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CASE REPORT

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

Vindhya Lakmali Miyanawala[1*†], Christian Beetz[2†], Samantha Waidyanatha[1], Sabine Schröder[2], Vasiliki Karageorgou[2], Claudia Cozma[2], Eresha Jasinge[1], Arndt Rolfs[2,3]

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With the recent advancements in sequencing technologies, variant detection has been replaced by variant interpretation as the major challenge in genetic diagnostics.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),

Correspondence to: Vindhya Lakmali Miyanawala
*Department of Chemical Pathology, Lady Ridgeway Hospital for Children, Colombo 08, Sri Lanka.
Email: vindhyalkm@gmail.com
Full list of author information is available at the end of the article.
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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

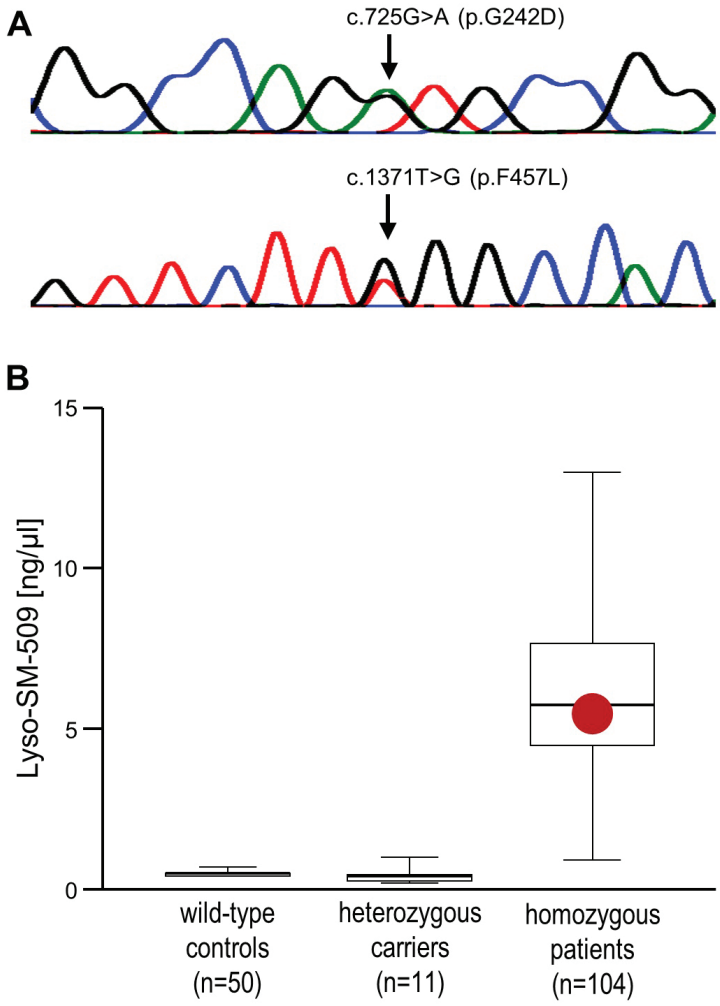

Figure 1. *Genetic and biomarker findings. (A) Sanger sequencing traces revealing heterozygosity for two single nucleotide SMPD1 substitutions, which are predicted to represent missense alteration. (B) Lyso-SM-509 values in controls, heterozygous carriers, and genetically confirmed patients. The value for the patient presented in the current study is depicted as a red circle.*

Table 1. *Allele frequencies and in silico prediction scores for the two SMPD1 variants identified by the present study. The prediction scores can range from 0 to 1, with higher scores indicating higher predicted pathogenicity.*

cDNA nomenclature	Protein nomenclature	Allele frequency in gnomAD	PolyPhen-2 prediction[a] (score)	SIFT prediction[b] (score)	MutationTaster prediction[c] (score)
c.725G > A	p.G242D	0.00	Possibly damaging (0.93)	Tolerated (0.10)	Disease-causing (0.99905)
c.1371T > G	p.F457L	0.00	Probably damaging (1.00)	Tolerated (0.05)	Disease-causing (0.99995)

[a]As determined on http://genetics.bwh.harvard.edu/pph2/ on 2019/10/04.

[b]As determined on https://sift.bii.a-star.edu.sg/ on 2019/10/04.

[c]As determined on http://www.mutationtaster.org/ on 2019/10/04.

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 lysosphingomyelin-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.

Author details

Vindhya Lakmali Miyanawala[1†], Christian Beetz[2†], Samantha Waidyanatha[1], Sabine Schröder[2], Vasiliki Karageorgou[2], Claudia Cozma[2], Eresha Jasinge[1], Arndt Rolfs[2,3]

1.Department of Chemical Pathology, Lady Ridgeway Hospital for Children, Colombo, Sri Lanka

2.CENTOGENE AG, Rostock, Germany

3.Rostock Medical University, Rostock, Germany

† These authors contributed equally.

References

1. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. 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 2015; 17(5):405–24; https://doi.org/10.1038/gim.2015.30

2. Cox TM. Biomarkers in lysosomal storage diseases: a review. Acta Paediatr Suppl 2005; 94(447):39–42; https://doi.org/10.1080/08035320510028085

3. Vanier MT. Niemann-Pick diseases. Handb Clin Neurol 2013; 113:1717–21; https://doi.org/10.1016/B978-0-444-59565-2.00041-1
4. McGovern MM, Dionisi-Vici C, Giugliani R, Hwu P, Lidove O, Lukacs Z, et al. Consensus recommendation for a diagnostic guideline for acid sphingomyelinase deficiency. Genet Med 2017; 19(9):967–74; https://doi.org/10.1038/gim.2017.7
5. Giese AK, Mascher H, Grittner U, Eichler S, Kramp G, Lukas J, et al. A novel, highly sensitive and specific biomarker for Niemann-Pick type C1 disease. Orphanet J Rare Dis 2015; 10:78; https://doi.org/10.1186/s13023-015-0274-1
6. Trujillano D, Oprea GE, Schmitz Y, Bertoli-Avella AM, Abou Jamra R, Rolfs A. A comprehensive global genotype-phenotype database for rare diseases. Mol Genet Genomic Med 2017; 5(1):66–75; https://doi.org/10.1002/mgg3.262
7. Wagner M, Tonoli D, Varesio E, Hopfgartner G. The use of mass spectrometry to analyze dried blood spots. Mass Spectrom Rev 2016; 35(3):361–438; https://doi.org/10.1002/mas.21441
8. Deodato F, Boenzi S, Taurisano R, Semeraro M, Sacchetti E, Carrozzo R, et al. The impact of biomarkers analysis in the diagnosis of Niemann-Pick C disease and acid sphingomyelinase deficiency. Clin Chim Acta 2018; 486:387–94; https://doi.org/10.1016/j.cca.2018.08.039
9. Elstein D, Mellgard B, Dinh Q, Lan L, Qiu Y, Cozma C, et al. Reductions in glucosylsphingosine (lyso-Gb1) in treatment-naive and previously treated patients receiving velaglucerase alfa for type 1 Gaucher disease: data from phase 3 clinical trials. Mol Genet Metab 2017; 122(1-2):113–20; https://doi.org/10.1016/j.ymgme.2017.08.005
10. Bauer P, Beetz C, Rolfs A. A call for considering biochemical concepts in clinical genetics research. J Biochem Clin Genet 2019; 2(1):1–2; https://doi.org/10.24911/JBCGenetics/183-1557963984