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Abstract

Niemann Pick (NP) disease is a genetically heterogeneous metabolic disorder, for which initial symptoms and age at onset vary widely. The interpretation of novel variants in NP genes is facilitated by the availability of biochemical follow-up assays. We present a patient, in whom two heterozygous missense variants of uncertain significance in SMPD1 could be re-classified as likely pathogenic based on determining blood-based levels of a recently described NP-specific biomarker.

Keywords	biomarker; enzymatic testing; Niemann-Pick disease; SMPD1, variant classification

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Sirs,

on behalf of all my co-authors, I submit a manuscript entitled “Metabolic biomarker testing facilitates genetic diagnosis of Niemann-Pick disease by enabling classification of novel *SMPD1* variants” by Miyanawala et al. We would greatly appreciate if you considered this manuscript for publication as *Brief Communication* in *Molecular Genetics and Metabolism*.

We present a patient for which clinical investigation had raised a suspicion of Niemann-Pick (NP) disease. Standard genetic work-up revealed heterozygosity of two novel missense variants in *SMPD1*. Phasing could not be determined, and *in silico* pathogenicity predictions were inconclusive. Subsequent biochemical analyses including both enzymatic testing and quantification of the NP-specific biomarker Lyso-SM509 eventually enabled a diagnosis of NP disease type A/B.

Our study highlights the difficulties with interpretation of novel genetic variants in rare, genetically heterogeneous genetic conditions. It also exemplifies that distinct biochemical follow up approaches are available to reach a diagnostic statement in metabolic disorders, and that disease-specific metabolic biomarkers represent an attractive alternative to enzymatic testing in this respect. We therefore believe that our study should be of high interest to the readership of *Molecular Genetics and Metabolism*.

As reviewers competent to evaluate our work we can suggest:

- Matthis Synofzik; Center for Neurology and Hertie-Institute for Clinical Brain Research, Tübingen, Germany; Email: matthis.synofzik@uni-tuebingen.de
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- Ari Zimran; Shaare Zedek Medical Center, the Hebrew University-Hadassah Medical School, Shmu'el Bait St 12, Jerusalem, Israel; Email: azimran@gmail.com

We look forward to hearing from you and thank you for your efforts on our behalf!

Yours sincerely,

Vindhya Lakmali Miyanawala

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

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Author contributions: VLM, SV, and EJ saw and clinically diagnosed the patient; CB, SS, VK and CC performed molecular, biochemical and *in silico* analyses; EJ and AR designed the study; VLM, CB, EJ and AR drafted the manuscript; all authors read and approved the final version

Abstract

Niemann Pick (NP) disease is a genetically heterogeneous metabolic disorder, for which initial symptoms and age at onset vary widely. The interpretation of novel variants in NP genes is facilitated by the availability of biochemical follow-up assays. We present a patient, in whom two heterozygous missense variants of uncertain significance in *SMPD1* could be re-classified as likely pathogenic based on determining blood-based levels of a recently described NP-specific biomarker.

Keyword: biomarker; enzymatic testing; Niemann-Pick disease; SMPD1, variant classification

1. 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 towards a standardization of the variant classification process [1]. They emphasize that comprehensive consideration of arguments for and against pathogenicity is especially crucial for variants that have never been seen or reported before. Along this line, they also recommend to not only use genetic, but also non-genetic types of evidence. Inherited metabolic disorders are frequently associated with measurable changes at non-genetic levels, but a meaningful implementation into diagnostic workflows needs to be optimized [2]. We present a patient, in whom such extended analyses were essential for reaching a diagnosis, and suggest metabolic biomarkers as an attractive alternative to enzymatic testing for this purpose.

2. Materials and methods

The patient presented at Lady Ridgeway Hospital for Children (Colombo, Sri Lanka) at ten months of age. Detailed clinical examination as well as laboratory follow-up were initiated. Genetic screening for variants in the 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, SIFT, Align GVD, 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].

3. Results

3.1. Clinical findings

A Sri Lankan male infant had been born by elective caesarian section due to oligohydramniosis and breech presentation to reportedly non-consanguineous parents at 35 weeks gestation. Apart from neonatal jaundice, developed at day 4 and treated with phototherapy, he appeared normal. Around age six months, the parents started to note abdominal distension and motor delay. At ten months of age, the patient presented with hypotonia, large Mongolian blue spots and slight facial dysmorphism (low set ears, elongated face). Ultrasound abdomen showed enlargement of spleen and liver with normal vasculature and no focal lesions. Biochemistry testing revealed elevated liver transaminases, cholesterol and triglycerides. Foamy macrophages were detected upon 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.

3.2. Genetic and biochemical findings

Genetic analysis of all three known NP genes revealed 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, gnomAD) as well as from our in-house database CentoMD® [6], and *in silico* pathogenicity predictions were inconsistent (Figure 1B). Parental samples 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 (VUSs). 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 the lyso-sphingomyelin 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. way above the maximum of 0.9 ng/µl seen in healthy controls. Both variants could therefore be re-classified as “likely pathogenic” (ACMG pathogenicity class 2), and a corresponding genetic diagnosis be issued.

4. 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). The classification of these variants as likely pathogenic, however, required efforts beyond gene sequencing, namely biochemical analyses. 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. Mass spectrometry-based quantification of disease-specific metabolites from DBSs overcomes many of these issues [7]. Indeed, a corresponding approach has already been successfully implemented in studies on e.g. Gaucher disease [8]. A wider application of this concept may eventually improve diagnosis of lysosomal storage disorders and additional metabolic conditions [2].

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Conflict of interest

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

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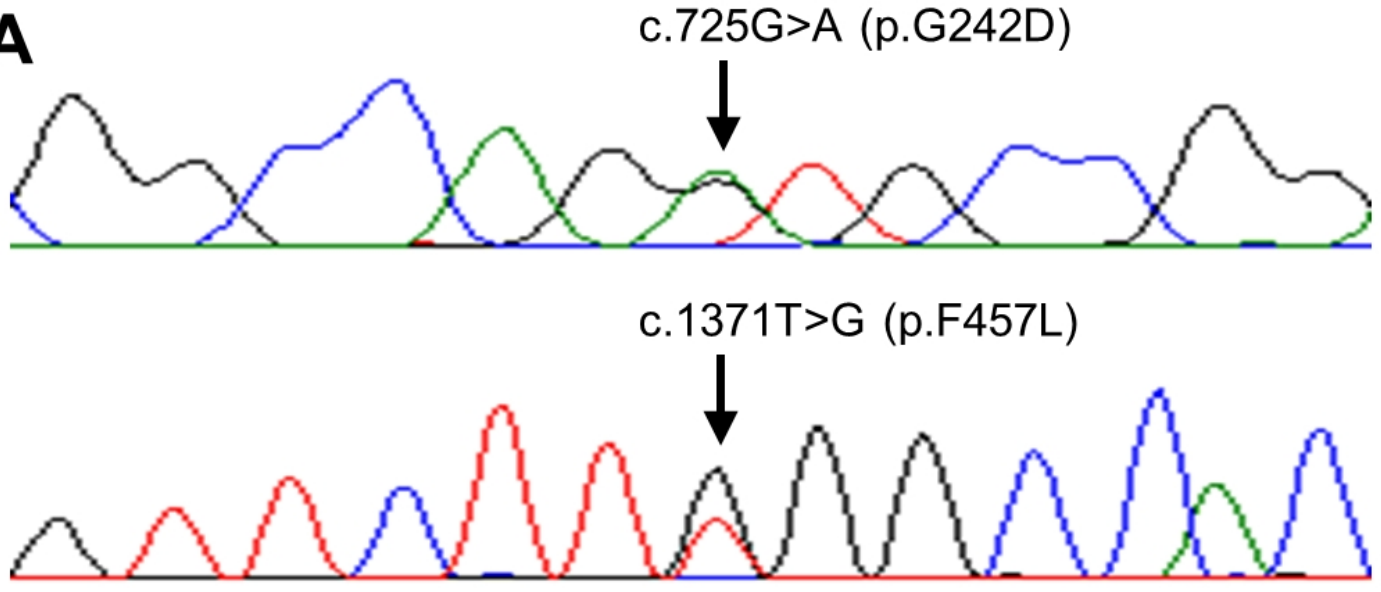
Figure legend

Figure 1: SMPD1 variants identified in the reported patient. **(A)** Sanger sequencing traces revealing heterozygosity for two single nucleotide substitutions, which are predicted to represent missense alteration. **(B)** *In silico* analysis of both alterations using four pathogenicity prediction tools.

A



B

	PolyPhen	SIFT	Align GVGD	Mutation Taster
p.G242D	possibly damaging	tolerated	tolerated	disease causing
p.F457L	probably damaging	deleterious	tolerated	disease causing