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Severe phenotype of Morquio A disease in a child with S287L N-acetylgalactosamine-6-sulfate sulfatase mutation

Case Report

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Abbreviations: denaturing high performance liquid chromatography, (DHPLC); glycosaminoglycans, (GAG); Mucopolysaccharidosis IVA, (MPS IVA); N-acetyl-galactosamine-6-sulfate sulfatase, (GALNS)

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Summary

Mucopolysaccharidosis IVA is caused by a deficiency of lysosomal N-acetyl-galactosamine-6-sulfate sulfatase (GALNS; E.C.3.1.6.4). One hundred fourty-eight GALNS mutations were described in patients with different phenotype severity. Our patient was a 6.5 year old boy with severe kyphoscoliosis and growth delay. He developed coarsening of the facial features and megalencephaly. Both corneas were cloudy. His gait was difficult with limited and painful movements in the hips, the spine and the knees. X-ray studies showed platispondily with ovoid vertebrae, bulging sternum and flaring of the rib cage. The long bones were short with irregular trabeculation. Metaphyses were widened, femoral head was flattened. The metacarpals had conical bases. Total excretion of glycosaminoglycans (GAG) in urine was increased. Thin layer chromatography of urinary GAG showed massive excretion of keratan sulphate. N-acetyl Galactosamine-6-sulphate sulfatase activity in leukocytes was low (0.7 nmol/MU17h/mg protein). DNA sequencing detected a S287L mutation (c.860C>Tc.860 C>T). This is the first GALNS mutation described in our population. The same mutation conferred a severe MPS IVA phenotype in a American, Austrian and Polish patients.

I. Introduction

Mucopolysaccharidosis IVA (MPS IVA; Morquio A disease; MIM#253000) is an autosomal recessive lysosomal storage disorder caused by a deficiency of lysosomal N-acetyl-galactosamine-6-sulfate sulfatase (GALNS; E.C.3.1.6.4) (Matalon et al, 1974). The enzyme deficiency results in the progressive accumulation of the undegraded substrates leading to a characteristic bone dysplasia. One hundred fourty-eight GALNS mutations and sixteen polymorphisms were documented (Tomatsu et al, 2005). We distinguish three forms of Morquio disease: severe, intermediate and mild forms. The phenotypes have a large diversity: from short trunk dwarfism, severe bone dysplasia, and a life span of 20-30 years, to mild bone dysplasia and visceral involvement (Tomatsu et al, 2005). About 70% of known lesions stem from missense mutations. It has been well documented that the patients' clinical severity correlates with the genotype, its effect on the tertiary structure, the level of conservation of altered amino acids, and the residual enzyme activity (Sukegawa et al, 2000). Some mutations are common in specific ethnic groups (Tomatsu et al, 2005).

In this article, we describe a boy with a severe MPS IVA phenotype caused by a S287L mutation (c.860C>Tc.860 C>T), the first described mutation in our population.

II. Case report

A 6.5 year old boy with short stature (-4.5 SD) progressively developed coarsening of the face (**Figure 1A**).

Macrocephaly, broad mouth, short and anteverted nose, widely spaced teeth, brittle and greyish enamel were evident. Intraocular pressure and hearing were normal, corneas were cloudy. The neck was short, kyphoscoliosis was pronounced (**Figure 1B**). He had progressive pains in the hips and the spine, the gait was difficult and he has recently begun to walk. Movements were limited and painful in the hips, the spine and the knees. In contrast, there was joint laxity in the wrists and the small joints.

Platispondily with ovoid vertebrae in anterior projection was seen on X-rays (**Figure 2A**). The sternum was bulging and the rib cage was flaring. The long bones were short, curved and with irregular trabeculation. Metaphyses were widened, the femoral head was flattened, the femoral neck was abnormal (**Figure 2B**). He had coxa valga and a knock-knee with medial spur of tibial metaphyses. The metacarpals had conical bases; hands were short and stubby (**Figure 2C**). MRI of the cervical spine showed no evidence for C1-C2 or C2-C3 subluxation. In addition, bone densitometry revealed reduced bone density. Mental development was normal. No cardiac anomalies or hepatomegaly were found.

Total excretion of glycosaminoglycans (GAGs) in urine (dimethylmethylene blue assay) was increased for the age group (16.2 mg/mmol creatinine; reference 7.6-9.2 mg/mmol creatinine) (de Jong et al, 1989). Thin layer chromatography of urinary GAG showed massive excretion of keratan sulphate (Humbel and Collart, 1975). N-acetyl galactosamine-6-sulphate sulphatase activity in leukocytes was low (0.7 nmol/MU17h/mg protein) compared to the controls (20-70 nmol/MU17h/mg protein) (Van et al, 1990). Analysis of GAGs in urine for the siblings and the parents was unavailable.

PCR amplification of the entire GALNS coding sequence and their flanking intronic regions was carried out on genomic DNA. PCR products were then subjected to denaturing high performance liquid chromatography (DHPLC) performed by a WAVE DNA fragment analysis system (Transgenomic, Omaha, Ne). Briefly, PCR products were denatured at 95 ºC for 5 min followed by gradual annealing to 50 ºC. Samples were automatically loaded on a DNAsep column (Transgenomic) and eluted with a linear acetonitrile gradient at the temperature for heteroduplex detection. The eluted DNA fragments were detected by an UV-C detector (Transgenomic). A S287L mutation (c.860C>Tc.860 C>T) was identified.

III. Discussion

The GALNS gene is located on chromosome arm16q24.3 (Masue et al, 1991; Tomatsu et al, 1992) and encodes 522 aminoacids, including a signal peptide of 26 residues. A total of 148 mutations included 103 missense mutations, eight nonsense mutations, 10 splice-site mutations, 20 small deletions, two large deletions and five insertions have been identified. In addition, sixteen polymorphisms producing amino acid changes have been identified (Tomatsu et al, 2005). A S287L mutation (c.860C>Tc.860 C>T) was previously described in American, Austrian and Polish populations to cause a severe clinical phenotype (Bunge et al, 1997; Tomatsu et al, 2004). The S287L mutation affects the amino acids (p.G96V), and is placed on exon 8. Interestingly, more than 5% of all mutations were 3 missense mutations, and the 10 most frequent mutations (in over seven mutant alleles) were represented by single nucleotide changes (except for c.334delG). Those ten mutations account for 35.3% of all described mutations (Tomatsu et al, 2005).

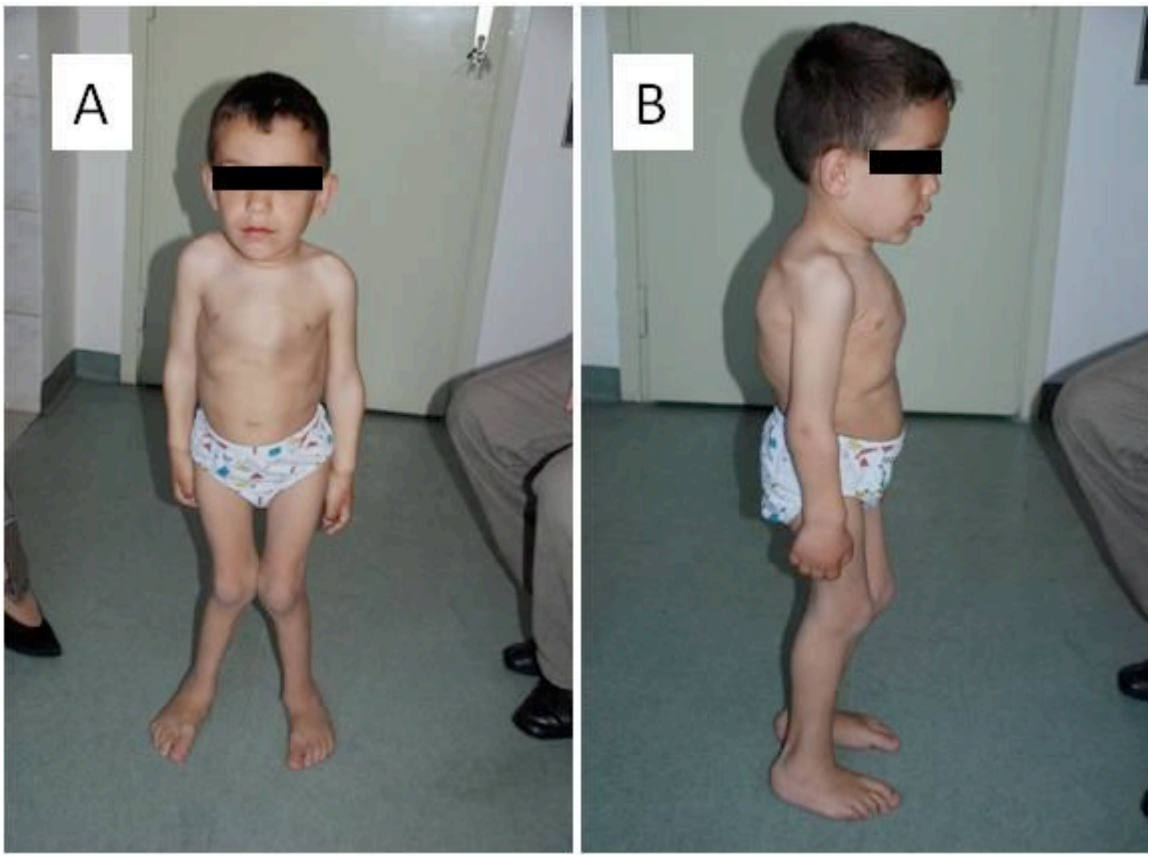

Figure 1. Photo of the patient. (**A**) Short neck, macrocephaly, coarse face with broad mouth, short and anteverted nose and widely spaced teeth. Genu vara. (**B**) Kyphosis, prominent sternum.

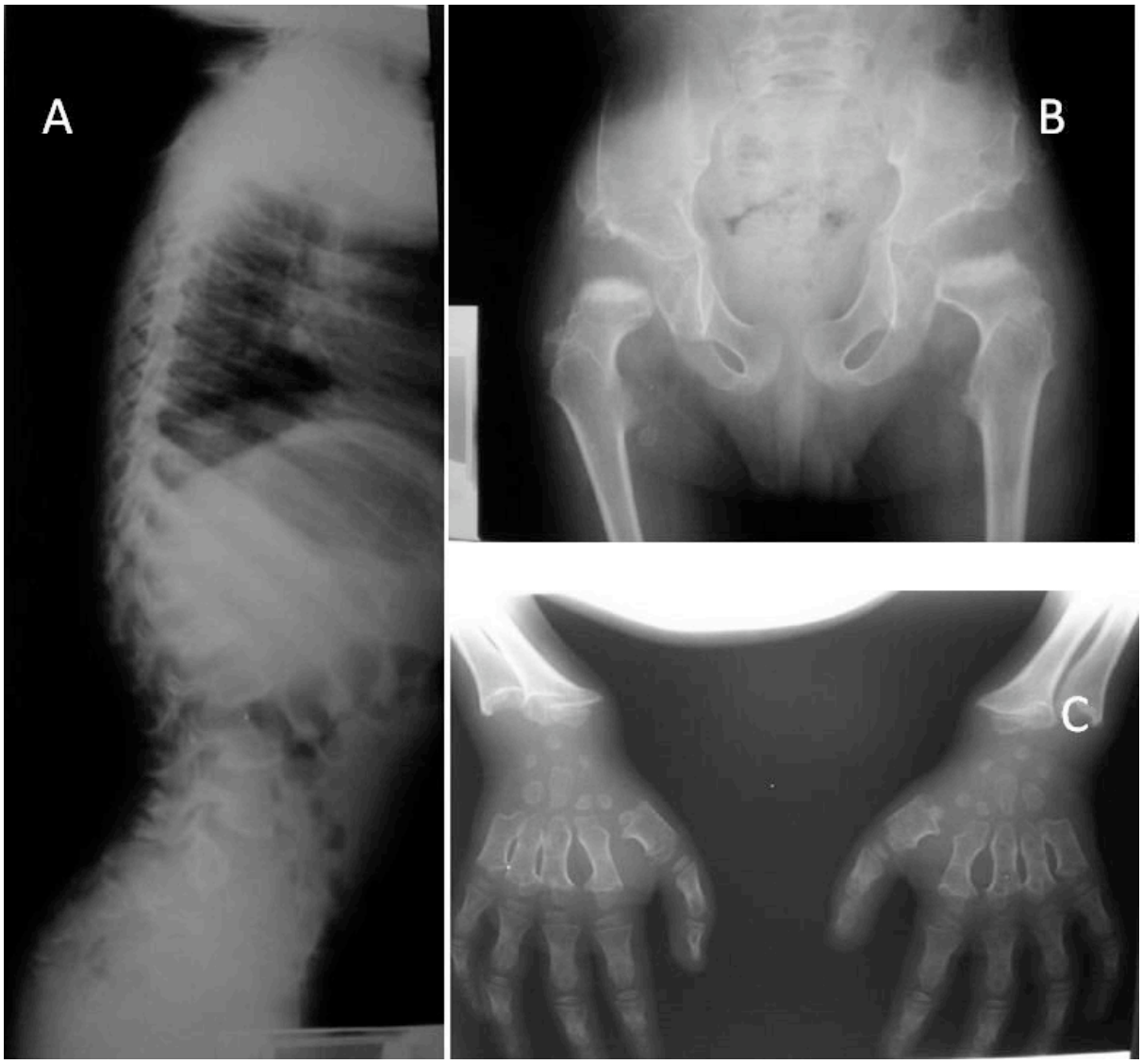

Figure 2. Bone X-ray. (**B**) Vertebra (lateral view): Platyspondyly with ovoid vertebrae and anterior projection and end-plates have irregular and rough surfaces. (**B**) Hip joints: Coxa valga, dysplastic and wave-like acetabula. (**C**) Hand: Hypoplastic scafoid and lunate bones. Madelung deformity of the radiocarpal articulation; and heads and bases of metacarpal bones spiky and mildly hypoplastic.

Our patient had normal intelligence, a severe form of bone dysplasia with marked platispondily, short neck and trunk plus severe kyphoscoliosis. In addition, joint restriction, especially in the hips was prominent and megalencephaly was also noted.

GALNS enzyme protein has been purified from human placenta as an oligomer with a molecular mass of 120 kDa, consisting of 40 and 15 kDa polypeptides linked by a disulfide bond (Masue et al, 1991). The model structure of GALNS has a monomeric from with two domains; the larger, N-terminal and the smaller, C terminal (Sukegawa et al, 2000). This model offerded the opportunity to analyze potential structural consequences of missense mutations in GALNS protein. Sukegawa K et al proposed three different reasons for the severe phenotype: destruction of the hydrophobic core or modification of the packing; removal of of a salt bridge to destabilize the entire conformation; modification of the active site (Sukegawa et al, 2000). S287L, which is a semi-conservative aminoacid change, affect the hydrophobic core and this substitution appears to break the packing and leading to a severe form of MPS IVA (Sukegawa et al, 2000).

MPS IVA is a rare disorder, and precise epidemiologic data are scare. The incidence of MPS IVA in Macedonia is unknown. However, this incidence was reported to be one per 76,000 live births in Northern Ireland (Nelson, 1997), one in 201 000 in Australia (Meikle et al, 1999), one in 450,000 live births in Portugal (Pinto et al, 2004). In Japan, the incidence was one in 500 000 live births (Tomatsu et al, 2005). For Tunisian MPS IVA patients, the incidence rate was approximately 2.8 in 100,000 live births (Laradi et al, 2006; Khedhiri, 2008).

Dental changes might be a prominent feature of MPS IVA. Our patients had brittle and thin dental enamel,

changes previously described by other authors (Levin et al, 1975; Beck et al, 1986; Nelson and Kinirons, 1988).

Although our patient was young he had corneal clouding further indicating the severity of the phenotype. However, no aortic regurgitation was present which might indicate that the severity of the symptoms is not always linked to all the systems involved. Born at term with unknown birth length, his growth velocity deteriorated since the age of 3. It also seems that the more severe form of the disease the earlier the start of the growth delay. So far, in spite of the severity of the phenotype the patient did not develop cord compression as the result of the severity of the defects of vertebrae. His history included frequent upper resiratory infections. The earliest signs of the disease were the thoracic changes: flaring of the ribs and prominent sternum.

In summary, it is obvious that the described S287L mutation (c.860C>Tc.860 C>T) in this boy resulted in an early and severe clinical phenotype.The identification of this mutation provide the opportunity to this family to have a prenatal diagnosis to ovoid another child suffering for this syndrome.

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