CASE REPORT

Identification of a homozygous deletion of the *NEU1* gene in a patient with type II sialidosis presenting isolated fetal ascites and central nervous system hypoplasia

Mitsiakos G, Gialamprinou D, Chouchou P, Chatziioannidis I, Karagianni P

2nd Neonatal Department and Neonatal Intensive Care Unit (NICU), Aristotle University, Papageorgiou General Hospital, Thessaloniki, Greece

Abstract

Background: Mutation of the *NEU1* sialidase gene is the etiology of sialidosis, a storage disorder with a plethora of systemic manifestations ranging from ocular abnormalities, bone pathologies, and ataxia (sialidosis type I) to mental decline and infantile death (sialidosis type II). Non-immune hydrops fetalis and isolated ascites are the most severe forms of sialidosis type II that manifests itself prenatally.

Case report: For the first time, we report congenital sialidosis with homozygous pathogenic deletion of the entire *NEU1* gene in a Greek neonate with hydrops fetalis, isolated ascites, central nervous system hypoplasia, and lethal progression. Genetic characterization of the patient showed one previously unreported deletion in the NEU1 gene.

Conclusion: Sialidosis type II should be considered in the differential diagnosis of neonatal hydrops fetalis of no immune causality or isolated fetal ascites. Genetic studying of the patient and the family by carrier detection is crucial to prevent missed diagnoses, while genetic counseling for following pregnancies is imperative. HIPPOKRATIA 2019, 23(4): 169-171.

Keywords: Sialidosis, *NEU1* gene mutations, hydrops fetalis, isolated ascites.

Corresponding author: Georgios Mitsiakos, MD, PhD, Associate Professor of Neonatology, 2nd Neonatal Department and Neonatal Intensive Care Unit, Papageorgiou General Hospital, Ring Road, 56429 Nea Efkarpia, Thessaloniki, Greece, tel: +302313323354, fax: +302313323360, e-mail: mitsiakos@auth.gr

Introduction

Sialidases or neuraminidases are hydrolytic enzymes whose function is to act in a catalytic way for the split of α-glycosidically linked final N-acetyl neuraminic acid from sialylated glycoconjugates[1]. In humans, lysosomal storage diseases are attributed to the deficiency of lysosomal neuraminidase (a-N-acetyl-neuraminidase, *NEU1* gene, OMIM: 608272). They thus are linked with two neurodegenerative diseases associated with deficits in lysosomal metabolic pathways, namely galactosialidosis and sialidosis, which are disorders considered secondary to lysosomal recessive mutations in the sialidase gene localized on chromosome 6p21.33[2-4]. Sialidase genetic aberrations result in abnormal intracellular accumulation of sialyl oligosaccharides, which can be traced in the urinary excretion. Phenotypes vary from the milder type I sialidosis (OMIM: 256550) or the so-called "cherry-red spot myoclonus" syndrome to sialidosis type II with an early-age onset which is manifested with facial dysmorphism, bone dysplasia, or neurodegeneration[5]. There are three different subtypes of sialidosis type II, which are contingent upon the beginning of the signs: i) congenital or hydropic, ii) infantile, and iii) juvenile[5]. The most severe form is the congenital sialidosis developing entirely prenatally, after the second trimester of pregnancy with non-immunological hydrops fetalis (HF) or isolated fetal ascites[6].

We present a neonate with sialidosis in the congenital form characterized by hydrops fetalis, isolated fetal ascites, and central nervous system (CNS) hypoplasia at birth in whom a new deletion variant in the *NEU1* gene was detected.

Description of case

The patient was a female newborn, the second birth of non-consanguineous Greek parents. The first child of the family was a 10-month-old female, phenotypically healthy. At the 30th week of gestation, HF was detected, and at 31st week of gestation delivery by elective cesarean section was conducted. She was of a normal female karyotype and normal body measurements. Mechanical ventilation was required after birth because of respiratory distress. Three diagnostic and therapeutic paracenteses were performed. She was hypotonic with mild generalized edema and isolated ascites. Distinctive facial dysmorphism features like coarse face, low nasal bridge, sparse hair, hypertelorism, skin redness, and midline abdominal hernia were identified. The liver was palpable at two finger breadths. Prenatal infection was ruled out, and any immunological causes of HF were excluded. The hemoglobin level was 14.3 g/dl, and the platelet count was normal. The serum albumin was 2.7 g/dl, and the serum

ascites albumin gradient (SAAG) was 1.2 g/dl (indicating portal hypertension). The abdominal ultrasound (US) showed isolated ascites, hepatomegaly, splenomegaly, and a mixed-echogenicity lesion in the right lobe of the liver. Cardiomegaly, atrial septal defect, and mitral and tricuspid valve regurgitation were found in the echocardiography. The cerebral US showed pachygyria, and the cerebral magnetic resonance imaging (MRI) revealed corpus callosum hypoplasia, continuing growth of cerebral parenchyma, and dilatation of the occipital horns of the lateral ventricles. The fundoscopy revealed a lack of pigment. Cytoplasmic vacuoles were detected in lymphocytes of the peripheral blood smear. The laboratory tests for metabolic disorders showed diffuse aminoaciduria. Plasma chitotriosidase activity was 435 nmol/ml/hr (normal range: 0-150 nmol/ml/hr). L-cell screen (Lysosomal enzyme activity in serum more than ten times the reference range; diagnosis in fibroblasts or amnion cells: same lysosomal enzymes deficient) was negative. Acid lipase activity, sphingomyelinase activity, β- glycosidase activity were within the normal ranges. Sialidase activity was 0.2 nmol/mg prot/hr (normal range: 4.2-22.2). Fibroblast cell culture -by measuring the activity of lysosomal enzymes- was found to be consistent with sialidosis type II. Full sequencing of the most common exons of the *NEU1* gene was performed, and a homozygous deletion encompassing the entire *NEU1* gene was detected. Both parents were heterozygous carriers. Her sister had a normal genotype. At the age of two months, she was below the 3rd percentile for all her measurements. The hepatomegaly and splenomegaly progressed, and pancytopenia was established. The patient died at four months of age due to cardiopulmonary failure after continued ventilatory care.

Discussion

Sialidase is the catalyst for the hydrolysis of final sialic acid remnants of glycoconjugates. In literature, three types of mammalian sialidases are mentioned: plasma membrane, enzymes localized in the cytosol, and the lysosomal enzymes. A hydrolase group with catalytic properties is formed by three differently expressed hydrolases: the glycosidase β-Galactosidase (β-GAL), protective protein/cathepsin A (PPCA), the serine carboxypeptidase, the sialidase, and Neuraminidase-1 (NEU1). Each of them causes three separate lysosomal storage disorders (LSDs): sialidosis or NEU1 deficiency, PPCA deficiency with a supplementary mixed β-GAL and NEU1 deficiency or galactosialidosis (GS), and GM1-gangliosidosis (GM1) or β-GAL deficiency[7]. The aforementioned are displayed as a disorder with an impact in multiple systems, in which internal organs and the nervous system are involved[7]. By sharing many clinical features LSDs -also including Gaucher disease, Niemann-Pick disease type A, Wolman disease, mucolipidosis II and sialluria- make the diagnosis for individuals challenging. To date, the number of mutations recorded exceeds 40 disease-causing genetic variations in the *NEU1* gene[7]. Once a *NEU1* gene mutation is found, the diagnosis is decisive, while the sequel of the separate variants and the intensity of signs and symptoms of sialidosis are closely connected[8]. As sialidosis type II is subdivided into three forms, the identification of each of them is founded on the time at the outbreak of the signs. Type II patients who tend to survive longer, create along time a progressive mucopolysaccharidosis-like phenotype; most frequent signs are coarse faces, organomegaly, multiple dysostosis, abnormalities in the spinal canal, mental deterioration, and myoclonus and cherry-red spot syndrome[9]. The subset with hydrops fetalis is linked to significant mutational modifications, which may result in the absolute lack of lysosomal sialidase and lethal development in utero or perinatal death. The latter, which is presented in utero and is characterized by hepatomegaly, hydrops fetalis, and ascites can be fatal at birth or ultimately after birth. The most common features are facial swelling, hernias, epiphyses, and periosteal deformities. Sialidosis II is mainly characterized by hepatosplenomegaly[10]. The peripheral blood's number of hematopoietic progenitors is increased along with the expansion in spleen cells and erythroblasts in liver tissue. A simultaneous decrease in these cells in Bone Marrow (BM) is noted. These findings point to hematopoiesis beyond bone sites being the reason for hepatosplenic hypertrophy in deficiencies in the *NEU1* gene[10,11].

There is a limited number of studies focusing on pathologic tissue findings distinct for congenital sialidosis. In a pregnancy that was ended at 20 weeks postmenstrual age, the examination of the fetus's affected tissues by light microscopy revealed cells with vacuolation in bone marrow, liver, brain, and kidney[10,12]. Vacuolated cells of the epithelium and the endothelium tissue of the choroid plexus and ependymal layer respectively were detected mostly in microglia leading to a prevalent inflammation of microglia progressively in fetus's CNS and especially in neonatal brain[13]. Furthermore, some findings suggest a potential deterioration resulting from the deposition of lysosomal substances in the tissues in the absolute lack of *NEU1* gene is accountable for the failure of the targeted organs involved[13-15]. In the presented case, the early development of symptoms combined with their severity, as well as the findings in screening tests, raised the index of suspicion for sialidosis type II. Hydrops fetalis, ascites, hepatosplenomegaly were predominant. Apart from distinct clinical signs, cytoplasmic vacuoles were detected in lymphocytes of the peripheral blood smear. Brain MRI showed corpus callosum hypoplasia, underdeveloped brain parenchyma, leucodystrophy, hydrocephalus, and reduced gyration. All consistent with abnormal CNS findings in these patients.

In our case, the patient had a novel deletion mutation in the *NEU1* gene, a homozygous deletion encompassing the entire *NEU1* gene. The NEU1 gene was analyzed by polymerase chain reaction (PCR) and sequencing of both DNA strands of the entire coding region, and the conserved exon-intron splice junctions' method was performed. The reference sequence is NEU1: NM_000434.3. We performed quantitative PCR assay (qPCR) by using six gene-specific amplicons encompassing the coding

exons 1 to 6 (or part of it) of the NEU1: NM_000434.3 gene. It is the first time, this variant was detected, and it is not described in Cento MDR 3.3. It is classified in Centogene medical database as pathogenic (class 1) according to the recommendations of the American College of Medical Genetics and Genomics (ACMG)[16]. The majority of mutations in congenital sialidosis are missense mutations that concern sialidase's impairment in the endoplasmic reticulum or Golgi particle[15]. Bonten et al proved that the residual potency of the mutant enzymes is seamlessly connected to both the clinical picture and the severity of the disease. Type II disease involves the inactive catalytic form of neuraminidase, whereas in mild type I disease, active catalytic neuraminidase is observed. Deletions in the *NEU1* gene leading to the complete absence of enzyme activity are directly related to delay in fetal development and/or death early after birth, as in the case of our patient[8]. As heterozygous carriers, parents are perfectly healthy, which is likely to mean that this deletion affects individuals only in a homozygous state. Our patient's severe phenotype was equally correlated to its genotype.

Nevertheless, evaluating genotype/phenotype correlation involves a more complicated process than a simple interpretation. Retrospective studies show high phenotype heterogeneity for the same mutation, even in patients of the same family[17]. Uhl et al identified an 11 kb deletion, including the whole coding and promoter genetic areas of the *NEU1* gene in two Turkish patients with sialidosis. Both patients deceased soon after birth. This depletion resulted in the fusion of exon 10 of the *CTL4* gene (OMIM: 606107), which is located 851 bp centromeric from *NEU1*, with the 3-prime UTR of *NEU1*. The expected *CTL4/NEU1* combining transcript was observed in one patient, but the other patient expressed a differently spliced CTL4 transcript that preserved intron 9 and stopped transcription before the combining site[17].

In conclusion, sialidosis type II should be considered as a potential in differential diagnostic procedure in a patient presenting with hydrops fetalis of no immune causality or isolated fetal ascites[18-20]. In sialidosis type II, when patients are presented with systemic disease, early-onset signs, and a lethal course, treatment development seems to be more demanding, suggesting an in utero therapeutic intervention if applicable. Alternatively, the genetic studying of the patient and the family by carrier detection is crucial to prevent missed diagnoses, while genetic counseling for following pregnancies is imperative.

Conflict of interest

None declared by authors.

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