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ORIGINAL ARTICLE

Caveolinopathy: Clinical, histological, and muscle imaging features and follow-up in a multicenter retrospective cohort

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Abstract

Background and purpose: *CAV3* gene mutations, mostly inherited as an autosomal dominant trait, cause various skeletal muscle diseases. Clinical presentations encompass proximal myopathy, distal myopathy, or isolated persistent high creatine kinase (CK) with a major overlapping phenotype.

Methods: Twenty-three patients with *CAV3* symptomatic mutations, from 16 different families, were included in a retrospective cohort. Mean follow-up duration was 24.2 ± 15.0 years. Clinical and functional data were collected during the follow-up. The results of muscle imaging, electroneuromyography, muscle histopathology, immunohistochemistry, and caveolin-3 Western blot analysis were also compiled.

Results: Exercise intolerance was the most common phenotype (52%). Eighty percent of patients had calf hypertrophy, and only 65% of patients presented rippling. One patient presented initially with camptocormia. A walking aid was required in only two patients.

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Electroneuromyography was mostly normal. CK level was elevated in all patients. No patient had cardiac or respiratory impairment. Muscle imaging showed fatty involvement of semimembranosus, semitendinosus, rectus femoris, biceps brachialis, and spinal muscles. Almost all (87%) of the biopsies were abnormal but without any specific pattern. Whereas a quarter of patients had normal caveolin-3 immunohistochemistry results, Western blots disclosed a reduced amount of the protein. We report nine mutations, including four not previously described. No phenotype–genotype correlation was evidenced.

Conclusions: Caveolinopathy has diverse clinical, muscle imaging, and histological presentations but often has limited functional impact. Mild forms of the disease, an atypical phenotype, and normal caveolin-3 immunostaining are pitfalls leading to misdiagnosis.

KEYWORDS

CAV3, caveolin-3, muscle imaging, myopathy, rippling muscle disease

INTRODUCTION

Mutations of *CAV3* gene coding for the caveolin-3 protein cause various skeletal muscle diseases [1]. Clinical presentations encompass proximal myopathy, distal myopathy, or isolated persistent high creatine kinase (CK) levels with a major overlapping phenotype [2]. Exercise-induced and/or postexercise myalgia are frequently the most prominent symptoms, and muscle hypertrophy is a common feature [2–4]. Since the limb girdle muscular dystrophy (LGMD) nomenclature revision in 2018, caveolinopathy is no longer classified as a cause of LGMD and phenotypes such as proximal motor deficit are classified as rippling muscle disease [4].

Whatever the phenotype, percussion-induced rapid contractions (PIRC) and muscle rippling are often present [5, 6]. Muscle rippling is a wavelike diffusion of electrically silent muscle contractions induced by stretching, affecting upper or lower limb muscles (Video S1).

Approximately 20 mutations have been reported, mostly with autosomal dominant inheritance [7].

Caveolin-3 is a muscle-specific caveolin protein isoform. By oligomerization, caveolin-3 molecules form caveolae, which are small omega-shaped invaginations of the plasma membrane and are involved in membrane trafficking of numerous signaling molecules. Caveolae also participate in the regulation of endocytosis and exocytosis, the regulation of signaling, and mechanotransduction (a mechanism that cells use to detect and respond to internal and external forces), as caveolae can break open in response to stretching of skeletal muscle fibers. They are also involved in muscle development and repair, lipid metabolism, and mitochondria homeostasis and interact with T-tubule proteins such as ryanodine receptor 1 (RYR1) and dihydropyridine receptor (DHPR) [7].

Here, we describe the various phenotypes and the disease evolution of 23 patients from 16 different families with *CAV3* mutations,

including four new mutations. We also focus on histological and muscle imaging findings and atypical clinical presentations.

METHODS

Between July 2021 and November 2021, we retrospectively reassessed the clinical and paraclinical data of all patients with caveolinopathy followed at four neuromuscular reference centers in France: Pitié-Salpêtrière, Raymond-Poincaré, Nancy, and Lille university hospital centers.

All patients with symptoms compatible with a caveolinopathy and a genetic confirmation (class 4 variants or above, following American College of Medical Genetics and Genomics [ACMG] guidelines) were included.

Detailed information on symptoms, onset age, neuromuscular examinations (including manual muscle testing, scored using the Medical Research Council scale), and rippling and/or PIRC presence and demographic information were collected. Functional outcomes were assessed using the Gardner–Medwin and Walton scale [8].

Muscle biopsies obtained at the time of diagnosis and processed according to standard methods were analyzed [9]. The following parameters were evaluated: presence of atrophic fibers, abundant centralized nuclei, necrotizing fibers, regenerating fibers, segmented fibers, inflammatory cells, core, mitochondrial anomaly (mitochondrial subsarcolemmal aggregate, ragged red fiber, or cytochrome c oxidase [COX] stain anomaly), vacuoles, and caveolin-3 immunohistochemistry (IHC). Muscle protein analysis was performed using the Western blot (WB) method in accordance with a previously described procedure [10]. Monoclonal antibodies used for WB were purchased from Novocastra (Newcastle, UK; www.novocastra.co.uk).

Genomic DNA was extracted from the patients' whole blood using standard procedures. Exons and flanking intronic sequences of *CAV3* gene (NM_033337.3) were sequenced using Sanger or next

generation sequencing. In line with ACMG guidelines, pathogenicity of novel variants was evaluated based on computational data, population data, and allelic data [11].

Additional data such as CK level, muscle imaging (muscles were assessed and categorized using the Mercuri score [12]), respiratory and cardiac evaluation, and neurophysiological studies were reported.

In accordance with French legislation, the study was approved by the local ethics committee and by the French data protection authority (Commission nationale de l'informatique et des libertés).

RESULTS

Patients' clinical and ancillary test findings are summarized in Table 1.

Clinical features

Twenty-three patients, from 16 families, with a mean age of 57.3 years (range = 20–85) are described. Mean age at onset of the first symptoms was 27.2 ± 18.8 years. One patient developed his

TABLE 1 Patients' characteristics.

Patient No.	Family	Variant	Gender	Native country	Age at first symptoms ^a	Age at last follow-up visit	Muscle weakness	Rest myalgia	Exercise intolerance
1	A	c.136G>A, heterozygous	F	France	43	73	N	N	Y
2	A	c.136G>A, heterozygous	M	France	~10	55	N	Y	Y
3	B	c.136G>A, heterozygous	M	France	~10	43	N	Y	Y
4	C	c.80G>A, heterozygous	F	Algeria	60	77	Proximal weakness	N	Y
5	D	c.194T>C, heterozygous	M	France	50	70	Proximal weakness	N	N
6	E	c.427_431del, homozygous	M	France	20	70	N	N	Y
7	F	c.427_431del, homozygous	M	Portugal	NS	49	N	N	N
8	G	c.80G>A, heterozygous	M	Belgium	12	45	N	N	Y
9	G	c.80G>A, heterozygous	F	France	NS	80	N	N	N
10 ^c	H	c.130G>A, heterozygous	F	France	39	69	Distal weakness	Y	N
11	H	c.130G>A, heterozygous	M	France	39	40	N	Y	Y
12	I	c.137C>T, heterozygous	F	France	~10	27	N	N	Y
13	F	c.99C>G, heterozygous	F	Portugal	40	54	N	Y	N
14	J	c.99C>G, heterozygous	M	Portugal	26	64	N	Y	N
15	K	c.172T>C, heterozygous	F	France	NS	62	N	Y	N
16	K	c.172T>C, heterozygous	F	France	NS	66	Proximal weakness	N	N
17	L	c.80G>A, heterozygous	F	France	13	18	N	Y	Y
18	M	c.80G>A, heterozygous	F	France	~10	49	N	Y	N
19	J	c.80G>A, heterozygous	F	Portugal	40	85	Distal weakness	Y	N
20	N	c.80G>A, heterozygous	M	France	64	79	Camptocormia/mild proximal weakness	N	N
21	O	c.241G>C, heterozygous	F	France	~10	21	N	N	Y
22	M	c.80G>A, heterozygous	F	France	~10	20	N	N	Y
23	P	c.80G>A, heterozygous	M	France	~10	17	N	Y	Y

Note: All patients presented heterozygous variants, except Patients 6 and 7. Age at first symptoms and age at last follow-up visit are expressed in years.

Abbreviations: ANL, abnormal; CK, creatine kinase; ENMG, electroneuromyography; F, female; GMW, Gardner–Medwin and Walton scale; HT, hypertrophy; IHC, caveolin-3 immunohistochemistry; M, male; N, no; NA, not available; ND, not done; NL, normal; NP, not performed; NS, not symptomatic; WB, caveolin-3 Western blot; Y, yes.

^aAge at first symptoms, excluding rippling and calf hypertrophy.

^bCK level (UI/L) in standard condition (without acute medical condition occurring or unusual exercise performed within the previous 72 h).

^cPatient 10 also carried ANO5 compound heterozygous variants c.191dup (p.Asn64LysfsTer15), a well-documented pathogenic variant, and c.155A>G (p.Asn52Ser), a variant of uncertain significance.

symptoms at 64 years of age. Nevertheless, symptoms such as myalgia started during childhood in almost half of the patients. Mean follow-up duration was 24.2 ± 15.0 years.

Patients' detailed clinical features are summarized in Table 2.

Six main clinical patterns emerged, although with clear overlapping in some patients: exercise intolerance, myalgia, isolated rippling phenomenon, predominantly proximal muscle weakness, predominantly distal muscle weakness, and camptocormia (Figure 1).

Exercise intolerance was present in 12 patients (52%). Five of them described a feeling of limb stiffness associated with the pain.

Eleven patients (48%) reported rest myalgia. Five patients (22%) had permanent myalgia, and six (26%) presented prolonged postexercise myalgia (lasting for >24 h).

Rippling phenomenon (see Video S1) was observed in two thirds of the patients, mostly in the thighs. Only one patient presented with spontaneous rippling and did not have PIRC.

Symmetric calf hypertrophy was noticed in >80% of the patients, associated with an Achilles tendon contracture in half of them. All 23 patients had preserved deep tendon reflexes.

Muscle weakness occurred during the disease course (mean follow-up = 24.2 ± 15.0 years) in nine patients (39%). It concerned lower limb proximal muscles and erector spinae in six and three

GMW scale	Calf HT	Rippling	CK ^b	ENMG	Biopsy	IHC	WB
1	N	Y	692	NL	ANL, atrophic fibers, nuclear centralization	ND	ND
0	Y	Y	600	NL	ANL, atrophic fibers	Irregular	Reduced
0	Y	N	769	NP	ANL, atrophic fibers	NL	NP
3	Y	Y	400	NL	NP	NP	NP
6	N	Y	1663	Myopathic pattern	ANL, atrophic fibers, nuclear centralization	Absence	Reduced
1	Y	Y	275	NP	ANL, atrophic fibers, nuclear centralization	Absence	NP
0	Y	Y	305	NL	NP	NP	NP
0	Y	Y	667	NL	NL	Absence	NP
0	N	Y	198	NL	ANL, atrophic fibers	Absence	NP
6	Y	N	1200	NL	ANL, atrophic fibers, nuclear centralization, cores	NL	Reduced
0	Y	Y	342	NP	NP	NP	NP
0	Y	N	211	NL	ANL, atrophic fibers, nuclear centralization	NL	NP
4	Y	Y	600	Myopathic pattern	NL	Irregular	Reduced
0	Y	N	439	NP	ANL, atrophic fibers, nuclear centralization	Absence	NP
0	Y	N	300	Myopathic pattern	ND	NP	Reduced
2	Y	N	1000	Myopathic pattern	ND	NP	Absence
0	Y	N	1700	NP	ANL, atrophic fibers, nuclear centralization	Absence	Absence
2	Y	Y	726	NP	ANL, atrophic fibers	NP	NP
3	N	N	283	NP	NP	NP	NP
3	Y	Y	476	NL	ANL, atrophic fibers, mitochondrial aggregates	Reduced	Absence
0	Y	Y	516	NL	ANL, atrophic and necrotizing fibers	Irregular	NP
1	Y	Y	750	NP	ND	NP	NP
1	Y	Y	1500	NL	NA	NP	Absence

patients, respectively. Facial muscles were always spared. None of them had swallowing or respiratory difficulties or sphincter dysfunction.

Functional consequences were limited, as all patients remained ambulant. Seventeen patients (74%) could climb stairs without banisters, and 21 patients (91%) walked without assistance; the remaining two patients (8%) walked with two sticks.

Functional consequences and disease evolution are summarized in [Figure 2](#).

One patient (Patient 20) had a peculiar phenotype. This Caucasian male, carrying a heterozygous c.80G>A (p.Arg27Gln) *CAV3* pathogenic variant, developed a progressive camptocormia at 64 years of age followed by a mild lower limb proximal weakness. He had symmetric calf hypertrophy, and rippling muscle and PIRC, both evidenced on thighs. He reported no exercise intolerance. At the last visit, aged 79 years and almost 15 years after the onset of the disease, the functional consequences were moderate. He was able to climb stairs with banisters and walked without assistance. CK level was 600–700 U/L. Neurophysiological studies revealed a myopathic pattern in proximal muscles of the lower limbs. His whole-body muscle magnetic resonance imaging (MRI) highlighted muscle fatty replacement especially in the thoracoabdominal paravertebral muscles, biceps brachii, abdominal muscles, hip adductors, and posterior compartment of the thigh. Histology of a deltoid muscle biopsy, performed at 72 years of age, revealed fibers of various diameters, without necrosis or regeneration, and subsarcolemmal mitochondrial aggregates with no COX-negative fibers. IHC revealed a marked reduction of caveolin-3 staining and absence of this protein on WB. The main differential diagnoses of camptocormia, such as parkinsonian syndromes, sporadic late onset nemaline myopathy, inclusion-body myositis, facioscapulohumeral dystrophy, and Pompe disease, were excluded.

Paraclinical investigations

All patients had elevated CK levels, and 13 patients (57%) presented with more than three times the upper limit of normal ([Table 3](#)). Mean CK level was 679 U/L (median=600 U/L, range=211–9500 U/L). No patients presented or described a previous episode of rhabdomyolysis. Two patients with exercise intolerance underwent a nonischemic forearm exercise test (grip test), which was normal [[13](#)].

Cardiac and respiratory examinations and electroneuromyography

Echocardiography (16 patients), electrocardiogram (18 patients), and functional respiratory investigations (nine patients) were normal for all these patients.

Electroneuromyography (ENMG) performed in 13 patients was normal in nine patients (69%). Four patients (31%) had a myopathic pattern (polyphasic short duration and low amplitude of motor unit potentials) in proximal muscles. Long and short exercise tests were normal.

Muscle MRI

Skeletal muscle imaging (MRI and/or computed tomography [CT]) was performed in 12 patients ([Figure 3](#)), five of whom underwent whole-body imaging (three with MRI and two with CT). In the latter patients, biceps brachii were affected with a mild fatty infiltration observed in two patients (Mercuri=2), whereas muscle imaging of the upper limbs was normal in the other three patients.

The lower limb muscles, studied in 12 patients, were abnormal in 10 patients, with a large range of severity. The thigh muscles were affected in seven patients; hamstrings were affected in all seven, mostly the semimembranosus and semitendinosus muscles, and quadriceps were also affected in four of these seven patients, mostly the rectus femoris muscle. In the legs, fatty involvement was observed in eight patients in gastrocnemii muscles and in soleus muscle in five of these latter patients. On the other hand, tibialis anterior muscles were spared in all patients.

Three of the five patients with whole-body imaging had moderate fatty infiltration (Mercuri=3) of their spinal muscle. Spinal muscles were globally involved without specificity except in one patient (Patient 10, 72 years old), who presented predominant involvement of the lumbar erector spinae muscles. Facial muscles had a normal appearance, and in addition, there was no hyperintensity on T2-weighted images.

Muscle histology

Deltoid muscle biopsies were performed in 15 patients and biceps brachii biopsies in one patient. Morphological analysis was abnormal in 87% of biopsies. Atrophic fibers were reported in 13 cases (87%) and profusion of internal nuclei in seven patients (47%; [Figure 4](#)). Additional abnormalities such as necrotizing and/or regenerating fibers and subsarcolemmal mitochondrial aggregates without ragged red fibers were noticed in some patients. Interestingly, core lesions were evidenced in the muscle biopsy of Patient 10 ([Figure 4](#)).

IHC of the caveolin-3 protein on muscle biopsy was performed in 13 patients. Immunostaining was absent in six patients (46%) and reduced or irregular in four patients 31% ([Figure 4](#)). The immunolabeling of caveolin-3 was normal in three patients (23%).

Western blot of the caveolin-3 protein on the muscle biopsy was performed in eight patients. Analyses were systematically abnormal; caveolin-3 was absent in half of the patients and greatly reduced in the remaining half. Of note, WB performed in Patient 10, with

TABLE 2 Demographic, clinical, and biological characteristics at last follow-up visit.

Demographic characteristics	
Mean age, years (SD)	57.3 (21.5)
Women/men, <i>n</i>	13/10
Age at onset, years, mean (SD) ^a	27.2 (18.8)
Clinical characteristics	
Exercise intolerance, <i>n</i> (%)	12 (52)
Myalgia, <i>n</i> (%)	18 (78)
Rest myalgia, <i>n</i> (%)	5 (22)
Prolonged postexercise myalgia (>24 h), <i>n</i> (%)	6 (26)
Rippling, <i>n</i> (%)	15 (65)
PIRC, <i>n</i> (%)	14 (61)
Motor deficit, <i>n</i> (%)	9 (39)
Calf hypertrophy, <i>n</i> (%)	19 (83)
Achilles tendon retraction, <i>n</i> (%)	9 (39)
Four limbs DTR preserved, <i>n</i> (%)	23 (100)
Functional impact	
GMW, mean (SD)	1.4 (1.9)
Blood biochemistry	
CK ^b , mean (SD)	677.9 (451)
Above the normal limit, <i>n</i> (%)	23 (100)
At least three times the upper limit, <i>n</i> (%)	13 (57)

Abbreviations: CK, creatine kinase; DTR, deep tendon reflex; GMW, Gardner–Medwin and Walton scale; *n*, number of patients; PIRC, percussion-induced rapid contractions.

^aAge at onset of first symptoms (excluding rippling and calf hypertrophy).

^bCK level (UI/L) in standard condition (without acute medical condition occurring or unusual exercise performed within the previous 72 h).

normal caveolin-3 immunolabeling, demonstrated a greatly reduced amount of the protein.

IHC of the dysferlin protein on muscle biopsy, performed in 10 patients, was systematically normal. No reduction of dysferlin was found on WB (three patients tested).

Genetic analysis

Eight different heterozygous pathogenic variants and one biallelic deletion in *CAV3* were found in our cohort (Figure 5).

Five variants, c.80G>A (p.Arg27Gln), c.136G>A (p.Ala46Thr), c.99C>G (p.Asn33Lys), c.137C>T (p.Ala46Val), and c.130G>A (p.Val44Met), evidenced in the heterozygous state in our cohort, had already been reported [14–18].

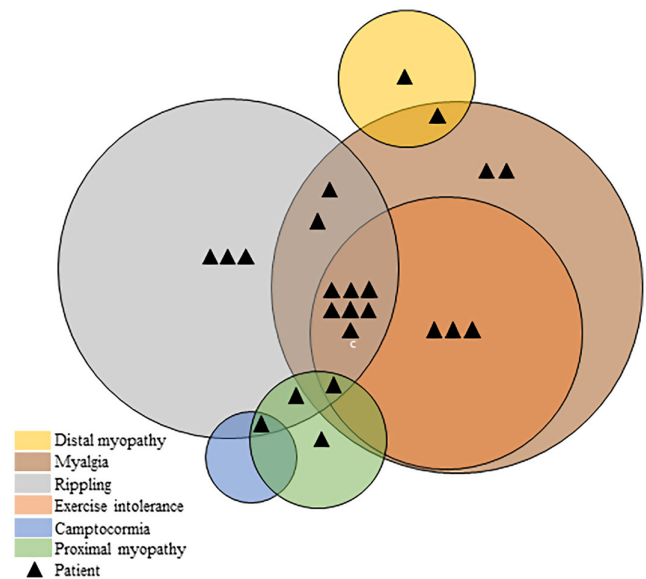


FIGURE 1 Schematic representation of clinical patterns in *CAV3*-mutated patients. Each circle corresponds to a clinical pattern. A clinical pattern is defined by a predominant symptom. Patients are represented by a triangle. When several symptoms are major in a patient, the triangle is placed at the intersection of the circles of each corresponding pattern. The size of each circle correlates to the number of patients with that pattern.

Variant c.80G>A (p.Arg27Gln) was the most prevalent variant in our cohort and was found in seven families, as previously reported [14].

The second most prevalent variant in our cohort was c.136G>A (p.Ala46Thr). It was observed in three of 23 patients (two families: Family A, Patients 1 and 2; and Family B, Patient 3).

Variants c.99C>G (p.Asn33Lys), c.137C>T (p.Ala46Val), and c.130G>A (p.Val44Met) were found in five patients, belonging to Families J, F, I, and H (Table 1).

We found four new pathogenic variants, comprising three missense variants, c.172T>C (p.Trp58Arg), c.194T>C (p.Phe65Ser), and c.241G>C (p.Gly81Arg), and one biallelic deletion, c.427_423del (p.Ile143_Lys144delinsGlyfsTer55). They were all absent from the gnomAD v3.1.2 database (<https://gnomad.broadinstitute.org/>).

Variant c.172T>C (p.Trp58Arg) was carried by Patients 15 and 16 (Family K). Caveolin-3 WB analysis was performed for Patient 16, showing a reduction of caveolin-3 protein levels. This variant had already been reported as pathogenic in the Leiden Muscular Dystrophy database (www.dmd.nl) but had not yet been published. Variant c.194T>C (p.Phe65Ser) was evidenced in Patient 5 (Family D), in whom the muscle IHC study showed an absence of caveolin-3 and a great reduction of the protein on WB. Variant c.241G>C (p.Gly81Arg) was found in Patient 21 (Family O), whose IHC study of

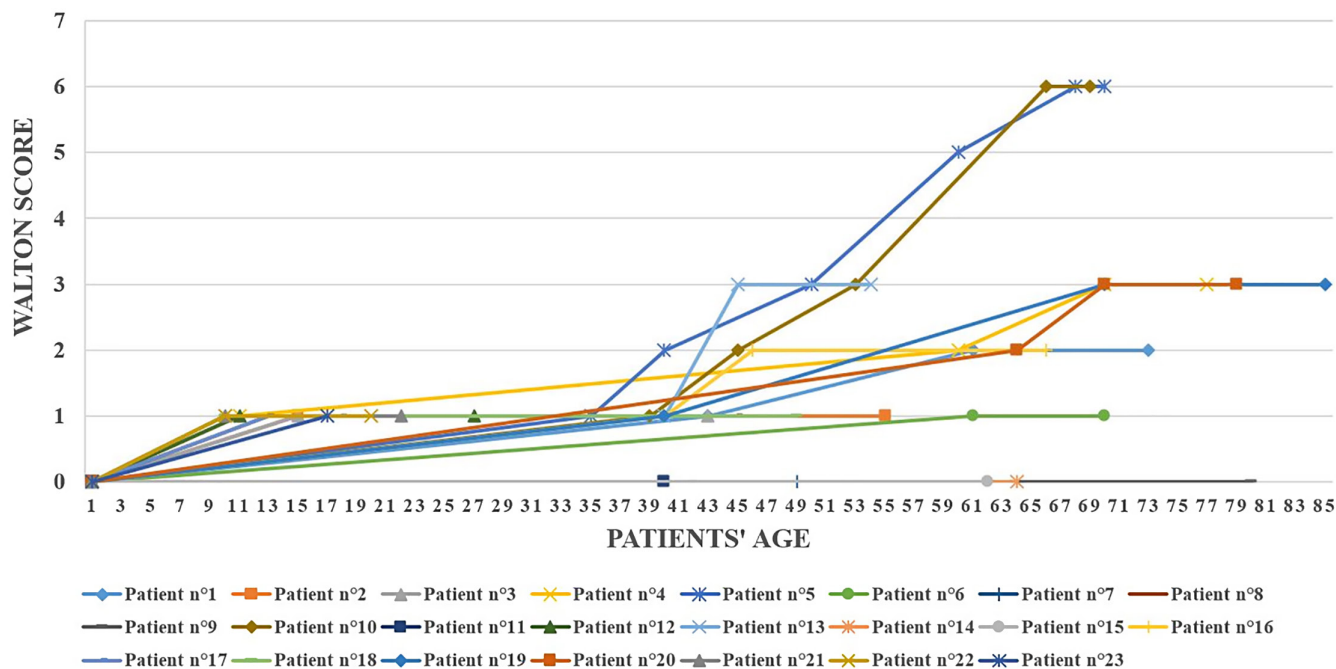


FIGURE 2 Functional consequences evolution.

caveolin-3 showed an irregular labeling. We considered these three variants likely pathogenic and classified them as class 4 following the ACMG criteria [11] because of their absence in the control population, their position in a functional domain of the protein, the absence or reduction of caveolin-3 in patients' muscle biopsy, and their pathogenic predictions based on bioinformatic missense variant predictors as shown in MobiDetails [19].

Homozygous deletion c.427_431del (p.Ile143_Lys144delins GlyX55) was carried by Patients 7 and 13 (Family F). This variant was also considered likely pathogenic and was classified as class 4 following the ACMG criteria [11], as it was absent in the control population and its frameshift variant status is predicted to cause a loss of function. Moreover, its pathogenicity is supported by a reduction of caveolin-3 immunolabeling on muscle biopsy.

In addition to the c.130 G>A (p.Val44Met) CAV3 heterozygous variant, Patient 10 carried ANO5 compound heterozygous variants c.191dup (p.Asn64LysfsTer15), a well-documented pathogenic variant [20], and c.155A>G (p.Asn52Ser), a variant of uncertain significance following the ACMG criteria [11], making it impossible to assert the influence of the ANO5 gene in the patient's phenotype.

Finally, we found no genotype/phenotype correlation, because clinical pattern and disease severity were highly variable between individuals carrying the same mutation even within the same family (Table 1).

DISCUSSION

Here, we report the largest cohort with muscle diseases due to CAV3 mutations to date. As previously reported, symptoms often began in childhood [5, 21]. However, CAV3 myopathy was finally confirmed only later, at a mean age of 49 years, highlighting the delay in diagnosing this disease. The main symptom was myalgia, often presenting as exercise intolerance, but which was sometimes misleading, as it appeared after prolonged exertion or at rest. Rippling muscle, a signature feature of the disease, was found in only two thirds of the cases, a finding quite similar to that in other cohorts, where it ranged from 55% to 80% [5, 22]. Because rippling may not be handicapping, many patients might not feel the need to consult.

Calf hypertrophy was found in 80% of patients, a feature also observed by Aboumoussa et al. [5]. In a context of exercise intolerance and elevated CK level, this feature may point toward this diagnosis, especially in patients with absence of weakness.

Although nine of our patients had lower limb muscle weakness after almost 3 decades of follow-up, only two patients of the 23 in our cohort required a walking aid, demonstrating that caveolinopathy is a quite benign myopathy. Functional consequences are rarely accurately reported in previous cohorts [1, 5, 6, 22]. However, Papadopoulos et al. reported that only one patient of six needed a walking aid [3]. The absence of respiratory or cardiac involvement is also a reassuring element in their prognosis.

TABLE 3 Ancillary tests and complementary/systemic investigations.

Test/investigation	n (%)
Neurophysiological exploration	
Four limbs electroneuromyography	13 (57)
Normal	9 (69)
Myopathic pattern observed in a muscle	4 (31)
Short and long exercise testing	4 (31)
Normal	4 (100)
Muscle histology	
Muscle biopsy	16 (70)
Atrophic fibers	13 (87)
Abundant centralized nucleus	7 (47)
Necrotizing fibers	1 (7)
Regenerating fibers	1 (7)
Segmented fibers	0 (0)
Inflammatory cells	1 (7)
Core	1 (7)
Mitochondrial anomaly	1 (7)
Mitochondrial subsarcolemmal aggregate	1 (7)
Ragged red fiber	0 (0)
COX stain anomaly	0 (0)
Muscle immunochemistry	13 (81)
Absence of caveolin-3	6 (46)
Reduction or irregular labeling of caveolin-3	4 (31)
Normal labeling of caveolin-3	3 (23)
Muscle immunoblots	8 (35)
Absence of caveolin-3	4 (50)
Very low level of caveolin-3	4 (50)
Normal presence of caveolin-3	0 (0)
Respiratory exploration	
Respiratory functional exploration	9 (39)
Normal	9 (100)
Cardiological exploration	
Electrocardiogram	18 (78)
Normal	18 (100)
Echocardiography	16 (70)
Normal	16 (100)

Note: Data represented as number (percentage) of patients.

Abbreviations: COX, cytochrome c oxidase; n, number of patients.

In our series, we could delineate the following six distinct although sometimes overlapping patterns, listed from mildest to the most disabling: rippling, exercise intolerance, myalgia, proximal muscle weakness, distal muscle weakness, and camptocormia, which

was not previously reported. Although we cannot exclude another rare cause for the major axial muscle involvement, muscle histological analysis and reduced caveolin-3 immunostaining support the caveolinopathy observed in the patient, who carried the common c.80G>A CAV3 variant [3, 5, 21]. Whole-body MRI in our series demonstrated a fatty replacement of erector spinae muscles in this patient with camptocormia but also in our patients with more common features, such as myalgia. It is interesting to note that a previous patient bearing the same heterozygous c.80G>A CAV3 variant has been reported with an isolated neck muscle weakness, but muscle imaging was not described [23].

Besides the paravertebral muscle involvement, the anterior and posterior compartment of thigh may be involved, mostly rectus femoris, semimembranosus, and semitendinosus muscle, extending in patients with lower limb weakness to biceps femoris, gracilis, soleus, and gastrocnemius, which may appear hypertrophic as the consequence of fatty replacement, as previously described [23]. The upper limbs were less affected, biceps brachialis being the most frequently involved. Whole-body muscle imaging, performed in some patients of our cohort, prompted us to observe that spinal muscles, especially erector spinae muscles, frequently presented with fatty involvement, even in patients without axial weakness. Studies with a larger number of patients are needed to confirm this imaging pattern of muscle involvement. Moreover, the fatty involvement of spinal muscles is noteworthy, as it also observed in other dystrophies, such as dystrophinopathies, facioscapulohumeral dystrophies, dysferlinopathies, and calpainopathies [24]. This paraspinal involvement is often neglected, because in the workup of muscle dystrophies, limb imaging is mostly performed.

CK levels were consistently elevated in our cohort, with half of the patients having CK levels more than three times the upper limit of normal, as observed in other cohorts [2, 5]. Although episodes of rhabdomyolysis or myoglobinuria may occur, they were not observed in our cohort [5, 6].

Neurophysiological explorations were rarely contributory; they were most often normal and sometimes suggestive of myopathy. As pointed out by others, ENMG is not very useful in the diagnosis of caveolinopathy, because a normal ENMG does not exclude this diagnosis, but is useful for differential diagnoses, such as myotonic disorders [6, 25].

The muscle histopathological findings were usually abnormal with nonspecific features, such as disseminated atrophic fibers. Moreover, histopathology may be misleading, as subsarcolemmal mitochondrial aggregates or even cores might be observed. The lack of muscle histology specificity was also reported in previous studies, which pointed to the occurrence of rimmed vacuoles or endomyosial inflammatory infiltration [3, 5, 26]. Altogether, this might also explain the delay in diagnosis, because some histological features might point to alternative diagnoses.

In contrast to many dominant conditions, a reduction in the quantity of or the absence of caveolin-3 has been systematically observed by IHC in reported cases, except in one patient with a

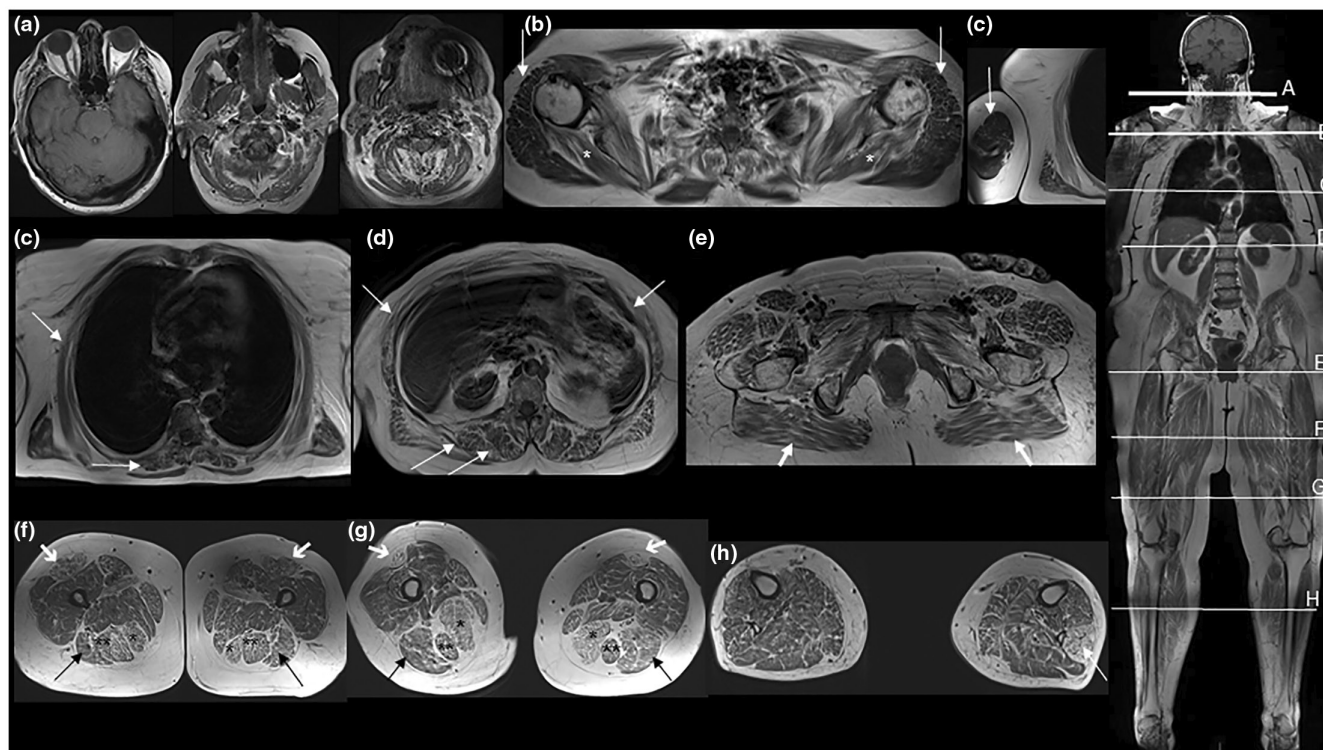


FIGURE 3 Whole-body magnetic resonance imaging of Patient 4, bearing the c.80G>A *CAV3* variant. T1-weighted turbo spin echo axial section, a–h, top to bottom (with coronal section correspondence on the right) shows moderate to significant fatty infiltration (Mercuri score = 3–4) with a symmetrical distribution. In the thigh, the rectus femoris (white arrow, f and g), semimembranosus (*, f and g), and semitendinosus (**, f and g) muscles present the most prominent fatty involvement, followed by the biceps femoris (black arrow, f and g), adductors, and glutei muscles (with arrow, e). In the shoulder girdle, note the involvement of the trapezius, supra- and infraspinatus (*, b), and deltoid muscles (white arrow, b). The biceps brachialis (white arrow, top right panel c) and the left fibularis longus (white arrow, h) are also mildly affected (might be a posttraumatic fatty infiltration for the left fibularis, considering the unilateral lesion), whereas the facial muscles are preserved. Paraspinal muscles, abdominal strap, and intercostal muscles (white arrows, middle left panel c and d) are undergoing mild fatty degeneration (grade 2).

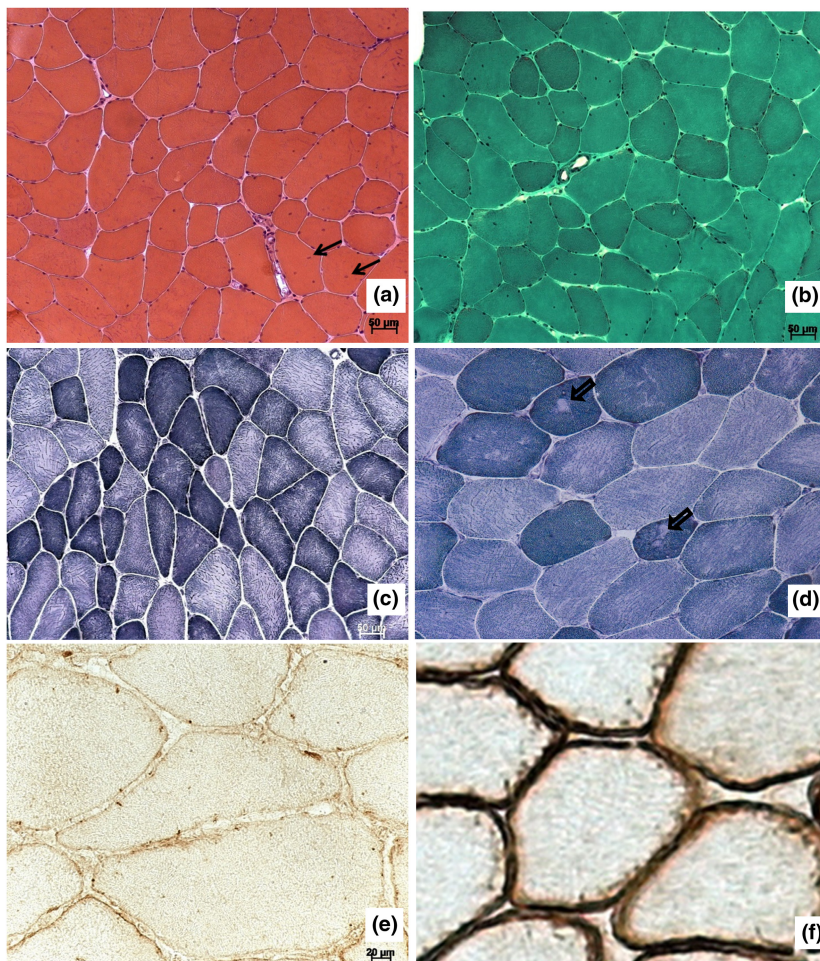
heterozygous pathogenic variant (c.233C>T) [10], although others have reported a reduction in caveolin-3 immunostaining with the same variant [26]. However, in our cohort, in a quarter of biopsies, IHC was normal, which could be misleading for the clinician, especially in those patients with only myalgia. However, caveolin-3 WB analysis performed on the muscle was systematically abnormal, and this is a reliable technique, as previously mentioned [1, 6].

Immune-mediated rippling muscle disease (iRMD) is a differential diagnosis for a patient presenting with rippling and elevated CK levels. This rare immune-mediated myopathy is most often associated with anti-cavin-4 antibodies [27]. The immunohistopathological analysis of biopsied muscle of patients with iRMD demonstrates a mosaic pattern of caveolin-3 deficiency [28]. On the contrary, in our cohort, reduction of caveolin-3 immunostaining was diffuse in *CAV3*-mutated patients. To our knowledge, caveolin-3 WB and muscle imaging have not been reported in iRMD. In contrast, calf hypertrophy is absent in iRMD, and symptoms tend to start earlier in our *CAV3*-mutated patients (median = 27 years old) than in iRMD patients (median = 60 years old). The frequent association with

myasthenia gravis is also a distinguishing feature in favor of iRMD [29]. On the contrary, a family history, identification of a *CAV3* gene pathogenic variant, and the fatty involvement on muscle imaging are supportive of a genetic cause of RMD.

We confirmed the previously reported pathogenic variants, among which the c.80C>G variant is the most common one [7], and we also described three new heterozygous pathogenic variants, c.194T>C (p.Phe65Ser), c.172T>C (p.Trp58Arg), and c.241G>C (p.Gly81Arg), associated with various phenotypes (myalgia, exercise intolerance, proximal myopathy). Moreover, we found a homozygous deletion, c.427_431del (p.Ile143_Lys144delinsGlyfsX55), associated with a mild phenotype, as the two siblings have only rippling muscle and calf hypertrophy. As in previous studies, no clear phenotype-genotype correlation was found, as patients with the same mutation, even within the same family, could present different clinical pictures [22]. Moreover, the biallelic frameshift mutation does not seem to be predictive of disease severity. Thus, additional genetic modifiers and/or environmental factors might explain the wide variability of the phenotype.

FIGURE 4 Muscle biopsy of Patients 5 and 10, showing transversal frozen sections of Patient 5 (a–c, e, f) stained with hematoxylin & eosin (a), modified Gomori trichrome (b), reduced nicotinamide adenine dinucleotide (NADH; c, d), and caveolin-3 immunostaining (e). Note the wide range of variation in fiber size, and that there are some internalized nuclei (a, arrows) without vacuole or protein aggregate (a–c). (e, f) Reduced staining of caveolin-3 is observed (e) compared with a control patient (f). (d) Muscle of Patient 10 showing cores (empty arrows show some examples) visible on reduced NADH staining.



Myalgia and exercise intolerance in *CAV3*-mutated patients are poorly understood and difficult to treat.

It was shown that muscle cells from *CAV3*-mutated patients had a 50% reduction in depolarization-induced Ca^{2+} influx, whereas there was no significant difference in DHPR or RYR1 channel expression level [30]. DHPR is a voltage-activated calcium channel, present in the transverse tubules responsible for both depolarization-induced Ca^{2+} influx and interaction with RYR1 for calcium release from the sarcoplasmic reticulum. Thus, the absence of caveolin-3 could affect DHPR and RYR1 distribution, leading to a less efficient coupling between DHPR and RYR1 and to a decrease in excitation-coupled Ca^{2+} influx, which may in part explain myalgia and the exercise intolerance observed in our patients.

On the other hand, the phenotype observed in our patients could also stem from a defective mechanoprotection of the sarcolemma. It has been shown that cells respond to mechanical stress by caveolae flattening and disassembly [31]. Moreover, the expression of a dystrophy-associated *CAV3* mutant in zebrafish led to sarcolemmal damage in response to vigorous muscle activity [32]. More recently, it has been shown that the regulation of mechanoprotection

by caveolae is directly coupled with specific intracellular signaling pathways leading to changes in gene expression and that this regulation is absent in *CAV3*-mutated patients [33].

CONCLUSIONS

Our study highlights the important clinical presentation heterogeneity from one patient to another with a *CAV3* mutation, already reported in the literature. Rippling, calf hypertrophy, and elevated CK level are major clinical features orienting the diagnosis. Although proximal or distal muscles might be involved, we also demonstrated that erector spinae muscles might undergo fatty degeneration, leading in some rare patients to camptocormia. In our series, electromyography and muscle biopsy were often poorly informative and could even be misleading. Thus, because immunostaining for caveolin-3 can be normal, immunoblot of caveolin-3 and subsequently *CAV3* gene analysis are mandatory for the diagnosis. We also highlight that caveolinopathy is in most cases a benign or mild myopathy even at advanced ages, thus explaining the diagnostic delay.

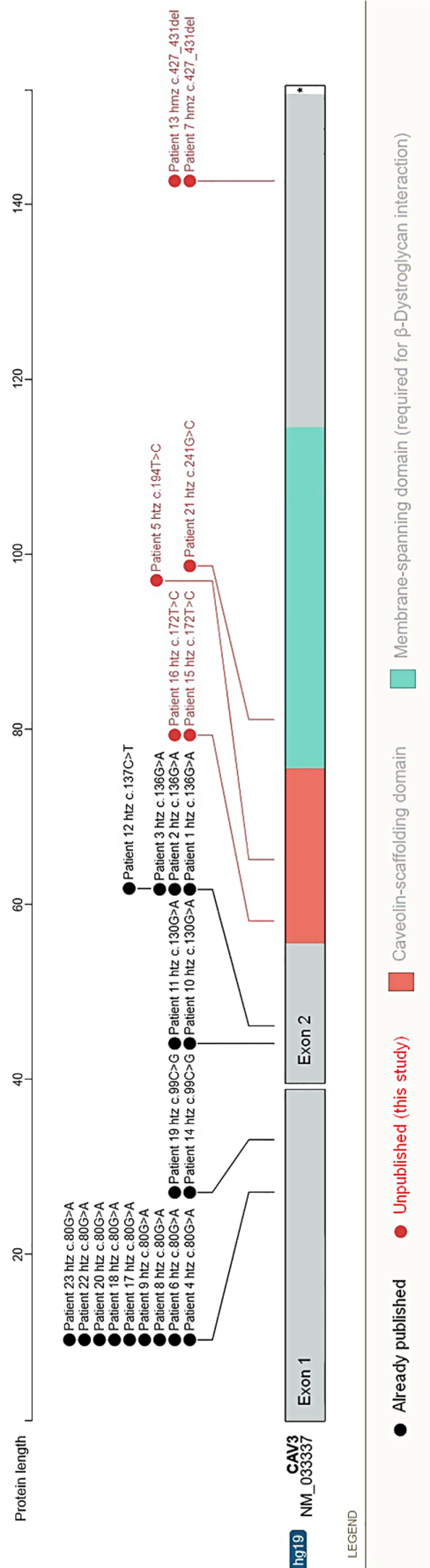


FIGURE 5 CAV3 variants in our cohort. CAV3 gene with already published (black) and as yet unpublished (red) variants are shown. Caveolin-scaffolding domain in red and membrane-spanning domain in green, also called WW-like domain, required for β -dystroglycan (DAG1) interaction, are shown. Figure was generated using St. Jude PeCan Data Portal.

AUTHOR CONTRIBUTIONS

Edouard Berling: Conceptualization; writing – original draft. **Camille VEREBI:** Writing – original draft; investigation. **Nadia Venturelli:** Investigation; writing – original draft. **Stéphane Vassilopoulos:** Writing – original draft. **Céline Tard:** Investigation. **maud michaud:** Investigation. **Savine Vicart:** Investigation. **Marion Masingue:** Investigation. **Norma Beatriz Romero:** Investigation. **Emmanuelle Lacene:** Investigation. **France Leturcq:** Investigation. **Bruno Eymard:** Investigation. **Pascal Laforêt:** Investigation. **Tanya Stojkovic:** Investigation; supervision.

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CONFLICT OF INTEREST STATEMENT

None of the authors has any conflict of interest to disclose.

DATA AVAILABILITY STATEMENT

Anonymized data not published within this article will be made available upon request from any qualified investigator.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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