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Acid Ceramidase Deficiency

New Insights on SMA-PME Natural History, Biomarkers, and *In Cell* Enzyme Activity Assay

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Abstract

Background and Objectives

Spinal muscular atrophy with progressive myoclonic epilepsy (SMA-PME) due to acid ceramidase deficiency is a rare disorder, allelic with Farber disease, resulting from recessive *ASAH1* variants. Patients present in early childhood with muscle weakness due to anterior horn degeneration and/or progressive drug-resistant myoclonic epilepsy. Death usually results from respiratory complications or status epilepticus during adolescence.

Methods

We identified 9 patients with SMA-PME from 5 different families followed in neurology, rehabilitation, and genetics departments of university hospitals in France and the United States. During disease progression, motor functional scores were assessed for seven of them and C26-ceramide quantification on dried blood spots (DBSs) was performed for 4 of them. An *in cell* assay, measuring the degradation rate of ceramides in living skin fibroblasts, was also performed in 2 patients. Finally, a literature review was conducted.

Results

Twelve years after the molecular characterization of SMA-PME, here we present the detailed history of 9 patients from 5 different families with 4 new *ASAH1* variants. The prospective follow-up for 4 of them allows us to evaluate the relevance of functional scales and of C26-ceramide assay on DBS, as a biomarker. In addition, an *in cell* assay could provide a more reliable level of the residual ceramidase activity. Based on a comprehensive literature review, we provide a detailed description of the natural history of the 44 patients with SMA-PME diagnosed to date and show a genotype-phenotype correlation for the 2 main variants and the disease onset.

Discussion

This study presents the detailed natural history of SMA-PME. Given the rarity of this disease and the current lack of a reliable biomarker for patient follow-up, this work may serve as a retrospective control group for future therapeutic trials.

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Supplementary Material

Introduction

Ceramides, composed of a fatty acid linked to a sphingoid base, have essential roles in cell signaling.[1] Acid ceramidase (ACDase) is a soluble lysosomal hydrolase, encoded by the *ASAH1* gene (8p22). It catalyzes the degradation of ceramides into fatty acids and sphingosine inside the lysosome.[2]

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Glossary

ACDase = acid ceramidase; **CNIL** = Commission for Information Technology and Civil Liberties; **DBS** = dried blood spot; **EM** = electron microscopy; **FD** = Farber disease; **GMFC-MLD** = Gross Motor Function Classification for Metachromatic Leukodystrophy; **HSCT** = hematopoietic stem cell transplantation; **MFM-32** = Motor Function Measurement–32 items; **SMA-PME** = spinal muscular atrophy with progressive myoclonic epilepsy.

Biallelic pathogenic variants in *ASAH1* cause 2 rare lysosomal storage diseases with ceramide accumulation: Farber disease (FD; MIM#228000) and spinal muscular atrophy with progressive myoclonic epilepsy (SMA-PME, MIM#159950). FD is an early-onset inflammatory lipogranulomatous disorder, characterized by a clinical triad of arthritis, subcutaneous nodules, and hoarseness in the first years of life, rapidly deteriorating with multiorgan failure.[3] In SMA-PME, symptoms usually begin in childhood with progressive proximal weakness and/or seizures (myoclonus, absences, atonic falls) that rapidly become drug-resistant. Death occurs in adolescence, due to status epilepticus and respiratory complications. To date, 35 patients from 29 families were reported with classic SMA-PME, confirmed by molecular diagnosis.[4-22] Subcutaneous and multiorgan involvement is prominent in FD while SMA-PME is characterized by CNS involvement. A clinical overlap is sometimes observed,[20-24] but the genotype-phenotype correlations in ACDase deficiencies remain poorly understood.[25,26]

In this study, we present the clinical data of 9 patients with SMA-PME, from 5 different families with prospective follow-up and functional assessments. We evaluate the relevance of C26-ceramide assay on dried blood spot (DBS) during the disease progression. Combined with a literature review of all previously reported patients, we present a precise description of the natural history of SMA-PME and report a genotype-phenotype correlation for the 2 main variants.

Methods

Standard Protocol Approvals, Registrations, and Patient Consents

Informed consent was obtained for genetic analyses. Collection of clinical data has been submitted to the noninterventional research ethics committee (CERNI/CEDIS) of Nantes University (reference number 27042020), with an information letter and a nonopposition form sent to the patient by the clinician. In addition, patients and their family signed a consent for prospective clinical data and research samples accepted by ethics committees (LeucoEpimar 2009-AU788-Commission for Information Technology and Civil Liberties [CNIL] 1406552; Operando 017-A01368-45).

Clinical data were obtained from physicians at university hospitals following patients with SMA-PME. Motor functional scores were longitudinally assessed. We chose the Gross Motor Function Classification for Metachromatic Leukodystrophy (GMFC-MLD),[27] validated for MLD and neurodegenerative disorders with childhood onset. The Motor Function Measurement–32 items (MFM-32), validated for the follow-up of patients with SMA,[28] was used by the physical therapist.

Molecular Data

Consents for genetic testing were obtained from each family. ASAH1 variants are annotated according to the reference transcript NM_177924.5. Patients P1, P2, P4, P5, P6, P8, and P9 were diagnosed by ASAH1-targeted Sanger sequencing. P3 was diagnosed by exome sequencing. P7 was diagnosed through sequencing of a panel of genes involved in monogenic epilepsies (eMethods). For each family, biparental segregation of ASAH1 pathogenic variants was confirmed by Sanger sequencing.

Enzymatic Determinations

For P1, P2, P3, P4, P7, and P8, ACDase activity was determined in vitro on leukocyte lysates, using the fluorogenic Rbm-14-12 substrate according to a previously published protocol.[29] For P5 and P6, the rate of in situ ceramide degradation was determined in living skin fibroblast cultures: after a pulse/chase with [^{3}H-*ceramide*]-sphingomyelin, cells were harvested and their lipid distribution was evaluated by radiochromatoscan analysis (eMethods).

Quantification of the biomarker C26-ceramide was performed on DBSs obtained from patients P1–4 during follow-up. All samples were analyzed by APTEEUS using liquid chromatography mass spectrometry (LC-MS/MS) (eMethods).

Literature Review

PubMed was consulted. Only clinical data from patients with molecular diagnosis were retained. A total of 44 patients from 37 families were identified in 21 publications. To prevent a potential bias due to preferential publication of atypical cases, we chose to classify patients as follows:

1. "Classic SMA-PME," presenting with loss of gait and/or epilepsy before 18 years and with no clinical feature of FD (absence of subcutaneous nodules and clinical visceral storage): 35 patients[4-16] met these criteria.
2. "Late SMA," presenting with slowly progressive muscle weakness, no loss of ambulation before adulthood, no myoclonus, no epilepsy, no deafness, nor cognitive impairment in adulthood: 4 patients[17-19] met these criteria.
3. "Atypical FD," defined at any age by the occurrence of non-neurologic FD features: 5 patients[20-24] met these criteria.

Statistics

To better document the natural history of SMA-PME, descriptive statistical data were calculated for the "classic SMA-PME" group, i.e., all 9 patients of our cohort and 35 of 44 patients from the literature, for a total of 44 patients in 34 families (eFigure 1). Descriptive and comparative statistics were obtained using Microsoft Excel and RStudio software, respectively. Cohort comparisons were made by the Chi-square or Fisher exact test for qualitative analyses (depending on the number of individuals) and by the Student *t* test (2 groups) or Kruskal-Wallis test (>2 groups) for quantitative analyses. Survival curves were calculated using the Kaplan-Meier method and compared using the log-rank test (Mantel-Cox) and Mantel-Haenszel method. A p value <0.05 was considered significant.

Data Availability Statement

The data that support the findings of this study are in the supplemental data section. Additional data are listed in eTables 1–4 and eFigures 1–4.

Results

Clinical Description of the 9 Patients of Our Series

Clinical symptoms are summarized in Table 1, and a detailed medical history is provided for each patient in eAppendix 1. Patients were aged from 5 to 16 years (y) at the last examination. In our series, the first symptoms were seizures (6/9), weakness (2/9), or tremor (1/9). During progression, 8 of 9 patients developed weakness at a mean age of 10 years, with documented anterior horn involvement in 8/8, and 5 of 9 lost their ability to walk at a mean age of 12.6 years. Epilepsy appeared in 7 of 9 patients at a mean age of 9.7 years, with myoclonic jerks (7/7), drop attacks (5/7), absences (4/7), and tonic-clonic seizures (2/7). Epilepsy became drug-resistant in 4 of 7 patients, on average 1.1 year after the first seizure. EEG showed slow waves and polyspike-waves (8/8), even before the first seizures (2/8). Two patients, including one aged 12 at the last examination, never developed epilepsy. Sensorineural deafness was observed in 5 of 9 patients and was clearly progressive in 1 of 5 (after 12 years). During infancy and early childhood, none had intellectual disability, but before the onset of weakness or epilepsy, 5 of 9 had learning difficulties and 4 of 9 presented behavioral disorders including attention deficit, anxiety, and mood disorder. Cognitive impairment was later observed in 4 of 9 patients (between 7 and 16 years of age; mean 11 years), followed by psychomotor regression in 3 of 9 patients (between 13 and 15 years of age; mean 14 years). Three patients died on average at 6 years after the first symptom, all from epileptic complications, associated in 2 of them with respiratory complications. We also observed digestive and urinary symptoms, respectively, in 3 and 2 of the 9 patients in our series, such as chronic diarrhea, constipation, and dysuria. None of these patients showed any clinical sign of FD.

Motor Functional Scores for Patient Follow-Up in SMA-PME

Longitudinal follow-up using the GMFC-MLD score was possible in 7 patients, completed by the MFM-32 for 3 patients. The GMFC-MLD score progression showed a two-step evolution, with mild and stable motor impairment during several years, followed by rapid deterioration over the last 2 years of life, at an average rate of 1.3 steps/year (estimated with P3, P4, P5, and P6). The evolution of MFM-32 score followed this trend, with impairment predominating on standing and transfer abilities (D1 score, eTable 2), relatively stable in P1, but with a dramatic deterioration in the last 2 years of life in P3 (Figure 1).

ASAH1 Variants

All families were Caucasian and nonconsanguineous. The mean age at genetic diagnosis was 10.6 years, with a mean time from onset to diagnosis of 3.3 years for index cases. Siblings P1–2 carry the missense variants c.526T > C p.(Trp176Arg) and c.134G > T p.(Gly45Val) in a compound heterozygous state. These variants had not previously been reported. P3 carries the c.918-2A > G variant, known to affect splicing of exon 12 leading to a premature stop codon p.(Glu306GlufsTer17),[14,23] and the recurrent missense variant c.456A > C p.(Lys152Asn), known to induce skipping of exon 6.[5] P4 carries the same c.456A > C p.(Lys152Asn) variant and a c.1098 + 1G > T p? splice variant, known to cause a 19–amino acid deletion in exon 13. Of interest, this c.1098 + 1G > T variant was reported only in FD.[30] Siblings P5–6 also carry the recurrent variant c.456A > C p.(Lys152Asn) and a previously unreported intragenic 9bp deletion c.548_556del p.(Lys183_Leu185del), resulting in an in-frame deletion of 3 amino acids. The siblings P7–9 carry the recurrent variant c.456A > C p.(Lys152Asn) and a nonsense variant c.186G > A p.(Trp62Ter) previously reported.[14] The biparental segregation was verified for each patient by Sanger sequencing.

Histologic Findings

Ten years before ACDase deficiency was associated with SMA-PME,[4] skin biopsies were obtained from siblings P5–6. Electron microscopy (EM) revealed, in P5 but not in P6, unexpected abnormalities suggestive of FD, namely typical "banana bodies" and "Farber bodies,"[31] as well as "zebra bodies," lysosomal inclusions also found in other sphingolipidoses[32,33] (Figure 2). EM findings of skin biopsies performed for P1–3 were normal.

In Vitro and *In Cell* Measurement of ACDase Activity

The classic in vitro determination of ACDase activity on leukocytes showed profoundly decreased values in the 7 patients tested (1.5%–8.3% of control values, Table).

For siblings P5–6, the degradation rate of ceramide was evaluated in skin fibroblast cultures. In normal cells (n = 5), the percentages of unhydrolyzed ceramide observed after a chase of 2 and 24 hours, respectively, were 38% ± 10% and

Table Clinical and Molecular Data in Our Series

Patient	1	2	3	4	5	6	7	8	9	
Family	**1**		**2**	**3**	**4**		**5**			**Total**
Sex	Female	Male	Female	Female	Male	Female	Female	Male	Female	6F/3M
Origin	France	France	France	France	France	France	United States	United States	United States	
Consanguinity	—	—	—	—	—	—	—	—	—	0/9
Age at onset	2 y 6 m	3 y 6 m	7 y	5 y	8 y	12 y	13 y	13 y	12 y	
First symptom	Muscle weakness	Muscle weakness	Falls due to seizures	Tremor, myoclonus	Myoclonus, absences	Tonic-clonic seizures	Myoclonus	Myoclonus	Myoclonus	
Age at diagnosis	6 y	4 y 5 m	10 y	11 y	10 y 11 m	15 y	14 y	13 y	11 y	
Last examination	10 y 6 m	4 y 10 m	14 y	13 y	13 y 4 m	16 y	15 y 10 m	14 y 11 m	12 y 11 m	
Death	—	—	15 y	—	14 y	18 y	—	—	—	3/9
Cause of death	—	—	Status epilepticus	—	Status epilepticus	Status epilepticus	—	—	—	
Neurodevelopment										
Developmental delay	—	—	—	—	—	—	—	—	—	0/9
Learning difficulties	+	+	+	+	+	+	—	—	—	6/9
Neuromuscular symptoms										
Muscle weakness	2 y 6 m	3 y 6 m	7 y	13 y	9 y	14 y	16 y	15 y	—	8/9
Walking difficulties	3 y	4 y	10 y	12 y	9 y	15 y	16 y	—	—	7/9
Gowers sign	7 y	4 y 10 m	11 y	n.a	9 y	n.a	No	No	No	4/7
Loss of ambulation	6 y	5 y	13 y	12 y	13 y	18 y	—	—	—	6/9
Swallowing difficulties	9 y	—	14 y	13 y	12 y	17 y	—	—	n.a	5/8
Epilepsy										
Myoclonic jerks	8 y	—	9 y	5 y	8 y	14 y	13 y	13 y	—	7/9
Drop attacks	10 y	—	7 y	9 y	—	12 y	16 y	—	—	5/9

Continued

Table Clinical and Molecular Data in Our Series *(continued)*

Patient	1	2	3	4	5	6	7	8	9	
Family	**1**		**2**	**3**	**4**		**5**			**Total**
Absences	—	—	7 y	9 y	8 y	14 y	—		—	4/9
Tonic-clonic	—	—	14 y	12 y	—	—	—	—	—	2/9
Status epilepticus	—	—	14 y	13 y	14y	18y	—	—	—	4/9
Other neurologic symptoms										
Tremor	3 y	4 y	9 y	5 y	9 y	13 y	14 y	15 y	—	8/9
Fasciculations	6 y	—	9 y	11 y	9 y	14 y	—	—	13 y	6/9
Pyramidal signs	7 y	4 y 10 m	14 y 9 m	—	n.a	n.a	—	—	—	3/8
Abolished DTR	—	—	13 y	—	n.a	17 y	—	—	—	2/8
Cognitive impairment	7 y	—	8 y	—	n.a	14 y	16 y	—	—	4/8
Behavioral problems	5 y	—	10 y	7 y	9 y	1 y	—	—	—	5/9
Other symptoms										
Hearing loss	—	—	6 y	6y	—	—	15	15	13	5/9
Scoliosis	6 y	—	—	13y	—	—	—	—	—	2/9
Aspiration pneumonia	—	—	14y	—	13y	—	—	—	—	2/9
Chronic constipation	8y	—	14y	—	—	—	—	—	—	2/9
Chronic diarrhea	—	—	—	12y	—	—	—	—	—	1/9
Chronic dysuria	—	—	11y	13y	—	—	—	—	—	2/9
Farber features[a]	—	—	—	—	—	—	—	—	—	0/9
Paraclinical findings										
Abnormal EEG[b]	+	n.a	+	+	+	+	+	+	+	8/8
Abnormal ENMG[c]	+	n.a	+	+	+	+	+	+	+	8/8

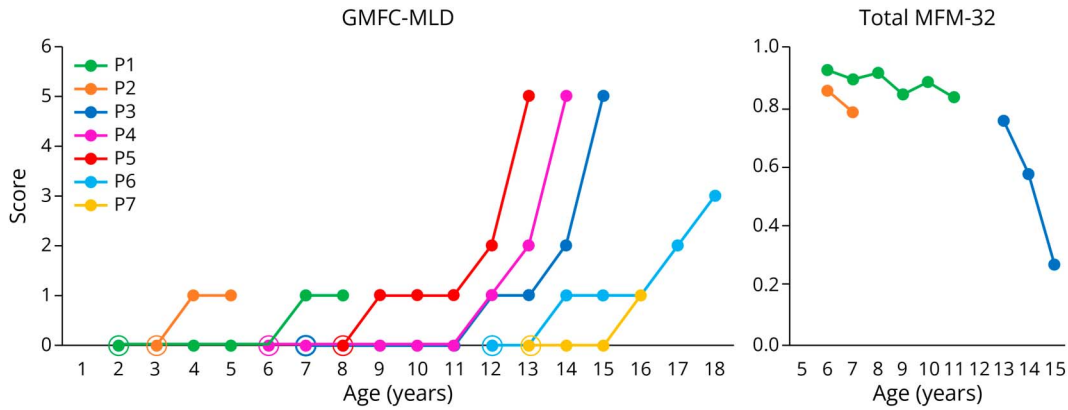
Continued

Table Clinical and Molecular Data in Our Series *(continued)*

Patient	1	2	3	4	5	6	7	8	9	
Family	**1**		**2**	**3**	**4**		**5**			**Total**
Abnormal MRI[d]	+	—	+	—	—	+	n.a	n.a	n.a	3/6
Skin biopsy	Normal	Normal	Normal	n.a	Farber bodies	Normal	n.a	n.a	n.a	
Enzyme-based test (%)										
Material	Leukocytes	Leukocytes	Leukocytes	Leukocytes	Fibroblasts	Fibroblasts	Leukocytes	Leukocytes	Leukocytes	
ACDase activity	4.5	3	1.6	1.5	Intermediate[e]	Intermediate[e]	7.2	8.3	7.3	
ASAH1 variants (NM_177924.4) c./p										
Maternal allele	c.526T>C p.(Trp176Arg)	c.526T>C p.(Trp176Arg)	c.918-2A>G p.(Glu306Glu fsTer17)	c.1098 + 1G>T p.(?)	c.548_556del p.(Lys183_Leu185del)	c.548_556del p.(Lys183_Leu185del)	c.456A>C p.(Lys152Asn)	c.456A>C p.(Lys152Asn)	c.456A>C p.(Lys152Asn)	
Paternal allele	c.134G>T p.(Gly45Val)	c.134G>T p.(Gly45Val)	c.456A>C p.(Lys152Asn)	c.456A>C p.(Lys152Asn)	c.456A>C p.(Lys152Asn)	c.456A>C p.(Lys152Asn)	c.186G>A p.(Trp62Ter)	c.186G>A p.(Trp62Ter)	c.186G>A p.(Trp62Ter)	

Abbreviation: DTR = deep tendon reflex; ENMG = electroneuromyogram; n.a = not available.
Among the neurologic symptoms, we have distinguished neurodevelopmental disorders preceding the first symptoms, cognitive impairment concomitant with the progression of the disease, and cognitive regression at a more advanced stage.
[a] Including subcutaneous nodules polyarthritis, painful swollen joints, hoarse voice, and hepatosplenomegaly.
[b] Including diffuse 2–4 Hz slow waves and generalized spike-wave discharges.
[c] Denervation pattern with anterior horn involvement.
[d] Including mild cortical, subcortical, and cerebellar atrophy.
[e] Based on the *in cell* assay (eMethods and eFigure 2).

Figure 1 Motor Functional Scores According to Patients' Age



Left, the gross motor function classification score from GMFC-MLD ranges from 0 to 6. Level 0: walking without support with normal performance. Level 1: walking without support with reduced performance. Level 2: walking with support. Level 3: walking with or without support not possible, but sitting without support and locomotion such as crawling or rolling possible. Level 4: sitting without support but no locomotion, or sitting without support not possible, but locomotion such as crawling or rolling possible. Level 5: no locomotion nor sitting without support, but head control possible. Level 6: loss of any locomotion, head and trunk control. The first circled point corresponds to the age at symptom onset. Right, the total Motor Function Measurement–32 items (MFM-32) score decreases from 100% to 0% with the progression of the motor impairment.

16% ± 9% (mean ± SD) while in typical Farber cells (n = 2), ceramide still constituted 75%–80% of radiolabeled lipids at both chase periods. After a 2-hour and 24-hour chase, respectively, the results found for P5 were 67% and 38% and for P6 were 60% and 55% (eFigure 2). A clear impairment in ceramide degradation was thus demonstrated, with, however, a significant level of residual enzyme activity compared with that observed in cells from patients with FD.

C26-Ceramide Levels in DBSs During Disease Progression

The concentration of C26-ceramide, considered as a good biomarker in FD,[34] was quantified in DBSs from P1–4 for 2.5 to 4 years by LC-MS/MS (Figure 3). C26-ceramide blood concentrations showed a slight increase in the last 6 months of follow-up in P1 and P2. P3 showed a progressive 1.7-fold increase in 1.5 years and then a dramatic decrease in her last 2 years of life, in parallel with her clinical deterioration. P4 showed a 2-fold increase in the last 2 years of follow-up. Of note, treatment with tocopherol, idebenone, levocarnil, and creatine did not result in any significant modifications in C26-ceramide level during the treatment period in P1–4 (eAppendix 1).

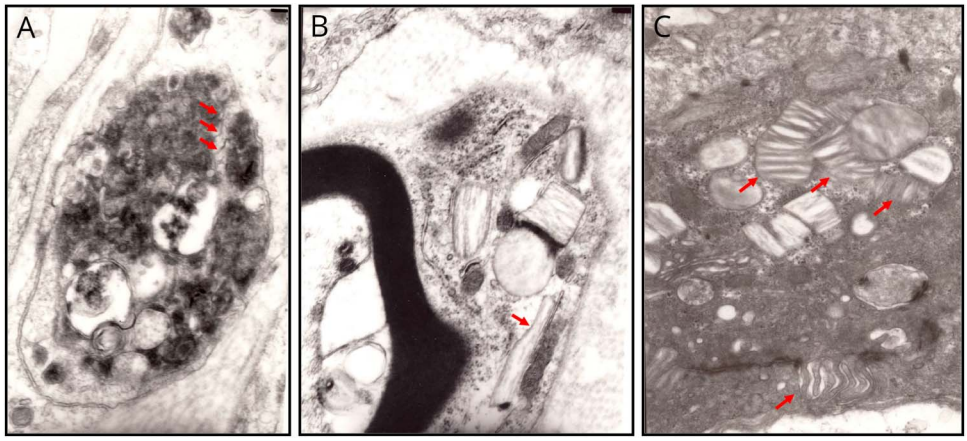
Natural History of Classic SMA-PME

In our analysis, we pooled the clinical data of our 9 patients with the 35 previously reported, for a total of 44 patients from 34 different families (eTable 1).

Clinical Analysis

The average age at onset was 5.6 years. The first reported symptoms were muscle weakness (62%), epilepsy (26%), tremor (7%), and deafness (5%) (Figure 4A).

Figure 2 Electron Microscopy (EM) Findings in Skin Biopsy of Patient P5



(A) "Farber bodies" (red arrows) appear as curvilinear tubular bodies of approximately 15-20 nm in diameter; ×24000. (B) "Banana bodies" (red arrows), also suggestive of FD, are large, clear, spindle-shaped vacuoles of approximately 2 μm; ×12000. (C) "Zebra bodies" (red arrows) are lysosomal inclusions also found in other sphingolipidoses, which appear as vacuoles with transverse membranes of approximately 2 μm; ×12000. FD = Farber disease.

Muscle weakness was reported at a mean age of 6.6 years, with a positive Gowers sign at around 7.8 years, swallowing difficulties at 14.3 years, and loss of walking at 15 years, i.e., 8.7 years after the first sign. Loss of ambulation had a mixed origin: muscle weakness, myoclonus, atonic seizures, and progressive proprioceptive ataxia.

Epilepsy, reported in 78.6% of patients, appeared on average at 8.8 years of age, with significant variability (3–15 years). The first seizures were myoclonic in 81% of cases, atonic in 28%, absences in 22%, and tonic-clonic in 9% (Figure 4B). Over the course of the disease, epilepsy became more complex and polymorphic, with combination of the different seizure types mentioned above (Figure 4C), having suggested De Vivo, Doose, or Jeavons syndromes. Myoclonus also evolved, becoming more frequent and disabling, affecting walking, and was sometimes triggered by movement, fatigue, emotion, or infections. The first tonic-clonic seizures generally occurred later, around 12.7 years of age, 2.8 years after the first seizure, and seemed to represent a turning point in the progression of the handicap. Drug resistance was established around 13.4 years of age, approximately 3 years after the first seizures. Ketogenic diet and vagus nerve stimulation were also ineffective in 5 and 2 patients, respectively. Status epilepticus occurred at 15.4 years on average, myoclonic seizures in 60% of cases, and tonic-clonic seizures in 40%.

Other neurologic symptoms reported over the course of the disease included tremor in 60% of patients (fine, at rest or action, predominantly in the extremities and fasciculations in 45% (mainly involving the tongue, but also the deltoids, triceps brachii, and quadriceps). Pyramidal signs were reported in 18% of patients, on average at the age of 8.1 years, while abolition of deep tendon reflexes was observed later (14.1 years), in 22%. Sensorineural hearing loss was reported in 30% of patients, diagnosed at around 11.2 years of age, mild or moderate, and possibly progressive (as for P3). Cognitive impairment of executive functions related to ideomotor slowing was reported in 27% of patients and identified at 11 years of age on average. Cognitive regression, reported in 16%, occurred around 14.3 years of age. Dystonia, hallucinations, abnormal oculomotor movements, ptosis, and exercise-induced myalgia were rarely reported.

Death, from respiratory causes in 56% of cases and from status epilepticus in 44%, occurred at around 17 years of age, with a significant variability (13–26 years). The mean delay between the first symptom and death was 10.3 years, and 50% of patients died before 18 years (Figure 4F).

For each symptom, mean ages at onset, quartiles, and extreme values are presented in Figure 4D, with precise values in eTable 1.

Electrophysiology

EEG showed diffuse 2–4 Hz slow waves and spike-waves (90% of patients), sometimes sensitive to intermittent light stimulation (24% of patients) and hyperpnea (12% of patients). These abnormalities also seemed to be present in patients without epileptic manifestations. Myoclonus was frequently observed on EEG, but electroclinical dissociation was sometimes reported, in favor of a mixed cortical and subcortical origin. Global slowing of EEG activity was reported in 25% of patients. Electroneuromyogram (ENMG) showed chronic denervation in all cases, and anterior horn involvement was evoked in at least 56% of cases.

Imaging

Brain MRI was normal in 70% of patients, but cortico-subcortical atrophy was reported in 23% and cerebellar atrophy in 17%. Spinal cord MRI revealed spinal cord atrophy in only 1 patient.

Muscle Biopsies

Histologic analysis found nonspecific neurogenic muscle atrophy in all reported cases (13/13). Mitochondrial DNA depletion with reduced respiratory chain activity was reported for 3 patients, without ragged red fibers nor COX-negative fibers.

Skin Biopsies

Histology of skin biopsies from 7 patients revealed nonspecific lamellar lysosomal inclusions ("zebra bodies") in four of them while banana/Farber bodies were observed only in 1 patient (P5; Figure 2).

Ophthalmologic Examinations

Bilateral optic atrophy was observed in only one 10-year-old patient.

Genotype-Phenotype Correlations

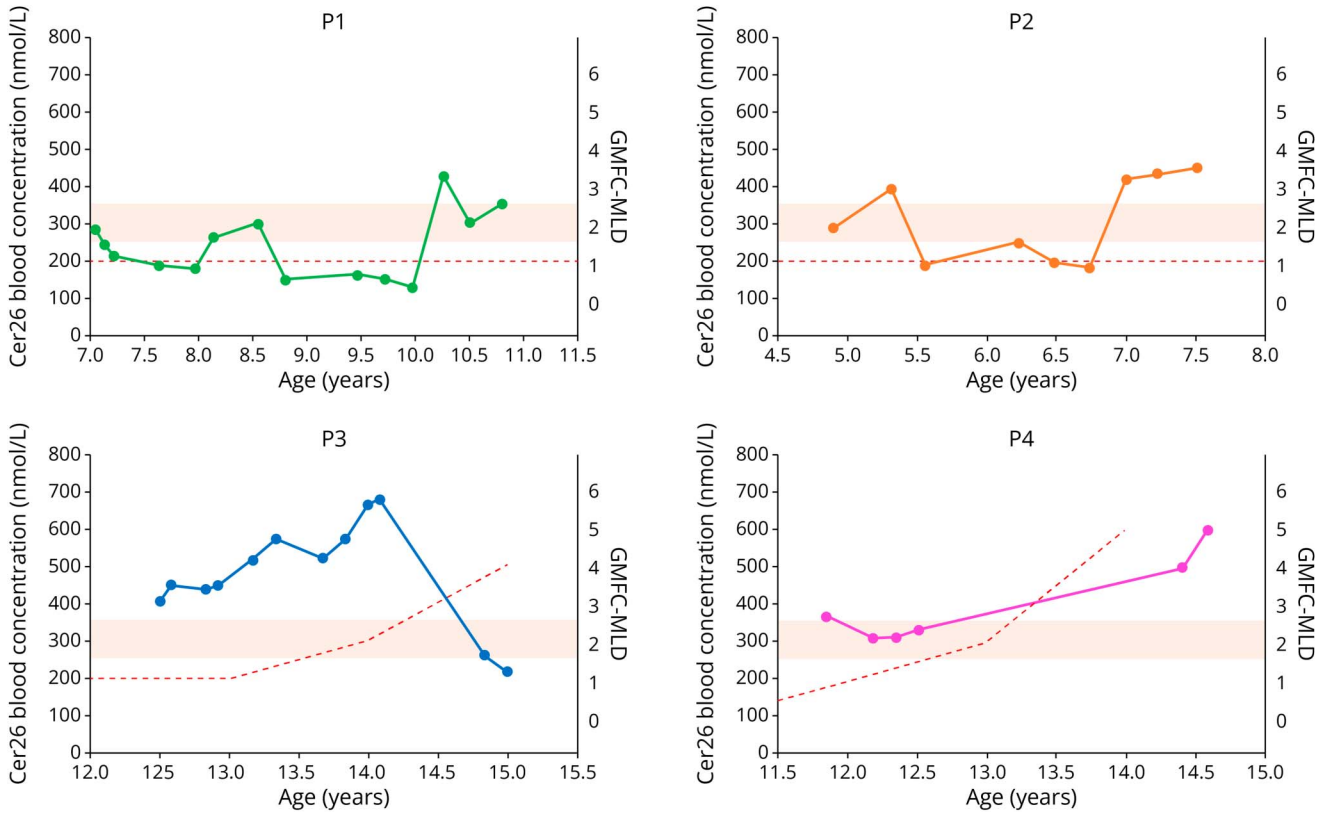
Variants in *ASAH1* were homozygous in 55% of patients, roughly concordant with parental consanguinity reported in 42% of patients. *ASAH1* variants associated with SMA-PME are shown in Figure 5A. Twenty patients carried the p.(Thr42Met) variant, and 14 the p.(Lys152Asn) variant, affecting, respectively, the α and β subunits. These 2 recurrent variations seem related to a founder effect in Middle Eastern and Caucasian populations, respectively.

To confirm the genetic diagnosis, ACDase activity was evaluated in 17 patients (on fibroblasts for 6/17, on leukocytes for 11/17) and found systematically decreased. The average residual in vitro activity was 16.5% on fibroblasts (range: 5.5–32) and 5.95% on leukocytes (range: 1.53–13.1).

The predominant p.(Thr42Met) variant, found in 20 of 35 patients (57%) in literature, was absent from our cohort while the p.(Lys152Asn), present in 7 of 9 patients (78%) in our series, was found in only 7 of 35 patients (20%) from the literature. It seemed that the patients in our cohort entered the disease state later and more frequently through epilepsy than through weakness in comparison with patients in literature (eTable 3).

We, therefore, hypothesized that a genotype-phenotype correlation could be at the origin of these discrepancies. Indeed, in

Figure 3 Evolution of C26-Ceramide Blood Concentration in Patients



C26-ceramide level (nmol/L) extracted from DBS discs and measured by LC-MS/MS for patients P1–4 during follow-up (left ordinate, spots, and solid lines), according to their age (abscissa) and the evolution of their GMFC-MLD scores (right ordinate, red dashed lines). The orange strip represents the mean control level of C26-ceramide ± 1 SD (mean = 305.38 nmol/L, SD = 57.30 nmol/L). DBS = dried blood spot.

patients with the p.(Lys152Asn) variant, the onset of the disease was more frequently associated with epilepsy compared with patients with the p.(Thr42Met) variant and those with other variants (57%, 5.0%, and 30% cases, respectively; p = 0.0014). In patients with the p.(Thr42Met) variant, the onset of the disease was more often associated with muscle weakness compared with patients with the p.(Lys152Asn) variant and those with other variants (21.4%, 75.0%, and 50.0%, respectively; p = 0.0083). We also confirmed that the p.(Lys152Asn) variant is strongly associated with sensorineural deafness compared with the p.(Thr42Met) variant and other variants (78.6%, 5.0%, and 10.0%, respectively; p = 0.00001), as previously hypothesized.[14] Although the first symptoms appear earlier in patients with the p.(Thr42Met) variant compared with patients with the p.(Lys152Asn) variant (p = 0.0084), we show no significant difference in survival (HR: 2.306 [0.6387–8.329] p = 0.2), nor in age at loss of ambulation (HR: 1.29 [0.46–3.61] p = 0.6) (Figure 5B, eFigures 3–4, eTable 4).

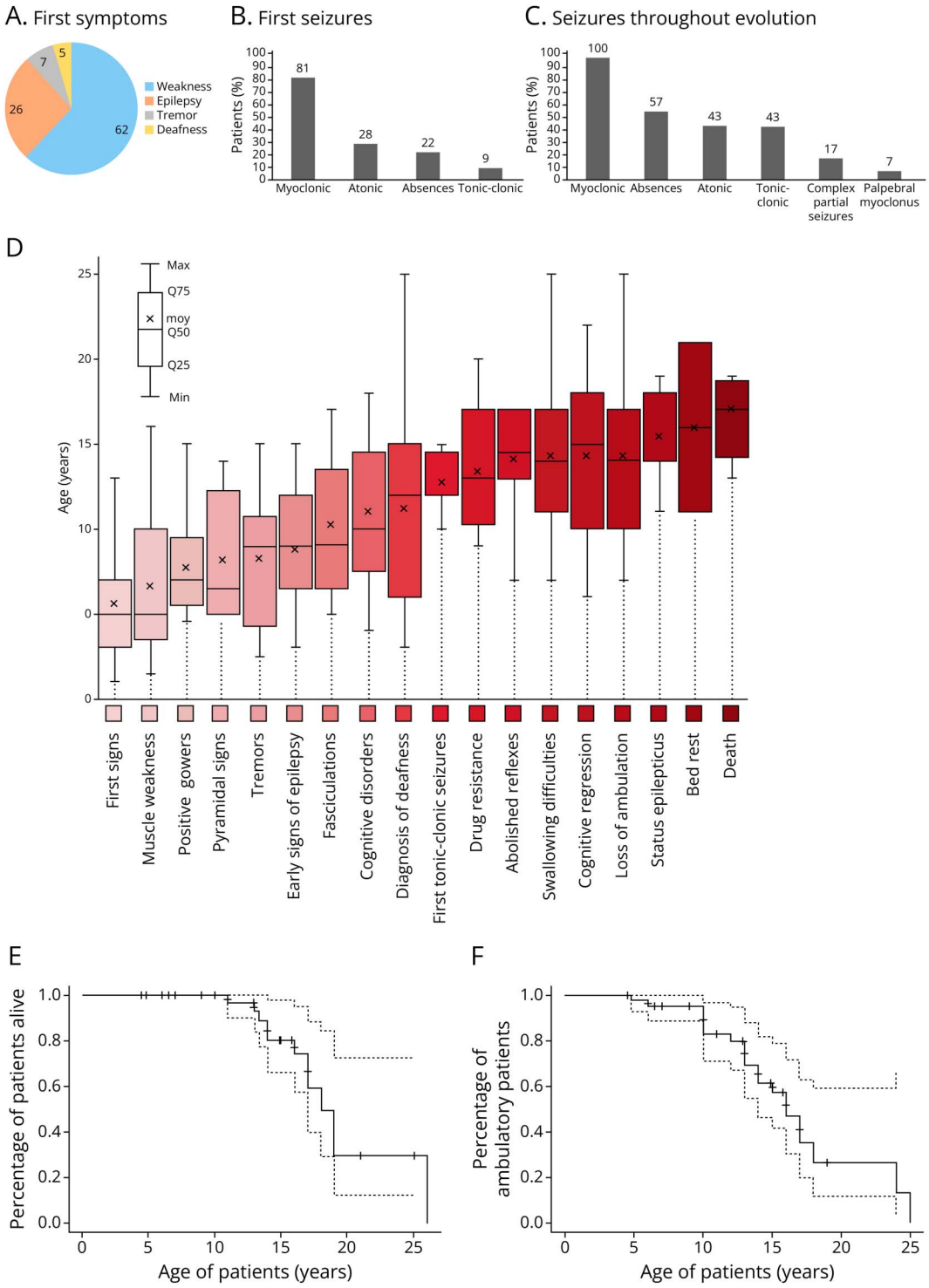
Discussion

We present in this work an in-depth clinical description of 9 patients, report 4 novel pathogenic variants associated with SMA-PME, and assess the relevance of the C26-ceramide as a biomarker for a prospective follow-up and of an *in cell* assay to determine the residual ACDase activity. In addition, the analysis of the previously reported patients with SMA-PME according to the data of our case series provides a statistical description of the disease natural history and brings new insights into genotype-phenotype correlations.

As previously reported, we observed in our patients a broad phenotypic spectrum and variability in disease progression. We highlight a dichotomy in the mode of onset, epilepsy or weakness. Age at onset ranged from 2.5 to 16 years, and the onset of epilepsy was inconstant and ranged from 5 to 14 years, as well as the loss of ambulation occurred at 5 years in P2 but maintained at 16 in P7. Intrafamily variability was also observed, with a faster progression of disability in P5 than in her sister P6 and almost 5 years of difference between their age at death.

We are also highlighting new symptoms: in addition to the neuromuscular, epileptic, and sensory phenotype, this series shows a probably underestimated incidence of digestive and urinary symptoms, not reported before, which may reflect progressive dysautonomia. Likewise, attentional deficit,

Figure 4 Natural History of 44 Patients With SMA-PME (9 Reported Here and 35 Studied From the Literature)

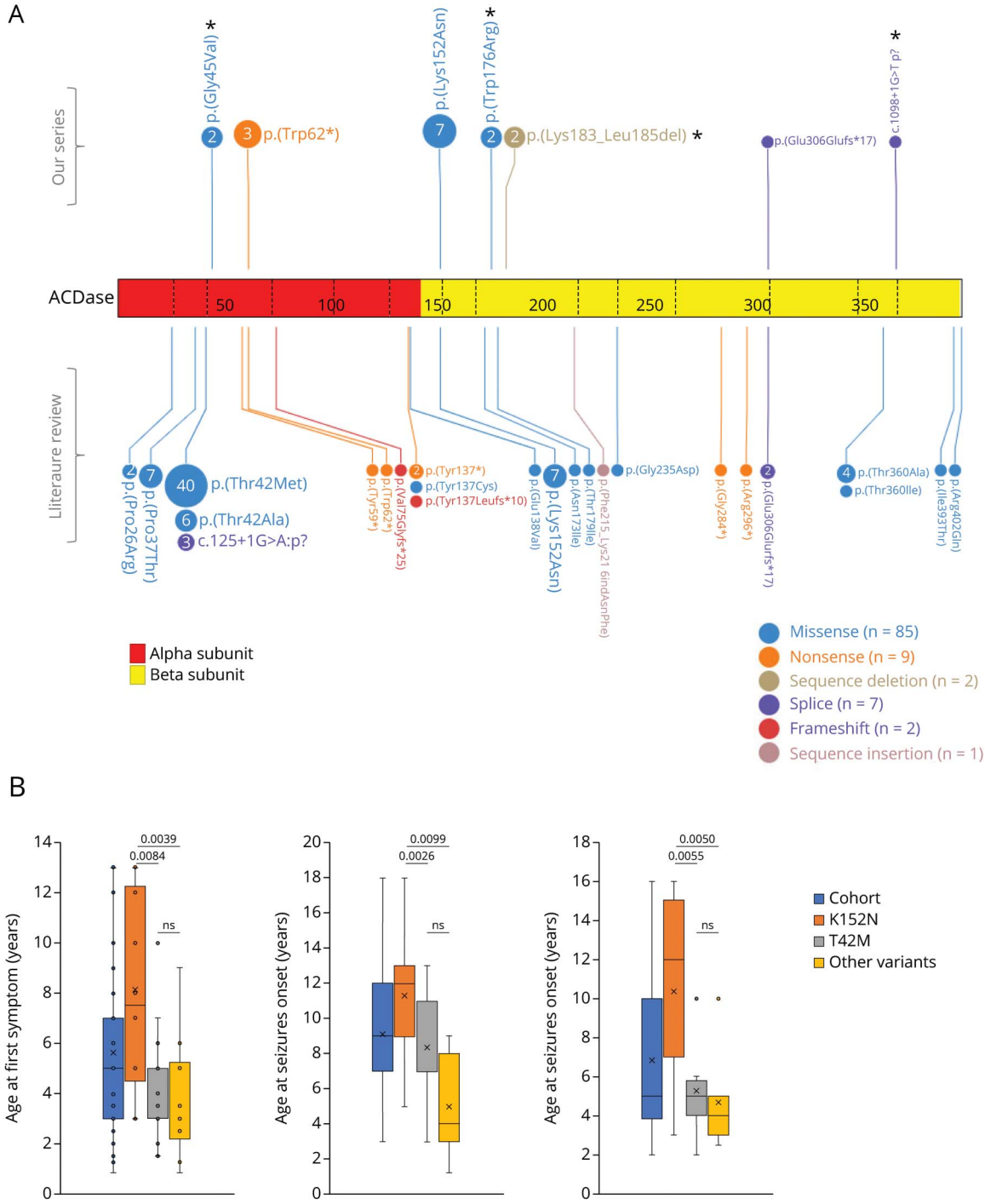


(A) First signs revealing SMA-PME. (B and C) Epileptic phenotype in SMA-PME: (B) types of seizures revealing epilepsy and (C) occurring during the course of the disease. (D) Symptoms in SMA-PME, ordered by age at onset. For each symptom, the cross represents the mean, the central bar represents the median, the peripheral bars represent the first and last quartiles, and the vertical lines represent the extreme ages. (E) Overall survival and (F) loss of ambulation in patients with classic SMA-PME, represented on the Kaplan-Meier curve. 95% CI: dotted line. SMA-PME = spinal muscular atrophy with progressive myoclonic epilepsy.

behavioral problems, and "multi-dys" learning disorders often precede muscle weakness and epilepsy and may not have been reported until now, considering normal neurodevelopment until school age in most cases.

Of interest, motor function assessments show a two-step progression, slowly progressive during several years and then markedly accelerated in the last 2 years of life. In some patients, the onset of tonic-clonic seizures and/or myoclonus

Figure 5 Schematic Representation of ACDase, the 27 Different Variants Found in Patients With SMA-PME, and Age at First Symptom of SMA-PME Depending on the Genotype of the Patients



(A) Heterodimeric structure of ACDase consists of α-subunit (13 kDa, residues 22–142) and β-subunit (40 kDa, residues 143–395), and the 27 different variants found in the 44 studied patients with SMA-PME. *Novel variants in SMA-PME. Created with ProteinPaint. (B) Genotype-phenotype correlation for the symptom onset, with T42M patients carrying at least one p.(Thr42Met) variant, K152N carrying at least one p.(Lys152Asn) variant, and others carrying none of these. Comparisons are made for (a) any symptom, (b) seizure, and (c) muscular weakness.

status epilepticus seems to coincide with this turning point toward accelerated disability and death. The cause of this subacute worsening is not understood but is reminiscent of the neuroinflammatory process observed in other lysosomal lipid storage diseases such as metachromatic leukodystrophy (MLD; MIM#250100) or Krabbe disease (KB; MIM#245200). Level of neuroinflammation and microglial activation is a key point for the decision to perform disease-specific treatments, such as hematopoietic stem cell transplantation (HSCT)[20] with allogenic donor or autologous ex vivo gene therapy and in vitro

gene therapy.[35] Such neuroinflammation could explain the poor or partial efficacy of HSCT in already symptomatic ACDase-deficient patient.[36]

The SMA and PME association seems specific enough to evoke the diagnosis: in our cohort, SMA-PME was clinically considered for 3 of 9 patients before genetic testing and then confirmed by targeted sequencing and biochemical analysis. The diagnostic delay of 3.3 years for index cases can be attributed to the multiplicity of differential diagnoses evoked before SMA and PME co-occur, which, therefore, depend on the mode of onset. When muscle weakness and anterior horn involvement are observed first, *SMN1*-related SMA is the main differential diagnosis. When epilepsy occurs first, differential diagnoses include various causes of myoclonus (juvenile myoclonic epilepsy, ceroid lipofuscinoses, mitochondriopathies such as Myoclonic Epilepsy with Ragged Red Fibers (MERRF), lysosomal storage diseases such as gangliosidoses or sialidoses, Lafora disease, Unverricht-Lundborg disease, dentato-rubro-pallido-luysian atrophy), atonic seizures (Doose syndrome), or atypical absences (GLUT1 deficiency).

By taking into account our series of 9 patients with 35 already reported patients, the significant differences in the prevalence of deafness, the age at and mode of onset, and the predominant genotype enabled us to demonstrate a statistically significant genotype-phenotype correlation for the 2 main variants (Figure 5B, eFigures 3–4, eTable 4). The p.(Thr42Met) substitution is associated with an earlier myopathic-like onset while the p.(Lys152Asn) variant is associated with deafness and a later epileptic onset. Despite different onset, the trajectories seem to converge: significant differences in the age at loss of walking or death were not observed (eFigure 4). Anecdotally, the p.(Thr42Ala) variant, although affecting the same residue as the predominant variant p.(Thr42Met), seems responsible for atypical forms of ACDase deficiency in 3 of 4 reported patients, including 2 late-onset SMA[18] and 1 late-onset FD.[22] Likewise, it is surprising to note that the only patient with visual impairment (bilateral optic atrophy) is the only with the p.(Arg402Gln) variant, at the ACDase C-terminus.[12]

It is hypothesized that the severity of ACDase deficiency correlates with residual enzymatic activity.[26] Indeed, (1) the *Asah1*−/− mouse model shows early embryonic lethality,[37] (2) no patient with FD nor SMA-PME carries truncating variants in the biallelic state, and (3) all patients with SMA-PME retain detectable residual ACDase activity.[26] In addition, the phenotypic continuum between FD and SMA-PME is increasingly documented: 4 patients have been reported with SMA and joint involvement, but no PME.[21-24] The observation of "Farber bodies" and "banana bodies" by EM in P5, having evoked FD,[31] fits into this framework (Figure 2). Moreover, motor neuronopathy, myoclonus, and seizures have also been reported in FD,[38] and some patients with FD who have undergone secondary HSCT develop neurologic symptoms with anterior horn involvement reminding SMA-PME.[20,36,39] Hence, SMA and PME could occur later in FD or could be in the background behind multivisceral involvement. Finally, a recently published mouse model demonstrated restricted spinal involvement.[40] This model is derived from the *Asah1*$^{P361R/P361R}$ mouse model for FD[35] after deletion of an intronic cassette, leading to increased residual *Asah1* mRNA expression. Thus, it seems that for the same variant, a variable level of *Asah1* expression can result in either FD or SMA phenotype in mice with neuroinflammation and microglial activation observed in the 2 models.[40,41] One can, therefore, imagine a threshold model below which substrate accumulation would be responsible for autoinflammation limited to the CNS in SMA-PME and above which the autoinflammatory process becomes subacute and systemic in FD.

A lower residual ACDase activity (<10%) would, therefore, be preferentially associated with the FD phenotype while a slightly higher, intermediate activity would be associated with SMA-PME, making these 2 conditions the extreme phenotypes of ACDase deficiency.[25] However, this hypothesis is challenged by several cases in our cohort and some previous series[5,14] because all patients who had an in vitro leukocyte ACDase activity assay were below this threshold. Of note, in all latter patients, ACDase was measured using the artificial fluorogenic Rbm 14-12 substrate[29] while variable and sometimes less reliable methods might have been used in some other publications.

Of interest, however, in situ measurements of ceramide degradation in living fibroblasts of P5 and P6 clearly showed a hydrolysis rate intermediate between that in FD and in control cell lines. Of note, using a similar methodological approach, an inverse correlation between residual activity and phenotype severity has been well documented in acid sphingomyelinase deficiency, separating the chronic-visceral and the neuronopathic forms.[42,43] In a previous study comparing ceramide degradation in 11 patients with FD,[38] the authors concluded that a correlation exists between undegraded ceramide levels and clinical severity, despite profound (<10% of normal) deficiency of ACDase activity measured in vitro. Although the exact numbers are not directly comparable because of some methodological differences, the proportion of residual ceramide observed in their oldest patients (death at 11 and 30 years) was of the same order of magnitude as that found for P5 and P6. Thus, while the in vitro assay remains of undeniable diagnostic value, the in situ assay in living cells could constitute an interesting functional test in the pathophysiologic investigations, or precisely quantify the level of ACDase activity restored after innovative therapies such as gene therapy.

C26-ceramide was proposed as a sensitive and specific biomarker in FD.[12,34] In our cohort, only a moderate C26-ceramide accumulation was observed in DBSs from patients with SMA-PME, in advanced stages of the disease. While P1 and P2 showed only a mild increase in C26-ceramide levels,

P3 and P4 showed a significant progressive accumulation (1.7-fold and 2-fold, respectively), concomitant with motor disability progression. P3 is also the only patient for whom C26-ceramide was quantified during the late phase of the disease. Surprisingly, the clinical worsening was associated with a significant decrease in C26-ceramide blood level, which remains unexplained, but could suggest the activation of an alternative catabolic pathway. However, this observation needs to be replicated in additional patients. In search of other biomarkers, lipidomic and cytokine profiles should also be evaluated prospectively in patients with SMA-PME.

In this study, we describe the clinical history of 9 patients with SMA-PME, report novel pathogenic variants, show a genotype-phenotype correlation for the 2 main variants, underline the need for precise level of residual ACDase activity, and evaluate the applicability of the C26-ceramide biomarker in the prospective follow-up of these patients. Based on the clinical history of 44 previously and newly reported patients, this study presents the detailed natural history of SMA-PME. Given the rarity of this disease, and in the absence of a reliable biomarker, this work may serve as a retrospective control group for future therapeutic trials.

Genetic analyses were subject to informed consent by the families after a specialized genetic consultation, in line with good ethical practice within the various centers. In France, this consent is edited for a given university hospital center, in accordance with bioethics laws and the General Data Protection Regulation (RGPD), framed by the National CNIL. Collection of clinical data has been submitted by Prof. Sandra MERCIER to the noninterventional research ethics committee (CERNI/CEDIS) of Nantes University (reference number 27042020), with an information letter and a nonopposition form sent to the patient by the clinician. In addition, patients and their family signed a consent for prospective clinical data and research samples accepted by ethics committees (LeucoEpimar 2009-AU788- CNIL 1406552; Operando 017-A01368-45).

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