Metabolic biomarker testing facilitates genetic diagnosis of Niemann–Pick disease by enabling classification of novel SMPD1 variants

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ABSTRACT

Background: Niemann–Pick (NP) disease is a genetically heterogeneous metabolic disorder caused by bi-allelic variants in NPC1, NPC2, or SMPD1, with initial symptoms and age at onset varying widely. The interpretation of variants in NP disease genes is challenging when these alterations have never been observed before, and when parental samples are not available.

Case Presentation: We clinically, genetically, and biochemically characterized an infant with a complex presentation and a negative family history. Clinical and paraclinical observations were consistent with NP disease. Genetic screening identified two previously unreported SMPD1 missense variants, which were initially classified as variants of unknown significance. Based on strongly increased plasma levels of lysosphingomyelin-509, both variants could be re-classified as likely pathogenic, thus establishing a diagnosis of NP disease type A/B.

Conclusion: A combination of genetics with biochemical approaches facilitates conclusive diagnosis of metabolic disorders including NP disease. Blood-based biomarkers are particularly promising in this respect.

Keywords: Biomarker, enzymatic testing, Niemann–Pick disease, SMPD1, variant classification.

Introduction

With the recent advancements in sequencing technologies, variant detection has been replaced by variant interpretation as the major challenge in genetic diagnostics. The guidelines proposed by the American Colleague of Medical Genetics (ACMG) represent the probably most widely recognized effort toward the standardization of the variant classification process (1). These guidelines emphasize that the comprehensive consideration of arguments for and against pathogenicity is crucial for variants that have never been previously observed or reported. Within this context, the guidelines also recommend to use both genetic and non-genetic types of evidence. While inherited metabolic disorders are frequently associated with measurable changes at the non-genetic level, a meaningful implementation into diagnostic workflows needs to be optimized (2). We present a patient, for whom such extended analyses were essential for reaching a diagnosis and suggest metabolic biomarkers as an attractive alternative to enzymatic testing for this purpose.

Case Presentation

The patient presented at Lady Ridgeway Hospital for Children (Colombo, Sri Lanka) at 10 months of age. Detailed clinical examination, as well as laboratory follow-up, was initiated. Genetic screening for variants in the Niemann–Pick (NP) genes SMPD1, NPC1, and NPC2 (3) was based on targeted next-generation sequencing of all exonic coding sequences and the neighboring >= 50 nucleotides. For in silico pathogenicity predictions, the online tools PolyPhen-2, Sorting Intolerant From Tolerant (SIFT),
and MutationTaster were applied. Biochemical analyses utilized dried blood spots (DBSs). They included standard testing of the enzymatic activity of acid sphingomyelinase (ASM) (4) and mass-spectrometry-based determination of the levels of lyso-SM-509 as described (5).

Results

Clinical findings

A Sri Lankan male infant had been born by elective caesarian section due to oligohydramnios and breech presentation to reportedly non-consanguineous parents at 35 weeks gestation. Apart from neonatal jaundice that developed at day four and was treated with phototherapy, the infant appeared healthy. Around the age of 6 months, the parents started to note abdominal distension and motor delay. At 10 months of age, the patient presented with hypotonia, large Mongolian blue spots, and slight facial dysmorphism (low set ears, elongated face). The family history for similar observations was negative. Ultrasound abdomen showed enlargement of the spleen and liver with normal vasculature and no focal lesions. Biochemistry testing revealed elevated liver transaminases, cholesterol, and triglycerides. Foamy macrophages were detected from bone marrow aspiration and trephine biopsy. Based on these findings, a clinical suspicion of Niemann–Pick disease was raised, and corresponding genetic analyses were initiated.

Genetic and biochemical findings

Genetic analysis of all three known NP genes revealed the heterozygous presence of the two SMPD1 variants c.725G>A and c.1371T>G (NM_00543.4) (Figure 1A). Both were predicted to entail missense alterations (p.Gly242Asp and p.Phe457Leu, respectively), were absent from public mutation and variation databases (ClinVar, HGMD, and gnomAD), as well as from our in-house database CentoMD® (6), and in silico pathogenicity predictions were inconsistent (Table 1). Parental samples

![Figure 1](image-url)
for determining an in cis or in trans constellation were not available. The variants could, at this stage, only be classified as variants of uncertain significance. Subsequent enzymatic testing showed the activity of the SMPD1-encoded ASM to be pathologically decreased to 0.4 µmol/l/h (normal >= 1.7 µmol/l/h). DBS-based quantification of lysophosphoglycero-509 (Lyso-SM-509), an increase of which has been shown to detect Niemann–Pick patients with high sensitivity (5), revealed a value of 5.5 ng/µl, i.e., far above the maximum of 0.9 ng/µl as seen in healthy controls (Figure 1B). Both variants could, therefore, be re-classified as “likely pathogenic” (ACMG pathogenicity class 2), and a corresponding genetic diagnosis be issued.

Discussion and Conclusions

Our study describes the steps that eventually led to the genetic diagnosis of Niemann–Pick disease type A/B (NP-A/B) in an infant with a clinically suspicious phenotype. It thereby extends the spectrum of published pathogenic SMPD1 variants from \( n = 254 \) to \( n = 256 \) (HGMD). By solely considering the genetic findings, however, the variants could only be classified as variants of uncertain significance. The major reasons for this were (i) the novelty of both variants and (ii) the unavailability of parental samples for phasing. Consequently, neither the absence of an effect nor a heterozygous in cis constellation could be excluded. The additional biochemical analyses were, therefore, critical for enabling classification as likely pathogenic. Both the lowered activity of ASM and the increased concentration of Lyso-SM-509 were supportive of pathogenicity. While ASM testing is the currently recommended standard for NP-A/B (4), it is prone to the general drawbacks of enzymatic testing that are related to pre-analytical measures, fluorescence measurement, enzyme stability, and assay duration. Despite having the potential of being less specific (5), mass spectrometry-based quantification of disease-associated metabolites from DBSs overcomes many of these issues (7). Indeed, the corresponding approach provided promising preliminary insights into NP (8) and is already a well-established part of studies on e.g., Gaucher disease (9). A wider application of this concept may improve the diagnosis of lysosomal storage disorders and additional metabolic conditions (10), and eventually enable comprehensive newborn screening.

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Declaration of conflicting interests

CB, SS, VK, CC, and AR are employees of CENTOGENE AG.

Ethical approval

The study is covered by ClinicalTrials.gov Identifier NCT01306604 (all information can be found on https://clinicaltrials.gov/ct2/show/NCT01306604).

Consent for publication

The family gave consent for publication of the genetic and biochemical findings.

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References

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