Molecular Genetics and Metabolism Reports 32 (2022) 100900

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: www.elsevier.com/locate/ymgmr

Short Communication

Acid sphingomyelinase deficiency: The clinical spectrum of 2 patients who carry the Q294K mutation and diagnostic challenges

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ARTICLE INFO

Keywords:
Acid sphingomyelinase deficiency type A/B
Intermediate-type acid sphingomyelinase deficiency
Lysosomal storage disorder
Niemann–Pick disease type A/B

ABSTRACT

Acid sphingomyelinase deficiency (ASMD) is caused by pathogenic variants in the *SMPD1* gene. This chronic, progressive, and potentially fatal condition requires prompt specialist care. The diagnosis of ASMD can be delayed or missed if patients that harbor the Q294K mutation undergo enzyme activity assessments that employ synthetic fluorometric substrates. Two case studies are presented, which illustrate the spectrum of disease in patients with a compound heterozygous Q294K pathogenic variant and the impact of false normal ASM activity results.

1. Introduction

Acid sphingomyelinase deficiency (ASMD) is a rare, debilitating, and potentially fatal lysosomal storage disorder (LSD) [1]. ASMD is caused by mutations in the *SMPD1* gene, which encodes acid sphingomyelinase (ASM). Reduced ASM activity leads to the progressive accumulation of sphingomyelin and other related lipids in organs [2].

To date, more than 200 *SMPD1* sequence variants have been associated with ASMD [2]. The range of mutations and other genetic and epigenetic factors results in a spectrum of disease trajectories and severity. Infantile neurovisceral ASMD (ASMD type A) is rapidly and uniformly fatal by the age of around 3 years [3]. These patients may exhibit symptoms including hepatosplenomegaly, fail to thrive or reach developmental milestones, and a proportion develop a cherry-red spot in the macula [3]. Chronic neurovisceral ASMD (ASMD type A/B) is thought to be an intermediate phenotype exhibiting slow progressive, variable visceral disease and neurodegeneration [4,5]. Chronic visceral disease (ASMD type B) has a slow progressive course with no, or little, neurologic involvement [6]; hepatosplenomegaly, indications of liver failure, reduced pulmonary function, elevations in serum triglycerides and LDL cholesterol, and a red/brown halo or cherry-red macular spot may also occur [7] ASMD was formally known as Niemann-Pick-A/B disease [6].

Management of ASMD aims to improve patient quality of life with palliative treatments and lifestyle modifications. Early access to a range of specialist medical practitioners is key to effectively managing the multisystem impact of ASMD.

Historically there have been no disease-specific treatments for ASMD, however, results from clinical trials in chronic non-neurological ASMD are encouraging [8–10]. Olipudase alfa was approved in Japan in March 2022.

Consensus recommendations for the diagnosis of ASMD suggest an ASM enzyme assay is performed prior to gene sequencing, which is considered diagnostic if two pathogenic variants of *SMPD1* are detected [11].

Q294K (also referred to as Q292K) [11,12] is a common chronic neurovisceral ASMD phenotype variant that is strongly linked with neurological involvement [4,5]. However, patients may be underdiagnosed when pseudo normal or even enhanced ASM activity is reported by ASM assays using synthetic fluorometric substrates [13–16].

Here we report the diagnostic challenges and disparate clinical course of two ASMD patients carrying the variant Q294K in heteroallelic form.

2. Patients

Patient 1 (a 21-year-old male) presented with splenomegaly and elevated chitotriosidase activity in early childhood. Gaucher's disease and ASMD were initially excluded by normal β-glucocerebrosidase activity in leukocytes and normal ASM activity assay [conducted with the

Abbreviations: HNP, 2-N-(hexadecanoyl)-amino-4-nitrophenyl phosphorylcholine; HMU-PC, 6-hexadecanoylamino-4-methylumbelliferylphosphorylcholine.

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https://doi.org/10.1016/j.ymgmr.2022.100900
Received 17 May 2022; Received in revised form 13 July 2022; Accepted 13 July 2022
Available online 19 July 2022

synthetic fluorometric substrate 2-N-(hexadecanoyl)-amino-4-nitrophenyl phosphorylcholine (HNP)] in leukocytes and fibroblasts. Niemann-Pick Disease Type C was diagnosed (aged 11) based on a variant filipin test, 1 mutation in the *NPC1*-gene (p.1007A), and clinical symptoms of splenomegaly and possible slow vertical saccades. Miglustat was initiated in 2012 but discontinued due to gastrointestinal symptoms and hand tremor. The patient has normal intelligence but developed psychiatric symptoms aged 13 and mild hepatomegaly. Additional genetic analysis (performed at the age of 15 because a second *NPC1* gene mutation could not be found) identified 2 mutations in the *SMPD1*-gene (Q294K and R293H), supporting a diagnosis of ASMD. Lung function tests were normal. However, a high-resolution CT of the lungs showed a granuloma in the right inferior lobe without signs of interstitial lung disease. The patient is currently stable and being treated for psychosis with amitriptyline.

Patient 2 (an 11-year-old female) presented with delayed speech development aged 4 years. Physical examination showed pronounced hepatosplenomegaly, unusual swelling of the cheeks and the periorbital tissue, abnormally thick scalp hair, and ataxia. Enzymatic testing for ASMD was not performed on this patient. Biochemical testing for other lysosomal storage diseases was normal. However, genetic analysis demonstrated 2 heterozygous mutations (p.Q294K and p.F482Wfs*20) in the *SMPD1*-gene, confirming a diagnosis of ASMD. Cognitive decline was exhibited from age 8 and epilepsy from age 9, which is stable under medication. A prolapsed mitral valve with moderate insufficiency and aortic insufficiency were noted following an ECG, and interstitial patterns were observed on chest X-rays. Currently, she suffers from epilepsy, spastic paraparesis, and severe mental retardation. An MRI showed white matter loss and severe cerebral atrophy.

3. Discussion

These case studies demonstrate the variability in disease course and severity of ASMD patients carrying the Q294K variant in heteroallelic form. Patient 1, who presented with a milder attenuated phenotype, carries R293H as a second variant. The question remains as to whether the psychiatric symptoms should be seen as a brain-organic manifestation of ASMD. Patient 2 has a more deleterious second mutation (p.F482Wfs*20) and exhibits a chronic-neurovisceral phenotype.

A study in Czech and Slovak patients revealed that Q294K is one of the most common variants in Eastern Europe [4]. Q294K present in either homoallelic or heteroallelic form was associated with a progressive neurovisceral phenotype [4]. Pavlů-Pereira et al. also observed that when the Q294K mutation was present in heteroallelic form, the clinical phenotype was dependent upon the second mutant allele [4]. Q294K in association with less deleterious, high ASM activity alleles resulted in a milder attenuated phenotype [4]. A large US-based study of ASMD type B patients revealed that neurologic abnormalities occurred in 30% of patients and that their disease course was either “severe and progressive or minor and static. [5]” Progressive disease was only seen in patients carrying the Q294K variant [5].

ASMD diagnosis can be delayed because patients present with multiple complex symptoms that overlap with other LSDs. Diagnosis of ASMD type B is further confounded by the wide spectrum of disease manifestations. Basic diagnostic tools, such as the ASM enzyme assay, are fundamental to prompt and accurate diagnosis.

Diagnostic ASM assays were rarely performed with radiolabeled sphingomyelin (SPM) (the natural substrate of ASM). Synthetic substrates were adopted as they provided a safer alternative [13]. However, the evaluation of patients with the Q294K variant demonstrated pseudo normal ASM activity in patients (who were subsequently discovered to have ASMD) when tests were conducted using the synthetic fluorometric substrates HNP [13] or 6-Hexadecanoylamino-4-methylumbelliferylphosphorylcholine (HMU-PC) substrates [14]. Harzer et al. have postulated that the substitution of a positively charged amino acid for one that is uncharged, such as the substitution of lysine for glutamine in the Q294K mutation, produced a defective enzyme (with reduced affinity for SPM) but with an unexpectedly high affinity for the fluorometric HNP substrate [13] Other *SMPD-1* variants can give rise to pseudo normal ASM assay results. This was observed in 2 ASMD type B patients (sisters) who were compound heterozygotes for p.C92W and p.P184L mutations when ASM activity was estimated using a synthetic short-chain C6-SPM substrate [15]. In our experience, ASMD diagnosis in patient 1 was delayed by pseudo normal ASM assay readouts obtained using a synthetic fluorometric (HNP) substrate.

To circumvent misdiagnosis, genetic sequencing, alternative methods, substrates, and assay conditions should be considered. Consensus guidelines have been drawn up that include best practice recommendations for ASM activity estimation; these state that tandem mass spectrometry (MS/MS) is the method of choice [11]. The synthetic substrate employed in the tandem mass spectrometry is a close analog of natural SPM that differs only in the length of its fatty acyl chain [16]. Tandem mass spectrometry analysis offers several advantages over fluorometric analysis:- i) an enhanced analytical range, ii) more accuracy in the lower ranges of detection, and iii) it can be multiplexed with other analytes to exclude differential diagnoses [11]. Dried blood spot screening arrays utilizing tandem mass spectrometry-based analysis have been developed for LSDs, including ASMD [16,17]. Van Diggelen et al. advocate that ASM assays should be carried out using synthetic substrates in the presence and absence of natural substrate SPM [14]. Synthetic substrate fatty acyl chain length and the type and concentration of surfactant used can influence ASM assay sensitivity and the propensity for false-negative readouts [15].

4. Conclusion

The wide spectrum of disease manifestation illustrated by these case studies in patients carrying the Q294K variant increases the challenge of diagnosing ASMD. This is compounded by pseudo normal results obtained from ASM assays employing fluorometric substrates, leading to substantial underdiagnosis in patients with the Q294K mutation. Best practice guidelines recommend the use of the tandem mass spectrometry ASM assay, which will provide a reliable diagnosis in Q294K-dependent cases. Missed ASMD diagnosis would deny patients prompt and appropriate care and may prevent access to treatment. Therefore, it is pivotal to carefully select the assay/methodology used to estimate ASM activity in this patient group.

Author statement

None.

Declaration of Competing Interest

YA and EM have received speaker honoraria from Sanofi. There are no other no competing interests.

Data availability

The data that has been used is confidential.

Acknowledgments

The authors respectfully acknowledge the two patients whose cases were presented in this manuscript. Writing support was provided by Julia Jenkins, Ph.D., of GK Pharmacomm Ltd. and funded via an unrestricted grant from Sanofi.

References

[1] M.M. McGovern, M.P. Wasserstein, B. Bembi, R. Giugliani, K.E. Mengel, M. T. Vanier, Q. Zhang, M.J. Peterschmitt, Prospective study of the natural history of

chronic acid sphingomyelinase deficiency in children and adults: eleven years of observation, Orphanet Journal of Rare Diseases. 16 (2021) 212, https://doi.org/10.1186/s13023-021-01842-0.
[2] C. Pinto, D. Sousa, V. Ghilas, A. Dardis, M. Scarpa, M.F. Macedo, Acid sphingomyelinase deficiency: a clinical and immunological perspective, Int. J. Mol. Sci. 22 (2021) 12870, https://doi.org/10.3390/ijms222312870.
[3] M.M. McGovern, A. Aron, S.E. Brodie, R.J. Desnick, M.P. Wasserstein, Natural history of Type A Niemann-Pick disease: possible endpoints for therapeutic trials, Neurology. 66 (2006) 228–232, https://doi.org/10.1212/01.wnl.0000194208.08904.0c.
[4] H. Pavlů-Pereira, B. Asfaw, H. Poupctová, J. Ledvinová, J. Sikora, M.T. Vanier, K. Sandhoff, J. Zeman, Z. Novotná, D. Chudoba, M. Elleder, Acid sphingomyelinase deficiency. Phenotype variability with prevalence of intermediate phenotype in a series of twenty-five Czech and Slovak patients. A multi-approach study, J. Inherit. Metab. Dis. 28 (2005) 203–227, https://doi.org/10.1007/s10545-005-5671-5.
[5] M. Wasserstein, A. Aron, S. Brodie, C. Simonaro, R. Desnick, M. Mcgovern, Acid sphingomyelinase deficiency: prevalence and characterization of an intermediate phenotype of NIEMANN-PICK disease, J. Pediatr. 149 (2006) 554–559, https://doi.org/10.1016/j.jpeds.2006.06.034.
[6] M.M. McGovern, R. Avetisyan, B.-J. Sanson, O. Lidove, Disease manifestations and burden of illness in patients with acid sphingomyelinase deficiency (ASMD), Orphanet J Rare Dis. 12 (2017) 41, https://doi.org/10.1186/s13023-017-0572-x.
[7] E.H. Schuchman, R.J. Desnick, Types A and B Niemann-Pick disease, Mol. Genet. Metab. 120 (2017) 27–33, https://doi.org/10.1016/j.ymgme.2016.12.008.
[8] M.P. Wasserstein, S.A. Jones, H. Soran, G.A. Diaz, N. Lippa, B.L. Thurberg, K. Culm-Merdek, E. Shamiyeh, H. Inguilizian, G.F. Cox, A.C. Puga, Successful within-patient dose escalation of olipudase alfa in acid sphingomyelinase deficiency, Mol. Genet. Metab. 116 (2015) 88–97, https://doi.org/10.1016/j.ymgme.2015.05.013.
[9] M.P. Wasserstein, G.A. Diaz, R.H. Lachmann, M.-H. Jouvin, I. Nandy, A.J. Ji, A. C. Puga, Olipudase alfa for treatment of acid sphingomyelinase deficiency (ASMD): safety and efficacy in adults treated for 30 months, J. Inherit. Metab. Dis. 41 (2018) 829–838, https://doi.org/10.1007/s10545-017-0123-6.
[10] G.A. Diaz, S.A. Jones, M. Scarpa, K.E. Mengel, R. Giugliani, N. Guffon, I. Batsu, P. A. Fraser, J. Li, Q. Zhang, C. Ortemann-Renon, One-year results of a clinical trial of olipudase alfa enzyme replacement therapy in pediatric patients with acid sphingomyelinase deficiency, Genet Med. 23 (2021) 1543–1550, https://doi.org/10.1038/s41436-021-01156-3.
[11] M.M. McGovern, C. Dionisi-Vici, R. Giugliani, P. Hwu, O. Lidove, Z. Lukacs, K. Eugen Mengel, P.K. Mistry, E.H. Schuchman, M.P. Wasserstein, Consensus recommendation for a diagnostic guideline for acid sphingomyelinase deficiency, Genet Med. 19 (2017) 967–974, https://doi.org/10.1038/gim.2017.7.
[12] M. Wasserstein, C. Dionisi-Vici, R. Giugliani, W.-L. Hwu, O. Lidove, Z. Lukacs, E. Mengel, P.K. Mistry, E.H. Schuchman, M. McGovern, Recommendations for clinical monitoring of patients with acid sphingomyelinase deficiency (ASMD), Mol. Genet. Metab. 126 (2019) 98–105, https://doi.org/10.1016/j.ymgme.2018.11.014.
[13] K. Harzer, A. Rolfs, P. Bauer, M. Zschiesche, E. Mengel, J. Backes, B. Kustermann-Kuhn, G. Bruchelt, O.P. van Diggelen, H. Mayrhofer, I. Krägeloh-Mann, Niemann-Pick disease type A and B are clinically but also enzymatically heterogeneous: pitfall in the laboratory diagnosis of sphingomyelinase deficiency associated with the mutation Q292 K, Neuropediatrics. 34 (2003) 301–306, https://doi.org/10.1055/s-2003-44668.
[14] O.P. van Diggelen, Ya.V. Voznyi, J.L.M. Keulemans, K. Schoonderwoerd, J. Ledvinova, E. Mengel, M. Zschiesche, R. Santer, K. Harzer, A new fluorimetric enzyme assay for the diagnosis of Niemann–Pick A/B, with specificity of natural sphingomyelinase substrate, J. Inherit. Metab. Dis. 28 (2005) 733–741, https://doi.org/10.1007/s10545-005-0105-y.
[15] W.-L. Chuang, J. Pacheco, S. Cooper, J.S. Kingsbury, J. Hinds, P. Wolf, P. Oliva, J. Keutzer, G.F. Cox, K. Zhang, Improved sensitivity of an acid sphingomyelinase activity assay using a C6:0 sphingomyelin substrate, Mol Genet Metab Rep. 3 (2015) 55–57, https://doi.org/10.1016/j.ymgmr.2015.04.001.
[16] F. Ghomashchi, M. Barcenas, F. Turecek, C.R. Scott, M.H. Gelb, Reliable assay of acid sphingomyelinase deficiency with the mutation Q292K by tandem mass spectrometry, Clin. Chem. 61 (2015) 771–772, https://doi.org/10.1373/clinchem.2014.236448.
[17] Y. Li, C.R. Scott, N.A. Chamoles, A. Ghavami, B.M. Pinto, F. Turecek, M.H. Gelb, Direct multiplex assay of lysosomal enzymes in dried blood spots for newborn screening, Clin. Chem. 50 (2004) 1785–1796, https://doi.org/10.1373/clinchem.2004.035907.