

Sanfilippo Syndrome: The Tale of a Challenging Diagnosis

Giuliana Baldini¹, José Fernando Palmejani¹, João Pedro Bonevechio Sant'Anna¹, Zumira Aparecida Carneiro¹, Roberto Giugliani^{2,3,4}, Catarina Pereira⁵, Claudia Cozma⁵, Cara O'Neill⁶ and Charles M. Lourenco¹ 

Abstract

Sanfilippo syndrome or mucopolysaccharidosis III (MPS III), includes a group of four autosomal recessive lysosomal storage disorders caused by deficient activity of enzymes involved in the catabolism of heparan sulfate. The four types of MPS III are recognized in accordance with the deficient enzyme, resulting in the accumulation of heparan sulfate with particularly deleterious effects in the central nervous system. The incidence of MPS III remains to be established in Latin American countries. We describe the journey of a patient with MPS IIIB whom, even in the presence of speech delay and deterioration, behavioral problems and motor incoordination, showed unaltered urinary glycosaminoglycans (GAGs) levels. An investigation for MPS was undertaken and enzyme analysis indicated a deficiency of alpha-N-acetylglucosaminidase, leading to the diagnosis of MPS IIIB. With the correct diagnosis, the patient's symptoms could be properly managed, and the parents received appropriate genetic counseling. The present case report reinforces the need of investigating MPS III in patients with language delay and/or regression, neurological impairment and behavioral alterations, even when urinary GAGs are within normal range. A definitive diagnosis ends the diagnostic journey and enables the medical team and family to provide a better care for the child.

Keywords

Sanfilippo syndrome, Mucopolysaccharidosis IIIB, heparan sulfate, neurological impairment, cognitive decline.

Introduction

Mucopolysaccharidoses (MPS) are a group of inherited disorders characterized by the tissue accumulation of glycosaminoglycans (GAGs) such as dermatan sulfate, heparan sulfate, keratan sulfate and chondroitin sulfate. MPS are classified in eleven subtypes in accordance with the specific lysosomal enzyme affected, showing variable phenotype, severity and progression [1].

Sanfilippo syndrome, or MPS type III, includes four autosomal recessive disorders, and the group is considered one of the most common types of MPS. The four subtypes of MPS III are caused by the deficiency of specific enzymes involved in the lysosomal catabolism of heparan sulfate, as follows: heparan N-sulfatase (MPS IIIA, OMIM #252900), alpha-N-acetylglucosaminidase (MPS IIIB, OMIM #252920), acetyl CoA alpha-glucosaminide acetyltransferase (MPS IIIC, OMIM #252930), and N-acetylglucosamine-6-sulfatase (MPS IIID, OMIM #252940) [2].

¹ Centro Universitário Estácio de Ribeirão Preto, Faculdade de Medicina, Ribeirão Preto, SP, Brazil.

² Hospital de Clínicas de Porto Alegre, Serviço de Genética Médica, Porto Alegre, RS, Brazil.

³ DR BRASIL Research Group, Porto Alegre, RS, Brazil.

⁴ Universidade Federal do Rio Grande do Sul, Departamento de Genética, Porto Alegre, RS, Brazil.

⁵ Centogene AG, Rostock, Germany.

⁶ Cure Sanfilippo Foundation, Columbia, SC, USA.

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Corresponding Author:

Charles M. Lourenço, Centro Universitário Estácio de Ribeirão Preto, Faculdade de Medicina, Rua Abrahão Issa Halack, 980, 14096-175, Ribeirão Preto, SP, Brazil.

Email: charles.lourenco@estacio.br.



The combined prevalence of the four subtypes of MPS III is estimated to 1 in 100,000 live births [3-5], with MPS IIIA and MPS IIIB being the most frequent subtypes worldwide [5]. In Northwest Europe, Australia and in the USA, MPS IIIA is the most frequent subtype of Sanfilippo syndrome while MPS IIIB has the highest prevalence in Southeast Europe, Taiwan, Japan and Brazil [6, 7]. MPS IIIC and D are significantly less prevalent globally [2, 3]. The onset of clinical manifestations is typically noted between one to six years of age [2, 4], with a shortened life expectancy leading to death often before adulthood [4].

Some of the most troublesome clinical manifestations of MPS III are mainly related to neurological disturbances (hyperactivity, sleep disorders, aggressiveness, neurodevelopmental delay, speech delay and/or regression and autistic-like behavior) [2, 4]. Similar to other MPS disorders, somatic manifestations, such as ear and throat infections, hearing loss, hepatomegaly, scoliosis and lordosis, and osteonecrosis of femoral head mimicking Legg-Calve-Perthes disease, are often found, though children with MPS III tend to have a less visually striking dysmorphic physical appearance [4, 8, 9].

This case report describes the journey of a patient that had clinical manifestations that strongly suggested MPS III in spite of normal levels of total urinary GAGs. The diagnosis allowed proper management for the patient and genetic counseling for the parents.

Case Report

A 5-year-old Caucasian girl, born to non-consanguineous parents, was referred to medical genetic evaluation due to speech delay and behavioral abnormalities.

The patient was born at term by an elective cesarean (51 cm of height and 2700 g weight) without complications during gestation or delivery. Parents denied other diseases and/or comorbidities and declared a negative familiar history for genetic conditions they knew. The patient had two healthy older siblings.

The child's history indicates that at the age of 3 years, she began experiencing a number of concerning symptoms.

These included language problems (difficulty in completing phrases), agitation, becoming fearless when facing dangerous situations (such as crossing a street with intense traffic), and motor incoordination in spite of hyperactive behavior. Parents noticed abdominal enlargement, and a splenomegaly was confirmed by ultrasonographic study at 3.6 years of age, which later spontaneously regressed. At medical consultation, physical evaluation revealed mild coarse face, hypertrichosis, normal joint mobility/no contractures.

As the patient did not display gross intellectual disability, she enrolled in a typical school setting at 2.5 years of age. However, the language difficulties led the school educators to refer her to a speech therapist, which, in turn, also referred her to a pediatric neurologist.

Suspecting a diagnosis of MPS III, the pediatric neurologist ordered urinary GAG test for screening purpose. The results of the urine GAGs, as collected at age of 4 years, were within normal limits, as shown in Table 1.

Table 1. Evaluation of urinary GAGs when patient was 4-year-old.

GAG evaluation	Results	Normal range
GAGs electrophoresis (qualitative analysis)	Normal pattern	Normal pattern
GAGs (quantitative analysis)	Patient: 123 µg/mg creatinine	<5 years: 64-127 µg/mg creatinine

As the clinical manifestations progressed, the patient was referred to a geneticist who suspected an attenuated form of MPS III. Of note, the patient still displayed fearless behavior, anthropomorphic measures within the normal range with mild dysmorphic signals, notably macrocephaly (Figure 1). The patient's growth status was: 1) body weight between P50 and P75 percentiles; 2) height between P75 and P90 percentiles and 3) head circumference between P50 and P75 percentiles. The curves used were obtained in the World Health Organization Child Growth Standards. No dysostosis multiplex was observed during clinical evaluation, however skeletal survey radiography was

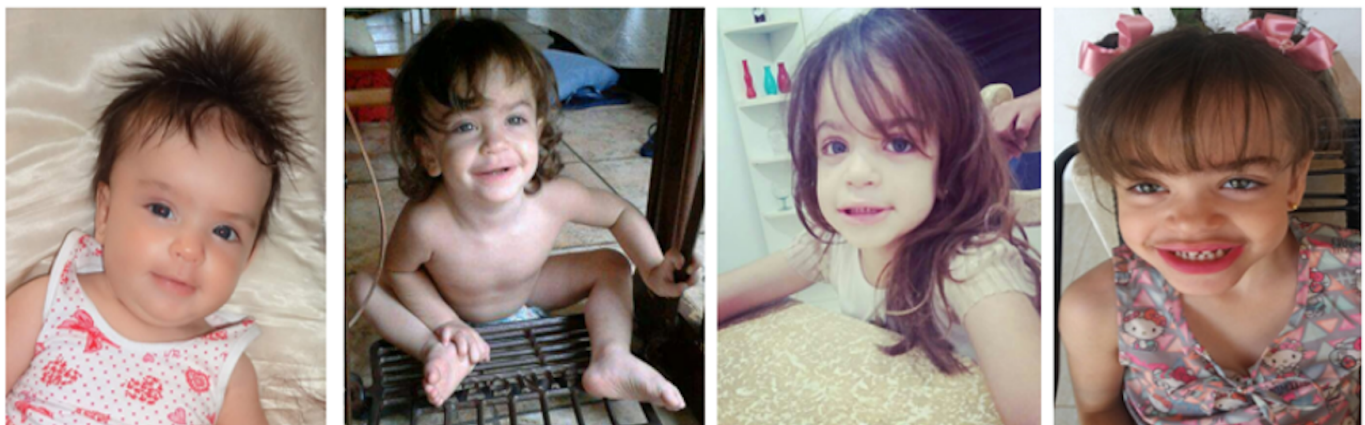


Figure 1. Patient at one, two, three and five years of age, showing the coarse facial features which are common in MPS.

not performed. Parents denied sleep disorders or epilepsy. The patient started receiving clonazepam to control hyperactivity. Testing was re-initiated at this time for urinary GAGs but also included a blood sample to assess the activity of enzymes related to for MPS III, which supported the diagnosis of MPS IIIB (see Table 2). Molecular genetics testing showed two pathogenic mutations in *NAGLU* gene, each one inherited from one of the parents, confirming the molecular diagnosis of MPSIIIB: c.1811C>T p.(Pro604Leu) and c.1597C>T p.(Arg533*), both in exon 6. Of note, both mutations have been previously described in the literature as pathogenic [10, 11].

Table 2. Evaluation of the activity of selected enzymes.

Enzyme	Activity	Normal range
heparan N-sulfatase (MPS IIIA)	9.2 nmol/17h/mg protein	5.5-24 nmol/17h/mg protein
alpha-N-acetylglucosaminidase (MPS IIIB)	Non detectable	0-34 nmol/17h/mg protein
N-acetylglucosamine-6-sulfatase (MPS IIID)	9.1 nmol/24h/mg protein	7-22 nmol/24h/mg protein

Discussion

The hallmark of MPS III is the degeneration of the central nervous system, leading to intellectual disability and hyperactivity. Taking this into account, this condition should be considered in the differential diagnosis in children presenting with behavioral disorders and developmental delays [12]. From a clinical perspective, MPS III has progressive stages, as follows: 1) a presymptomatic stage lasts from the birth to two years of age in which the child displays normal neuro-psychomotor development [2, 4, 13]; 2) the stage 1 begins around 1-2 years of life and is characterized by the onset of the development delay (speech delay and/or regression and impaired development) [4, 14]; 3) the stage 2 includes a delayed development accompanied by sleep disorders and challenging behaviors (i.e., autistic-like behaviors, fearless behavior, aggression and hyperactivity) from the third to the seventh year of life [4, 11, 14]; 4) in the last stage, progressive mental deterioration is observed, followed by a reduction in the behavioral problems, loss of motor abilities, swallowing difficulties and spasticity [4, 11, 15].

The neurological component of MPS III is a particularly impairing aspect of this multisystemic disease. Even though there is deposition of heparan sulfate in all cell types, it is particularly toxic to the neurons and glia, leading to oxidative stress and neuroinflammation [4, 16-18]. More specifically, the underdegraded heparan sulfate molecules can simultaneously lead to neuronal apoptosis and microglia-mediated phagocytosis, culminating in neurodegeneration [19-21].

If there is suspicion of MPS III, it is critical to measure both urinary GAGs and blood enzyme activity levels. This will ensure that a false negative urine result will not delay diagnosis and will

also allow for subtype analysis via multiplex enzyme activity testing [2, 22]. Of note, it is not uncommon that patients with MPS III as well as MPS VI display unaltered urinary GAGs, reinforcing the need of evaluation of enzyme activity and/or perform molecular genetic studies to confirm the diagnosis. Recently, Sabir and colleagues [23] have proposed the establishing of a neonatal screening for MPS in Morocco measuring the urinary GAGs by the DMB assay. The authors detected these solutes were stable for a maximal 7 weeks at 40°C, highlighting the necessity of evaluating not only GAGs but also enzymatic activity simultaneously.

Molecular genetics testing showed two mutations in the exon 6 of the *NAGLU* gene previously described as pathogenic c.1811C>T p.(Pro604Leu) [11] and c.1597C>T p.(Arg533*) [10]. The c.1811C>T mutation has been reported in two Tunisian homozygous patients who had severe phenotype including skeletal abnormalities, which was not found in the case reported here [11]. From a biochemical perspective, the mutation produced a substitution of leucine for the wild-type proline at amino acid 604 of the *NAGLU* protein. Such replacement may lead to destabilizing conformational changes in the native *NAGLU* protein fold [24]. The c.1597C>T mutation has also described in two patients (one in homozygous state and other in a compound heterozygous state) with severe phenotypes. This mutation creates a premature STOP codon and the truncated protein is formed with less than 211 amino acids, probably being a non-functional product [10]. In our patient, although these mutations produced a null enzymatic activity, the phenotype of the patient is slowly progressive in opposition to what was found in other studies [10, 11]. Collectively, these molecular data suggest that a prediction of genotype-phenotype relation in MPS IIIB is complicated by numerous polymorphisms that may potentially modulate the disease severity.

Currently, other methods of diagnosis can be employed in order to shorten the time of diagnosis. In fact, with the advance of the next generation sequencing, the use of genetic panels to accelerate the diagnosis of rare diseases or neurological disorders induced by unknown causes with onset during childhood have become progressively popular. In Europe and in the USA, genetic panels that test for almost all lysosomal disorders are already available, thus allowing a timely diagnosis more frequently. Nevertheless, these panels are not yet commonly employed in Brazil, where the diagnosis of Sanfilippo disorder (as well as other lysosomal storage diseases) is primarily obtained by biochemical methods.

Since the speech development is generally delayed compared to motor development, it is not uncommon that patients with MPS IIIB may be misdiagnosed with idiopathic developmental delay, attention deficit/hyperactivity disorder or even autism spectrum disorders. Within this context, our case report reinforces the need to include MPSIIIB as a differential diagnosis in patients with autistic-like behavior, hyperactivity or development regression, particularly with language difficulties as well as hypertrichosis [25].

Even though there is no specific treatment available for MPS IIIB so far (several therapeutic strategies are presently in clinical trials), there are some pharmacological and behavioral strategies that could be implemented to slow the disease progression and improve the quality of life of the patients [2, 4, 11, 14, 25-27]. A timely diagnosis is critical to enroll patients in clinical trials, as there are experimental therapies such as enzyme replacement and gene therapy which are currently under investigation. Additionally, the correct diagnosis is necessary to allow adequate genetic counselling to be provided to the family.

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Ethics Approval and Consent to Participate

The Ethical Committee from the Centro Universitário Estácio De Ribeirão Preto approved the study under the #170623317700005581 protocol. The patient consented with the publication of this case report.

Authors' Contributions

GB, JFP, JPBS, ZAC and CML conceived the manuscript and drafted the Portuguese version. RG, CC, CP, CO and CML revised the text critically. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

CP and CC are employers of Centogene AG, Rostock, Germany. GB, JFP, JPBS, ZAC, RG, CO and CML declare no conflict of interest. All fees received by CML are donated to the CML Medical Foundation for Research and Genetic Diagnosis Support for families with unknown genetic disorders.

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