Case Report

Gonca Kılıç Yıldırım*, Coşkun Yarar, Berna Şeker Yılmaz and Serdar Ceylaner

Niemann–Pick type C disease with a novel intronic mutation: three Turkish cases from the same family

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Abstract

Objectives: Niemann–Pick type C (NPC) disease is a rare progressive neurodegenerative condition that is characterized by the accumulation of cholesterol, glycosphingolipids, and sphingosine in lysosomes. Patients have various systemic and neurological findings depending on their age at onset. This disease is caused by the autosomal recessive transmission of mutations in the *NPC1* and *NPC2* genes; patients have mutations mainly in the *NPC1* gene (95%) and the majority of them are point mutations located in the exonic regions.

Case presentation: Here, we presented three cousins with hepatosplenomegaly and progressive neurodegeneration who were diagnosed with visceral-neurodegenerative NPC disease. Their parents were relatives, and they had a history of sibling death with similar complaints. Bone marrow smear showed foamy cells in patient 1. Vertical supranuclear gaze palsy was not present in all cases. Sphingomyelinase (SM) activities were almost normal to exclude NPA or NPB. Filipin staining was performed in patient 2 and showed a massive accumulation of unesterified cholesterol The *NPC1* gene analysis of the three patients showed a novel homozygous c.1553+5G>A intronic mutation. cDNA analysis was performed from the patient 3 and both parents. It was observed that exon 9 was completely skipped in the homozygous mutant baby. Both the normal and the exon 9-skipped transcripts have been detected in the parents.

Conclusions: When combined with the filipin staining and the patients' clinical outcomes, this mutation is likely to be deleterious. Moreover, cDNA sequencing supports the pathogenicity of this novel variant.

Keywords: filipin test; neurodegeneration; Niemann-Pick Type C (NPC); novel mutation; *NPC1*gene.

Introduction

Niemann–Pick type C (NPC) (OMIM#257220; OMIM #607625) disease is an autosomal recessive neurovisceral disorder due to defect in cellular cholesterol trafficking. The incidence of NPC disease is estimated at 1:100,000–120,000 live births. Mutations of *NPC1* (95%) and *NPC2* (4–5%) genes lead to impaired intracellular transport of unesterified cholesterol and glycosphingolipids in the liver, spleen, and brain [1]. Patients have various systemic and neurological findings depending on their age at onset [2]. It is often characterized by hepatosplenomegaly and progressive neurological dysfunction. NPC disease is classified on the age at onset of neurological symptoms and there is a correlation between the age of onset of the neurological symptoms and the general development of the disease [3]. Patients have mutations mainly in the *NPC1* gene and the majority of them are point mutations located in the exonic regions. Up to date, 552 *NPC1* and 29 *NPC2* variants have been reported [2].

Here, we present three cousins with a novel homozygous intronic site mutation in the *NPC1* genes that were diagnosed as visceral-neurodegenerative NPC disease.

***Corresponding author: Gonca Kılıç Yıldırım**, Assistant Professor, MD, Division of Child Nutrition and Metabolism, Department of Paediatrics, Faculty of Medicine, Eskisehir Osmangazi University, Meselik campuse, Odunpazari, Eskisehir, Turkey, Phone: +90 222 2392979 2763, +90 532 7485817, Fax: +90 222 22900644, E-mail: goncaklch@yahoo.com. https://orcid.org/0000-0001-6769-667X

Coşkun Yarar, Division of Pediatric Neurology, Department of Paediatrics, Faculty of Medicine, Eskisehir Osmangazi University, Eskisehir, Turkey. https://orcid.org/0000-0001-7462-4578

Berna Şeker Yılmaz, UCL Institute of Child Health, Genetics and Genomics Medicine, London, UK. https://orcid.org/0000-0003-0425-0341

Serdar Ceylaner, Intergen Genetics Centre, Ankara, Turkey. https://orcid.org/0000-0003-2786-1911

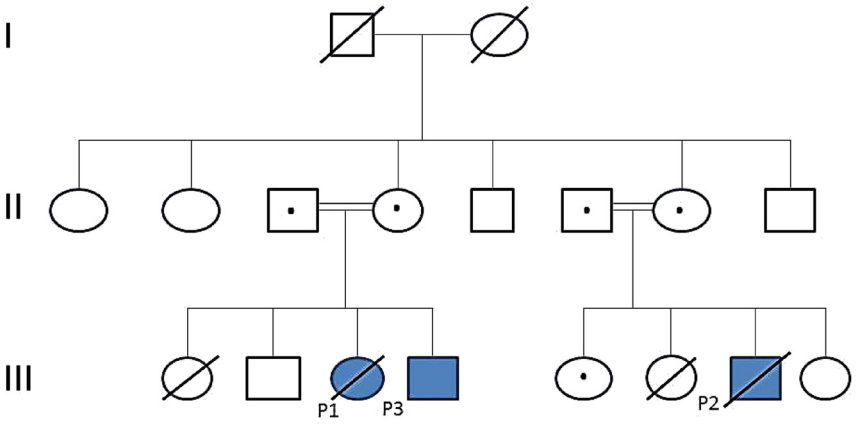
Case descriptions

Patient 1

Patient 1 was a female born at term. She has been displaying delayed neuromotor development since she was six months of age. Her parents were consanguineous (Figure 1) and their first child died at the age of 3.5 years with a suspicion of NP disease due to hepatosplenomegaly and foamy cells in the bone marrow smear. She was initially admitted due to a lung infection at 18 months of age, and massive splenomegaly was detected. The neurological examination revealed poor head control, generalized hypotonia, and increased deep tendon reflexes (DTRs). She couldn't follow objects or speak meaningful words. Vertical supranuclear gaze paralysis (VSGP) and cherry-red spot weren't present. The Denver developmental screening test-II showed retardation in all areas. Electroencephalography and visual evoked potential studies were normal, however cranial MRI showed bilateral periventricular white matter lesions and brain atrophy (Figure 2A, B). Bone marrow smear showed foamy cells. Although sphingomyelinase (SM) activity was slightly below normal (2.05 nmol/h/mg protein-NR: 7.73 ± 3.08), there wasn't any molecular defects detected in the *SMPD1* gene. Therefore, the suspected diagnosis was NPC and genetic analysis showed a homozygous mutation c.1553+5G>A on the *NPC1* gene, at the donor splice site in intron 9. The promoter region, all exons and exon-intron junction regions of *NPC1* gene were studied with next-generation sequencing (NGS). Her parents were found to be carriers of the same mutation. Butyldeoxynojirimycin (Miglustat [ZavescaTM; Actelion Pharmaceuticals Ltd.]) treatment was started, nevertheless gastrointestinal complaints, mainly diarrhea, occurred. Neuromotor impairment increased gradually and the patient died at three years of age because of a severe respiratory infection.

Patient 2

The patient was referred for splenomegaly at two months of age. He was the third child of consanguineous parents and the cousin of the patient 1 (Figure 1). The family's second child had died at three years old due to unknown causes. He had splenomegaly (2 cm below the costal margin) at admission. He also had cholestatic jaundice with elevated conjugated bilirubin (total bilirubin 8.8 mg/dL, direct bilirubin 5.33 mg/dL) and transaminases levels (ALT 51 U/L, AST 268 U/L, ALP 1,653 U/L, GGT 367 U/L). The neurological examination was normal as well as the cranial MRI. VSGP and cherry-red spot weren't reported. There weren't any foamy cells in the bone marrow smear. SM activity was in normal ranges (8.93 nmol/17 h/mg protein-NR: 7.73 ± 3.08). The filipin staining on cultured fibroblasts from a skin biopsy was definitely pathological (Laboratoire de Biologie Medicale Multi Sites du Chu de Lyon) and showed a massive accumulation of unesterified cholesterol (Figure 3). Genetic analysis showed the same homozygous mutation c.1553+5G>A on the *NPC1* gene as patient 1. His parents were carriers of this mutation. With supportive therapies, his cholestatic jaundice was resolved within three months. Miglustat was initiated at six months of age. He started crawling at one year and walking at the age of 18 months. However, control cranial MRI showed bilateral periventricular white matter lesions (Figure 2C, D). At the



P1: Patient 1, P2: Patient 2, P3: Patient 3

Figure 1: The family tree of the consanguineous family.

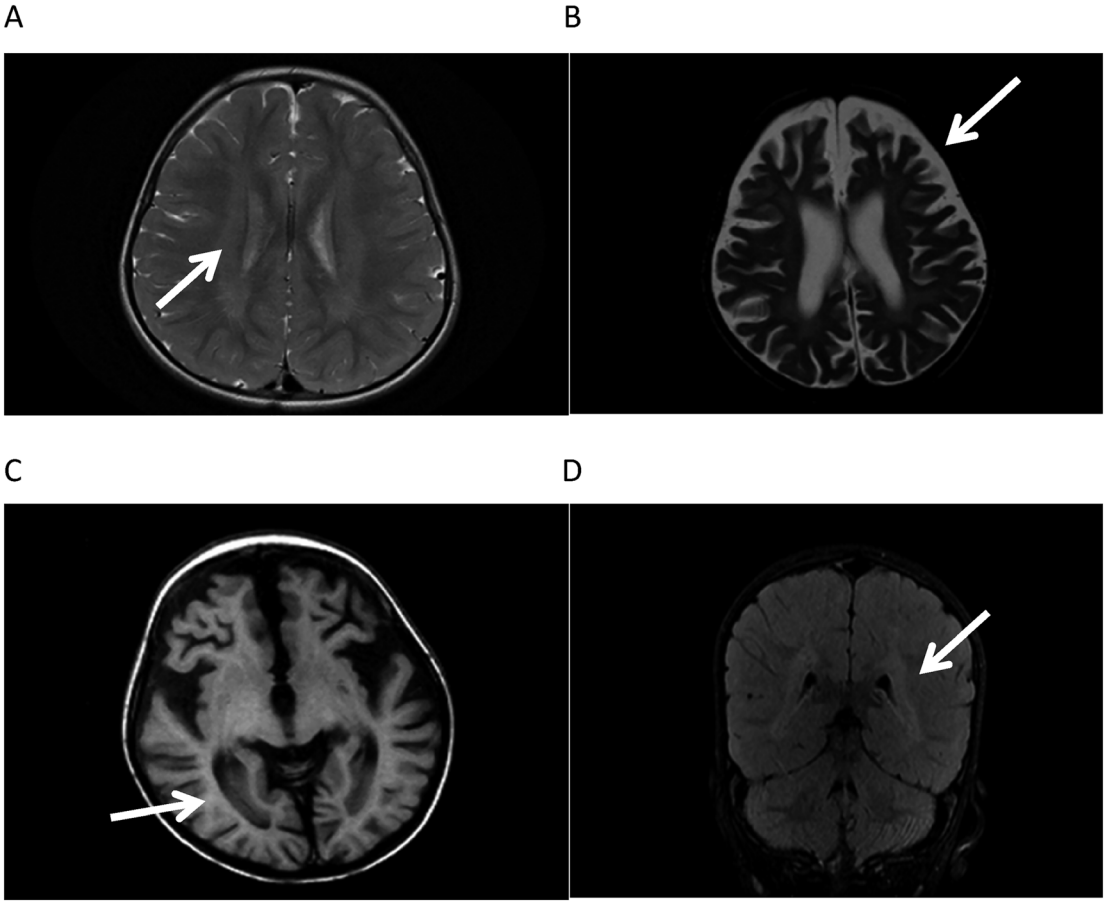


Figure 2: Brain MRI images of patients 1 and 2. (A–B) (patient 1): (A) hyperintensity in periventricular white matter (axial, T2), (B) diffuse brain atrophy, more prominent in the frontotemporal (axial, T1). (C–D) (patient 2): (C) hyperintensity in periventricular white matter (axial, T2), (D) hyperintensity in periventricular white matter (coronal flair).

age of 18 months, he was able to pronounce 14–15 meaningful words. Meanwhile, he had hepatomegaly and splenomegaly, 8 and 10 cm below the costal margins, respectively. His gross motor abilities were delayed and DTRs were hyperactive. Significant rough tremor started to affect both hands and head. Gastrointestinal intolerance was the main side effect of the miglustat treatment, as expected. He underwent several hospitalizations due to respiratory infections. Neurological deterioration continued over time with a worse outcome. Therefore, percutaneous endoscopic gastrostomy was performed due to the swallowing dysfunction. He died at 3.5 years of age due to the respiratory failure. The family later had a healthy baby with preimplantation genetic screening.

Patient 3

Patient 3 was a male admitted to our hospital when he was two months of age. He was the brother of the patient 1 (Figure 1). Despite the intrauterine diagnosis, his family's religious convictions prevented the pregnancy from being terminated. He was born at term with a weight of 2,700 g. He was icteric on physical examination. He had hepatomegaly (5 cm below the costal margin), splenomegaly (7 cm below the costal margin), and umbilical hernia. His neurological examination was normal. He had cholestatic jaundice with elevated conjugated bilirubin (total bilirubin 6.55 mg/dL, direct bilirubin 3.56 mg/dL) and transaminases levels (ALT 110 U/L, AST 334 U/L, ALP 990 U/L, GGT 541 U/L). The Lyso-SM level was found to be 7.30 nmol/L (NR<3.40), the Lyso-SM 509 level was 1,618 nmol/L (NR: 1.00–33.00) and the Lyso-SM 509/Lyso-SM ratio was increased to 221.64 (NR: 1.70–28.00) (Pediatric Metabolism Laboratory at Gazi University Faculty of Medicine, Turkey). cDNA sequence analysis of NPC1 gene with NGS showed the skipping of exon 9 due to the presence of the homozygous mutation c.1553+5G>A. Figure 4 refers to the genomic DNA NGS. Both the normal and the exon 9-skipped transcripts have been detected in the

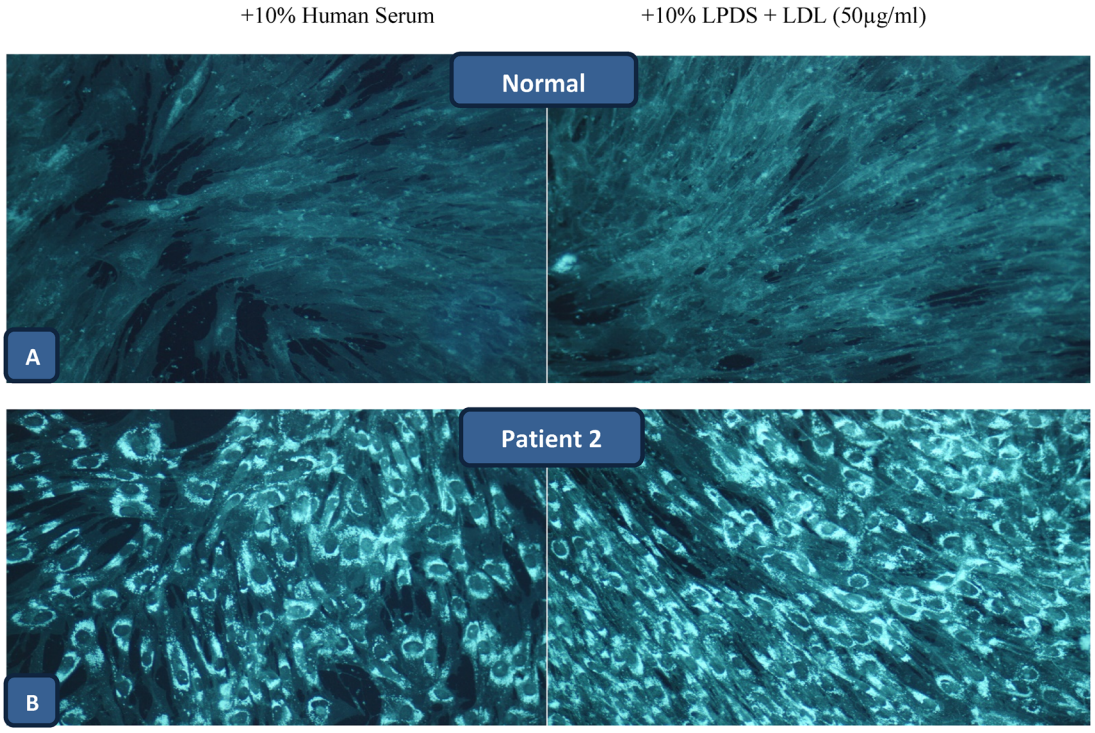


Figure 3: Filipin staining in fibroblasts from Patient 2 and normal cell appearance. (A) Normal cells, (B) visualization of unesterified cholesterol by filipin staining in fibroblasts from patient 2 with *NPC1* mutation. LPDS, lipoprotein-deficient serum; LDL, low-density lipoproteins.

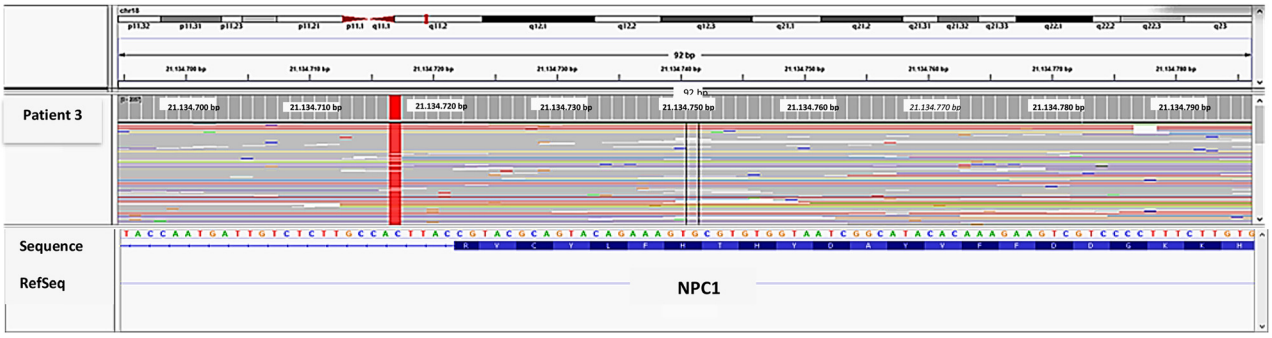


Figure 4: The mutation analysis of patient 3 with next-generation sequencing (NGS).

parents (Figure 5). There is poor coverage for exon 8 and 10. Miglustat and supportive treatments were initiated.

Discussion

The course of the NPC disease varies widely, even among siblings with same genotypes and similar biochemical phenotypes. In contrast, neurological involvement, characterized by the age of onset of neurological symptoms, and determining the prognosis of the disease, is usually constant within a family, with genotype–phenotype correlations [4]. NPC is categorized by the age of onset of neurological manifestations as follows: a) early-infantile (<2 years), b) late-infantile, and c) juvenile (6–15 years), and d) adult (>15 years) [2, 3]. However, visceral manifestation can be observed in all forms of the disease, although not constant, and are not predictive of the prognosis. The cases reported here displayed an early-infantile form.

We suspected NP disease in our patients due to their family histories and clinical findings. In patient 1, bone marrow smears showed foamy cells, but the SM activity was not low enough for the diagnosis of NPA and NPB. Furthermore, foamy cells were absent and SM activity was normal in

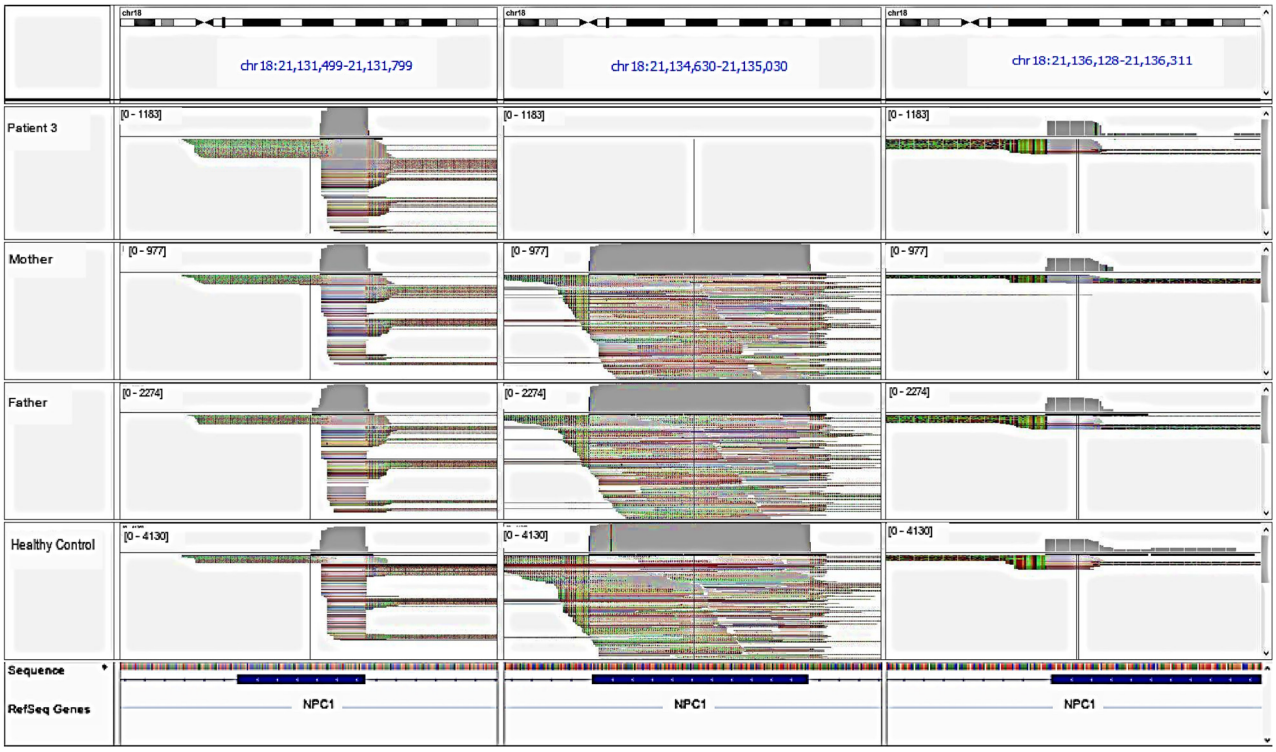


Figure 5: cDNA analysis of patient 3 and his family [exon 9 was completely skipped in the homozygous mutant new baby. Both the normal transcript and the skipped exon 9 transcripts have shown in the parents (heterozygous). The control sample is completely normal].

patient 2. We were able to do an oxysterol assay only for the patient 3, and we discovered that they had all increased. Filipin staining was not successful in patient 1 due to the contamination of the skin fibroblast culture. The filipin test was definitely pathological in the patient 2. Molecular analysis of the patients revealed the homozygous *NPC1* (NM_000271.5): c.1553+5G>A mutation in intron 9. cDNA sequence analysis with NGS was performed from RNA samples of patient 3, his parents and a healthy control independent of the family to investigate the functional effect of c.1553+5G>A variant on splicing. As a result, it was observed that exon 9 was completely skipped in the homozygous mutant baby (Figure 3). Both the normal transcript and the skipped exon 9 transcripts have shown in the parents (heterozygous). The control sample is completely normal. Unfortunately, coverage analysis has not been performed. The *NPC1* (NM_000271.5): c.1553+5G>A splicing variant which causes a truncated protein p. Val443Glyfs*7 had not been reported in any database to date. It is predicted that the nucleotide change will lead to the production of a truncated protein p.(Val443Glyfs*7). The functional analysis performed in patient 3 and his parents, the filipin staining test performed in patient 2 and the patients' clinical outcomes led us to demonstrate the pathogenic effect of the c.1553+5G>A mutation. The Human Gene Mutation Database (www.hgmd.cf.ac.uk), listed 552 *NPC1* and 29 *NPC2* variants. For *NPC1*, 357 were missense mutations and 48 were splicing mutations. Few intronic disease-causing mutations have been reported in NPC1 patients and in most of them, the effect on RNA splicing was predicted from genomic DNA without functional analysis [5–9].

Abnormal saccadic eye movements are often an early neurological sign, but usually not the earliest, and can be initially absent, as in the cases reported and the initial defect occurs in the vertical plane [10]. Subsequently, both horizontal and vertical movements become irregular, and complete SGP develops. Although, the most frequent neurological finding is VSGP, it is difficult to recognize in early infancy, and commonly detected from the late-infantile ages. The vertical gaze paralysis has been reported only in the 63% of NPC early-infantile patients [3, 10].

Oxysterols (mainly cholestane-3ß,5α,6ß-triol), Lyso-SM 509/N-palmitoyl-O-phosphocholine serine (PPCS) and some bile acids are sensitive and specific biomarkers of NPC; measurement of plasmatic biomarkers is now indeed the first-line screening test for NPC [11, 12]. Lyso SM and Lyso SM 509 were performed in patient 3 and both biomarkers were increased compared to normal ranges. Foamy cell identification in tissues is helpful but not necessary for a conclusive diagnosis. A filipin test for the

accumulation of free-cholesterol on cultured fibroblasts is currently a sensitive and specific assay, but it isn't considered as a first-line test for diagnosis, which must be confirmed by mutation analysis in all cases.

Treatments include supportive therapies and substrate inhibition therapy with miglustat, which is the first and only approved targeted therapy for NPC [3]. A number of experimental therapy trials are currently at different stages of clinical development. These include arimoclomol as a molecular chaperone, hydroxypropyl-beta-cyclodextrin which decreases storage and a natural aminoacid acetyl-L-leucine [3, 13]. Gene therapy is an emerging strategy for the disease and several preclinical studies are ongoing [3]. All pre-symptomatic individuals should be regularly assessed by a neurologist and/or metabolic specialist so that treatment can be considered at the early stages of neurological presentation. Héron B et al. [14] assessed the response of 20 French infants to treatment with miglustat. The NPC impairment scores were increased or remained stable in 75% of patients with late-infantile onset, but no patients with early-infantile onset type had a successful neurological improvement. About 1/9 infants who were treated before four years of age showed a stabilization. We started miglustat treatment on patient 1 because of her severe neuromotor retardation. Treatment should begin at or before the onset of neurological symptoms in patients who don't have neurological findings but have a confirmed family history and an expected disease course. For patient 2, due to the accelerated decline of his deceased cousin, miglustat was begun at six months of age, which was prior to the onset of neurological findings. But neurological impairment worsened during the treatment.

Conclusions

Although there is currently no cure for this disease, identifying the index case is essential to provide a chance to have healthy children by genetic counseling to the affected families. The filipin staining has not been considered anymore the first-line test for diagnosis of NPC because is invasive and time-consuming. However, it still represents a valuable method to confirm the diagnosis in the presence of a variant of unknown significance. We aim to add the novel homozygous c.1553+5G>A mutation to the literature.

What is new?

- A novel mutation of the *NPC1* gene in intron 9 was identified.

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