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Late Infantile Metachromatic Leukodystrophy Due to Novel Pathogenic Variants in the *PSAP* Gene

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Abstract

Impairment of saposin B causes rare atypical metachromatic leukodystrophy (MLD). It is encoded (together with saposin A, C, and D) by the *PSAP* gene. Only ten pathogenic variants were described in the PSAP gene in MLD patients to date. We report on two novel variants in the *PSAP* gene - c.679_681delAAG in the saposin B encoding exon 6 and c.1268delT in the saposin D encoding exon 11 in a patient with MLD. We discuss the fact, that variants resulting in PSAP null allele can be shared in patients with the deficit of other saposins (A–D) or whole prosaposin. The patient's phenotype depends then on the nature of the second allele - atypical Gaucher disease in case of saposin A, MLD in case of saposin B, and Krabbe disease in case of saposin C impairing mutations. The clinically most severe prosaposin deficit is caused by the presence of two PSAP null alleles. Thus, the assessment of a variant impact is needed to prevent delayed diagnosis or misdiagnosis in patients with *PSAP* mutations.

Keywords Metachromatic leukodystrophy · Saposin B deficiency · *PSAP* gene

Introduction

Metachromatic leukodystrophy (MLD) is an autosomal recessive neurodegenerative disorder with progressive demyelination and dysfunction of the central and peripheral nervous system. The disease is caused by mutations in the *ARSA* gene which encodes the lysosomal enzyme arylsulfatase A, or, more rarely, by mutations in the *PSAP* gene affecting function of saposin B. The *PSAP* gene (MIM #176801, 10q22.1, 14 exons, GenBank accession numbers NM_002778.2 and NP_002769.1) encodes a 524 amino-acid precursor protein prosaposin (pSap), that is cleaved to four small glycoproteins—saposins (SapA, B, C, and D) (Fig. 1a) (Kishimoto et al. 1992). Saposins are sphingolipid activator proteins required for the function of specific lysosomal hydrolases. Mutations in the *PSAP* gene can cause a deficiency of either the entire pSap protein (null alleles) or an individual saposin. Patients with two null alleles present with pSap deficiency and fatal infantile lysosomal storage disease (Elleder et al. 2005; Motta et al. 2016). Patients with at least one allele with a mutation impairing only isolated saposins present with disorders resembling impairment of particular hydrolases. Defects of SapB result in MLD due to impaired degradation and accumulation of cerebroside-3-sulfate (sulfatide), which is in the normal state catabolized by the arylsulfatase A (Wrobe et al. 2000). Defects in SapA and SapC lead to atypical Krabbe and Gaucher diseases, respectively (Spiegel et al. 2005; Vaccaro et al. 2010). SapD deficiency was reported only in mice and resembled Farber disease (Matsuda et al. 2004).

While more than 250 ARSA causative variants were described to date (Bohringer et al. 2017; Cesani et al. 2016; Golchin et al. 2017; Shahzad et al. 2017; Stoeck et al. 2016), only 10 PSAP pathogenic variants found in 26 MLD patients

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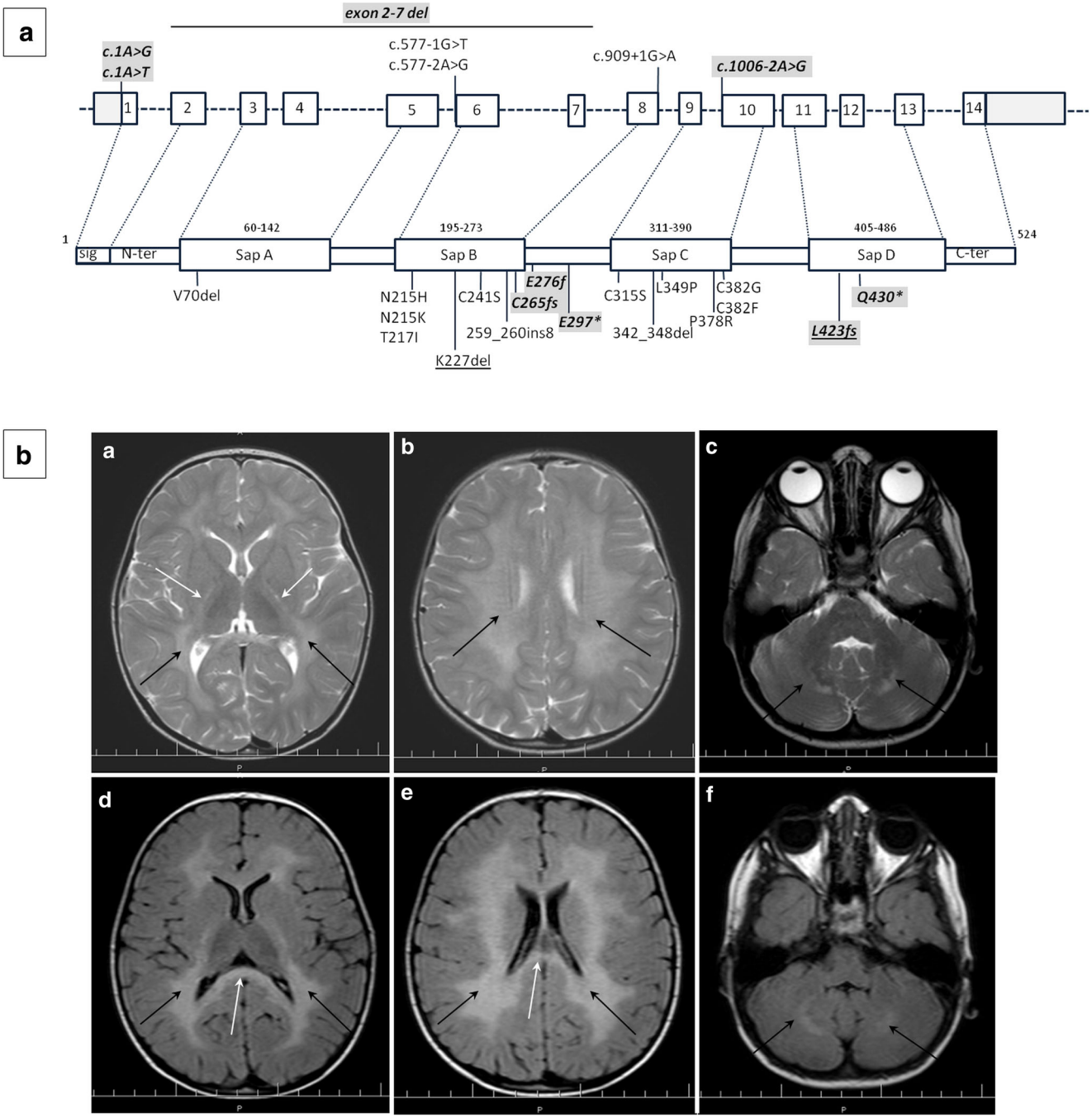

Fig. 1 **a** Variants reported in patients with MLD, Krabbe disease, Gaucher disease, or prosaposin deficiency. Variants in shaded frame result in a null allele. Underlined variants were identified in this study. **b** Diffuse white matter changes, hypersignal intensity in T2-weighted images (a, b, c – black arrows) and FLAIR (d, e, f – black arrows) localized supra - and infratentorially on both sides, saving the juxtacortical region and U-fibers, pathological findings in the dorsal capsula interna (white arrows a), corpus callosum (white arrows d, e)

were published in the literature (reviewed in (Cesani et al. 2016)). Majority of them, 17 patients, presented with the late infantile form, 6 with juvenile–onset disease, and 1 with adult MLD; 2 patients were reported as being still asymptomatic.

Here, we describe a case of a patient with late infantile MLD due to two novel compound heterozygote mutations, one in the SapB portion of the *PSAP* gene and the other in a SapD encoding exon, but resulting in a null allele, presumably.

Case Description

We report a boy from the first pregnancy of non-consanguineous parents. The mother suffered from gallbladder colics during gestation. He was born on term, birth weight 3380 g, length 50 cm; post-natal adaptation period and psychomotor development until the age of 22 months were nearly normal, including speech and walking development (he walked clumsily but without support).

At the age of 2 years, he was examined by a neurologist for the first time for walking disturbance. Findings suggesting spastic diparesis were dominant in neurological examination, evolving into tetraparesis with psychomotor regression over the next 10 months. Brain findings included diffuse hypersignal intensity changes in T2-weighted and FLAIR images supra- and infratentorially on both sides, saving the juxtacortical region and U-fibers. Pathological findings were in the dorsal capsula interna, corpus callosum, and white matter of both cerebral hemispheres. These findings were consistent with MRI changes in MLD (Fig. 1b).

Cerebrospinal fluid examination revealed mildly increased protein content of 0.696 g/l (reference range 0.190–0.310) and slightly elevated lactate—2.55 mmol/l (reference range 1.20–2.10); other parameters were within reference values. Electromyography examination showed combined axonal-demyelinating polyneuropathy; abdomen ultrasound was normal. We focused on the determination of enzymatic levels of arylsulphatase A activity in leukocytes, which was 60.9 nmol/15 h/mg (reference ranges 50.0–125.0 nmol/15 h/mg). Urinary excretion of sulfatides was clearly increased (qualitative analysis—26.0 nmol/l (reference ranges 6.36–13.55), quantitative

Table 1 Characteristics of the *PSAP* alleles up to date

Location	DNA[a]	Protein	Variant type	Domain	Patient phenotype	Null allele	Reference
Exon1	c.1A > G	p.Met1Val	Start loss	Init. codon	MLD, Gaucher	**Y**	(Deconinck et al. 2008; Tylki-Szymanska et al. 2007)
Exon1	c.1A > T	p.Met1Leu	Start loss	Init. codon	Gaucher	**Y**	(Elleder et al. 2005; Tylki-Szymanska et al. 2007)
Exon 3	c.207_209del	p.Val70del	Deletion	SapA	Krabbe	N	(Spiegel et al. 2005)
Intron 5	c.577-1G > T	p.Asp193_ Ile240del; p.Asp193_ Gln199del	Splicing	SapB	MLD	N	(Henseler et al. 1996)
Intron 5	c.577-2A > G	p.Asp193_ Ile240del; p.Asp193_ Gln199del	Splicing	SapB	MLD	N	(Kuchar et al. 2009)
Exon 6	c.643A > C	p.Asn215His	Missense	SapB	MLD	N	(Wrobe et al. 2000)
Exon 6	c.645C > A	p.Asn215Lys	Missense	SapB	MLD	N	(Regis et al. 1999) (Deconinck et al. 2008)
Exon 6	c.650C > T	p.Thr217Ile	Missense	SapB	MLD	N	(Kretz et al. 1990)
Exon 6	c.679_681delAAG	p. Lys227del	Deletion	SapB	MLD	N	This study
Exon 7	c.722G > C	p.Cys241Ser	Missense	SapB	MLD	N	(Holtschmidt et al. 1991)
Exon 7–8	c.777_778ins24[b]	p.Met259_ Gln260ins8	Splicing	SapB	MLD	N	(Zhang et al. 1991)
Exon 8	c.794delG	p.Cys265Leufs*10	Frameshift	SapB	pSap-d	**Y**	(Hulkova et al. 2001)
Exon 8	c.828_829delGA	p. Glu276Aspfs*27	Frameshift	LR	MLD, pSap-d	**Y**	(Kuchar et al. 2009; Motta et al. 2016)
Exon 8	c.889G > T	p.Glu297*	Nonsense	LR	pSap-d	**Y**	(Motta et al. 2016)
Intron 8	c.909 + 1G > A	p.Gln260_Lys303del	Splicing	SapB + LR	MLD	N	(Siri et al. 2014)
Exon 9	c.943 T > A	p.Cys315Ser	Missense	SapC	Gaucher	N	(Vaccaro et al. 2010)
Intron 9	c.1006-2A > G	p.?	Splicing	SapC	pSap-d	**Y**	(Kuchar et al. 2009)
Exon 10	c.1024_1044del	p.Phe342_Lys348del	Deletion	SapC	Gaucher	N	(Vaccaro et al. 2010)
Exon 10	c.1046 T > C	p.Leu349Pro	Missense	SapC	Gaucher	N	(Tylki-Szymanska et al. 2007)
Exon 10	c.1133C > G	p.Pro378Arg	Missense	SapC	Gaucher	N	(Kang et al. 2018)
Exon 10	c.1144 T > G	p.Cys382Gly	Missense	SapC	Gaucher	N	(Tamargo et al. 2012)
Exon 10	c.1145G > T	p.Cys382Phe	Missense	SapC	Gaucher	N	(Tamargo et al. 2012)
Exon 11	c.1268delT	p.Leu423Argfs*40	Frameshift	SapD	MLD	**Y**	This study
Exon 11	c.1288C > T	p.Gln430*	Nonsense	SapD	Gaucher	**Y**	(Diaz-Font et al. 2005)
Exons 2–7	Deletion of exons 2–7	–	Frameshift	–	Gaucher	**Y**	(Kang et al. 2018)

[a] Numbering according to GenBank RefSeq NM_002778.2, where c.1 position corresponds to the A of the initiation codon AUG

[b] The insertion is a result of a deep intronic C to A transversion creating a new acceptor splice site and formation of a new 24 bp exon

Init. initiation, *LR* - linking region, *MLD* metachromatic leukodystrophy, *pSap-d* prosaposin deficiency, *N* no, *Y* yes

analysis 5623 nmol/l (reference ranges 73–326). The patient was strongly suspect of SapB protein deficiency.

Currently, at the age of 4 years, the boy suffers from profound psychomotor regression with minimal motor activity, pronounced spastic paraparesis, and no verbal expression; he is relatively well responding to speech and sounds and has no swallowing difficulties. There are no epileptic seizures present.

Genetic testing by Sanger sequencing of the patient's DNA revealed the presence of compound heterozygous variants c.679_681delAAG (p.Lys227del) and c.1268delT (p.Leu423Argfs*40) in the *PSAP* gene (Supplementary Figure). Segregation analysis in both parents confirmed variant c. 679_681delAAG in the father, and variant c.1268delT in the mother.

The variant c.679_681delAAG (p.Lys227del) is located in exon 6 and causes deletion of lysine 227 in SapB protein. This variant was found only in one heterozygous individual in the GO ESP project database (Exome Variant Server) but is absent from ExAC and gnomAD databases (Exome Variant Server; Lek et al. 2016). It has not been described in relation to a disease. In silico tool PROVEAN capable of assessment of deletions (Choi and Chan 2015) predicts this variant to be deleterious (score −9.19, cutoff −2.5). Additionally, as we have found this variant in *trans* with another pathogenic allele and the patient's phenotype corresponds with MLD caused by mutations in the *PSAP* gene, it can be classified as likely pathogenic according to ACMG guidelines (Richards et al. 2015).

The variant c.1268delT is located in exon 11 encoding the SapD protein. It causes the p.Leu423Argfs*40 frameshift resulting in a premature termination codon. This variant leads to a null allele as such aberrant transcript should be degraded in the process of nonsense-mediated decay. This variant has not been previously reported in patients nor publicly available population databases. Together with the patient's specific phenotype, there is enough evidence to classify this variant as pathogenic (classification according to (Richards et al. 2015)).

Both variants were added to the locus-specific database LOVD (www.lovd.nl/PSAP, IDs PSAP_000017 and PSAP_000018) and submitted to ClinVar (www.ncbi.nlm.nih.gov/clinvar/, accession numbers SCV000809015 and SCV000809016).

Discussion

The patient discussed in this report was highly suspected of MLD due to deficiency of saposin B protein according to clinical findings and biochemical studies. This diagnosis was confirmed by sequencing analysis of the *PSAP* gene where we have found two variants.

In the published cohort of 26 PSAP – MLD patients (Cesani et al. 2016), only two cases were compound heterozygotes carrying a SapB impairing variant and a null allele: p.Asn215Lys + p.Met1Val (Deconinck et al. 2008) and p.Asp193_Ile240del; p.Asp193_Gln199del + p.Lys275fs (Kuchar et al. 2009) with the former one being the only case with a mutation outside the SapB encoding exons 6–8. Here, we describe another such case, one single amino acid deletion located in exon 6 encoding SapB and one frameshift variant located in the exon 11 encoding SapD. The phenotype of the patient confirms that the frameshift variant c.1268delT (p.Leu423fs) in exon 11 results in a null allele and he suffers from an isolated SapB deficiency due to the p.Lys227del allele in exon 6. Importantly, further *PSAP* null alleles were described in prosaposin or SapC deficiency (Table 1) and could be possibly involved also in MLD if combined with a mutation within the SapB portion of the gene. On the other side, some of the splicing mutations in the *PSAP* gene cause only isolated saposin deficit due to skipping of exons causing in-frame deletions (Table 1, Fig. 1). Therefore, it might be necessary to analyze the effect of novel splicing mutations on the mRNA level to confirm the genetic etiology of the disease definitively.

Furthermore, it might be noteworthy that all three reported patients with one null allele presented with the late infantile form of MLD, as well as the majority of patients with both mutations impairing selectively the SapB protein. The clinical course of their disease was similarly severe, without substantial differences.

In conclusion, our data update the *PSAP* gene mutation list and show a new example of how a mutation in a non-SapB exon can contribute to isolated SapB deficiency. Awareness of this might be crucial to prevent delayed diagnosis or misdiagnosis and to promptly provide an accurate genetic counseling to families.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

Bohringer J et al (2017) Enzymatic characterization of novel arylsulfatase a variants using human arylsulfatase A-deficient immortalized mesenchymal stromal cells. Hum Mutat 38:1511–1520. https://doi.org/10.1002/humu.23306

Cesani M, Lorioli L, Grossi S, Amico G, Fumagalli F, Spiga I, Filocamo M, Biffi A (2016) Mutation update of ARSA and PSAP genes causing metachromatic Leukodystrophy. Hum Mutat 37:16–27. https://doi.org/10.1002/humu.22919

Deconinck N, Messaaoui A, Ziereisen F, Kadhim H, Sznajer Y, Pelc K, Cécile Nassogne M, Vanier MT, Dan B (2008) Metachromatic leukodystrophy without arylsulfatase a deficiency: a new case of saposin-B deficiency. Eur J Paediatr Neurol 12:46–50. https://doi.org/10.1016/j.ejpn.2007.05.004

Diaz-Font A, Cormand B, Santamaria R, Vilageliu L, Grinberg D, Chabas A (2005) A mutation within the saposin D domain in a Gaucher disease patient with normal glucocerebrosidase activity. Hum Genet 117:275–277. https://doi.org/10.1007/s00439-005-1288-x

Elleder M, Jeřábková M, Befekadu A, Hřebíček M, Berná L, Ledvinová J, Hůlková H, Rosewich H, Schymik N, Paton BC, Harzer K (2005) Prosaposin deficiency – a rarely diagnosed, rapidly progressing, neonatal neurovisceral lipid storage disease. Report of a further patient. Neuropediatrics 36:171–180. https://doi.org/10.1055/s-2005-865608

Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA (URL: http://evs.gs.washington.edu/EVS/. Accessed July 2018)

Golchin N, Hajjari M, Malamiri RA, Aminzadeh M, Mohammadi-Asl J (2017) Identification of a novel mutation in ARSA gene in three patients of an Iranian family with metachromatic leukodystrophy disorder. Genet Mol Biol 40:759–762. https://doi.org/10.1590/1678-4685-GMB-2016-0110

Henseler M, Klein A, Reber M, Vanier MT, Landrieu P, Sandhoff K (1996) Analysis of a splice-site mutation in the sap-precursor gene of a patient with metachromatic leukodystrophy. Am J Hum Genet 58:65–74

Holtschmidt H, Sandhoff K, Kwon HY, Harzer K, Nakano T, Suzuki K (1991) Sulfatide activator protein. Alternative splicing that generates three mRNAs and a newly found mutation responsible for a clinical disease. J Biol Chem 266:7556–7560

Hulkova H et al (2001) A novel mutation in the coding region of the prosaposin gene leads to a complete deficiency of prosaposin and saposins, and is associated with a complex sphingolipidosis dominated by lactosylceramide accumulation. Hum Mol Genet 10:927–940

Choi Y, Chan AP (2015) PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels. Bioinformatics 31:2745–2747. https://doi.org/10.1093/bioinformatics/btv195

Kang L, Zhan X, Ye J, Han L, Qiu W, Gu X, Zhang H (2018) A rare form of Gaucher disease resulting from saposin C deficiency. Blood Cells Mol Dis 68:60–65. https://doi.org/10.1016/j.bcmd.2017.04.001

Kishimoto Y, Hiraiwa M, O'Brien JS (1992) Saposins: structure, function, distribution, and molecular genetics. J Lipid Res 33:1255–1267

Kretz KA, Carson GS, Morimoto S, Kishimoto Y, Fluharty AL, O'Brien JS (1990) Characterization of a mutation in a family with saposin B deficiency: a glycosylation site defect Proceedings of the National Academy of Sciences of the United States of America 87:2541–2544

Kuchar L et al (2009) Prosaposin deficiency and saposin B deficiency (activator-deficient metachromatic leukodystrophy): report on two patients detected by analysis of urinary sphingolipids and carrying novel PSAP gene mutations. Am J Med Genet A 149A:613–621. https://doi.org/10.1002/ajmg.a.32712

Lek M et al (2016) Analysis of protein-coding genetic variation in 60,706 humans. Nature 536:285–291. https://doi.org/10.1038/nature19057

Matsuda J, Kido M, Tadano-Aritomi K, Ishizuka I, Tominaga K, Toida K, Takeda E, Suzuki K, Kuroda Y (2004) Mutation in saposin D domain of sphingolipid activator protein gene causes urinary system defects and cerebellar Purkinje cell degeneration with accumulation of hydroxy fatty acid-containing ceramide in mouse. Hum Mol Genet 13:2709–2723. https://doi.org/10.1093/hmg/ddh281

Motta M, Tatti M, Furlan F, Celato A, di Fruscio G, Polo G, Manara R, Nigro V, Tartaglia M, Burlina A, Salvioli R (2016) Clinical, biochemical and molecular characterization of prosaposin deficiency. Clin Genet 90:220–229. https://doi.org/10.1111/cge.12753

Regis S, Filocamo M, Corsolini F, Caroli F, Keulemans JL, van Diggelen OP, Gatti R (1999) An Asn > Lys substitution in saposin B involving a conserved amino acidic residue and leading to the loss of the single N-glycosylation site in a patient with metachromatic leukodystrophy and normal arylsulphatase A activity. Eur J Human Genet: EJHG 7:125–130. https://doi.org/10.1038/sj.ejhg.5200266

Richards S et al. (2015) 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 Genetics in medicine : official journal of the American College of Medical Genetics 17:405–424 doi:https://doi.org/10.1038/gim.2015.30

Shahzad MA, Khaliq S, Amar A, Mahmood S (2017) Metachromatic leukodystrophy (MLD): a Pakistani family with novel ARSA gene mutation. J Mol Neurosci: MN 63:84–90. https://doi.org/10.1007/s12031-017-0959-0

Siri L, Rossi A, Lanza F, Mazzotti R, Costa A, Stroppiano M, Gaiero A, Cohen A, Biancheri R, Filocamo M (2014) A novel homozygous splicing mutation in PSAP gene causes metachromatic leukodystrophy in two Moroccan brothers. Neurogenetics 15:101–106. https://doi.org/10.1007/s10048-014-0390-4

Spiegel R, Bach G, Sury V, Mengistu G, Meidan B, Shalev S, Yona Shneor, Mandel H, Zeigler M (2005) A mutation in the saposin A coding region of the prosaposin gene in an infant presenting as Krabbe disease: first report of saposin A deficiency in humans. Mol Genet Metab 84:160–166

Stoeck K, Psychogios MN, Ohlenbusch A, Steinfeld R, Schmidt J (2016) Late-onset metachromatic leukodystrophy with early onset dementia associated with a novel missense mutation in the arylsulfatase a gene. J Alzheim Dis: JAD 51:683–687. https://doi.org/10.3233/JAD-150819

Tamargo RJ, Velayati A, Goldin E, Sidransky E (2012) The role of saposin C in Gaucher disease. Mol Genet Metab 106:257–263. https://doi.org/10.1016/j.ymgme.2012.04.024

Tylki-Szymanska A et al (2007) Non-neuronopathic Gaucher disease due to saposin C deficiency. Clin Genet 72:538–542. https://doi.org/10.1111/j.1399-0004.2007.00899.x

Vaccaro AM, Motta M, Tatti M, Scarpa S, Masuelli L, Bhat M, Vanier MT, Tylki-Szymanska A, Salvioli R (2010) Saposin C mutations in Gaucher disease patients resulting in lysosomal lipid accumulation, saposin C deficiency, but normal prosaposin processing and sorting. Hum Mol Genet 19:2987–2997. https://doi.org/10.1093/hmg/ddq204

Wrobe D, Henseler M, Huettler S, Pascual Pascual SI, Chabas A, Sandhoff K (2000) A non-glycosylated and functionally deficient mutant (N215H) of the sphingolipid activator protein B (SAP-B) in a novel case of metachromatic leukodystrophy (MLD). J Inherit Metab Dis 23:63–76

Zhang XL, Rafi MA, DeGala G, Wenger DA (1991) The mechanism for a 33-nucleotide insertion in mRNA causing sphingolipid activator protein (SAP-1)-deficient metachromatic leukodystrophy. Hum Genet 87:211–215