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Clinical report

The early detection of Salla disease through second-tier tests in newborn screening: How to face incidental findings

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ABSTRACT

We describe here a 34 months child, practically asymptomatic which presented with high levels of free sialic acid in urine by biochemical detection in second-tier tests newborn screening and with two disease causing mutations in SLC17A5 gene. *SLC17A5* mutation analysis showed p.Tyr306* previously decribed and the novel mutation p.Leu167Pro. This early onset diagnosis allowed us to perform a fast and accurate genetic counseling to the family, helped to better understanding the natural history of this rare disease and probably it could promote cost reduction in future diagnostic tests in the hypothetic case of starting symptoms without diagnosis established. Moreover, an early diagnosis could save family from a long period of time until achieving a definitive diagnostic and to develop an early symptomatic and supportive management of patient to attenuate, as much as possible, disease complications. But, above all, this case illustrates the huge ethical dilemma which arises from any secondary finding (second tier) in newborn screening.

1. Introduction

The phenotypes of the allelic disorders of free sialic acid metabolism – Salla disease (mildest phenotype; OMIM:604369), intermediate severe Salla disease, and infantile free sialic acid storage disease (ISSD; OMIM:269920) - range from mild to severe. All are neurodegenerative disorders resulting from increased lysosomal storage of free sialic acid [Aula and Gahl, 2001; Lemyre et al., 1999]. Onset varies from apparently unaffected in the newborn period to prenatal severely affected. Salla disease is characterized by normal appearance and neurologic findings at birth, following by slowly progressive neurologic deterioration resulting in mild to moderate psychomotor retardation, spasticity, athetosis, and epileptic seizures [Alajoki et al., 2004; Renlund et al., 1983; Varho et al., 2002]. Muscular hypotonia is often recognized at first, approximately at the age of six months. One third of affected children learn to walk. Speech can be limited to single words but understanding of oral communication is good. Slow developmental progress often continues until the third decade, after which regression can occur. Some individuals with Salla disease present later in life with spasticity, athetosis, and epileptic seizures, becoming nonambulatory and nonverbal. At the other extreme of wide range phenotypes is ISSD characterized by severe developmental delay coarse facial features, hepatosplenomegaly, and cardiomegaly, leading to death in early childhood.

This disorder results from defective free sialic acid transport out of lysosomes as a consequence of mutations in *SLC17A5* (OMIM:604322), encoding the lysosomal transport protein sialin.

Abbreviations: cDNA, DNA complementary to RNA; CHX, cycloheximide; DBS, dried blood spots; ESI-MS/MS, electro spray ionization tandem mass spectrometry; ISSD, infantile free sialic acid storage disease; LSD, lysosomal storage disorders; NBS, newborn screening; NMD, nonsense-mediated mRNA decay; PTC, premature termination codon; TLC, thin-layer chromatography.

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Mutations in *SLC17A5* lead to defective sialin and elevated intra-lysosomal free sialic acid.

Sialic acid storage disease is a very rare disorder. ISSD has been identified in only a few dozen infants worldwide. Salla disease occurs mainly in Finland and Sweden and has been reported in approximately 150 people [Aula et al., 2000; Erikson et al., 2002].

Management is symptomatic and supportive: rehabilitation to optimize mobility and communication, adequate nutrition, and standard treatment of seizures. This pathology is inherited in an autosomal recessive manner.

We describe a 34 month-old girl with mild clinical symptoms until now but diagnosed of Salla disease in the neonatal period thanks to second-tier tests of newborn screening and confirmed by genetic testing.

2. Methods

2.1. Free sialic acid measurement

2.1.1. Newborn screening using ESI-MS/MS

The newborn screening (NBS) lab in Galicia (N.W. Spain) receives at the same time dried blood spots (DBS) and urine dried samples of all newborns in Galicia collected at the third day of life. In those blood samples in which acylcarnitines or amino acids profile are abnormal, urine sample is used as a multi-parametric second tier test employing electro spray ionization tandem mass spectrometry (ESI-MS/MS), including the determination of creatinine, organic acids, acylcarnitines and amino acids [Rebollido-Fernandez et al., 2012]. This protocol enables to focus on the diagnostic of the first NBS sample and possibly opens up, the detection of pathologies not included in the main NBS panel.

2.1.2. Measurement of free sialic acid using TLC/spectrophotometry

In order to confirm the results obtained in NBS, a urine sample was adjusted to a creatinine content of 10 μg and spotted onto a precoated silica gel 60 thin-layer chromatography (TLC) plates (Merck) and developed three times in butanol-acetic acid-water (2:1:1 by vol). The plate was sprayed with orcinol-Fe^{3+}-HCl stain (Bial's reagent) to detect sialic acid- containing compounds and free sialic acid. The band corresponding to free sialic acid was scraped and the color extracted by butanol. The content of free sialic acid was then quantified using a spectrophotometer at 580 nm [Tsai and Marshall, 1979].

2.2. Mutation screening: analysis of cDNA and gDNA

After informed consent was received from the parents, a skin biopsy was obtained from the patient and fibroblasts were cultured as described [Macías-Vidal et al., 2009]. To detect possible mutations whose mRNAs are candidate to suffer nonsense-mediated mRNA decay (NMD) process, fibroblasts were treated with cycloheximide (CHX) (Sigma, St. Louis, MO) according to the protocol previously described [Macías-Vidal et al., 2009].

To identify mutations in *SLC17A5* gene (reference sequence NM_012434.4), sequence analysis of its cDNA was performed. The changes identified were confirmed by sequencing the corresponding gDNA region. RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR) were performed using standard methods. *SLC17A5* cDNA was amplified in 3 overlapping PCR fragments using self-designed primers (www.bioinformatics.nl/primer3plus/). PCR products were screened for mutations by DNA sequencing using the Big Dye Terminator Cycle Sequencing v3.1 kit (Applied Biosystems, Foster City, CA), according to the manufacturer's instructions. The sequencing reactions were run on an ABI Prism® 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA).

gDNA was extracted from whole blood by standard methods. Both *SLC17A5* exons 3 and 7 and their intron boundaries were amplified using self-designed primers. PCR products were purified and sequenced as described in the preceding paragraph.

3. Results

3.1. Case report

Newborn girl, with unrelated parents and 2 healthy sisters, was born at 37 weeks by caesarean section, also carried out on previous births. Birth weight was 3285 g, length was 49 cm, head circumference was 35 cm (25–50 percentile), and Apgar score was 9/10/10. The neonatal screening tests taken on the third day of life showed a high value of tyrosine for which, a second level multi-parametric testing was performed by MS/MS in urine dried sample to rule out type I tyrosinemia. An elevated excretion of 4-hydroxyphenil lactic acid and free sialic acid (371 μmol/mol creatinine; p99:224.55) was detected, but without appreciable elevation of succinylacetone. That's why it was recommended to evaluate protein intake, provide vitamin C and retake samples a week later. In this second sample was determined that blood tyrosine and urine 4-hydroxyphenil lactic acid had normalized (so this case was considered as a transient neonatal tyrosinemia), but sialic acid remained elevated (372 μmol/mol creatinine; cut off < 224).

We confirmed these pathological values quantifying free sialic acid by thin layer chromatography/spectrophotometry which confirmed a high level of sialic acid (216 μmol/mmol creatinine and 189 μmol/mmol creatinine one year later. Age matched control range was 2–95 and 2,5–78 respectively). Sialic acid levels in parents and sisters were within the normal range.

3.2. SLC17A5 mutation analysis

The *SLC17A5* gene was analyzed using both cDNA and gDNA (Fig. 1A). To determine whether the transcripts encoded were targeted by the NMD process, we analyzed mRNA before and after CHX treatment.

As shown in Fig. 1A, classic sequencing analysis revealed that patient carried one previously described nonsense mutation, p.Tyr306* and one new missense mutation, p.Leu167Pro. The c.918T $>$ G change in exon 7 led to a premature termination codon (PTC) in the amino acid 306, which was only detectable upon CHX-treatment, suggesting degradation by the NMD mechanism. The individual's second mutation was a c.500T $>$ C in exon 3, this change was observed in homozygosity in untreated cDNA sample. The analysis of the gDNA sample from the child confirmed the compound heterozygous state.

Next generation sequencing analysis of a lysosomal storage disorders (LSD) panel (included in the research lysosomal storage disorders project funded by FIS PI10/01193) sustained the same result (data not shown). Each mutation was present in one parent (Fig. 1A) and family co-segregation was shown.

3.3. Follow-up

She started walking at 11 months and her first words were at 12 months. At 24 months of age biochemical analysis showed a slight increase in liver enzyme levels: GOT: 40U/L (range 0–25U/L), GGT: 122U/L (range: 5–38U) and LDH: 409U/L (range: 140–310). Cerebral and abdominal ultrasound, and bone X-ray, did not shown anomalies. At 34 months of age somatometric parameters were