A Novel Mutation (p.Met1?) of a Cuban Patient in the NAGLU Gene with Mucopolysaccharidosis IIIB

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Abstract

Mucopolysaccharidosis III (MPSIII) or Sanfilippo syndrome is an autosomal recessive disorder of lysosomal metabolism. MPS III is caused by mutations in genes that encode for the enzymes involved in the degradation of heparan sulfate. It is classified into 4 subtypes (MPSIII A-D). MPS IIIB is induced by mutations in the gene encoding the alpha-N-acetylglucosaminidase enzyme. We report a 6-year-old boy with phenotypic findings of Sanfilippo syndrome type B, such as mild coarse facie, clear corneas, hirsutism, hepatomegaly, mild joint stiffness and mild dysostosis multiplex. He also presents frequent upper respiratory infections, bilateral hearing loss, sleep disturbances, progressive neurologic deterioration and behavioral problems. He is compound heterozygous for the *NAGLU* gene (c.503G>A; p.Trp168Ter/ c.3G>A; p.met1?). One of the mutation was described in two patients before. A novel pathogenic variant was detected.

Keywords: Mucopolysaccharidosis, Sanfilippo syndrome, *NAGLU*, new mutation.

Introduction

Sanfilippo syndrome or Mucopolysaccharidosis type III (MPS III) is a complex of clinical conditions that are divided into four subtypes (MPS 3A-D; OMIM #252900 - #252940). MPS III is caused by mutations in one of the four gene that codes to lysosomal enzymes involved in the degradation of heparan sulfate [1,2]. This genetic defect, have an autosomal recessive inheritance [3]. The central nervous system is predominantly afected. It is characterized by progressive mental deterioration and behavioral disturbances. However, patients with MPS III have less marked facial and skeletal findings than other types of MPS, which often leads to a significant delay in diagnosis (4).

According to published reports, it was found that the combined prevalence of MPS at birth was 1.53 per 100,000 live births in Japan and Switzerland [5]. The frequency of MPS IIIB is variable in different populations studied, ranging from 1: 139 000 to 1: 5 000 000 live births [6]. It is caused by a mutation in both alleles of the gene that codes for the enzyme alpha-N-acetyl-D-glucosaminidase (NAGLU;E.C. 3.2.1.50), which is located on chromosome 17q21.1 [7].

More than 160 different mutations have been reported, recognized as causative variants of the disease [8]. Taking into account the difficulties in clinically distinguishing the 4 subtypes of MPS III, the diagnosis should be made by enzymatic activity assay. The wide allelic heterogeneity that characterizes this defect causes difficulties in the genotype-phenotype correlation [9], so its diagnostic confirmation requires the molecular detection of the responsible mutation. We aim to report a cuban MPS case, where we identified a new mutation in the *NAGLU* gene. Clinical, radiological, biochemical and molecular investigations have allowed us to establish a definitive diagnosis in these case.

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Case Report

A 6-year-old boy born at full term by caesarean section. He presented a prenatal history of hypogastria and oligohydramnios during the second and third trimesters of gestation, respectively. There is no positive family history for genetic conditions. He is the only child of nonconsanguineous parents. At birth, mensurations corresponding to a normal newborn were observed. He presented a psychomotor development consistent with his age, up to 6 months of age. Subsequently, a progressive slowing down in the acquisition of skills began, fundamentally in the language and control of the bladder sphincter. He started with nasal obstruction, frequent rhinorrhea and recurrent gastric colic since stard the lactation stage. Recurrent respiratory infections, such as moderate otitis media, adenoiditis and severe pneumonias with pleuritis and pericarditis began around 4 months of age. Significant episodes of sleep apnea were also associated, requiring adenotonsillectomy at 2 years of age. Physical examination revealed generalized hirsutism, with mild coarse facies, arched and populated eyebrows but without synophrys, clear corneas, narrowing of the anthelix, broad root and nasal bridge and small nasal tip at 3 years. In addition, he had low posterior hair line of the neck, usually semi-open mouth, with slightly protruding tongue, short neck, broad thorax, and globular abdomen with hepatomegaly.

The patient exhibited broad hands, mild joint stiffness of the fingers and elbows, and mild dysostosis multiplex (Figure 1).

There was a trend towards high height and overweight until age 6, which started a slowdown in growth in the pondoestatural development. The cephalic circumference increased postnatally above the mean, without ever surpassing the 2 standar desviation. No ophthalmological or cardiovascular alterations have been observed through the fundus and echocardiogram, respectively. Abdominal ultrasound showed hepatomegaly and absence of splenomegaly. The IgM, IgG and IgA concentrations and lymphocyte subpopulations have been normal; only CD19 was found slightly diminished. We also observed hight levels of advance products of protein oxidation and malonylaldehide. This result sugest us protein and lipid oxidative damage at this patient. In the neurocognitive area, the patient has evolved from the presence of a slight delay in psychomotor development (3 years), to alterations in the areas of behavior, language and attention, with significant troubles in his cognitive performance at present. Currently, there is high pitched voice, third person language which a regression in this skill, difficulties in pronunciation and hyperactive behavior, with a tendency to aggression, sleep disturbances, polyphagia and bruxism. Bladder sphincter control has not yet been achieved and despite not yet convulsive seizures, EEG showed global cortical-subcortical dysfunction with active paroxysmal disorder in the parieto-occipital regions bilaterally.

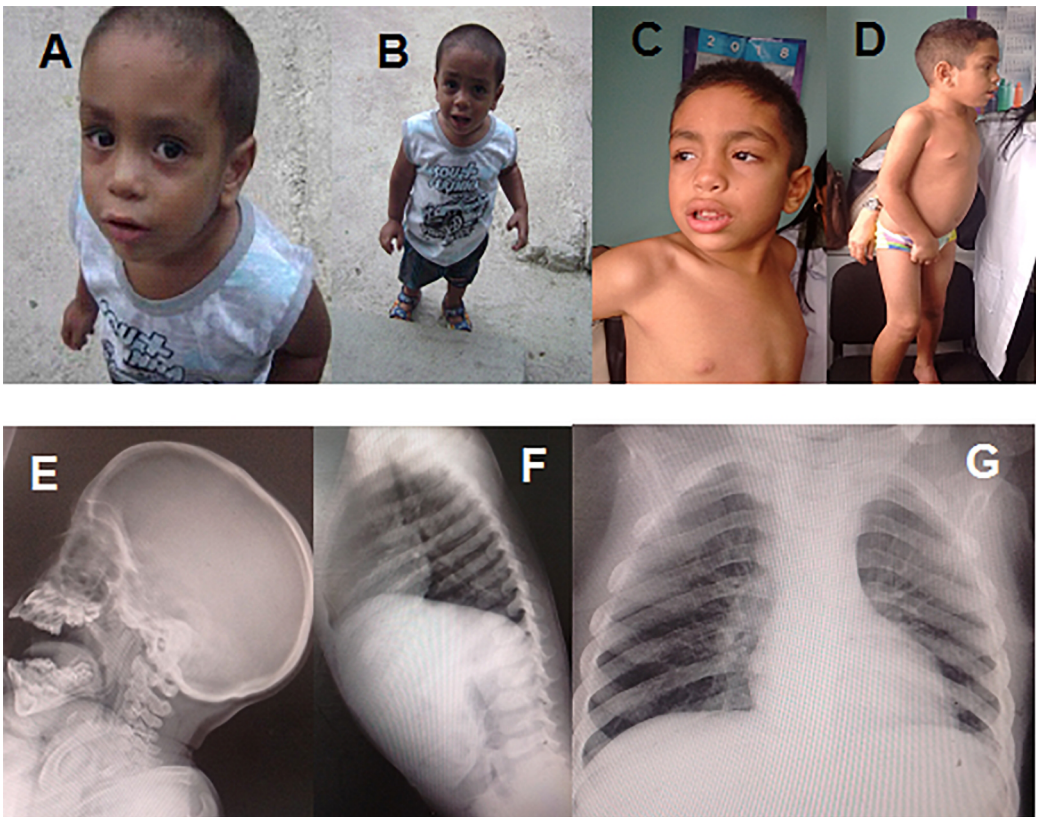

Figure 1. Clinical-radiological findings of the affected patient. A-B) Patient at 2 years old with discreet coarse facial features, broad nasal root without sinofris, depression of the nasal bridge with small nose and anteverted nares. Claw hand trend. C-D) Patient at 6 years with more marked dysmorphic signs. E-G) Mild signs of multiple dysostosis.

He presents moderate to severe bilateral hearing loss showed by performance of auditory evoked potentials (PEATC and PEAee). He was treatted. Risperidone (1ml/day) was used for treatment of behavioral problems. Unfortunately, melatonin could not be incorporated into the treatment. However risperidone improved sleep disturbances but did not modify the patient´s hyperactive, irritable or aggressive behavior.

The facial findings, the hepatomegaly and the incipient neurodegeneration at three years old let us suspected MPS. The biochemical diagnosis was made in the Biochemical Genetics Department at the National Center of Medical Genetics. The algorithm established for the confirmation of these diseases was followed. The positive results of the qualitative studies in urine by means of the Berry test and the precipitation reaction with the bromide-cetyl-trimethyl-ammonium, and the identification of the heparan sulfate as the GAG excreted in urine samples collected during 24 hours using thin-layer chromatography (TLC), supported clinical suspicion and suggested MPS III as a possible diagnosis. The activity of NAGLU enzyme in leukocytes was low in the patient but the activity of arylsulfatase was normal [10,11]. A Dried Blood Spot sample was also analyzed in the Rede MPS Brasil laboratory, as a possible external quality control. The result obtained was similar (Table 1).

The molecular study was carried out on the Diagnostic Center of Molecular Diseases of the Autonomous University of Madrid when he was four years old. Genomic DNA was used for the identification of the mutations and was carried out using massive sequencing with the TruSightOne clinical exome sequencing panel and the virtual capture of the genes related to MPS, for its bioinformatic analysis.

The selected variants were confirmed by conventional sequencing of Sanger using genomic DNA from the patient and their parents. Through this study, a heterozygous genotype was obtained for the *NAGLU* gene (NM_000263.3; NP_000254.2), identifying a mutation previously described for this disease (p.Trp168Ter) and a new allelic variant (p.Met1?) Functional prediction of the new "in silico" mutation was made, using the MutationTaster (http://www.mutationtaster.org/) and mutalyzer 2.0.28 (https://mutalyzer.nl/) programs. These predictors resulted in a high score indicative of pathogenic mutations (Table 2).

Discussion

Sanfilippo syndrome type B is a widely studied lysosomal condition. Mutations that damage the protein NAGLU, cause a severe neurological disease, progressive neurological deterioration being the most characteristic symptomatology of this type of MPS [12]. When interpreting the results of molecular analysis in the patient we found that the allelic variant (c.503G> A, p.Trp168Ter) was previously described in two spain patients by Coll et al in 2001. This allelic variant is recognized as pathogenic and it is associated with a severe phenotype. Unfortunately, the main clinical manifestations of these patients were not described by the authors [13].

This mutation causes a change in the amino acid sequence due to the early appearance of a stop codon. As a consequence of this, a protein with only 168 amino acid is synthesized instead of a 743 amino acid one. The other allelic variant found (c.3G> A, p.Met1?), not previously reported until this study. We were not able to carry out a study of this mutation in a control population, but this variant was not found in ExAC browser (http://exac.broadinstitute.org) neither in the 1000 Genomes project data base (https://www.internationalgenome.org/). We did not find it in the HGMD or ClinVar databases either. We considere as a new variant.

A single nucleotide variant in the same codon also was reported in ClinVar data base, but the mutation is diferent (NM_000263.4(*NAGLU*):c.2T>C (p.Met1?); rs1013345784) (https://www.ncbi.nlm.nih.gov/clinvar/). A patient diagnosed with MPS III from Tunisia is reported with a similar mutation (c.2T>C; p.Met1?) but in another gene (*SGSH*) [14]. These findings allows us to reinforce the idea that this allelic variant could be related to an Sanfilippo syndrome phenotype, taking into account that both patients (patient from Tunisia/ index case) present clinical manifestations similar and compatible with a severe form of this disease (Table 3).

Table 1. Result of the NAGLU and ARSB activity enzymes of the patient.

Sample	Laboratory	NAGLU	Reference Values	ARSB	Reference values
Leukocite	NCMG	1,9 **nmol/h/mg proteína** 19%	20,0-97.6 **nmol/h/mg proteína >30% RCA**	109 **nmol/h/mg proteína** 100% :	>51 **nmol/h/mg proteína >30% RCA**
DBS	Rede MPS Brasil	ND	0,96-5,80 nmol/h/ml	18,0 nmol/h/ml	5.3-22 nmol/h/ml

Legend: DBS: Dried blood spot; ND: undetectable; RCA: Control related activity.

Table 2. Results of in silico mutation prediction analysis (c.3G>A, p.Met1?).

Algoritm	Prediction	Score
Mutation Taster	Pathogenic. Disease causing.	1.00
Mutalyzer	Pathogenic. Disease causing.	1.00

Table 3. Clinical features of a Tunisian and Cuban patients with MPS III.

Clinicals features	Tunisian patient	Cuban patient
Type of MPS	MPSIII A	MPSIII B
Consanguinity	+	
Age diagnosis (year)	3	4
Mental disability	+	+
Behavioral problems	+	+
Dysmorphic features	+	+
Macrocrania	+	
Hirsutism	+	+
Organomegaly	+	+
Squeletal abnormalities	+	+
Deafness	+	+
Seizures		
Hernia	+	+
Diarrhea	+	
Recurrent infections	+	+
Others	-	Clear corneas

The bioinformatic prediction indicates that this allelic variant could be a pathogenic change. The repercussion that this mutation must have caused changes in the enzymatic phenotype is a change in the primary structure of NAGLU protein by loss of the initiation codon (Kozak consensus sequence); in the translation it would then be used as the initial codon, one that is towards the 5`UTR region or an AUG codon that appears more distant in the coding zone. In any case, a shift of the reading frame affecting the function of the mentioned enzyme is predicted. The finding of a decreased activity of NAGLU in leukocytes suggests that the combination of both mutations are the cause of the disease in the patient. The genetic study of the parents confirmed that the index case presents different pathological alleles and the parental origin of each variant detected. The known variant turned out to be of paternal origin, and maternally the variant not previously reported. The discrete facial findings, hepatomegaly, language disorders and the severe behavior problems of the patient correspond to the diagnosis of Sanfilippo B syndrome. We consider that this patient has a severe form of MPS IIIB because the rapidly progressive neurological deterioration and the destructive behavior. His genotype and the low value of enzymatic activity of NAGLU confirm that idea.

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Ethics Approval and Consent to Participate

For the accomplishment of this work we obtained written confirmation from the parents to use the information contained in the Clinical History, photos and images of the patient for its use with scientific and didactic purposes.

Declaration of Conflict Interest

The authors declare that there are no interest conflicts with respect to the authorship or publication of this article.

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