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CLN3-Associated NCL Case with a Preliminary Diagnosis of Niemann Pick Type C

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Established Facts

- The clinical findings of neuronal ceroid lipofuscinosis 3 (NCL3), like progressive vision loss between 5 and 10 years of age, loss of motor coordination around the age of 10–12 years, mental decline, seizures, behavioral abnormalities such as hallucinations and/or other neuropsychiatric symptoms, were compatible with the presented case.
- A novel biomarker, lyso-sphingomyelin-509 (LysoSM-509), has been established for the primary diagnosis of Niemann Pick Type C.

Novel Insights

- LysoSM-509 is a biomarker which is elevated especially in Niemann Pick Type C.
- We can consider that a high LysoSM-509 level might also be an indicator of NCL, especially NCL type 3.

Keywords
CLN3 · LysoSM-509 · Juvenile neuronal ceroid lipofuscinosis

Abstract
Introduction: Neuronal ceroid lipofuscinoses (NCLs) are a broad class of inherited lysosomal storage disorders. Known mutations in at least 13 different genes can result in NCL with variable ages of onset, symptoms, and pathologic findings. Generally, these patients experience cognitive and motor decline, seizures, visual impairment, and premature death. Pathologically, NCL patients display heterogeneous histologic abnormalities, but consistently exhibit neuronal loss, reactive gliosis, and lysosomal accumulation of autofluorescent storage material or lipopigment. Juvenile-onset NCL has been classically referred to as Batten disease. By far the most prevalent NCL is *CLN3*-associated disease. It is an autosomal recessive condition that is usually caused by mutations in the ceroid-lipofuscinosis, neuronal 3 (*CLN3*) gene. *CLN3* encodes battenin, a ubiquitously expressed trans-

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membrane protein of unknown function that is associated with cellular homeostasis and neuronal survival. The initial clinical symptom of *CLN3*-associated NCL is central vision loss, which is usually detected between 4 and 9 years of age. Seizures typically begin early in the second decade of life, and affected individuals rarely live beyond their mid-20ies. ***Case Presentation:*** Herein, we describe a 16-year-old patient with *CLN3*-related juvenile NCL with a preliminary diagnosis of Niemann Pick Type C disease. The proband showed characteristic clinical signs, including epilepsy, ataxia, psychomotor regression, dementia, and visual impairment with an unusual elevation of lyso-sphingomyelin-509 (LysoSM-509; 812 nmol/L, normal 1–33 nmol/L). A homozygous NM_001042432.2(CLN3):c.233dup (p.Thr80fs) variant was detected at exon 4 of *CLN3*. Diagnosis of NCL was difficult due to the pronounced elevation of LysoSM-509. ***Discussion:*** LysoSM-509 is a biomarker which is elevated especially in Niemann Pick Type C. We can consider that a high LysoSM-509 level might be also an indicator of NCL, especially NCL type 3.

Introduction

Neuronal ceroid lipofuscinoses (NCLs) are a broad class of inherited lysosomal storage disorders. Known mutations in at least 13 different genes can result in 14 NCLs with variable ages of onset, symptoms, and pathologic findings. Generally, these patients experience cognitive and motor decline, seizures, visual impairment, and premature death [Cotman and Lefrancois, 2021]. Pathologically, NCL patients display heterogeneous histologic abnormalities, but consistently exhibit neuronal loss, reactive gliosis, and lysosomal accumulation of autofluorescent storage material or lipopigment. Juvenile-onset NCL has been classically referred to as Batten disease [Gowda et al., 2020]. By far the most prevalent NCL is *CLN3*-associated disease. It is an autosomal recessive condition that is usually caused by mutations in the ceroid-lipofuscinosis, neuronal 3 (*CLN3*) gene. *CLN3* encodes battenin, a ubiquitously expressed transmembrane protein of unknown function that is associated with cellular homeostasis and neuronal survival [Abdennadher et al., 2021]. The initial clinical symptom of *CLN3*-associated NCL is central vision loss, which is usually detected between 4 and 9 years of age [Singh et al., 2021]. Seizures typically begin early in the second decade of life, and affected individuals rarely live beyond their mid-20ies [Kuper et al., 2021]. Herein, we describe a 16-year-old patient with *CLN3* disease.

Case Presentation

Here, we describe a 16-year-old girl with *CLN3*-associated NCL who has visual complaints and ataxic gait. Past and family histories were unremarkable except for parental consanguinity.

Her vision progressively worsened by 10 years of age and was finally diagnosed as retinitis pigmentosa. Progressive vision loss in the previously healthy patient was accompanied by personality changes, behavioral problems, and slow learning. Psychoneurological manifestations including a gradual loss of social adaptation with frequent and abrupt mood changes were observed by the parents. These symptoms were replaced within 4 years by even more obvious psychomotor degeneration, leading to convulsive seizures and a persistent decrease in cognitive activity, followed by the loss of acquired skills including walking and loss of speech skills. She remained well otherwise until age 14 years when she experienced 2 generalized seizures. Brain MRI at that time revealed significant, generalized cortical atrophy (cerebral and cerebellar cortical atrophy) (Fig. 1). Now, at the age of 16, she is blind, severely intellectually impaired, and suffers from epilepsy. She was started on levetiracetam which was successful in controlling the seizures. Biochemical investigations including serum very-long-chain fatty acids, phytanic and pristanic acids, vitamin B12 and E levels, urine oligosaccharide profile, plasma amino acid chromatography, and urine organic acid profile, tandem mass analysis were all unremarkable in our patient. Further evaluation for an inherited metabolic disease revealed increased lyso-sphingomyelin (LysoSM; 10.2 nmol/L, normal <3.40 nmol/L) and extremely high levels of LysoSM-509 (812 nmol/L, normal 1–33 nmol/L) values in lysosphingolipid analysis. LysoSM-509 elevation led us to evaluate this patient for Niemann Pick type C (NPC). Written informed consent was obtained from the parents of the patient. Sanger sequencing for *NPC* was performed due to LysoSM-509 elevation, and revealed normal results. Chromosomal microarray analyses using the Infinium CytoSNP-850K v1.2 BeadChip (Illumina, Inc., San Diego, CA, USA) revealed no chromosomal abnormalities. Whole-exome sequencing (WES) using IDT xGen Exome Research Panel v2 was performed. The Novaseq (Illumina) platform was used in accordance with the manufacturer's instructions for WES. FASTQ files were analyzed on QCIAU1.6 and the annotation of VCF files was completed with Qiagen Ingenuity Variant Analysis and Clinical Insight Interpretation.

A homozygous NM_001042432.2(CLN3):c.233dup (p.Thr80fs) variant was detected at exon 4 of *CLN3*. According to the American College of Medical Genetics and Genomics (ACMG) AMP variant interpretation guideline, the variant was interpreted as "pathogenic." Via segregation analysis, both parents were found to be heterozygous carriers.

Discussion

NCLs are a group of rare genetic metabolic neurodegenerative disorders historically referred to as "Batten disease" caused by a buildup of ceroid lipofuscin in the brain. NCLs affect 2–4 in 100,000 births. Cardinal pathologic features of NCL are toxic levels of protein aggregates

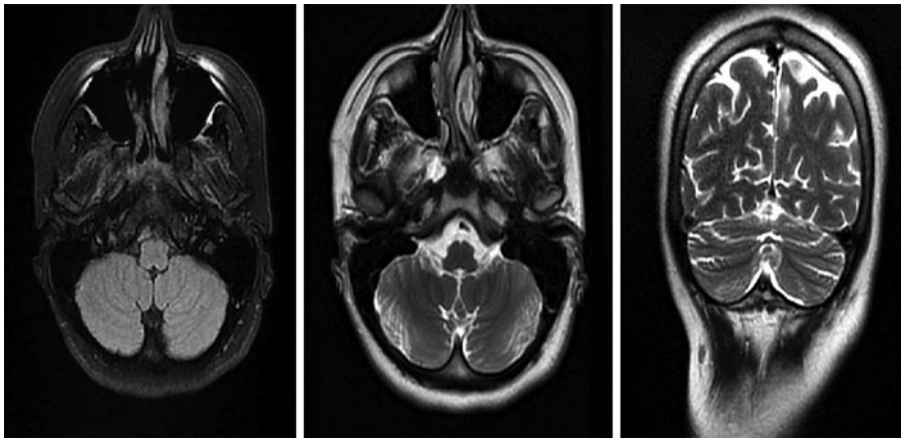

Fig. 1. Cerebral and cerebellar atrophy in T1A axial section and T2A axial and sagittal images of the 16-year-old patient.

in the central nervous system, especially aggregates of lipopigments (lipofuscin) within lysosomes. Aggregated lysosomal lipofuscin affects the neuronal cytoskeleton and cellular trafficking, resulting in neuronal loss [Specchio et al., 2021].

NCLs are characterized by seizures and progressive neurologic deterioration, which results in dementia, ataxia, visual failure, and various forms of abnormal movement. Though the underlying mechanism is not clear, pathogenic variants in 13 different genes (*PPT1*, *TPP1*, *DNAJC5*, *CLN3*, *CLN5*, *CLN6*, *MFSD8*, *CLN8*, *CTSD*, *GRN*, *ATP13A2*, *CTSF*, and *KCDT7*) cause 14 NCL types [Kose et al., 2021]. All NCL types are still fatal, and there is no curative treatment except in NCL2. Enzyme replacement therapies are tried for treatment of the NCL group as in all lysosomal storage disorders. In 2017, the FDA approved cerliponase alpha (Brineura® BioMarin Pharmaceutical) as the first treatment option to stop the progression of NCL2 [Iwan et al., 2021].

NCLs are classified as congenital, infantile (INCL), late infantile (LINCL), juvenile (JNCL), and adult (ANCL) NCLs according to the clinical presentation age of the disorder [Masten et al., 2021].

CLN3 is a multipass transmembrane protein that primarily localizes to endosomes and lysosomes. CLN3 disease, classic juvenile autosomal recessively inherited form typically begins with progressive visual loss between 5 and 10 years of age. Around the age of 10–12 years, children start to manifest loss of motor coordination, mental decline, and seizures may occur. Later, behavioral abnormalities can develop. In some cases, disease symptoms can be accompanied by hallucinations and/or other neuropsychiatric symptoms. Death usually occurs in the third decade of life [Gilani et al., 2021].

Niemann Pick type C (NPC) can also present challenges to diagnosis due to its wide variability in terms of clinical severity and the age of onset. NPC can be categorized into early/late-infantile, juvenile, and adolescent/adult-onset forms to aid in disease management, genetic counseling and prognosis. The juvenile form with onset between 6 and 15 years of age is the most common and so-called "classic" form of the disease. Deficiency in NPC proteins impairs cholesterol movement from the lysosome. The consequent lysosomal accumulation of unesterified cholesterol is the hallmark of the NPC cellular defect and is accompanied by dysregulation of cholesterol homeostatic pathways. Laboratory testing options have been limited. Until recently, the standard for diagnosis of NPC disease was filipin staining of unesterified cholesterol in cultured skin fibroblasts obtained from suspected patients. The filipin test identifies approximately 80–85% of "classical" NPC patients; however, the test does not provide a clear result in patients with variant phenotypes and is also susceptible to false positives. Other disadvantages of the filipin assay are its invasiveness, high cost, and long turnaround times (>7 weeks). For these reasons, filipin staining is no longer considered a first-line test for NPC diagnosis. Oxysterols, the products of cholesterol oxidation, have received much attention as biochemical markers in NPC but can also be elevated in other disorders.

Both the juvenile form of NCL (CLN3) and NPC are lysosomal storage disorders caused by trafficking defects. In the juvenile form, patients affected by both disorders often have completely normal early development. They later exhibit impaired attention and executive function, slowing of learning progress in school, and coordination and motor praxis dysfunction. Over time, clumsiness increases, with falls and difficulty in running due to a variable

combination of progressive dyspraxia, ataxia, and dystonia. Cognitive abilities regress with loss of previously learned skills. Cataplexy, with or without narcolepsy, and seizures may develop. Dysarthria and dysphagia become prominent, and patients often stop talking and require gastrostomy feeding. The lifespan is quite variable, with death in the second to fourth decade of life [Berry-Kravis, 2021].

LysoSMs may be useful biomarkers for the identification of several sphingolipidoses. A novel biomarker, LysoSM-509, has been established for the primary diagnosis of NPC [Bulut et al., 2022]. LysoSM-509, a modified species of LysoSM could be measured easily in Ankara, Turkey. In our patient, we measured very high LysoSM-509 levels and suspected NPC, but we could not confirm the presumptive diagnosis by molecular genetic analysis. NPC was ruled out, and we performed clinical exome sequencing with a suspicion of neurometabolic disease. The diagnosis of NCL was based on clinical, biochemical, neuroradiological, and molecular genetic analyses [Gardner and Mole, 2021].

Based on the fact that consanguineous marriages are more common in Turkey than in western societies, it is easily predicted that this disease group is more common in our country than it is seen. The variant which was first described in a patient from Turkey caused serious changes in protein structure. The detection of the same variant in 2 different unrelated families showed that there may be a founder effect for this variant in Turkey [Kousi et al., 2012].

In conclusion, we present a patient from consanguineous parents with clinical MRI and molecular genetic analysis confirming the diagnosis of JNCL. Knowing the specific type of NCL helps in predicting the natural history of the disease and genetic counseling. LysoSM-509 is a biomarker which is elevated especially in NPC. We can consider that a high LysoSM-509 level might be an indicator of NCL especially NCL type 3. LysoSM-509 should be measured in larger cohorts of patients with CLN3 and other CLN types.

Acknowledgement

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Statement of Ethics

This study was exempt from ethical approval procedures, being a case report of a single patient whose parents provided verbal consent to participate in the study, and gave written consent to have the case published. Informed consent for genetic analysis and publication of clinical reports and photographs was obtained from the patient's parents in compliance with the national ethics regulation. There is no name or number indicating the patient's identity.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Çiğdem Seher Kasapkara: collection of clinical and laboratory data of the patient, manuscript writing. Ahmet Cevdet Ceylan: molecular analyses and interpretation. Oya Kıreker Köylü, Burak Yürek, Burcu Civelek Ürey, Mehmet Gündüz: collection of clinical data. Deniz Yılmaz: collection of neurological data of the patient.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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