              |
| III.2       | 18.1       | 1.43        | 14       | 53% (SD=8)     |                              |
The severity of Fabry disease in this girl is in accordance with the enzyme activity which was found to be reduced to levels seen in males with Fabry disease. In addition, the plasma concentration of lysoGb3, which is the deacylated form of Gb3 and is formed in αGal A deficient cells, was exceptionally high. In most female heterozygotes, plasma lysoGb3 is low at birth and only gradually increases with age. Of interest, a recent study revealed that high plasma lysoGb3 levels in male Fabry patients is related to a high risk for cerebrovascular white matter lesions.

The high frequency of moderate and even severe disease expression in female Fabry heterozygotes compared to heterozygotes in e.g. the other X-linked lysosomal storage disorder, mucopolysaccharidosis type II (MPS-II, Hunter Syndrome) is remarkable. In MPS-II, heterozygotes are almost always unaffected. Several theories have been proposed to explain the variable disease penetrance in females with X-linked diseases. X-inactivation pattern as an explanation of disease severity in female Fabry patients has been suggested previously. Dobrovolny et al demonstrated that heterozygotes with a significantly inactivated wild-type allele (skewing 75:25 and more towards the mutated gene) tended to have a more rapid clinical evolution of the disease. One might argue that skewed X-inactivation favoring the presence of cells expressing the mutated allele may explain the presence of clinical symptoms in Fabry heterozygotes. However, in one study, the distribution of X-inactivation patterns in heterozygotes did not differ from controls. Another possible mechanism in Fabry may be a lack of metabolic cross-correction. Lysosomal enzymes excreted by cells can be taken up by deficient cells and may subsequently correct the enzyme deficiency to some extent. This phenomenon has been proposed to explain why in MPS-II heterozygotes in general do not express the disease phenotype. Although intercellular transfer of αGal A and metabolic cross-correction have been reported in vitro, apparently this mechanism is insufficient to prevent storage and clinical symptoms in a considerable proportion of Fabry heterozygotes. An explanation may be that formation of a specific metabolite in αGal A deficient cells could potentially affect αGal A competent cells. Indeed it has been shown that lysoGb3, formed in αGal A deficient cells, inhibits residual αGal A activity. Furthermore lysoGb3 promotes smooth muscle cell proliferation and altered podocyte behavior in vitro. Both are known cell-types involved in the pathogenesis of Fabry disease.

The family history presented here is remarkable for the high prevalence of cerebrovascular complications. The mother of the child showed clinical symptoms of a TIA at the age of 40 and both she and the grandmother suffered from sudden loss of vision of unknown origin, which might be caused by a vascular occlusion. Sudden loss of visual acuity has been previously reported in Fabry disease patients. In addition to the endothelial accumulation of Gb3, other factors may play a role in the pathogenesis of cerebral pathology including WMLs in Fabry patients, such as increased homocysteine concentration and genetic modifiers such as IL-6, eNOS, factor V and protein Z polymorphism, which were not studied in this family.
An extensive workup in the 13 year old patient did not reveal any of the more common prothrombotic disorders other than Fabry disease, apart from smoking.

This case study has limitations. Imaging of the brain was not done at an early age in the mother and aunt, and therefore early presence of asymptomatic CNS lesions cannot be excluded. Furthermore, the pattern of X-inactivation was determined only in leukocytes and it is uncertain whether the same 100% skewed inactivation pattern is present in all cell types and tissues, including the endothelial and subendothelial cells in the brain. Finally, although extensive studies did not reveal any risk factors for stroke, we did not study all potential genetic modifiers reported by Altarescu and co-workers.

We demonstrate that WML including a silent cerebral infarction may occur at an early age in females with Fabry disease. This supports the inclusion of brain imaging by MRI for assessment and follow-up of Fabry patients from an early age and independent of gender. We also showed that ERT in combination with anti-platelet therapy did not prevent progression of the CNS disease in this young Fabry patient. It has been suggested previously that ERT may not be very effective in reducing the incidence of TIAs or strokes in patients with advanced Fabry disease. Future studies need to address the benefit of timely initiation of stroke prevention by other therapeutic interventions in addition to ERT.

REFERENCES

12. Maier EM, Osterrieder S, Whybra C et al. Disease manifestations and X inactivation in...


