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RESEARCH NOTE

Novel compound heterozygous mutation in *NPC1* gene cause Niemann–Pick disease type C with juvenile onset

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Abstract. Niemann–Pick disease type C (NPC) is a progressive lysosomal storage disorder caused by mutations in the *NPC1* (in 95% of cases) or *NPC2* (in ∼5% of cases) genes, inherited in an autosomal recessive manner. We report the case of a 38-year-old woman with learning disorder from her first year of schooling, and could notice slow progressed cognitive impairment, social withdrawal, apathy, handwriting alterations, deterioration of language skills and dysphagia. Brain magnetic resonance imaging showed severe cerebellar atrophy, hypoplasia of the corpus callosum, asymmetric lateral ventricular enlargement, and severe enlargement of frontal and parietal subarachnoid spaces. Next generation sequencing for NPC genes (*NPC1* and *NPC2*) detected compound heterozygous mutations in *NPC1* gene, including c.1553G > A (p.Arg518Gln), paternally inherited, and c.1270C > T (p.Pro424Ser) maternally inherited. The first mutation has been already described in literature and correlated to NPC, while the second mutation is still unknown. Moreover, filipin test and quantification of plasma oxysterols confirmed NPC diagnosis. We can suggest the missense mutation c.1270C > T (p.Pro424Ser) as a new causative mutation of NPC.

Keywords. cognitive impairment; Miglustat; missense mutation; Niemann–Pick disease type C; *NPC1* gene.

Introduction

Niemann–Pick disease type C (NPC) is a progressive lysosomal storage disorder. The clinical presentation is a miscellaneous of neurological and systemic symptoms hesitating in premature death (Imrie *et al*. 2015; Winstone *et al.* 2017). Although NPC has been historically considered as a childhood-onset disease, to date, a greater proportion of adult-onset cases are being detected (Imrie *et al*. 2015). Approximately, the incidence of NPC ranges from 1/100000 to 1/120000 live births (Koens *et al*. 2016; Nadjar *et al*. 2018). NPC is notoriously caused by mutations in the Niemann–Pick C1 protein (*NPC1*) (in 95% of cases) or Niemann–Pick C2 protein (*NPC2*) (in ∼5% of cases) genes, inherited in an autosomal recessive manner (Koens *et al*. 2016; Nadjar *et al.* 2018; Sun 2018; Sobrido *et al*. 2019). *NPC1* and *NPC2* encode intracellular transporter proteins involved in cholesterol and lipid intracellular trafficking (Benussi *et al*. 2018; Polese-Bonatto *et al*. 2019). Loss of function in both proteins produces accumulation of cholesterol and sphingolipids in the late endosomal/lysosomal intracellular compartment with lipid storage, most prominently in the liver, spleen, and central nervous system (Koens *et al*. 2016; Evans and Hendriksz 2017; Benussi *et al.* 2018). For both adolescent and adult-onset disease, the cognitive and motor development are normal during early stages of life; however, some patients may present clinical signs before the onset of the disease, such as haepatomegaly or splenomegaly with spontaneous remission in childhood; learning disorder; and hearing defect since childhood (Sun 2018; Geberhiwot *et al*. 2018). Successively, cognitive impairment and neuropsychiatric symptoms predominate (Manganelli *et al.* 2014; Benussi *et al*. 2018). Typical neurological symptoms include progressive ataxia/dystonia,

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vertical gaze palsy, dysarthria, dysphagia, gelastic cataplexy; treatment resistant cognitive decline and atypical psychiatric symptoms (psychosis, depression, behavioural problems, language impairments, deficits in social cognition, cognitive impairment) can also occur (Koens *et al*. 2016; Nadjar *et al.* 2018; Benussi *et al*. 2018; Pineda *et al.* 2018; Rego *et al.* 2019). Although the filipin staining of fibroblast cells is considered, the gold standard tests for the diagnosis of NPC, a valuable biomarker to screen patients with possible NPC is quantification of plasma oxysterols (Benussi *et al*. 2018; Sobrido *et al*. 2019).

To date, genetic testing for NPC includes full gene sequencing of the *NPC1* and *NPC2* (Nadjar *et al*. 2018; Geberhiwot *et al.* 2018; Sobrido *et al*. 2019). Moreover, by identifying preclinical cases is important for early intervention and therapy (Evans *et al*. 2019). In fact, miglustat is approved for specific treatment of NPC. Management of NPC is otherwise supportive and aimed to treat symptoms and improve quality of life (Winstone *et al*. 2017; Nadjar *et al.* 2018; Pineda *et al.* 2018; Pineda *et al.* 2019). Here, we report a novel compound heterozygous mutation of the *NPC1* gene in a patient with juvenile-onset NPC. In particular, we detected a missense mutation c.1270C > T (p.Pro424Ser) in NPC1 linking for the first time with NPC. Since NPC is an inherited autosomal recessive disorder, the detected *NPC1* missense mutation in compound heterozygousity with the already known pathogenic missense mutation c.1553G > A (p.Arg518Gln) account for the diagnosis of NPC; the pathogenic role of the latter has been described in literature (Zhang *et al*. 2014). In addition, the strong correlation between clinical and instrumental findings confirmed the pathogenic role of missense mutation c.1270C > T (p.Pro424Ser).

Case report

We report the case of a 38-year-old woman not born from consanguineous parents. The patient presented with a normal perinatal history and psychomotor development. However, learning disorders were reported from the first year of school. Throughout her childhood and adolescence, she revealed slow progressive cognitive impairment, social withdrawal, apathy and handwriting alterations. At 28 years of age, patient reported a worsening of symptoms and a gradual onset of deterioration of language skills and dysphagia, thus, she came to our observation at the age of 38 years. At clinical examination, the neuropsychiatric assessment revealed dysmetria, ataxia, motor slowing, hyporeflexia in the four limbs, fatuous smile, mild-drooling, oral-motor movements and facial mimic impairment, slow speech, and problems recalling words. Blood test for hepatic and renal functionality, metabolic blood and urine screening resulted to be within the normal range. Abdomen ultrasonography was normal. Electromyography of upper and lower limbs showed neurogenic involvement and reinnervation signs, while nerve conduction velocity was within the normal range. Hearing test showed bilateral mild hearing loss; brainstem auditory evoked potentials were abnormal with absence of complex I, III, and V. Electroencephalogram showed a diffuse slowing of background activity. Brain magnetic resonance imaging (MRI) showed severe cerebellar atrophy, hypoplasia of the corpus callosum, asymmetric lateral ventricular enlargement, and severe enlargement of frontal and parietal subarachnoid spaces (figure 1). Based on the instrumental, clinical and neurophysiological findings (tables 1 & 2), next generation sequencing (NGS) for NPC genes (*NPC1* and *NPC2*) was performed. Informed consent was obtained from patient and both parents prior to the

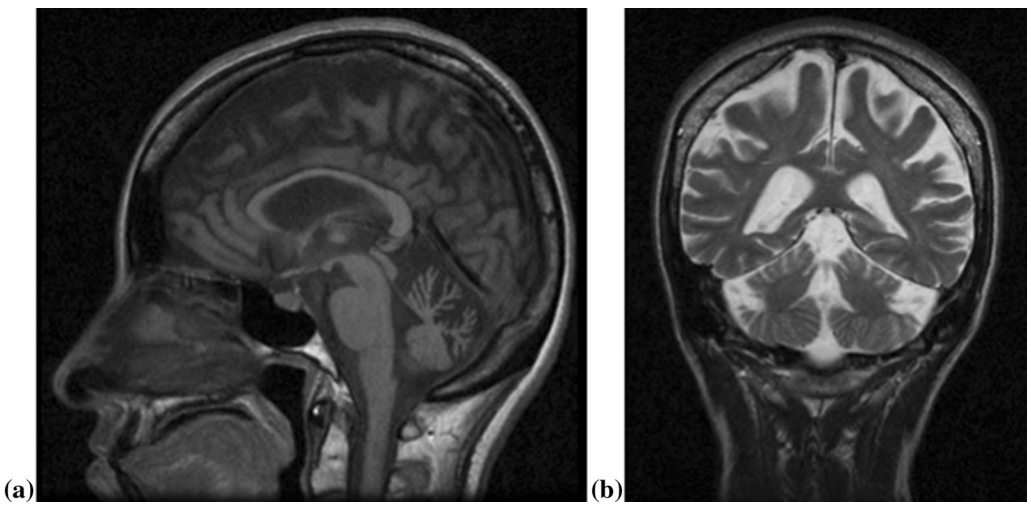

Figure 1. Sagittal (a) and coronal (b) brain MRI views. Severe cerebellar atrophy, hypoplasia of the corpus callosum, asymmetric lateral ventricular enlargement, and severe enlargement of frontal and parietal subarachnoid spaces were detected.

beginning of the study. Genomic DNA was isolated from peripheral blood leucocytes by using standard procedures. Custom Ion Torrent panel (*NPC1* and *NPC2* genes) was designed (design cover 100%) with Ampliseq Designer software using GRCh37 (hg19) as references. Emulsion PCR was performed using Ion Chef according to manufacturer's instructions. Quality control of all libraries was performed on Qubit 2.0 Fluorometer. Ampliseq Design Samples were subjected to the sequencing protocol using Ion 520 chips and Ion S5 systems. Sequencing reads were aligned using the Torrent mapping alignment program (TMAP). Data of runs were processed using the Ion Torrent Suite 5.0, VariantCaller 5.0, Coverage Analysis 5.0 and the Ion Reporter (Thermo Fisher Scientific) and/or wANNOVAR tools. Sanger confirmation were performed using ABI3130 DNA (Life Technologies). NGS analysis detected compound heterozygous mutations in *NPC1* gene, including c.1553G > A (p.Arg518Gln), paternally inherited, and c.1270C > T (p.Pro424Ser) maternally inherited. Both mutations were located in lumenal domains of the NPC1 protein. The first mutation has been already described in literature and correlated to NPC, while the second mutation is still unknown. Moreover, this variant was not detected in a population screening of 50 healthy controls (100 alleles) of the same geographic area, ruling out the possibility of a frequent population polymorphism, although it was identified (rs143797098) with a frequency of 0.0002 (Yoruba in Ibadan, Nigeria) within the healthy population (1000 Genome Project: http://www.1000genomes.org/). In addition, the most predictive test considered the mutation as deleterious (table 3). Quantification of plasma oxysterols and filipin test in fibroblasts confirmed NPC diagnosis. In fact, quantification of plasma oxyterols using liquid chromatography–tandem mass spectrometry (LC–MS/MS)-based bioanalytical methods showed increased level of cholestane-3β,5α,6β-triol (revealed value 80.5 ng/mL; normal value < 34.8); moreover, filipin test in fibroblasts detected the presence of fluorescent perinuclear vesicles (Jiang *et al.* 2011; Takamura *et al.* 2013). The presented case study has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Additional informed consent was obtained from the parents to include their clinical and laboratory data in this article.

Table 1. Clinical features of NPC compared to our patient.

Clinical feature	Clinical features of NPC patient	Clinical features of our patient
Abnormal gait / ataxia	+	+
Developmental delay	+	−
Developmental regression	+	+
Learning disorders	+	+
Language impairments	+	+
Seizures	+	−
Vertical supranuclear gaze palsy	+	−
Dysphagia	+	+
Cataplexy	+	−
Dysarthria	+	+
Hypotonia	+	−
Hypertonia	+	−
Psychiatric symptoms	+	+

NPC, Niemann–Pick disease type (Iodice *et al*. 2015; Koens *et al*. 2016; Sobrido *et al*. 2019; Polese-Bonatto *et al*. 2019; Rego *et al*. 2019).

Table 2. Results of instrumental investigations in NPC compared to our patient.

	Instrumental investigations in NPC	Instrumental investigations in our patient
Electromyography	Normal or motor and sensory polyneuropathy	Neurogenic suffering and reinnervation signs NCV: normal values
Visual evoked potentials	Normal	Not performed
Somatosensory evoked potentials	Normal	Not performed
Brainstem auditory evoked potentials	Abnormal in all patients with both peripheral and central impairment of auditory pathway	Abnormal (absence of I, III and V complex)
Electroencephalogram	Slowing of alpha activity with an increase in slow frequency activity	Diffuse slowing of background activity
Abdomen ultrasonography	Hepatomegaly and/or splenomegaly	Normal
Brain MRI	Atrophy or white matter T2 signal increase on MRI	Severe cerebellar atrophy, hypoplasia of the corpus callosum, asymmetrical lateral ventricular enlargement, and severe enlargement of frontal and parietal subarachnoid spaces

NPC, Niemann–Pick disease type C; NCV, nerve conduction velocity (Bagel *et al*. 2013; Esposito *et al*. 2019).

Table 3. *In silico* analysis of the NPC1 missense mutation (c.1270C>T (p.Pro424Ser)).

Predictive test	Prediction/score/PHRED-scaled
CADD_phred	18.71 (>30 highly pathogenic; > 20 pathogenic)
DANN_score	0.991 (range from 0 to 1)*
FATHMM_pred	DAMAGING
GERP++_RS	5.53 (range from − 12.3 to 6.17)*
LRT_pred	Deleterious
M-CAP_pred	DAMAGING
MetaSVM_pred	TOLERATED
MetaLR_pred	DELETERIOUS
MutationAssessor_pred	Low (nonfunctional)
MutationTaster_pred	DAMAGING
PROVEAN_pred	NEUTRAL
Polyphen2_HDIV_pred	BENIGN
SIFT_pred	TOLERATED
SiPhy_29way_logOdds	19.819 (range from 0 to 37.9718)*
fathmm-MKL_coding_pred	DAMAGING

*A larger number indicates a higher probability to be damaging.

Discussion

NPC is a progressive lysosomal storage disorder with variable manifestations and degenerative course; this autosomal-recessive hereditary disorder (Vanier 2010) is characterized by the accumulation of sphingomyelin in reticuloendothelial and parenchymal tissues; therefore, identifying preclinical cases is important for prevention and therapy (Kawazoe *et al*. 2018). Miglustat is an inhibitor of glycosphingolipids biosynthesis, it reduces lipid storage and cellular pathology in the brain, and therapeutic effects already quoted. It is considered the only specific therapy of NPC because it is able to stabilize or partially improve visceral, neurological and psychiatric symptoms (Evans and Hendriksz 2017; Nadjar *et al*. 2018; Pineda *et al*. 2019). With exception for hepatomegaly/splenomegaly (that sometimes is reported in spontaneous remission in childhood) and vertical supranuclear gaze palsy (reported in 71% of cases) our patient showed several typical clinical features of NPC disease, such as cognitive impairment, neurological/psychiatric symptoms, brain MRI abnormalities, and auditory deficit (Winstone *et al* 2017). Moreover, neurophysiological investigations suggested the suspected NPC. Finally, the detected compound heterozygous mutations and biochemical findings confirmed the diagnosis. In fact, the strong correlation between clinical, biochemical and instrumental findings, in addition to the new detected missense mutation, confirmed the initial diagnostic hypothesis and the pathogenic role of c.1270C > T mutation. Therefore, we can suggest the missense mutation c.1270C > T (p.Pro424Ser) as a new causative mutation of NPC. According to the clinical characteristics and the age of onset neuropsychiatric symptoms, we classified our case as juvenile onset of NPC. Further investigations are needed to understand if the detected mutation can influence the tardive onset or the expression of some clinical aspects (slight symptoms) of our patient. Early genetic diagnosis would provide the rationale to start miglustat therapy, undoubtedly improving patient's quality of life. This is one of the few target-gene studies useful to increase the number of mutations and extend the clinical features associated to *NPC1* gene variants. Genetic counselling should be extended to family members to assess the carrier status and establish the genetic risk.

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