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Brief communication

An index case for the attenuated end of the mucopolysaccharidosis type VI clinical spectrum

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Abstract

Mucopolysaccharidosis type VI (MPS VI, Maroteaux–Lamy syndrome, McKusick #253200) is a lysosomal storage disorder that is caused by a deficiency in the lysosomal exohydrolase *N*-acetylgalactosamine-4-sulphatase (4-sulphatase, EC 3.1.6.1). We report a patient with no obvious clinical signs of MPS VI that has 5% of normal 4-sulphatase catalytic capacity. This patient represents an index case for the attenuated end of the MPS VI clinical spectrum.

Keywords: Lysosomal storage disorder; Mucopolysaccharidosis type VI; 4-Sulphatase protein; 4-Sulphatase activity; Attenuated clinical presentation

Introduction

Mucopolysaccharidosis type VI (MPS VI, Maroteaux–Lamy syndrome, McKusick #253200) is an autosomal recessive lysosomal storage disorder (LSD) that results from a deficiency in the lysosomal enzyme, *N*-acetylgalactosamine-4-sulphatase (4-sulphatase, arylsulphatase B, EC 3.1.6.1). A 4-sulphatase deficiency leads to the intracellular accumulation and urinary excretion of undegraded/partially degraded dermatan sulphate and chondroitin sulphate glycosaminoglycans. MPS VI has an incidence of approximately 1 in 248,000 live births in Australia, which compares to an incidence of 1 in 9000 for LSD as a group [1].

MPS VI patients can present within a spectrum of clinical phenotypes. The classical symptoms of MPS VI include short stature, hepatosplenomegaly, dysostosis multiplex, joint stiffness, corneal clouding, cardiac abnormalities, and facial dysmorphia [2]. Severely affected patients suffer early onset of these symptoms with rapid disease progression, while patients at the attenuated end of the clinical spectrum have a later onset and variable clinical presentation. In the severe form of MPS VI, death usually occurs in the early teenage years due to respiratory and cardiac problems, while in patients with the attenuated form of this disorder, lifespan can be up to 50 or more years in some cases. In this study, a 44-year-old Dutch woman with no obvious clinical signs of MPS VI was characterised with reduced levels of 4-sulphatase protein and activity.

Materials and methods

Patient description

The patient presented clinically with halitosis, slight photophobia, dyslexia, and mild hearing loss. The patient was 172 cm high and weighed 74 kg. There were

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neither corneal deposits nor cataracts, and ocular pressures were within normal limits. The eyes were straight with a full range of movement and fundus examination was unremarkable. The photophobia was concluded to have a cortical basis and was corrected by the prescription of heavily tinted glasses. Examination of joint mobility showed hands, wrists, elbows, and knees with a full range of movement. The shoulders had a possible, slight limitation of extension. Hips had a possible, slight limitation of abduction and the neck had full range of movement. The face appeared normal and the body had no hirsutism. A slight (1 cm) hepatomegaly was observed with no spleen enlargement. Echocardiogram examination and Doppler flow studies were normal. A routine examination of a blood film revealed leukocytes with obvious inclusion bodies, which was suggestive of a LSD. However, radiological examination revealed no obvious skeletal abnormalities.

Biochemical determinations

Urinary mucopolysaccharide was defined by a previously described method [3]. Lysosomal enzyme activities were determined by either radiolabelled trisaccharide assay (iduronate-2-sulphatase, β-D-glucuronidase, α-L-iduronidase [4]), or fluorogenic assay (β-hexosaminidase [5]) or immune capture using a fluorogenic substrate (4-sulphatase [6]). 4-Sulphatase protein [7], catalytic capacity [7], and protein processing [8] were as previously described.

Molecular genetic analysis

Molecular genetic analysis was performed on DNA extracted from the patient's skin fibroblasts. Exons 1–8, including the exon/intron boundaries of the 4-sulphatase gene (ARSB), were amplified by polymerase chain reaction using 250 ng of genomic DNA and intronic primer pairs, as previously described [9].

Results

Biochemical analyses

A urinary mucopolysaccharide screen demonstrated a mucopolysaccharide pattern consistent with MPS. A mild dermatan sulphaturia was noted for this patient [7], with 1 g of dermatan sulphate per mole of creatinine, compared to normal controls (>6 years-old, $n=20$) who had little or no detectable dermatan sulphate with <0.7 g per mole of creatinine.

Analysis of lysosomal enzyme activities on skin fibroblast lysates from this patient showed 73 nmol min^{-1} mg^{-1} of β-hexosaminidase, compared to a normal control range of 30–700 nmol min^{-1} mg^{-1}; 31.3 pmol min^{-1} mg^{-1} of iduronate-2-sulphatase, compared to a normal control range of 15–77 pmol min^{-1} mg^{-1}; 175 pmol min^{-1} mg^{-1} of α-L-iduronidase, compared to a normal control range of 150–980 pmol min^{-1} mg^{-1}; and 14 nmol min^{-1} mg^{-1} of β-glucuronidase, compared to a normal control range of 2–30 nmol min^{-1} mg^{-1}. The patient had a reduced 4-sulphatase protein of 2.5 ng mg^{-1} (compared to a mean of 75.8 ng mg^{-1} and range of 38–112 ng mg^{-1} for $n=10$ normal controls), and a reduced 4-sulphatase activity of 145.8 pmol min^{-1} mg^{-1} (using immune capture and the fluorogenic substrate 4-methylumbelliferyl sulphate, which compared to a mean of 7524 pmol min^{-1} mg^{-1} and a range of 4427–15,538 pmol min^{-1} mg^{-1} for $n=10$ normal controls), which was pathognomonic of MPS VI. Hence, the 4-sulphatase protein and activity results for this patient were comparable to other MPS VI patients, who had a range of not detectable—5.7 ng mg^{-1} for 4-sulphatase protein ($n=40$) and not detectable—318 pmol min^{-1} mg^{-1} for 4-sulphatase activity ($n=10$). Enzyme kinetic analysis on fibroblast extracts from the patient [10], demonstrated a normal K_m/V_{max} for the residual 4-sulphatase. The patient's 4-sulphatase catalytic capacity (the amount of 4-sulphatase activity per cell [7]) was 5% of that detected for normal controls, but this was greater than the range of not detectable-1.4% observed for 15 other MPS VI patients. Analysis of 4-sulphatase protein biosynthesis showed reduced levels of 4-sulphatase protein, but relatively normal protein processing of the residual protein to a mature form [8]. These results were consistent with a residual level of catalytically normal 4-sulphatase.

Mutation analyses

The patient was shown to have two novel ARSB missense mutations; P313A, which was detected in two other MPS VI patients with a severe clinical phenotype (unpublished observations) and P481L, which was unique in this patient. Two non-pathogenic ARSB mutations were also detected, V358M and IVS5-27 a → c, which had minimal impact on 4-sulphatase protein and activity in expression cell lines [9,11]. The P313A mutation was expressed in CHO-K1 cells to confirm pathogenicity, as previously described [9], and demonstrated 1.6 ng mg^{-1} of mutant 4-sulphatase protein, but no detectable 4-sulphatase activity (compared to a wild-type expression cell line, which produced 313 ng mg^{-1} of 4-sulphatase protein and 16,450 pmol min^{-1} mg^{-1} of 4-sulphatase activity). The P481L mutation was expressed in CHO cells using the same methodology and demonstrated 60 ng mg^{-1} of mutant 4-sulphatase protein (19% of the wild-type control), with 2773 pmol min^{-1} mg^{-1} of 4-sulphatase activity (17% of the wild-type control). The specific activity of this mutant protein was comparable to the wild-type

control 4-sulphatase. The P481L mutation was therefore responsible for the residual 4-sulphatase activity in the patient, contributing to the near normal clinical presentation.

Discussion

The biochemical and immunochemical characterisation of this patient was consistent with that observed for MPS VI patients, but there were no clearly recognisable clinical signs of MPS VI at the time of diagnosis. This patient represents an index case for the extreme attenuated end of the MPS VI clinical spectrum. A preliminary referral to the significance of this patient was recorded on the OMIM database under MPS VI (http://www.ncbi.nlm.nih.gov/entrz/query.fcgi?db+OMIM#253200). We concluded that a 5% level of catalytic capacity [7] was sufficient to avoid the onset of most MPS VI clinical symptoms. This was supported by a similar observation in MPS VI cats [12,13]. The latter study showed that MPS VI cats with the genotype D520N/D520N had an attenuated clinical presentation and that they produced 4.6% of the level of 4-sulphatase detected in normal control cats. This residual level of 4-sulphatase activity was sufficient to avert the onset of degenerative joint disease in these cats [12,13]. For treatment regimens like enzyme replacement therapy, which is currently in phase III clinical trial for MPS VI [14,15], these results indicated that correction to a level of 5% of normal control activity in each affected cell should prevent the onset of MPS VI pathogenesis. For enzyme replacement therapy, this critical threshold level of 4-sulphatase activity would ideally be implemented before irreversible pathology and before the accumulation of a level of substrate that would alter the dynamic balance between residual or replacement enzyme and substrate, thereby preventing the progression of MPS VI pathogenesis.

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