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.]

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 isofrom 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.
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, catalepsy, 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.

### Table 2. Genomic findings

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

HGVS, Human Genome Variation Society.
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 oxyysterol 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. All authors contributed to the reviewing of the final version.

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REFERENCES

Carstea ED, Morris JA, Coleman KG, Loftus SK, Zhang D, Cummings C, Gu J, Rosenfeld MA, Pavan WJ, Krizman DB, et al. 1997. Niemann–Pick C1 disease gene: homology to mediators of cholesterol homeostasis. Science 277: 228–231.

Coonrod EM, Margraf RL, Russell A, Voelkerding KV, Reese MG. 2013. Clinical analysis of genome next-generation sequencing data using the Omicia platform. Expert Rev Mol Diagn 13: 529–540.

Degtyareva AV, Mikhailova SV, Zakharova EY, Tumanova EL, Puchkova AA. 2016. Visceral symptoms as a key diagnostic sign for the early infantile form of Niemann–Pick disease type C in a Russian patient: a case report. J Med Case Rep 10: 143.

Di Rocco M, Dardis A, Madeo A, Barone R, Fiumara A. 2012. Early miglustat therapy in infantile Niemann–Pick disease type C. Pediatr Neurol 47: 40–43.

Fernandez-Valero EM, Ballart A, Iturriaga C, Lluch M, Macias J, Vanier MT, Pineda M, Coll MJ. 2005. Identification of 25 new mutations in 40 unrelated Spanish Niemann–Pick type C patients: genotype–phenotype correlations. Clin Genet 68: 245–254.

Héron B, Valayannopoulos V, Baruteau J, Chabrol B, Ogier H, Latour P, Poblet-Baladera D, Eyet D, Labarthe F, Maurey H, et al. 2012. Miglustat therapy in the French cohort of paediatric patients with Niemann–Pick disease type C. Orphanet J Rare Dis 7: 36.

http://evs.gs.washington.edu/EVS/. 2016. NHLBI, Exome Variant Server, GO Exome Sequencing Project (ESP). Retrieved 2016, from http://evs.gs.washington.edu/EVS/.

Jahnova H, Dvorakova L, Vlaskova H, Huklova H, Poupetova H, Hrebicek M, Jesina P. 2014. Observational retrospective study of a large cohort of patients with Niemann–Pick disease type C in the Czech Republic: a surprisingly stable diagnostic rate spanning almost 40 years. Orphanet J Rare Dis 9: 140.

Karczewski KJ, Weisburd B, Thomas B, Solomonson M, Ruderfer DM, Kavanagh D, Hamamsy T, Lek M, Samocha KE, Cummings BB, et al. 2016. The ExAC browser: displaying reference data information from over 60,000 exomes. Nucleic Acids Res 45: D840–D845.

Köhler S, Vasilevsky NA, Engelstad M, Foster E, McMurry J, Aymé S, Baynam G, Bello SM, Boerkoel CF, Boycott KM, et al. 2017. The Human Phenotype Ontology in 2017. Nucleic Acids Res 45: D865–D876.
Kwon HJ, Abi-Mosleh L, Wang ML, Deisenhofer J, Goldstein JL, Brown MS, Infante RE. 2009. Structure of N-terminal domain of NPC1 reveals distinct subdomains for binding and transfer of cholesterol. Cell 137: 1213–1224.

Liu B, Turley SD, Burns DK, Miller AM, Repa JJ, Dietschy JM. 2009. Reversal of defective lysosomal transport in NPC disease ameliorates liver dysfunction and neurodegeneration in the npc1−/− mouse. Proc Natl Acad Sci 106: 2377–2382.

Miller NA, Farrow EG, Gibson M, Willig LK, Twist G, Yoo B, Mars T, Corder S, Krivohlavek L, Walter A, et al. 2015. A 26-hour system of highly sensitive whole genome sequencing for emergency management of genetic diseases. Genome Med 7: 100.

Naureckiene S, Sleat DE, Lackland H, Fensom A, Vanier MT, Wattiaux R, Jadot M, Lobel P. 2000. Identification of HE1 as the second gene of Niemann–Pick C disease. Science 290: 2298–2301.

Park WD, O’Brien JF, Lundquist PA, Kraft DL, Vockley CW, Karnes PS, Patterson MC, Snow K. 2003. Identification of 58 novel mutations in Niemann–Pick disease type C: correlation with biochemical phenotype and importance of PTC1-like domains in NPC1. Hum Mutat 22: 313–325.

Patterson MC, Vecchio D, Prady H, Abel L, Wraith JE. 2007. Miglustat for treatment of Niemann–Pick C disease: a randomised controlled study. Lancet Neurol 6: 765–772.

Patterson MC, Mengel E, Vanier MT, Schwierin B, Muller A, Cornelisse P, Pineda M; NPC Registry investigators. 2015. Stable or improved neurological manifestations during miglustat therapy in patients from the international disease registry for Niemann–Pick disease type C: an observational cohort study. Orphanet J Rare Dis 10: 65.

Pentchev PG, Comly ME, Kruth HS, Vanier MT, Wenger DA, Patel S, Brady RO. 1985. A defect in cholesterol esterification in Niemann–Pick disease (type C) patients. Proc Natl Acad Sci 82: 8247–8251.

Richards S, Aziz N, Bale S, Bik D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, et al. 2015. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17: 405–424.

Scott C, Ioannou YA. 2004. The NPC1 protein: structure implies function. Biochim Biophys Acta 1685: 8–13.

SNOMED. 2016. SNOMED CT Systematized Nomenclature of Medicine-Clinical Terms. http://www.ihtsdo.org/snomed-ct, from http://www.ihtsdo.org/snomed-ct.

Sokol J, Blanchette-Mackie J, Kruth HS, Dwyer NK, Amende LM, Butler JD, Robinson E, Patel S, Brady RO, Comly ME, et al. 1988. Type C Niemann–Pick disease. Lysosomal accumulation and defective intracellular mobilization of low density lipoprotein cholesterol. J Biol Chem 263: 3411–3417.

Vanier MT. 2010. Niemann–Pick disease type C. Orphanet J Rare Dis 5: 16.

Wenger DA, Barth G, Githens JH. 1977. Nine cases of sphingomyelin lipidosis, a new variant in Spanish-American Children. Juvenile variant of Niemann–Pick Disease with foamy and sea-blue histiocytes. Am J Dis Child 131: 955–961.

Willig LK, Petkink JE, Smith LD, Saunders CJ, Thiffault I, Miller NA, Soden SE, Calcici JA, Herd SM, Twist G, et al. 2015. Whole-genome sequencing for identification of Mendelian disorders in critically ill infants: a retrospective analysis of diagnostic and clinical findings. Lancet Respir Med 3: 377–387.

Yang H, Robinson PN, Wang K. 2015. Phenolyzer: phenotype-based prioritization of candidate genes for human diseases. Nat Methods 12: 841–843.

Zervas M, Somers KL, Thrall MA, Walkley SU. 2001. Critical role for glycosphingolipids in Niemann–Pick disease type C. Curr Biol 11: 1283–1287.