β -Galactosidosis in Patient with Intermediate GM1 and MBD Phenotype

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Abstract A 5-year-old girl with clinical and biochemical phenotypes encompassing both GM1-gangliosidosis (GM1) and Morquio B disease (MBD) is described. Mild generalized skeletal dysplasia and keratan sulfaturia were consistent with a diagnosis of MBD, while developmental delay and GM1-specific oligosacchariduria were consistent with GM1 gangliosidosis. No observable β -galactosidase activity was detected in leukocytes, and two mutations, p.R201H (c.602G>A) and p.G311R (c.931G>A), were identified by gene sequencing. The R201H substitution has been previously reported in patients with both GM1 and MBD, and G311R is a novel mutation. Our patient represents a further example of the clinical heterogeneity that can result from mutations at the β -galactosidase locus.

Introduction

GM1-gangliosidosis (GM1) and Morquio B disease (MBD) are biochemically and phenotypically distinct lysosomal storage disorders caused by a deficiency in β -galactosidase (β -Gal, EC 3.2.1.23) activity, due to mutations in the *GLB1* gene (Suzuki et al. 2001). GM1 is a neurodegenerative disorder characterized by the accumulation of GM1 ganglioside in nervous tissue, and can be categorized into three phenotypic variants according to age of onset and severity of symptoms. The infantile form (Type I), shows early and rapid psychomotor deterioration, generalized central nervous

system involvement, hepatosplenomegaly, cardiomyopathy, facial dysmorphism, and skeletal dysplasia (Suzuki et al. 2001). The late infantile/juvenile (Type II) and adult (Type III) forms display a progressive neurologic disease in childhood or early adulthood with localized skeletal and nervous system involvement, such as gait and speech disturbance (Suzuki et al. 2001). In contrast, patients with MBD retain neurological functions, but develop generalized skeletal dysplasia, keratan sulfaturia and corneal clouding (Suzuki et al. 2001). However, the clinical demarcation between GM1 and MBD can be obscured as in some patients displaying mental regression and the skeletal abnormalities of MBD (Giugliani et al. 1987; Mayer et al. 2009).

More than 130 sequence alterations in the *GLB1* gene have been identified so far, but our understanding of their effects on β -gal biosynthesis and function is still limited, and only a few may be predictive for one of the GM1 subtypes or MBD (Brunetti-Pierri and Scaglia 2008; Hofer et al. 2010). The tertiary structure of human GLB1 has recently been resolved, providing some insight into the bases of GM1 and MBD (Ohto et al. 2011). Based on crystallographic modeling, structural changes effecting β -gal protein folding, catalytic activity, substrate binding, and aggregation with lysosomal protective proteins have been predicted for various GLB1 mutations, but have not resulted in definitive phenotype classifications between the different diseases (Morita et al. 2009; Caciotti et al. 2011; Ohto et al. 2011).

We present a patient with intermediate clinical and biochemical phenotype between GM1 and MBD. The patient exhibits both skeletal disease and developmental delay, has deficient β -galactosidase enzyme activity, and has increased excretion of both GM1-specific oligosaccharides and keratan sulfate. Two mutations were identified by gene analysis, p.R201H (c.602G>A) and p.G311R (c.931G>A). R201H has been previously reported in both GM1 and MBD, and in the

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intermediate GM1 and MBD phenotype (Caciotti et al. 2005; Paschke et al. 2001). G311R is a novel mutation involving a highly conserved amino acid residue, and is predicted to affect the β -gal catalytic site. This patient represents a further example of the problematic partition between GM1 and MBD, and highlights the need for further characterization of the *GLB1* gene and its substrate specificities.

Clinical Report

The patient is a 5-year-old girl with developmental delay who was referred to our metabolic clinic following the identification of cloudy corneas by ophthalmological exam. Her early cognitive development had been typical although she took her first steps at 18 months of age. At 2½ years of age her gait was noted to be slightly unsteady. At that time a prominence at the thoracolumbar junction was also observed. Generally, she experienced good health without hospitalization, major illness or surgery.

At the time of our evaluation the patient was of normal stature, weight and head circumference for age. No cardiac abnormalities or organomegaly were appreciated on exam. Full range of motion was present in the fingers, wrists, elbows and knees. A developmental evaluation showed mild learning difficulties and evidence of processing delays. There was no recognized history of developmental regression. Neurological consultation noted mildly decreased muscle tone. An x-ray evaluation of the spine demonstrated mild beaking of L1 and L2. No radiologic abnormalities were observed in the long bones of the arm or in the wrist and hand.

Laboratory Analysis

Thin-layer chromatography (TLC) of urinary oligosaccharides showed an abnormal pattern characteristic of GM1. Interestingly, urine mucopolysaccharide TLC showed increased excretion of keratan sulfate, typical of patients with MBD, not GM1. β -gal activity in leukocytes using the artificial substrate 4 MU- β -D-galactopyranoside (Mayo Medical Laboratories) revealed no detectable enzyme activity, and molecular testing of the *GLB1* gene (Emory Genetics) identified two mutations: a previously reported p.R201H (c.602G>A) and a novel mutation p.G311R (c.931G>A). Molecular analysis of the patient's parents confirmed biparental inheritance; the father had a c.931G>A mutation and the mother a c.602G>A mutation.

Discussion

Deficiency in the activity of β -gal is expressed clinically and biochemically as GM1 and MBD. Classically, individ-

uals with GM1 exhibit neurological deterioration and GM1-specific oligosacchariduria, without keratan sulfaturia, while those with MBD demonstrate normal intelligence, skeletal dysplasia, and keratan sulfaturia (Suzuki et al. 2001). β -gal catalyzes the removal of the β -linked galactose residue from its natural substrates, including ganglioside GM1, oligosaccharides, and keratan sulfate, and the differing clinical and biochemical phenotypes have been ascribed to the different substrate specificities of the mutant enzymes (Hofer et al. 2010).

Enzyme and mutation analysis have provided little insight into predicting the probable course and outcome of the disease. Residual β -gal enzyme activity of mutations expressed in vitro correlate fairly well with severity of disease, but the phenotypes of most compound heterozygous genotypes remain difficult to predict (Callahan 1999; Santamaria et al. 2007). Additionally, while it has been shown that most MBD patients carry a common mutation (p.W273L), only a few of the over 100 mutations known in GM1 can be related to a specific phenotype. Certain mutations have also been identified in both GM1 and MBD, and the same genetic assessment has been shown in patients who exhibited different symptoms, further complicating possible prognoses in these individuals (Kaye et al. 1997; Bagshaw et al. 2002; Roze et al. 2005; Santamaria et al. 2007; Caciotti et al. 2011).

The clinical and biochemical phenotypes of our patient obscure the lines between GM1 and MBD. Her mild skeletal abnormalities and keratan sulfaturia support the diagnosis of MBD, while her mild cognitive delay and GM1-specific oligosacchariduria support type II or III GM1. Whereas some mutations in *GLB1* can be predictive of the disease, the R201H mutation identified in this patient has been described in both GM1 type II/III and in MBD (Ishii et al. 1995; Kaye et al. 1997; Morrone et al. 2000; Paschke et al. 2001; Caciotti et al. 2005, 2011; Santamaria et al. 2006; Hofer et al. 2009). Interestingly, a patient homozygous for R201H was classified as MBD while other reports on heterozygous patients described more severe GM1 phenotypes (Santamaria et al. 2006; Morrone et al. 2000; Santamaria et al. 2007). Additionally, this mutation has been identified in two patients exhibiting skeletal changes of MBD together with neurological impairment (Caciotti et al. 2005; Paschke et al. 2001). Similar to our patient, both were heterozygous for the R201H mutation, and at least one was shown to have keratan sulfaturia and oligosacchariduria (Caciotti et al. 2005; Paschke et al. 2001). The R201H mutation is believed to cause a small conformational change, preventing the aggregation of β -gal with a lysosomal protective protein and resulting in its premature degradation (Morita et al. 2009; Ohto et al. 2011). In contrast to previous reports that describe residual enzyme activity for R201H, our patient had undetectable activity. The varied enzyme activity

and phenotypes of individuals with R201H could be explained by the specific counter allele. The precursors derived from R201H would be prematurely degraded while those derived from the counter allele would predominantly reach the lysosomes and thus become determinant for the enzyme activity and phenotype.

The second mutation found in our patient (p.G311R) has not been reported in patients with MPS or GM1, nor has it been documented as a variant in the general population (www.ncbi.nlm.nih.gov/dbSNP; SIFT; PolyPhen). It is predicted to be causative of disease, however, due its location within the TIM barrel domain, a region of β -gal responsible for catalysis (Ohto et al. 2011). The Gly311 residue is also highly conserved between species and when replaced by a bulky, positive arginine residue in the vicinity of the catalytic site, may possibly weaken the enzyme-substrate interaction. The catalytic (Glu268, Glu188) and galactose binding (Tyr83, Asn187, Tyr333, Glu129, Ala128) residues of β -gal have been identified, but additional residues and/or domains may in addition be involved in the degradation of keratan sulfate (McCarter et al. 1997; Ohto et al. 2011). A mutation affecting the Tyr333 residue of the β -gal catalytic site (Y333H) has previously been observed in siblings with intermediate GM1 and MBD features together with keratan sulfaturia and GM1-specific oligosacchariduria (Giugliani et al. 1987; Mayer et al. 2009). Since our patient shows intermediate phenotype and both oligosaccharide and keratan storage, G311R could affect the ligand binding and/or catalytic activity of β -gal, and thus its specificity for both keratan sulfate and ganglioside GM1. Further characterization of mutant gene product, particularly for substrate specificity, will be required to clarify the pathogenesis as well as the prognoses of the diverse β -galactosidase disorders.

Synopsis

We present a case report of a 5-year-old girl with clinical and biochemical phenotypes encompassing both GM1-gangliosidosis (GM1) and Morquio B disease (MBD).

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