Probable Diagnosis of a Patient with Niemann–Pick Disease Type C: Managing Pitfalls of Exome Sequencing

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Received: 12 September 2017 / Revised: 23 January 2018 / Accepted: 29 January 2018 / Published online: 17 February 2018
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Abstract Here, we present a case of a 31-year-old man with progressive cognitive decline, ataxia, and dystonia. Extensive laboratory, radiographic, and targeted genetic studies over the course of several years failed to yield a diagnosis. Initial whole exome sequencing through a commercial laboratory identified several variants of uncertain significance; however, follow-up clinical examination and testing ruled each of these out. Eventually, repeat whole exome sequencing identified a known pathogenic intronic

variant in the *NPC1* gene (NM_000271.4, c.1554-1009G>A) and an additional heterozygous exonic variant of uncertain significance in the *NPC1* gene (NM_000271.4, c.2524T>C). Follow-up biochemical testing was consistent with a diagnosis of probable Niemann–Pick disease Type C (NP-C). This case illustrates the potential of whole exome sequencing for diagnosing rare complex neurologic diseases. It also identifies several potential common pitfalls that must be navigated by clinicians when interpreting commercial whole exome sequencing results.

Communicated by: Robert Steiner

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Case Report

A 31-year-old man of Puerto Rican and Mexican ancestry with history of obesity and dyslipidemia presented for evaluation after a fall. Childhood was notable for delayed language acquisition, with speech onset around age 3. Symptoms began in his early 20s with gait difficulty. He and his family also noted slowed mentation, difficulty following instructions, poor memory, impaired manual dexterity with difficulty writing, intermittent slurred speech, difficulty swallowing, and arm tremors. He reported no family history of early dementia or ataxia. Examination demonstrated deficits in orientation, recall, calculations, and visuospatial skills; slowed horizontal saccades and saccadic dysmetria; focal hand dystonia; hyperreflexia; and wide-based unsteady gait. There was no hepatosplenomegaly noted on exam. Symptoms progressed over the next 5 years, and he developed depression and apathy. Cognitive impairments, primarily frontal and executive, persisted. Serial examination revealed vertical supranuclear gaze palsy, dysarthria, spasticity, and progressive ataxia with appendicular dysmetria.

Laboratory studies including blood counts, comprehensive metabolic panel, TSH, B12, vitamin E, RPR, HIV, sedimentation rate, antinuclear antibodies, angiotensin converting enzyme, protein electrophoresis, ceruloplasmin, copper levels, anti-GAD, antigliadin antibodies, urine heavy metals, very long chain fatty acids, plasma amino acids, urine organic acids, urine sulfatides, lactate, pyruvate, and WBC enzyme hexosaminidase A, arylsulfatase, TPP-1, and PPT-1 were all within normal limits. Lipids were notable for mildly elevated triglycerides and low HDL. MRI of the brain showed generalized cerebral atrophy (Fig. 1). Single gene testing for Huntington disease, Huntington disease-like 2, spinocerebellar ataxia type 17, and dentatorubral-pallidoluysian atrophy did not reveal any pathogenic variants. Chromosomal microarray did not detect any copy number variants or regions of homozygosity. Duo exome sequencing (sample submitted with unaffected mother serving as control to interpret identified variants) was submitted to a commercial laboratory in 2014. The reported results listed a suspected pathogenic heterozygous variant in the *FXN* gene (NM_000144.4, c.494G>A, p.Arg165His, associated with Friedreich ataxia) and four heterozygous variants of uncertain significance (VUS): *ALDH5A1* (NM_001080.3, c.886G>A, p.Ala296Thr, associated with succinic semialdehyde dehydrogenase deficiency), *ITPR1* (NM_002222.5, c.4573delC, p.Leu1525fs, associated with spinocerebellar ataxia 15/29, Gillespie syndrome), *SACS* (NM_014363.5, c.9562T>C, p.Phe3188Leu, associated with spastic ataxia Charlevoix-Saguenay type), and *NDUFS7* (NM_024407.4, c.439G>A, p.Val147Met, associated with Leigh syndrome). The variants in *ITPR1*, *SACS*, and *NDUFS7* were maternally inherited. Follow-up *FXN* GAA triplet repeat testing was normal.

Repeat exome sequencing on a blood sample from the patient was performed 16 months later at a different commercial laboratory. This confirmed the previously noted *ITPR1* gene variant (c.4573delC), now described as likely

pathogenic. The *SACS* VUS (c.9562T>C) was again reported. The variants previously reported in *FXN* and *NDUFS7* were detected, but after discussion with the company's medical board committee, were not reported because the phenotypic overlap with the patient was felt to be too low. The *ALDH5A1* variant was either not detected or filtered out at an early step in the analysis pipeline. A heterozygous deep intronic variant, known to be pathogenic, was identified in the *NPC1* gene (NM_000271.4, c.1554-1009G>A, associated with Niemann–Pick disease type C [NP-C]) (Abela et al. 2014; Rodríguez-Pascau et al. 2009). An additional heterozygous VUS in the *NPC1* gene (NM_000271.4, c.2524T>C, p.Phe842Leu) was detected, as well as a heterozygous VUS in the *VCP* gene (NM_007126.3, c.811+2T>C, p.?, associated with ALS, inclusion body myopathy, and frontotemporal dementia). Multiple in silico models predict the *NPC1* VUS to be deleterious (Provean prediction “Deleterious,” score −5.67; SIFT prediction “Damaging,” score 0.003; and PolyPhen-2 prediction “Probably damaging,” score 1.0). Subsequent metabolic testing revealed elevations in oxysterols (cholestane-3 β ,5 α ,6 β -triol [0.05 nmol/mL, ref. <0.02] and 7-ketocholesterol [0.13 nmol/mL, ref. <0.05]) and lysosphingomyelin-509 (90.48, ref. <30), consistent with a diagnosis of probable NP-C. Miglustat was prescribed, and the patient's family reported improvements in his cognition and stabilization of his other neurologic deficits.

Discussion

NP-C is a rare autosomal recessive lysosomal lipid storage disorder resulting from biallelic pathogenic variants in *NPC1* or *NPC2* (Mengel et al. 2013). Neurologic manifestations include gelastic cataplexy, dementia, vertical supranuclear gaze palsy, ataxia, tremor, and dystonia. Additional psychiatric and visceral (hepatosplenomegaly) manifestations are common. The combination of visceral, neurologic, and psychiatric manifestations suggests the diagnosis (Wijburg et al. 2012). Our patient did not have classical visceral manifestations of NP-C but did have dyslipidemia and obesity, which have been linked to *NPC1* variants (Garver et al. 2010; Liu et al. 2017).

The true incidence of NP-C is not known and likely underestimated, in part due to the difficulty in diagnosing milder forms of the disease. In adult-onset NP-C, the presenting symptoms, such as mild cognitive impairment, ataxia, tremor, and dystonia, are nonspecific and overlap with numerous more common neurologic diseases. As such there is frequently a considerable delay between onset of symptoms and diagnosis (Vanier et al. 2016). This patient experienced this process first hand as he was seen by numerous physicians without diagnosis before whole exome sequencing finally provided the clue.

Fig. 1 Neuroimaging findings. T1-weighted MRI images from presentation (a) and 5 years later (b) show slowly progressive global cerebral atrophy. Arrowheads demonstrate widening of cerebral sulci

A number of laboratory techniques exist to aid in the diagnosis of NP-C. Until recently, the most extensively used was filipin staining. This technique involves growing patient derived skin fibroblasts in culture and then staining with the fluorescent antibiotic filipin, which binds to cholesterol. Controls show few cells staining positive whereas cells from NP-C patients typically show perinuclear staining due to a defect in intracellular cholesterol transport. While this assay is well validated, it is invasive, time consuming, and requires rigorous technical expertise. In addition, a minority of NP-C patients show a variant profile of staining that can overlap with healthy controls or NP-C heterozygous carriers (Vanier and Latour 2015). More recently, several new biochemical diagnostic techniques have been developed that are noninvasive, easier to perform, and lower cost. These are now recommended as first-line screening tests for patients in whom a diagnosis of NP-C is suspected (Patterson et al. 2017). Here, we tested for both oxysterol and lysosphingolipids, levels of which have been shown to be specifically elevated in NP-C (Vanier et al. 2016). However, these tests cannot differentiate NP-C from Niemann–Pick types A and B, and values for heterozygous carriers are not well defined. Therefore, confirmation of the diagnosis with either filipin staining or genetic testing is recommended even in patients with elevated biomarkers. In our case, although genetic testing was carried out, sequencing of parents to determine the phase of the *NPC1* variants could not be performed. Therefore, it was not possible to determine whether the patient was a compound heterozygote or a carrier of a complex allele. In addition, the patient's surrogate decision maker declined confirmatory filipin staining. For these reasons, the patient was classified as “probable” NP-C.

After making the diagnosis of probable NP-C, we discussed the diagnosis with the laboratory that performed the initial exome sequencing. They confirmed that intronic regions of *NPC1* were not sequenced and the exonic variant (c.2524T>C) was detected but was not reported because the variant was heterozygous and of uncertain significance. This omission led to a significant diagnostic delay of almost 2 years as testing was completed to rule out the other reported variants of uncertain significance. Repeat exome sequencing crucially included sequencing of known pathogenic intronic variants and detected a pathogenic variant (c.1554-1009G>A) that causes abnormal splicing of *NPC1* mRNA, resulting in inclusion of a pseudoexon and subsequent transcript degradation (Rodríguez-Pascau et al. 2009). The exonic variant (c.2524T>C) remains classified as of “uncertain significance” (<https://medgen.medizin.uni-tuebingen.de/NPC-db2/>). This variant is rare, with an allele frequency of 0.00003612 from the gnomAD browser

(<http://gnomad.broadinstitute.org/>). In addition, multiple in silico models predict the allele to be deleterious. Therefore, this case provides initial evidence that this variant could play a pathogenic role (assuming in *trans* inheritance). However, complex alleles with two variants on the same allele have been reported in other lysosomal storage disorders. Since sequencing of the *NPC1* variants from the parents of this patient was not performed, we cannot rule out the possibility of one complex allele with both identified variants and an additional unidentified variant in the other *NPC1* allele.

This patient also has a novel variant in the *ITPR1* gene. Variants in *ITPR1* lead to autosomal dominant spinocerebellar ataxia type 15/16. While progressive adult-onset ataxia with tremor is consistent with the SCA-15/16 phenotype (Storey 1993), prominent cognitive and psychiatric involvement and vertical supranuclear gaze palsy are not. Inheritance of this *ITPR1* variant from an unaffected mother also argues strongly against pathogenicity. Although penetrance of the disease is not fully known, it is expected to be high. However, very late onset cases have been reported and thus we cannot fully exclude the possibility that the variant is pathogenic, but the patient's mother remains presymptomatic (Storey 1993).

This case illustrates the difficulties in diagnosing rare neurologic diseases in patients with nonspecific or complex neurological symptoms. Whole exome sequencing and other next-generation sequencing technologies hold great potential as diagnostic tools. For adult-onset cerebellar ataxias, whole exome sequencing has been shown to yield clinically relevant genetic information in up to 60% of patients, with a definitive diagnosis obtained in 21% of tested patients (Fogel et al. 2014). Such a diagnostic yield far exceeds that of most other clinically available diagnostic tests; however, interpreting exome sequencing is not without its pitfalls. A typical exome will identify 20–25,000 “rare” variants that are then filtered using algorithms to identify only variants that result in sequence changes predicted to be deleterious. For commercial exomes, this smaller pool of variants (still typically numbering in the 100–1,000 s) is then manually annotated and only variants thought to have phenotypic overlap with the clinical information provided are reported back as results. Ultimately, the results obtained rely on the clinical judgment of those doing the annotation and as such, the output depends significantly on the quality of the phenotyping provided by the ordering physician. Our case illustrates the pitfalls of this process clearly, as the first exome detected, but did not report, the exonic variant in *NPC1*, leading to significant diagnostic delay. Conversely, the second exome detected, but did not report (due to low

phenotypic overlap), several of the variants that were reported initially. Of note, both laboratories were supplied similar clinical information.

With increasing use of exome and genome sequencing, and data sharing through public databases, interpretation of sequence variants is evolving rapidly. This illustrates the need to resequence or reinterpret results when there is clinical uncertainty. Phenotyping – informed by personal and family history, clinical exam, imaging, and laboratory testing – remains key to diagnosis and interpretation of sequence variants.

Synopsis

Whole exome sequencing is a powerful tool for diagnosing inherited metabolic disorders with complex neurologic phenotypes such as NP-C, but results obtained through commercial laboratories must be interpreted carefully and always in the appropriate clinical context.

Compliance with Ethics Guidelines

Author Contributions

William Zeiger – Conceptualization of the study; interpretation of the data; and drafting and revising the manuscript for intellectual content.

Nasheed I. Jamal – Conceptualization of the study; interpretation of the data; and revising the manuscript for intellectual content.

Maren T. Scheuner – Analysis and interpretation of the data; and revising the manuscript for intellectual content.

Patricia Pittman – Conceptualization of the study.

Kimiyo M. Raymond – Analysis and interpretation of the data; and revising the manuscript for intellectual content.

Massimo Morra – Analysis and interpretation of the data; and revising the manuscript for intellectual content.

Shri K. Mishra – Conceptualization of the study; analysis and interpretation of the data; and revising the manuscript for intellectual content.

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William Zeiger.

Competing Interests

Dr. Zeiger declares that he has no conflict of interest.

Dr. Jamal declares that she has no conflict of interest.

Dr. Scheuner declares that she has no conflict of interest.

Ms. Pittman declares that she has no conflict of interest.

Dr. Raymond declares that she has no conflict of interest.

Dr. Morra is an employee of Personalis, Inc.

Dr. Mishra declares that he has no conflict of interest.

The authors confirm independence from any sponsors and the content of the article has not been influenced by any sponsors.

Study Funding

No targeted funding reported.

This article does not contain any studies with human or animal subjects performed by any of the authors.

Patient Consent

This case report was carried out in accordance with the guidance of the office of research and development of the Veterans Health Administration. No identifiable patient information is included in this report.

Ethical Approval

Not required for this type of study.

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