Review article

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Spinal muscular atrophy associated with progressive myoclonus epilepsy

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ABSTRACT – A rare syndrome characterized by lower motor neuron disease associated with progressive myoclonic epilepsy, referred to as "spinal muscular atrophy associated with progressive myoclonic epilepsy" (SMA-PME), has been described in childhood and is inherited as an autosomal recessive trait. SMA-PME is caused by mutation in the *ASAH1* gene encoding acid ceramidase. Ceramide and the metabolites participate in various cellular events as lipid mediators. The catabolism of ceramide in mammals occurs in lysosomes through the activity of ceramidase. Three different ceramidases (acid, neutral and alkaline) have been identified and appear to play distinct roles in sphingolipid metabolism. The enzymatic activity of acid ceramidase is deficient in two rare inherited disorders; Farber disease and SMA-PME. Farber disease is a very rare and severe autosomal recessive condition with a distinct clinical phenotype. The marked difference in disease manifestations may explain why Farber and SMA-PME diseases were not previously suspected to be allelic conditions. The precise molecular mechanism underlying the phenotypic differences remains to be clarified. Recently, a condition with mutation in CERS1, the gene encoding ceramide synthase 1, has been identified as a novel form of PME. This finding underlies the essential role of enzymes regulating either the synthesis (CERS1) or degradation (ASAH1) of ceramide, and the link between defects in ceramide metabolism and PME.

Key words: spinal muscular atrophy, myoclonus, Farber disease, progressive myoclonus epilepsies

Progressive myoclonus epilepsy (PME) (Minassian *et al.*, 2016) represents a heterogeneous group of epilepsies characterized by myoclonic and generalised seizures with progressive neurological deterioration (Girard *et al.*, 2013). A rare syndrome characterized by lower motor neuron disease associated with progressive myoclonic epilepsy (SMA-PME) has been described in childhood (Jankovic & Rivera, 1979; Haliloglu *et al.*, 2002). This condition is inherited as an autosomal recessive trait and the disease-causing gene has been recently identified. SMA-PME is caused by mutation in the *ASAH1* gene, which encodes acid ceramidase (Zhou *et al.*, 2012). A total of 11 affected individuals have been reported thus far, leading to a relatively precise phenotypic characterization (Zhou *et al.*, 2012; Dyment *et al.*, 2014, 2015; Rubboli *et al.*, 2015; Giráldez *et al.*, 2015).

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Mutations in the same gene are responsible for Farber disease, a very rare autosomal recessive condition with a distinct clinical phenotype, resulting from marked reduction or complete lack of acid ceramidase activity (Sugita *et al.,* 1972; Levade *et al.,* 2009). The marked difference in disease manifestations may explain why Farber and SMA-PME diseases were previously not suspected to be allelic conditions.

Several therapeutic strategies have been adopted to treat Farber disease, including allogeneic bone marrow transplantation and introduction of wild-type human acid ceramidase cDNA, using recombinant oncoretroviral or lentiviral vectors. The latter approach performed in non-human primates was recently shown to result in increased acid ceramidase activity. This underlines the importance of screening for *ASAH1* or acid ceramidase activity in patients with undiagnosed SMA or PME, for the purposes of diagnosis and future treatment.

Clinical description of SMA-PME

Based on the clinical description of cases reported to date ($n = 11$), SMA-PME is mainly characterized by progressive lower motor neuron degeneration followed by progressive myoclonic epilepsy (*table 1*) (Jankovic & Rivera, 1979; Haliloglu *et al.,* 2002; Zhou *et al.,* 2012; Dyment *et al.,* 2014, 2015; Rubboli *et al.,* 2015; Giráldez *et al.,* 2015).

Lower motor neuron disease manifestations

Early development milestones are usually normal, with an ability to walk unaided between 14 to 17 months of age. Progressive symmetric weakness and muscle atrophy of the lower and then the upper limbs are the main clinical features, usually occurring between 2.5 to 6 years of age (*table 1*). The patients begin to show slowness of gait and difficulties in standing from the sitting position. The motor deficit is purely peripheral, with a decrease in or absence of deep tendon reflexes. The disease is progressive, leading to the inability to stand up from the floor, the inability to sit unsupported, a lack of head control, difficulty swallowing, fasciculations of the tongue, severe scoliosis, and subsequent respiratory insufficiency. Electromyography (EMG) shows a chronic denervation process while muscle biopsy shows neurogenic atrophy, although there are no changes suggestive of a mitochondrial disorder.

However, in 1 of these 11 patients, atonic and absence seizures and myoclonic jerks were the main and first clinical features. Once the *ASAH1* gene mutations were identified, an EMG was performed, showing evidence of motor neuron disease despite only mild proximal muscle weakness (Dyment *et al.,* 2014, 2015).

Progressive myoclonus epilepsy characteristics

Later on, myoclonic epilepsy is observed from 3 to 12 years of age (*table 1*). This is characterized by brief myoclonic seizures without loss of consciousness, generalized epileptic seizures and myoclonic jerks, myoclonic seizures, absences with head drop or postural lapses in the upper limbs, atonic seizures, or upper limb myoclonic jerks. The EEG shows subcortical myoclonic epileptiform abnormalities which are sensitive to hyperventilation, paroxysmal activity consisting of frequent, diffuse bursts of sharp waves and polyspike and wave complexes, bursts of generalized spike- and polyspike-and-wave complexes associated with myoclonic phenomena, generalized polyspike-and-wave discharges, or interictal bursts of posterior delta activity and ictal generalized spike-wave and polyspike-wave discharges. Brain MRI is most often normal or displays mild supratentorial and subtentorial cortical atrophy. Myoclonic seizures are most often refractory to antiepileptic drugs.

Other symptoms

Variable degrees of cognitive impairment occur. When reported, cognition is usually mildly impaired. Neither skin, joint abnormalities, or hoarseness of the voice, as observed in Farber disease, has been reported. Ophthalmological examination did not reveal corneal opacities or cherry red spots.

SMA-PME is caused by mutations in *ASAH1*

This condition is inherited as an autosomal recessive trait. Genome-wide linkage analysis combined with exome sequencing in two multiplex families and one sporadic case suffering from childhood SMA-PME revealed mutations in *ASAH1* which were responsible for the disease (Zhou *et al.,* 2012). *ASAH1,* located on chromosome 8, was the only gene to have a mutation that was shared by the unrelated affected individuals. The same missense mutation in exon 2 of *ASAH1* (NM_177924.3; c.125C>T; p.Thr42Met) was found in the affected individuals. The p.Thr42Met missense substitution affected an evolutionarily conserved amino acid among different species and was predicted to be damaging. In 2 families, both parents were heterozygous and affected siblings were homozygous for this missense mutation. In one family, the affected child carried compound heterozygous

Table 1. Literature review.

	Zhou *et al.*, 2012			Rubboli *et al.*, 2015			Dyment *et al.*, 2014 & 2015	Giráldez *et al.*, 2015
	Family D	**Family ITA**	**Family ITB**	**Case 1**	**Case 2**	**Case 3**		
Nb. of affected individuals	3	2	1	1	1	1	1	1
MOTOR DEVELOPMENT								
Ability to walk (m.)	14	Normal	Normal	Normal	17 (abnormal deambulation)	Not reported	Not reported	17 (unsteady gait)
Age of onset of weakness (y.)	5	4 to 5	5	6	2,4	3	?	3
Muscle symptoms	Proximal weakness	Progressive muscle weakness	Progressive muscle weakness	Progressive muscle weakness	Progressive muscle weakness and limb tremor	Mild proximal weakness	Mild proximal muscle weakness	Proximal muscle weakness
EMG	Chronic denervation process	Denervation process	Denervation-reinnervation process	Chronic denervation process	Chronic denervation process	Chronic denervation process	Evidence of motor neuron disease	Chronic denervation process
Muscle biopsy	Neurogenic atrophy	Denervation process	Denervation-reinnervation	Neurogenic damage	Neurogenic damage	Neurogenic damage	nd	Neurogenic atrophy
EPILEPSY								
Age of onset (y.)	7	12	10	8	3	12	10	7
Clinical symptoms	Brief myoclonic seizures without loss of consciousness	Generalized epileptic seizures and myoclonic jerks	Loss of consciousness associated with myoclonic jerks	Brief episodes of impairment of consciousness associated with "jerks" at the upper limbs	Staring and myoclonic jerks	Myoclonic seizures, absences with head drop or postural lapses at the upper limbs	Absence and atonic seizures; frequent myoclonic jerks	Multiple and brief absences, upper limb myoclonic jerks and/or head nodding episodes

Table 1. (*Continued*)

	Zhou *et al.*, 2012			Rubboli *et al.*, 2015			Dyment *et al.*, 2014 & 2015	Giráldez *et al.*, 2015
	Family D	**Family ITA**	**Family ITB**	**Case 1**	**Case 2**	**Case 3**		
EEG	Subcortical myoclonic epileptiform abnormalities sensitive to hyperventilation	Not reported	Paroxysmal activity consisting in frequent diffuse bursts of sharp waves and poly-spike and wave complexes	Bursts of generalized spike- and polyspike-and-wave complexes associated with myoclonic phenomena	Similar to case 1	Similar to case 1	Generalized polyspike and wave discharges	Frequent interictal bursts of posterior delta activity and ictal generalized spike-wave and polyspike-wave discharges
Brain MRI	Normal	Normal	Normal	Normal	Diffuse supratentorial and subtentorial cortical atrophy	Normal	Normal then mildly increased size of the III and IV ventricles and mild volume loss	Normal
EVOLUTION	Progressive	Progressive	Progressive	Progressive	Progressive	Progressive	Not reported	Progressive
Age of death (y.)	13-17	na	15	19	na	na	na	na
ASAH1 MUTATION								
Nucleotide (NM_177924.3)	c.125C>T; homozygous	c.125C>T; homozygous	c.125C>T; *ASAH1* deletion	c.125C>T; homozygous	c.223_224insC; c.125C>T	c.177C>G; c.456A>C	c.850G>T; c.456A>C	c.125C>T; lack of the other allele (deletion ?)
Protein	p.Thr42Met	p.Thr42Met	p.Thr42Met	p.Thr42Met	p.Val75Alafs*25; p.Thr42Met	p.Tyr59*; p.Lys152Asn	p.Gly284*; p.Lys152Asn	p.Thr42Met
Acid ceramidase activity (% of normal value)	32%	32%	32%	Not reported	Not reported	Not reported	5.5%	Not reported

Nb.: number; y. : year; m. :month ; nd : not done; na: not applicable

mutations, the c.125C>T missense mutation on one allele and a deletion of the whole gene on the other. Transient expression of the mutant cDNA in immortalized fibroblasts derived from an individual with Farber disease with very low acid ceramidase activity (less than 3.5 per cent of control value), revealed a mild reduction in acid ceramidase activity when compared to that of the wild type cDNA (about 32 per cent of the control value) (Zhou *et al.,* 2012). To analyze the effect of *ASAH1* loss-of-function *in vivo*, a morpholino antisense oligonucleotide of the *ASAH1* ortholog was used to knock down *ASAH1* in zebrafish embryos. Analysis of this model revealed a marked defect in motor neuron axonal branching associated with a significant increase in apoptosis in the spinal cord (Zhou *et al.,* 2012).

For other SMA-PME patients reported to date, *ASAH1* mutations were identified using sanger or whole-exome sequencing (Rubboli *et al.,* 2015; Dyment *et al.,* 2014, 2015; Giráldez *et al.,* 2015). The same c.125C>T mutation was identified in three additional patients, and other mutations including missense, non-sense, or large deletion of *ASAH1* were identified (*table 1*). Although acid ceramidase activity was reported *in vitro* to be mildly reduced as the consequence of the p.Thr42Met mutation (Zhou *et al.,* 2012), acid ceramidase activity in patient fibroblasts carrying the compound *ASAH1* heterozygous mutation (p.Gly284*; p.Lys152Asn) was markedly reduced (5.5 per cent of normal activity), similar to that observed in Farber patients. Surprisingly, this patient did not develop manifestations of Farber disease (Dyment *et al.,* 2014, 2015).

Pathogenesis of SMA-PME is linked to *ASAH1* gene mutations

Ceramide is synthesized in the endoplasmic reticulum and transported by the ceramide-transfer protein, CERT, to the trans-Golgi membrane where it is converted to sphingomyelin by the sphingomyelin synthase-1,2. Ceramide and the metabolites (sphingosine and sphingosine 1-phosphate) participate in various cellular events as lipid mediators. The catabolism of ceramide in mammals occurs in lysosomes through the activity of ceramidase. Three different ceramidases (acid, neutral, and alkaline) have been identified and characterized according to optimum pH and primary structure; these include the acid (ASAH1), neutral (ASAH2), and alkaline ceramidases (ASAH3). The three families of ceramidase appear to play distinct roles in sphingolipid metabolism.

The enzymatic activity of acid ceramidase is deficient in two rare inherited disorders, Farber disease and SMA-PME. Importantly, in addition to the role of acid ceramidase in ceramide catabolism, the enzyme may have other functions depending on its subcellular location and the local pH. The other putative functions of ASAH1 may account for the clinical spectrum of the disease associated with *ASAH1* mutation. Refined characterization of acid ceramidase activity in the subcellular domain should contribute to a better understanding of the genotype-phenotype correlation.

Ceramides are the precursors to complex sphingolipids, which are important for normal functioning of both the developing and mature brain. Recently, a mutation in CERS1, the gene encoding ceramide synthase 1, has been identified as a novel cause of PME (Vanni *et al.,* 2014). This data underlies the essential role of enzymes in terms of regulating the synthesis (CERS1) or degradation (ASAH1) of ceramide and the link between ceramide metabolism defects and PME.

Molecular basis of Farber versus SMA-PME diseases

Farber disease is a rare and severe autosomal recessive lysosomal storage disorder (Levade *et al.,* 2009) which, like SMA-PME, is linked to the disease gene, *ASAH1*. It is characterized by a marked deficiency in acid ceramidase activity (Sugita *et al.,* 1972). Farber disease is characterized by a severe early onset (from 2 weeks to 4 months of age) of a unique triad of clinical manifestations including painful and progressively deformed joints, subcutaneous nodules, and progressive hoarseness as the result of laryngeal involvement. The illness advances rapidly, with progressive neurological deterioration, leading to death at a mean age of 1.45 years. At late stages of disease progression, 13 per cent of Farber disease patients display hypotonia and muscular atrophy with reduced or absent deep tendon reflexes and signs of denervation, as observed on EMG. These data strongly suggest that while a dramatic reduction in acid ceramidase activity leads to Farber disease, a milder reduction in enzymatic activity leads to a later onset of symptoms restricted to spinal cord motor neurons and subsequently other areas of the CNS responsible for PME. However, as reported recently (Dyment *et al.,* 2014, 2015), one patient presenting with features of PME, with mild motor weakness, had a marked reduction in acid ceramidase activity, similar to that observed in Farber patients, but did not present any manifestations of Farber disease, suggesting that additional factors are likely to be involved in the different phenotypic expression.

Animal models and therapeutic research strategies

Previously, homozygous *ASAH1* knockout mouse embryos were shown not to live beyond the four-cell stage, indicating that acid ceramidase plays an essential role during development (Eliyahu *et al.,* 2007). Recently, a knock-in mouse model was created by introducing a single-nucleotide mutation, identified in human Farber patients, into the murine *ASAH1* gene (Pro362Arg) (Alayoubi *et al.,* 2013). Homozygous mutant mice displayed Farber disease manifestations and died within 7-13 weeks. Treating mutant mice during the neonatal period with a single injection of lentivector expressing human acid ceramidase diminishes the severity of the disease. It would be of great interest to determine whether, using this model, partial correction of acid ceramidase deficiency could prevent both Farber and SMA-PME disease manifestations or only the Farber phenotype, which should reinforce the hypothesis that the clinical expression of the disease correlates with residual acid ceramidase activity. This model should contribute to a better understanding of the pathogenesis of Farber and SMA-PME diseases and represents a valuable tool towards developing therapeutic strategies.

While bone marrow transplantation has been reported to be effective in relieving joint contractures and subcutaneous nodules in a patient with Farber disease (Yeager *et al.,* 2000), the affected individual developed progressive muscle weakness and features consistent with the occurrence of lower motor neuron involvement, the main clinical features found in SMA-PME individuals. More recently, a preclinical gene therapy study for Farber disease involving a lentiviral vector was performed in non-human primates. Acid ceramidase activity was detected above normal levels in various cell types, including bone marrow cells, spleen and liver 1 year after lentiviral vector transduction (Walia *et al.,* 2011).

Genetic counselling

The identification of the *ASAH1* gene mutations found in SMA-PME patients has greatly improved diagnostic testing and family-planning options of SMA-PME family members. Because the risk of recurrence of the disease in the sibling of an affected child is high (1/4), information should be given to parents early after diagnosis. Explanations regarding molecular pathology, risk evaluation, and prenatal testing possibilities should be given to the parents and their relatives within the context of a genetic counselling visit.

Conclusion

Mutations in *ASAH1,* which encodes acid ceramidase, are the major cause of SMA-PME. Based on an overview of patients reported to date, the main clinical characteristic features are onset, most often within the first 6 years of age, characterized by lower motor neuron disease leading to progressive muscle weakness, followed by the occurrence of clinical and EEG characteristics of myoclonus epilepsies, and a progressive course. This data underlies the link between ceramide metabolism defects and PME. Taking into account ongoing therapeutic research for Farber disease, a disease allelic to SMA-PME, screening for *ASAH1* or acid ceramidase activity should be proposed for the diagnosis and future treatment of patients with PME or SMA. □

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References

Alayoubi AM, Wang JC, Au BC, *et al*. Systemic ceramide accumulation leads to severe and varied pathological consequences. *EMBO Mol Med* 2013; 5: 827-42.

Dyment DA, Sell E, Vanstone MR, *et al*. Evidence for clinical, genetic and biochemical variability in spinal muscular atrophy with progressive myoclonic epilepsy. *Clin Genet* 2014; 86: 558-63.

Dyment DA, Tétreault M, Beaulieu CL, *et al*. Whole-exome sequencing broadens the phenotypic spectrum of rare pediatric epilepsy: a retrospective study. *Clin Genet* 2015; 88: 34-40.

Eliyahu E, Park JH, Shtraizent N, He X, Schuchman EH. Acid ceramidase is a novel factor required for early embryo survival. *FASEB J* 2007; 21: 1403-9.

Giráldez BG, Guerrero-López R, Ortega-Moreno L, *et al*. Uniparental disomy as a cause of spinal muscular atrophy and progressive myoclonic epilepsy: phenotypic homogeneity due to the homozygous c.125 C>T mutation in ASAH1. *Neuromuscul Disord* 2015; 25: 222-4.

Girard JM, Turnbull J, Ramachandran N, Minassian BA. Progressive myoclonus epilepsy. *Handb Clin Neurol* 2013; 113: 1731-6.

Haliloglu G, Chattopadhyay A, Skorodis L, *et al*. Spinal muscular atrophy with progressive myoclonic epilepsy: report of new cases and review of the literature. *Neuropediatrics* 2002; 33: 314-9.

Jankovic J, Rivera VM. Hereditary myoclonus and progressive distal muscular atrophy. *Ann Neurol* 1979; 6: 227-31.

Levade T, Sandhoff K, Schulze H, Medin JA. Acid ceramidase deficiency: Farber lipogranulomatosis. In: Valle D, Beaudet AL, Vogelstein B, Kinzler KW, Antonarakis SE, Ballabio A, eds. *Scriver's OMMBID (online metabolic and molecular bases of inherited disease)*. New-York: McGraw-Hill, 2009.

Minassian BA, Striano P, Avanzini G. Progressive Myoclonus Epilepsies: State-of-the-Art. *Epileptic Disord* 2016; 18(Suppl. 2): S1-158.

Rubboli G, Veggiotti P, Pini A, *et al*. Spinal muscular atrophy associated with progressive myoclonic epilepsy: a rare condition caused by mutations in *ASAH1*. *Epilepsia* 2015; 56: 692-8.

Sugita M, Dulaney JT, Moser HW. Ceramidase deficiency in Farber's disease (lipogranulomatosis). *Science* 1972; 178: 1100-2.

Vanni N, Fruscione F, Ferlazzo E, *et al*. Impairment of ceramide synthesis causes a novel progressive myoclonus epilepsy. *Ann Neurol* 2014; 76: 206-12.

Walia JS, Neschadim A, Lopez-Perez O, *et al*. Autologous transplantation of lentivector/acid ceramidase-transduced hematopoietic cells in nonhuman primates. *Hum Gene Ther* 2011; 22: 679-87.

Yeager AM, Uhas KA, Coles CD, Davis PC, Krause WL, Moser HW. Bone marrow transplantation for infantile ceramidase deficiency (Farber disease). *Bone Marrow Transplant* 2000; 26: 357-63.

Zhou J, Tawk M, Tiziano FD, *et al*. Spinal muscular atrophy associated with progressive myoclonic epilepsy is caused by mutations in *ASAH1*. *Am J Hum Genet* 2012; 91: 5-14.