COLD SPRING HARBOR
Molecular Case Studies

RAPID COMMUNICATION

Rapid whole-genome sequencing identifies a novel homozygous *NPC1* variant associated with Niemann–Pick type C1 disease in a 7-week-old male with cholestasis

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Abstract Niemann–Pick type C disease (NPC; OMIM #257220) is an inborn error of intracellular cholesterol trafficking. It is an autosomal recessive disorder caused predominantly by mutations in *NPC1*. Although characterized as a progressive neurological disorder, it can also cause cholestasis and liver dysfunction because of intrahepatocyte lipid accumulation. We report a 7-wk-old infant who was admitted with neonatal cholestasis, and who was diagnosed with a novel homozygous stop-gain variant in *NPC1* by rapid whole-genome sequencing (WGS). WGS results were obtained 16 d before return of the standard clinical genetic test results and prompted initiation of targeted therapy.

[Supplemental material is available for this article.]

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Ontology terms: abnormal cholesterol homeostasis; clinodactyly of the 5th finger; foam cells with lamellar inclusion bodies; generalized neonatal hypotonia; hepatosplenomegaly; prolonged neonatal jaundice

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CASE PRESENTATION

A 2.7-kg male infant was born at 38 wk via cesarean section for breech position to healthy nonconsanguineous Hispanic parents. There was no known consanguinity per parental report; however, the families of the mother and father were from the same small hometown in Mexico. He was admitted at 7 wk of age for evaluation of persistent jaundice and poor weight gain. On examination, he was thin and jaundiced with soft hepatosplenomegaly, clinodactyly, and diffuse hypotonia, but no other neurologic abnormalities. Growth parameters met criteria for failure to thrive (Supplemental Data 1). Initial serum tests were aspartate aminotransferase (AST) 349 U/l (20–60 U/l), alanine aminotransferase (ALT) 125 U/l (5–48 U/l), γ-glutamyl transferase (GGT) 277 U/l (10–100 U/l), alkaline phosphatase 1106 U/l (145–320 U/l), total bilirubin 3.9 mg/dl (0.1–1.0 mg/dl), direct bilirubin 2.6 mg/dl (0.0–0.3 mg/dl), and lactic acid 2.7 mmol/l (0.7–2.1 mmol/l). Tests for infectious causes of liver disease, autoimmune hepatitis, α-1 antitrypsin deficiency, and thyroid disease were negative. Serum bile acids were not obtained. Technetium 99 hepatobiliary scan revealed a normal hepatic uptake and excretion into the small bowel. A liver biopsy was performed, which was significant

for marked intrahepatic cholestasis, abundant extramedullary hematopoiesis, and giant cell hepatitis. Although an abdominal ultrasound revealed a 7 × 8 mm hyperechoic mass suggestive of a hemangioma, this was not present upon follow-up magnetic resonance imaging (MRI). Because of the concern for an intrahepatic hyperechoic liver mass, α-fetoprotein (AFP) level was obtained and elevated at 189,222.7 ng/ml (1.6–4.5 ng/ml). AFP-L3, which is the isoform associated with hepatocellular carcinoma, was within normal limits. Thus the elevation in AFP was likely reflective of hepatitis. The infant was clinically stable and discharged. However, he was readmitted 4 d later because of rising AFP levels of >200,000 ng/ml. On hospital day 9 of readmission consent was obtained for rapid whole-genome sequencing (WGS) on the proband alone. Electron microscopy of the liver biopsy later identified concentric lamellar bodies (Supplemental Data 2), highly suggestive of, but not specific for, Niemann–Pick disease. (See Table 1.)

TECHNICAL ANALYSIS AND METHODS

A blood sample was collected and underwent sequencing on a HiSeq X instrument (Illumina). Rapid alignment and nucleotide variant calling was performed using the Dragen (Edico Genome) hardware and software (Miller et al. 2015). Sequence yield was 170.4 Gb, resulting in 4,613,310 distinct variant calls (Supplemental Data 3). Large regions of homozygosity were noted (Supplemental Data 4). Although the patient's parents denied consanguinity, it was presumed that they have a shared ancestry based on sequencing results. Variants were annotated and analyzed in Opal Clinical (Omicia) (Coonrod et al. 2013). Initially, variants were filtered to retain those with allele frequencies of <1% in the Exome Variant Server, 1000 Genomes Samples, and Exome Aggregation Consortium database (http://evs.gs.washington.edu/EVS/ 2016; Karczewski et al. 2016). A cholestasis gene panel was built in Phenolyzer (Yang et al. 2015) using Human Phenotype Ontology (HPO) (Köhler et al. 2017) and Systematized Nomenclature of Medicine-Clinical Terms (SNOMED-CT [SNOMED]). This panel included 382 genes related to the HPO terms neonatal cholestatic liver disease (HP:0006566), conjugated hyperbilirubinemia (HP:0002908), and hepatomegaly (HP:0002240). Variants were further filtered to retain those mapping to these 382 genes, yielding 29 variants that fit an autosomal recessive inheritance pattern. No variants in these genes that fit a dominant inheritance pattern were found. Manual curation revealed one variant as likely pathogenic (zero strong, three moderate, and three supporting criteria; Supplemental Data 4, 5) by American College of Medical Genetics and Genomics (ACMG) guidelines (Richards et al. 2015) and likely causative of the infant's illness. (See Table 2.)

VARIANT INTERPRETATION

The c.2713 C>T (p.Gln905Ter) is a novel stop-gain variant that is predicted to result in premature truncation of the NPC1 protein by 30%. The *NPC1* gene is one of two genes known to cause Niemann–Pick disease type C (NPC). More than 200 pathogenic variants have been described in *NPC1*. This individual was homozygous for c.2713 C>T, which was located within a region of homozygosity in Chromosome 18. The Gln905 amino acid residue is highly conserved. Although this particular variant has not been reported in the literature, pathogenic missense, and stop-gain variants have been widely reported (Park et al. 2003; Scott and Ioannou 2004; Fernandez-Valero et al. 2005; Jahnova et al. 2014). Based on the combined evidence, this variant was classified as likely pathogenic for Niemann–Pick type C1 disease.

Table 1. Phenotypic features

Niemann–Pick type C disease	Proband (II-1)	Relevance/alternate explanation
Vertical supranuclear gaze palsy	No	
Hepatomegaly	Yes	
Neonatal jaundice	Yes	
Fatal liver failure in infancy	No	
Splenomegaly	Yes	
Dysphagia	No	
Hypotonia	Yes	
Developmental delay	No	
Dysarthria	No	
Loss of speech	No	
Mental deterioration	No	
Dementia	No	
Spasticity	No	
Dystonia	No	
Seizures	No	
Cerebellar ataxia	No	
Cataplexy	No	
Neuronal loss, particularly of cerebellar Purkinje cells	No	
Neurofibrillary tangles	No	
Poor school performance	n/a	
Behavioral problems	n/a	
Psychosis	n/a	
Foam cells on bone marrow biopsy	n.d.	
"Sea blue" histiocytes	Yes	On liver biopsy
Fetal ascites	No	
Normal or mildly reduced sphingomyelinase activity	n.d.	
Low cholesterol esterification rates	n.d.	
Abnormal cholesterol homeostasis	Yes	Elevated plasma oxysterols (noted postdiagnosis)
Foam cells in visceral organs and CNS	n.d.	
Foam cells contain polymorphic cytoplasmic inclusions consisting of lamellar osmiophilic membranes on electron microscopy	Yes	
Novel clinical features		
Elevated α-fetoprotein	Yes	
Bilateral kidney lesions	Yes	Focal medullary non-enhancement on MRI
Clinodactyly	Yes	

The list of clinical features are based on the OMIM clinical synopsis related to *NPC1* gene (#257220; Niemann–Pick disease, type C1).
CNS, central nervous system; n/a, not available; n.d., not determined; MRI, magnetic resonance imaging.

Table 2. Genomic findings

Gene	Genomic location	HGVS cDNA	HGVS protein	Zygosity	Parent of origin	Variant interpretation
NPC1	Chr18:21119857 (on Assembly GRCh38)	NM_000271.3 c.2713 C>T	p.Gln905Ter	Homozygous	Both	Likely pathogenic

HGVS, Human Genome Variation Society.

NPC is a rare autosomal recessive disorder of lysosomal lipid metabolism. Prevalence is estimated at 1:100,000 (Vanier 2010). NPC is caused by biallelic mutations in either *NPC1* (18q11–18q12, referred to as type C1) or *NPC2* (14q24.3, referred to as type C2) (Carstea et al. 1997; Naureckiene et al. 2000). The NPC1 protein is an important transmembrane protein for intracellular sorting of cholesterol and glycosphingolipids (Pentchev et al. 1985; Sokol et al. 1988; Kwon et al. 2009). Loss of function of either NPC1 or NPC2 protein disrupts normal intracellular cholesterol trafficking, resulting in accumulation of cholesterol within the lysosomes and relative cholesterol deficiency in other cellular regions such as the cellular membrane.

NPC has a spectrum of phenotypic features that vary by age of presentation and timing of systemic and neurologic involvement. Many children and adults present with a progressive neurodegenerative disorder characterized by progressive gait abnormalities, dystonia, cataplexy, seizures, vertical supranuclear palsy, dysphagia, dysarthria, and dementia (Vanier 2010). Hepatomegaly and splenomegaly are present in most affected children and can precede neurologic symptoms. Neonates can present severe cholestatic liver disease and do not typically manifest neurologic abnormalities during the neonatal period (Wenger et al. 1977). Risk of mortality is high because of acute liver failure or respiratory failure secondary to cholesterol infiltration of the organs.

A provisional diagnosis was made 6 d after commencing the sequencing run. The research protocol under which the proband received rapid WGS was approved by the Food and Drug Administration and local Institutional Review Board. It requires confirmation by a clinically accepted standard before reporting, except in cases of actionable diagnoses where major morbidity or likelihood of mortality is likely during confirmatory testing. Given that targeted therapies with the potential to delay onset of neurologic symptoms or affect course of disease are available for this diagnosis, results were immediately relayed to the primary physicians caring for the patient. Clinical panel testing was sent to Invitae at the same time as enrollment for rapid WGS, which includes the *NPC1/NPC2* sequencing with deletion/duplication studies. The laboratory was contacted with our findings and the *NPC1* variant was confirmed by this clinical test and reported back 16 d after provisional reporting of rapid WGS results.

Specific treatment of NPC was promptly started with miglustat (Zavesca, Actelion Pharmaceuticals Ltd), which competitively inhibits glucosylceramide synthase. This enzyme is needed to produce glycosphingolipids and decreases the rate of glycosphingolipid glycosylceramide formation within neurons (Patterson et al. 2015). Miglustat has been proposed to delay the onset of neurodegeneration in animal models of NPC1 and is approved in Europe for the treatment of NPC (Patterson et al. 2007). In one study it was shown to promote increased survival in mice with *NPC1*-associated Niemann–Pick disease (Zervas et al. 2001). Plasma oxysterols were checked before initiation of therapy and found to be elevated, with subsequent decline on therapy (Supplemental Data 7). The patient was also referred to the National Institutes of Health (NIH) Observational Study of the Natural History of NPC, which offers specific testing and specialized resources for patients and families affected by NPC.

In addition, an application to start 2-hydroxypropyl-β-cyclodextrin (HPβCD) therapy was submitted as part of an expanded access investigational new drug (IND) for compassionate use in patients with NPC1. HPβCD is the only drug that has been proven to ameliorate neurodegeneration and prolong the life span of NPC1 mice (Liu et al. 2009) and thus far has shown promising results in a small cohort of older NPC1 patients (World 2017 posters). Diagnosis of NPC1 in infants before development of neurologic disease is uncommon (Degtyareva et al. 2016). A literature review identified only two prior cases of patients diagnosed with NPC in infancy who started miglustat therapy upon diagnosis. Patient 1 was homozygous for the p.Tyr1019Cys variant and started on therapy at the age of 7 mo. Patient 2 was a compound heterozygote with p.Pro1007Arg and p.Thr1205Lys variants and started on therapy at the age of 19 mo. After 7 and 6 yr of miglustat therapy, respectively, both patients remain free of neurologic manifestations (Di Rocco et al. 2012). In the current patient, plasma oxysterol levels (a biomarker for NPC) have declined, however this may not be related to miglustat therapy. Liver transaminase levels have also improved. It has been suggested that miglustat may be more effective if used to prevent, rather than treat, neurologic manifestations in infantile-onset Niemann–Pick type C1 (Di Rocco et al. 2012; Héron et al. 2012). Intrathecal HPβCD therapy is also likely to have a more pronounced disease-modifying effect if started before significant loss of Purkinje neurons has occurred. The window of opportunity to delay or prevent the irreversible effects of a rare mutation is often small, underscoring the clinical utility of rapid WGS in neonatal intensive care unit (NICU) and pediatric intensive care unit (PICU) infants (Willig et al. 2015).

This case demonstrates the clear clinical utility of sequencing beyond a more restricted panel of cholestasis genes. Rapid sequencing not only assures that the child is on the correct therapy but has the potential to avoid unnecessary procedures. This case was not enrolled until 7 wk of age as we had not started rapid sequencing at the time of diagnosis. It is reasonable to presuppose that if we had performed rapid testing earlier we would have been able to avoid both the morbidity of liver biopsies and decreased total hospital charges from ~$160,000.00 to $80,000.00. This compares favorably with the $7,000.00 estimated cost of singleton rapid WGS. Although it may have been less expensive to send oxysterols at presentation followed by targeted panel testing, the combined turnaround time would have been much longer (estimated at 39 d compared with 6 d for WGS). Thus, even with a less expensive test, the patient would likely have remained in the hospital longer accruing additional charges. It underscores the importance of a rapid diagnosis in getting a child started on therapy before classical signs and symptoms of neurological injury have occurred.

SUMMARY

We describe a novel homozygous stop-gain variant (c.2713 C>T, p.Gln905Ter) in *NPC1*, a gene well known to cause Niemann–Pick disease type C. To our knowledge, this is the youngest patient presenting with cholestasis diagnosed with this disease, which was made possible by performing rapid WGS. In addition, this diagnosis allowed for early intervention of targeted therapy prior to the onset of obvious neurologic symptoms. It is expected that this early intervention will significantly improve the quality of life of this patient by delaying the neurodegenerative component of this disease.

ADDITIONAL INFORMATION

Data Deposition and Access

The ClinVar accession number is SCV000538195 (https://www.ncbi.nlm.nih.gov/clinvar/).

Ethics Statement

Informed and signed consent forms were obtained for all sequenced individuals of this study. The project is approved by Institutional Review Board of the University of California at San Diego under protocol #160468 and has received nonsignificant risk status in a pre-Investigational Device Exemption submission to the Food and Drug Administration.

Author Contributions

A.H. contributed to manuscript preparation and phenotyping. K.W. contributed to manuscript preparation and clinical implementation. S.C. contributed to variant interpretation and manuscript preparation. S.N. contributed to clinical implementation and manuscript preparation. J.B. contributed to clinical implementation and manuscript preparation. P.O. contributed to clinical implementation and manuscript preparation. S.B. contributed to Bioinformatics study. D.D. contributed to supervision and manuscript preparation. S.K. contributed to supervision and manuscript preparation. RCIGM Investigators contributed to process development, infrastructure deployment, and maintenance. All authors contributed to the reviewing of the final version.

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Competing Interest Statement

The authors have declared no competing interest.

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