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RESEARCH REPORT

Lyso-Gb3 Indicates that the Alpha-Galactosidase A Mutation D313Y is not Clinically Relevant for Fabry Disease

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Abstract The X-chromosomal-linked lysosomal storage disorder Fabry disease can lead to life-threatening manifestations. The pathological significance of the Fabry mutation D313Y is doubted, because, in general, D313Y patients do not present clinical manifestations conformable with Fabry disease. This is in contrast to the analysis of the alpha-galactosidase A activity, which is reduced in D313Y patients. We report a comprehensive clinical, biochemical and molecular genetic analysis of two patients with a D313Y mutation. The alpha-galactosidase A activity was reduced in both patients. No Fabry symptoms or Fabry organ involvement was detected in these patients. The new biomarker lyso-Gb3, severely increased in classical Fabry patients, was determined and in both patients lyso-Gb3 was below the average of a normal population.

Our data for the first time not only clinically but also biochemically supports the hypothesis that the D313Y mutation is not a classical one, but a rare variant mutation.

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Report

Fabry disease is an X-chromosomal-linked lysosomal storage disorder caused by decreased activity of the enzyme alpha-galactosidase A (Desnick et al. 1995). Classical Fabry disease mainly affects three organs: the kidney, the heart and the central nervous system (Whybra et al. 2009). This can lead to life-threatening manifestations at end stage of the disease including severe cardiomyopathy with arrhythmias, renal failure and stroke (Whybra et al. 2009). Besides the classical form of the disease, in recent years, so-called Fabry organ-variants have been described which mainly affect only the heart or the kidney. Moreover, some reports suggest that also non-organ-affecting variants, which do not alter organ function, may exist (Linthorst et al. 2010; Houge et al. 2011). These non-organ-affecting variants tend to have a higher frequency in the normal population than the classical private Fabry mutations, which are often only found in one single family (Linthorst et al. 2010). The variant mutation D313Y (located on an exon) seems to be such a mutation. The allele frequency in the normal population of D313Y is approximated as high as 0.5 % (Yasuda et al. 2003). Initially the D313Y mutation was described as causing classical Fabry disease by Eng et al. in 1993 (Eng et al. 1993), although Eng et al. did not include extensive organ characterisation in their study. Newer reports doubted the pathological significance of this special mutation, even the

possibility that D313Y is a polymorphism was discussed (Froissart et al. 2003; Yasuda et al. 2003). In some of the studies, the alpha-galactosidase A activity in patients with the D313Y mutation was decreased, in others it was not (Froissart et al. 2003; Yasuda et al. 2003; Baptista et al. 2010; Gaspar et al. 2010; Wozniak et al. 2010). Because of the phenotypic and biochemical partly discrepant results, a large Portuguese stroke study screening for Fabry disease comes to the conclusion, however, that the pathogenicity of the mutation D313Y cannot be conclusively determined without additional information (Baptista et al. 2010).

We report a comprehensive clinical, biochemical and molecular genetic analysis of two patients with a pure D313Y mutation (out of our cohort of 175 Fabry patients). The study conformed to the principles outlined in the Declaration of Helsinki and the locally appointed ethics committee has approved the research protocol. The referral to our Fabry centre took place because of the query for initiation of enzyme replacement therapy in a 20-year-old female patient with proven D313Y mutation. The patient had been screened for Fabry disease in another university hospital because of skin lesions that were found on the arms and legs, but also on the trunk and face. Her only clinical symptom was unspecific pain manifested in her arms. A reduced alpha-galactosidase A activity of 0.35 nmol/min/mg protein (normal: 0.4–1.0 nmol/min/mg protein) and the described D313Y mutation were detected. Subsequently, an extensive family pedigree and testing of first-grade relatives was performed. The results showed no mutation in the mother's gene with a normal alpha-galactosidase A activity with 0.52 nmol/min/mg protein. The father, however, had a D313Y mutation and a slightly reduced alpha-galactosidase activity of 0.32 nmol/min/mg protein. We conducted a detailed clinical analysis of the two family members with proven mutations in our centre (Table 1). Neither the 20-year-old female nor her father (53 years old) showed any signs or organ manifestations linked to classical Fabry manifestation. The only clinical diagnosis which could be made in the father was a long-lasting and excessive nicotine abuse. In the young female, a new dermatological examination with skin biopsies gave evidence for the presence of keratosis pilaris rubra atrophicans, but no evidence of typical Fabry angioma. In addition to alpha-galactosidase A activity and molecular testing, we determined lyso-Gb3 in these two patients. For lyso-Gb3, lyso-Ceramide had been used as reference items (Matreya LLC, Pleasant Gap, PA, USA) and D5-Fluticasone Propionate (EJY Tech, Inc., Rockville, MD, USA) were used as internal standards. The method was performed analogous to the method published in Tanislav et al. (2011). The lyso-Gb3 in the daughter was measured 0.22 ng/ml, which is below the average of a normal population (around 0.4 ng/ml) (Tanislav et al. 2011). No lyso-Gb3 was detectable in her father's blood.

Table 1 Clinical characteristics of the two Fabry patients with the D313Y mutation

	Daughter	Father
Age (years)	20	53
Alpha-galactosidase activity (nmol/min/mg protein)	0.35	0.32
LysoGb3 (ng/ml)	0.22	0
BMI (kg/m²)	25.0	22.1
Heart rate (/min)	60	62
Blood pressure (mmHg)	115/65	116/82
Cardiac assessment		
LVED wall-thickness (mm)	7	8
Left ventricular mass (g/m²) (Devereux formula)	42	62
EF (%)	69	69
Diastolic function	Normal	Normal
Strain rate lateral wall (s^{-1})*	1.3	1.2
MRI	No LE	No LE
Oedema	No	No
Renal assessment		
Creatinin plasma (mg/dl)	0.7	0.8
DTPA-clearance (ml/min)	123	124
Proteinuria	No	No
Albuminuria	No	No
Neurological assessment		
Stroke	No	No
Transient ischaemic attack	No	No
Pain	Yes, atypical	No
Acroparaesthesia	No	No
Symptoms		
Abnormal sweating	No	No
Heat or cold intolerance	No	No
Sudden deafness	No	No
Angiokeratomata	0	0
Dyspnoea on exertion	No	No

BMI body mass index, *DTPA* diethylene triamine pentaacetic acid, *EF* ejection fraction, *Gb3* globotriaosylceramide, *GFR* glomerular filtration rate, *LE* late enhancement, *LVED* left ventricular end-diastole, *MRI* magnetic resonance tomography

*Normal absolute values for systolic strain rate in the lateral wall (regional myocardial function) > 1.1

The detailed analysis of our two patients provides clear evidence that the mutation D313Y causes a pseudodeficiency of the alpha-galactosidase A, not associated with Fabry disease. Even in the 53-year-old father (an age at which male Fabry patients often die), no Fabry-associated manifestations could be determined. Thus, our data challenge the usual way to establish the diagnosis in Fabry disease (Havndrup et al. 2010; Weidemann and Niemann 2010): When a male patient shows decreased alpha-galactosidase A

activity (like our male patient), the diagnosis is regarded as proven. In female patients with borderline alpha-galactosidase A activity (like in our female patient), genotyping with the search for a Fabry-related mutation is demanded (Weidemann and Niemann 2010). However, these two conditions are perfectly met in our patients with the D313Y mutation. Thus, our female and male patients are very good examples that the pure assessment of the alpha-galactosidase A activity in combination with genotyping is not sufficient for diagnosing Fabry disease (which is especially a problem for the future in screening studies (Linthorst et al. 2010; Houge et al. 2011). In contrast, lyso-Gb3 seems to be the biochemical key for the clinical classification of an unclear alpha-galactosidase A mutation. In 2008, the new biomarker lyso-Gb3, a degradation product of the stored lyso-Gb3, was proposed for the first time to be a hallmark of Fabry disease by Aerts et al. (2008). They could show that lyso-Gb3 was elevated in patients with classical Fabry disease. Although the pathophysiological role of lyso-Gb3 in Fabry disease is not elucidated and mechanisms in Fabry organ complications involve multiple mechanisms beyond lyso-Gb3 (Schiffmann 2009; Auray-Blais et al. 2010; Brakch et al. 2010), the results of the study by Aerts et al. suggested that lyso-Gb3 plays an important role as a factor in the pathogenesis and progression of the disease (Aerts et al. 2008). Moreover, the same group also demonstrated little to normal values in atypical mutations (van Breemen et al. 2011). In our two patients, only a very low or no lyso-Gb3 at all was detectable, indicating that there was no classic organ involvement in these patients – as proven by our clinical characterisation. The explanations for this might be as follows: (1) In general, mutated and often misfolded alpha-galactosidase A proteins do not reach the lysosomes but are stuck in the endoplasmatic reticulum. However, there is evidence that the D313Y mutated alpha galactosidase A proteins do reach the lysosomes (Yasuda et al. 2003). (2) In addition, a normal activity of the D313Y-mutated protein was found in the lysosomes and a pseudo-reduced activity in plasma by Yasuda et al. (2003). This is due to the pH dependency of the D313Y-mutated alpha galactosidase A protein which results in a normal activity in the acid lysosomes and a reduced activity in the neutral plasma. (3) The mutation is located at some distance from the active site of the protein and the dimer interface. (4) The asparagine 313 has expressed its carboxyl-end to the exterior (hydrophilic fraction), while the rest of the side chain is in a hydrophobic environment (Froissart et al. 2003; Yasuda et al. 2003). Therefore, Yasuda et al. concluded that an exchange from asparagine to tyrosine could be tolerated without greater damage for protein activity (Yasuda et al. 2003). This all leads to a residual high activity of alpha-galactosidase A in the lysosomes leading to only small amounts of Gb3 storage. Because Lyso-Gb3 is a degradation product of Gb3 the residual activity of alpha-galactosidase A also leads to only a small amount of lyso-Gb3, which indicates a non-organ-affecting variant.

In conclusion, our data strengthen the hypothesis that the D313Y mutation is not a classical phenotype mutation but a rare variant mutation located on an exon as described by Froissart, Yasuda and Desnick. For the first time this assumption can also be supported biochemically by the measurement of the new marker lyso-Gb3. There are several other mutations (e.g. R112H, c.593C4T) where a discrepancy between positive standard diagnostic tests and lack of manifest disease have been shown (Aerts et al. 2008; Houge et al. 2011). It is a task for the future to perform lyso-Gb3 and other biochemical testing in various Fabry mutations, which are described as atypical in the literature. Of course, we cannot deny – due to the small number of our patients' cohort, the main limitation of our study – that there might be some patients with D313Y, where the biochemical analysis (enzyme activity and lyso-Gb3) might argue for a mild phenotype. This has to be discussed since it might be possible that the clinical spectrum of resulting consequences in some mutations reflects a continuum instead of a yes/no spectrum. The biochemical analysis of Fabry patients is even more complicated by the missing standardisation of measurements of the biomarkers involved in the diagnosis of Fabry disease. The standardisation is a major task for the future.

Limitations: The small number of our patient's cohort, leading to the diagnostic consequences explained in the last paragraph of the discussion.

Take Home Message

Our Lyso-Gb3 analysis indicates that the alpha-galactosidase A mutation D313Y is not clinically relevant for Fabry disease and questions the usual way to diagnose Fabry disease.

Conflict of Interest

None declared.

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