Phenotype variability and natural history of X-linked myopathy with excessive autophagy

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ABSTRACT

Objective: X-linked myopathy with excessive autophagy (XMEA) linked to the *VMA21* gene leads to autophagy failure with progressive vacuolation and atrophy of skeletal muscles. Current knowledge of this rare disease is limited. Our objective was to define the clinical, radiological and natural history of XMEA.

Methods: We conducted a retrospective study collecting clinical, genetic, muscle imaging and biopsy data of XMEA patients followed in France and reviewed the literature for additional cases.

Results: Eighteen males had genetically confirmed XMEA in France, carrying four different *VMA21* variants. Mean age at disease onset was 9.4±9.9 (range 1-40) years. In 14/18 patients (77.8%) onset occurred during childhood (<15 years), however in four patients the disease started in adulthood. Patients had anterior and medial compartment thigh muscle weakness, distal contractures (56.3%), elevated CK levels (1287.9±757.8 U/l) and autophagic vacuoles with sarcolemmal features on muscle histopathology. Muscle MRI (n=10) showed a characteristic pattern of lower limb muscle involvement. In 11 patients, outcome measures were available for an average follow-up period of 10.6±9.8 years and six of them show disease progression. Mean change of functional outcomes was 0.5±1.2 points for Brooke and 2.2±2.5 points for Vignos score, 7/16 patients (43.8%) needed a walking aid and 3/16 (18.8%) were wheelchair-bound (median age of 40 years old, range 39-48). The variant c.164-7T>G was associated with a later onset of symptoms. Respiratory insufficiency was common (57.1%) but cardiac involvement rare (12.5%).

Interpretation: XMEA has variable age of onset, but a characteristic clinical, histopathological and muscle imaging presentation, guiding the diagnosis. Although slowly, motor disability progresses with time, and relevant genotype-phenotype correlations will help design future clinical trials.

Keywords: X-linked myopathy with excessive autophagy, VMA21, muscle biopsy, muscle MRI

Introduction

X-linked myopathy with excessive autophagy (XMEA) (OMIM 310440) is a monogenic disorder characterized by a progressive vacuolation and atrophy of skeletal muscles. XMEA is caused by pathogenic variants in the *VMA21* gene at Xq28, resulting in a reduced expression of the vacuolar ATPase assembly factor 21 (VMA21) protein, a key proton pump for endo-lysosomal acidification, with consequent impairment of lysosomal function leading to autophagy failure.[1] From a pathological point of view, this leads to the accumulation of cytoplasmic vacuoles in muscle cells, described by Sugie et al as autophagic vacuoles with sarcolemmal features (AVSF).[2]

First reported by Kalimo et al in 1988,[3] the disease was initially described as a childhood-onset, slowly progressive, proximal-predominant myopathy, sparing cardiac and respiratory muscles. Individual case reports and small patient series have been published since. Nevertheless, a comprehensive description of a large series of patients with XMEA is lacking and the natural history of the disease is mostly unknown. Finally, although potential treatment strategies in myopathies with AVSF already exist, such as targeting autophagy through the overexpression of Vps15 + PIK3C3,[4] no current disease-modifying therapy is available for XMEA.

In this multicentric retrospective study, we describe the clinical, electrophysiological, histopathological and muscle MRI features as well as the progression of the disease in 18 patients with genetically confirmed XMEA followed in France, the largest cohort reported so far. In addition, the literature was carefully revisited to include an additional eight genetically confirmed XMEA patients for a pooled analysis.

Patients and Methods

Data from the French patient series originated from the electronic clinical records of Pitié-Salpêtrière Hospital in Paris and seven other neuromuscular reference centers in France (Angers, Bordeaux, Lyon, Marseille, Nantes, Rennes, Strasbourg) (n=18).

The following data were collected: "first progressive symptom" as the first progressive motor deficit, leading the patient (or parents) to request a medical advice; age at onset; neonatal or

infantile manifestations before the first progressive symptom; age at first examination; neurological examination (including Medical Research Council scale, MRC); functional data (Brooke and Vignos scores), need for a walking aid or loss of ambulation; electroneuromyography (ENMG) findings; initial creatine kinase (CK) level (normal <200U/l); and histopathological data from muscle biopsies. Cardiac and respiratory examinations were also reviewed. Respiratory insufficiency was considered moderate if vital capacity (VC) was 50-79% of the populations' theorical value and severe if it was less than 50%.

Furthermore, in all patients, lower limb or whole-body muscle MRI had been performed as part of the routine diagnostic process. T1-weighted (T1W) and short-tau inversion recovery (STIR) sequences were analyzed and scored by the same radiologist (IA), with experience in muscle imaging. Images were scored using the Mercuri muscle MRI staging[5] as follows: 1. normal appearance; 2. early moth-eaten appearance, with scattered small areas of increased signal or with numerous discrete areas of increased signal with beginning confluence, comprising less than 30% of the volume of the individual muscle (mild involvement); 3. late moth-eaten appearance, with numerous discrete areas of increased signal with beginning confluence, comprising 30% to 60% of the volume of the individual muscle (moderate involvement); 4. an end-stage, washed-out or fuzzy appearance due to confluent areas of increased signal, with muscle replaced by increased density connective tissue and fat and only a rim of fascia and neurovascular structures distinguishable (severe involvement).

VMA21 (NM_001017980.4) gene variants were analyzed through Sanger sequencing or a dedicated next generation sequencing (NGS) gene panel. Each variant was classified following the American College of Medical Genetics (ACMG) classification.[6]

For all but two patients (patients 12 and 18, lost to follow-up), follow up data were collected and reviewed, focusing on the appearance of new symptoms and the variation of functional scores.

A review of the literature was performed using the PubMed tool to explore the Medline database and identify all reports of XMEA patients. The keywords "XMEA" and "X-linked myopathy with

excessive autophagy" and the filter "human" identified 12 case reports and/or series accounting for 38 XMEA patients,[3,7–17] among whom 15 had a genetic confirmation.[9–12,14–17] Seven of these genetically confirmed XMEA patients from the literature review (Chabrol et al. 2001, Mercier et al. 2015, Pegat et al., 2022) were already part of our French series;[8,17,18] therefore, eight genetically confirmed XMEA patients from the literature were finally added, for a total of 26 patients to run a pooled analysis.

In accordance with French legislation, the study was registered with and is accessible to the French data protection authority (CNIL, Commission Nationale de l'Informatique et des Libertés).

Statistical Analysis

Data analysis was performed using GraphPad Prism 10.0.3. Mean and median values, standard deviation (SD), range of quantitative variables and absolute or relative frequencies of categorical variables were reported. Association was studied through the Mann-Whitney test (continuous dependent variables) and the Fisher's exact test (categorical dependent variables). Kaplan-Meier curves and log-rank test were used to estimate the probability of needing a walking aid. Statistical significance was established at $p \leq 0.05$.

Results

Clinical Features at Onset

Eighteen male patients from 13 different families were included in the French series (Table 1). Fourteen patients (77.8%) had an onset of the first symptom during childhood or adolescence (age <15 years), with a mean age of 5±2.6 years, while four (22.2%) had an onset during adulthood (≥15 years), with a mean age of 25.3±10.4 years. Of the four latter, one patient had a very late onset of symptoms (40 years).

A symmetrical, proximal-predominant lower limb muscle weakness was the first progressive symptom in all patients, with psoas and quadriceps muscles being most frequently weak at the first examination. MRC scores for limb muscle weakness at first examination are shown in Figure 1. Distal limb contractures were frequent (56.3%), scapula alata was present in four patients (22.2%)

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and no facial weakness or ptosis was observed (Table 1). Functional outcomes at first examination showed a median Brooke score of 1 (range 1-3) and a median Vignos score of 2 (range 1-6), and all patients were ambulatory without the need of walking aids. No patient showed respiratory insufficiency or cardiac disease at onset.

Regarding psychomotor development, one patient had language delay and one patient had mild intellectual disability, although no intellectual quotient tests were performed. MRI imaging for the second patient did not reveal cortical brain atrophy or white matter abnormalities.

The mother of the siblings P1, P2 and P3 as well as the one of P13 and the one of P16, three probable (although unconfirmed) female carriers of *VMA21* variants, were explored by experts in neuromuscular disorders (MS, ESC and TS), showing no signs nor symptoms of the disease. However, no other test was performed (CK levels or muscle MRI).

Disease Progression.

Data at last examination were available for 16/18 patients (mean age of 36.1±17 years); the median Brooke score was 3 (mean 2.8±2.3, range 1-6) and the median Vignos score at last examination was 6 (mean 4.7±2.6, range 1-9).

In 11/18 patients, two Brooke and Vignos scores were available at different time points (with a mean elapsed time between measurements of 10.6±9.8 years): mean change was 0.5±1.2 points for the Brooke score and 2.2±2.5 points for the Vignos score. In 5/11 patients these scores were stable, although in two cases follow-up was less than 2 years.

Regarding ambulation, seven (43.8%) patients needed a walking aid at a median disease duration of 15 years (range 2-29) and three (18.8%) patients were wheelchair-bound at a median disease duration of 37 years (range 35-47) (and a median age of 40 years old, range 39-48).

Respiratory insufficiency was reported in 8/14 patients (57.1%) at a median age of 31.5 years old (range 15-45) and occurred at a median of 9 years (range 5-28) after onset of the disease. Four patients needed non-invasive ventilation (NIV) by the last examination. Pulmonary function tests were available in 4/8 patients with respiratory insufficiency, showing a restrictive pattern. Three of

these patients had moderate restrictive respiratory insufficiency (VC theoretical value=70% at 15 years old, 64% at 24 years old and 70% at 35 years old respectively) and one patient had a severe restrictive respiratory insufficiency (VC theoretical value=22% at 52 years old), the latter requiring NIV since asymptomatic respiratory acidosis was diagnosed on routine arterial blood gases.

Only two patients developed cardiac disease: patient 7 developed a type 1 atrioventricular block and right bundle branch block at 37 years old requiring a pacemaker implantation at 43 years old after an episode of syncope. Patient 17 developed a hypertrophic cardiomyopathy at the age of 63 years in the context of arterial hypertension.

Muscle-MRI.

Ten patients had undergone a lower limb or whole-body muscle MRI, with a mean age at muscle MRI of 28.8±11.2 years and a mean disease duration of 19.5±12.1 years (Figure 2). The muscle MRI findings of *VMA21* patients can be described as follows:

- A predominant muscle degeneration of the anterior and medial compartment of the thigh, with relative sparing of rectus femoris, tensor fasciae latae, adductor longus and gracilis muscles (as in patients 2, 8, 10 and 11).

- Earlier involvement of biceps femoris brevis (as in patients 2, 8 and 9) compared to the rest of the proximal lower limb posterior muscles.

- Earlier involvement of soleus and both gastrocnemius medialis and lateralis in the distal compartment with relative sparing of tibialis anterior muscle (as in patients 1, 5 and 6)

This common MRI muscle involvement can be observed in Figure 3. As expected, patients with an earlier onset of symptoms and longer disease duration before muscle MRI had more severe muscle involvement on MRI. Similarly, patients with a higher Vignos score showed more severe muscle involvement on imaging.

Other Paraclinical Findings.

The mean CK value at the first examination was 1287.9±757.8 U/l (range=380-2900), therefore no patient had normal CK levels at the first examination. When available (n=6), patients' ENMG

examinations showed the typical features of a myopathic process without signs of neuropathic involvement and myotonic discharges were found in two patients (33.3%).

Muscle biopsy was performed in 15 patients and disclosed a myopathic process with muscle fiber splitting and numerous cytoplasmic rimmed vacuoles expressing dystrophin and other sarcolemmal proteins on immunostaining, indicating ongoing autophagy. Only one patient showed signs of necrosis/regeneration. In 4/6 (66.7%) biopsies where a reaction for complement deposit was performed, a deposition of C5b-9 at the muscle fiber surface membrane and in the cytoplasmic rimmed vacuoles was observed. Immunostaining for TDP43, a DNA-binding protein whose aggregates have been described in other myopathies with rimmed vacuoles, and p62, a protein involved in the autophagy pathway, was performed in four muscle biopsies, confirming p62 aggregates (1/4), TDP43 inclusions (1/4) or both (2/4).

Phenotype-genotype correlations.

Regarding *VMA21* gene variants, most patients carried splicing variants (17/18, 94.4%): nine patients from six different families carried the c.163+4A>G variant, seven patients from five different families carried the c.164-7T>G variant and one recently reported patient who is also part of the French series carried the splicing variant c.164-20T>A.[17] Only one patient carried a missense variant (c.272G>C, p.Gly91Ala). The probable effects of these mutations are summarized in Table 2.

When comparing the two most frequent variants (c.163+4A>G and c.164-7T>G), patients carrying the c.164-7T>G had a later age at onset of symptoms (median 6, range 5-40 years vs median 3, range 1-18 years respectively; *p*=0.03). Concerning disease progression, no patient carrying the c.164-7T>G lost ambulation during follow-up, however the log-rank test was non-significant, possibly due to low sample size. When available, the Brooke and Vignos scores seemed to progress more steeply for patients with the c.163+4A>G variant compared to those with the c.164-7T>G (Figure 4). Finally, patients with the c.164-7T>G variant also had less muscle fat transformation on

muscle MRI (Figure 2). This was particularly the case for patient 11, who underwent the MRI 19 years after the onset of symptoms (Figure 3).

Literature Review and Pooled Analysis

Thirty-eight XMEA patients from the literature were reviewed and the summarized data are presented in Supplementary material.

Globally, genetically confirmed (n=26) vs unconfirmed patients (n=23) were similar, with an almost identical age at onset of symptoms (10.2±12.8 vs 10.9±7.4 years) and a predominantly proximal lower limb girdle weakness. Muscle biopsies of genetically confirmed vs unconfirmed patients also showed similar myopathic abnormalities with AVSF, bearing in mind that histopathology has been the standard for XMEA diagnosis in the past.

We then proceeded to run a pooled analysis of the 18 patients from the French series and the eight additional genetically confirmed *VMA21* patients from the literature, for a total of 26 patients (Table 1). The pooled analysis confirmed the clinical and paraclinical findings from the French series described above, with an anterior compartment proximal lower limb weakness, frequent distal contractures (43.5% since some patients had both finger flexor and ankle contractures), moderate elevations of CK levels (1440.7±977.4 U/l) and a muscle biopsy showing in all available cases (n=22) a myopathic pattern with AVSF. Details are shown in Table 1.

Mean age at onset of symptoms was 10.2±12.8 years (range 1-55). We could again define two subgroups of patients: a larger one (20/26, 77%) comprising patients with an onset during childhood or adolescence (<15 years), with a mean age at onset of 4.5 ±2.6 years, and a smaller one (6/26, 23%) comprising patients with an adulthood onset (≥15 years), with a mean age at onset of 29.2 ±15.2 years. In the latter subgroup, an additional patient with a very late onset of the disease (55 years) had been reported by Crockett et al.[10]

In terms of disease progression in the pooled analysis, eight patients (30.8%) needed a walking aid at a median of 15 years (range 2-29) after disease onset, and four (15.4%) were wheelchair-bound at

a median of 36 years (range 19-47) after disease onset. Only one additional patient with respiratory insufficiency and no patients with cardiac involvement were reported in the literature.

Discussion

This study presents by far the largest series of XMEA patients to date, depicting the full landscape of clinical findings, muscle involvement on MRI, natural history of the disease and relevant genotype-phenotype correlations. While XMEA has been so far considered a childhood-onset myopathy, we show that an adult onset is relatively frequent (almost a quarter of patients). Therefore, it is important to consider XMEA in the differential diagnosis of adult-onset vacuolar myopathies and limb girdle muscular dystrophies (LGMD). Despite this variable age at onset, the first symptom was almost systematically a proximal lower limb weakness involving the anterior and medial compartment of the thighs. Half of the patients presented with distal limb contractures, particularly in the Achilles tendons. All patients were male and three females, parents of VMA21 patients, had a normal neuromuscular examination. No symptomatic female patient with *VMA21* gene variants has been described to date, contrary to other X-linked myopathies like female carriers of Duchenne muscular dystrophy/Becker muscular dystrophy (DMD/BMD).[19]

Importantly, we show that the motor function, especially Vignos score of lower limbs, can deteriorate with time in more than half of patients, leading to walking difficulties. Although the rate of disease progression may vary between carriers of the two most frequent *VMA21* variants (c.163+4A>G and c.164-7T>G), patients in both groups can progress with time. Therefore, all patients with XMEA need to be monitored from a neuromuscular point of view. Also, we identified that respiratory insufficiency is more frequent than previously reported, being present in more than half of the patients during follow-up, sometimes requiring non-invasive ventilation, and should be monitored as well.

Given that V-ATPases are implicated in lysosomal function and autophagy, a process which is crucial in all cell types, extra skeletal muscle involvement could be expected in XMEA as in other autophagic vacuolar myopathies, such as Pompe or Danon disease.[20,21] On the contrary, multiorgan

involvement does not seem to be frequent if present at all. Only two patients had cardiac manifestations (hypertrophic cardiomyopathy and an atrioventricular block) and in the first case it was in the context of hypertension. In terms of central nervous system involvement, mild psychomotor delay was reported in two patients, although we lack a systematic cognitive assessment. Therefore, the restricted skeletal muscle involvement in XMEA could be the result of specific skeletal muscle-related altered lysosomal functions, such as sarcolemma repair, and/or differently expressed VMA21 isoforms, like the VMA21-120 isoform, which is predominantly expressed in skeletal muscle, as has been recently described.[22]

Regarding the ancillary investigations, XMEA does not show specific findings in ENMG studies aside from classic myopathic features with sometimes spontaneous activity such as myotonic discharges, as already described in other autophagic vacuolar myopathies.[23] Fortunately, muscle biopsy oriented the diagnosis in all patients from both the French series and case reports from the literature, showing hallmark findings (autophagic vacuoles with sarcolemmal feature or AVSF) of XMEA. Not surprisingly, in all cases there was a positive reaction for sarcolemmal components on the membrane of rimmed vacuoles and sometimes, but not systematically as we have shown, complement C5b-9 deposit within vacuoles and p62 and TDP43 aggregates. It is worth mentioning that XMEA patients with or without a genetic confirmation, had a similar age at onset of symptoms and a similar clinical phenotype, which *a priori* supports the notion that several of these patients without a genetic analysis most likely do carry *VMA21* pathogenic variants. However, it is also possible that in some of these patients the disease is not linked to the *VMA21* gene, as others and ourselves have failed to identify *VMA21* mutations in some individuals with AVSF.[24] Therefore, the histopathological findings of AVSF are not 100% specific to *VMA21* gene variants and other genes implicated in sarcolemmal membrane homeostasis and lysosomal function should consequently be explored in *VMA21*-negative XMEA patients.

Muscle MRI is an important tool in the differential diagnosis and follow-up of patients with myopathies, however reports of muscle imaging in XMEA are scarce.[18] We showed that VMA21-

linked XMEA patients have consistent findings on muscle MRI which can help guide the genetic diagnosis: a predominant muscle degeneration of the anterior and medial compartment of the thigh, with relative sparing of the rectus femoris, tensor fasciae latae, adductor longus and gracilis muscles, early involvement of biceps femoris brevis in the posterior compartment of the thigh, and a more posterior than anterior compartment muscle involvement in the leg (Figure 3). This pattern of muscle involvement on muscle MRI is somewhat different from that of BMD, a more frequent X-linked myopathy with anterior and medial compartment of the thigh motor deficit, where the semimembranosus and biceps femoris longus are more severely damaged than in XMEA.[25] In BMD, mean fat fraction calculated using the Dixon technique and T2 measurements have already been shown to be an excellent marker of disease severity, correlating with disability.[26] In line with this, we also observed that patients with more severe muscle involvement on MRI had longer disease duration before muscle MRI and higher (more severe) lower limb motor function Vignos score. Thus, muscle MRI should also be explored as a surrogate outcome measure of disease progression in XMEA patients.

Finally, pathogenic variants in the *VMA21* gene commonly affect splicing and are located in intron 2 in the case of the most common variants (c.163+4A>G and c.164-7T>G). Importantly, the c.164-7T>G variant was associated with a later onset of symptoms when compared to patients carrying the c.163+4A>G variant. Furthermore, no patient with this variant were wheelchair-bound and both patients with the c.164-7T>G who underwent an MRI were among the three least affected XMEA patients in our study. Interestingly, another *VMA21* variant, c.164−6T>G, located only one nucleotide away from the c.164−7T>G variant, causes a severe congenital autophagic vacuolar myopathy (CAVM) with respiratory failure.[27] This very striking phenotype disparity can be at least partially explained by the fact that the c.164-6T>G variant leads to greater reductions of both VMA21 transcript and protein than the c.164-7T>G variant, likely due to a more severe impact on splicing failure.[22] We speculate by analogy that the difference in onset of disease and progression between the carriers of the c.163+4A>G and c.164-7T>G variant observed in our study might be

due to difference in residual protein expression, however other alternatives like a differential expression of other genes, or even epigenetic factors modulating transcriptional or translational regulators, could also influence the observed differences in onset of the disease.

In conclusion, XMEA linked to *VMA21* gene variants can have a variable age of onset, but a highly characteristic clinical, histopathological and muscle imaging presentation, helping to guide the molecular diagnosis. Although slowly, motor disability progresses with time and respiratory involvement is more common than previously thought. A better understanding of the natural history of the disease and of specific genotype-phenotype correlations, and the potential of muscle MRI as a biomarker of the disease are important considerations for clinical trial readiness in XMEA.

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FIGURE LEGENDS

FIGURE 1: Clinical presentation of VMA21-linked XMEA patients. A.1) Heatmap showing the detailed MRC muscle power scale score results of muscle testing in 13 patients. Gray squares indicate that data were unavailable. A.2) Summary of clinical muscle involvement in XMEA, with a predominant hip flexion (psoas), knee extension (quadriceps) and hip adduction (adductor muscles) motor weakness, milder deltoid and triceps muscle weakness in the upper limbs as well as distal lower limb involvement.

FIGURE 2: Heatmap representation of the lower limb muscle MRI Mercuri score for fat degeneration in VMA21-linked XMEA patients. The heatmap color represents the Mercuri score, ranging from light green (low) to dark green (high). Gray squares indicate that data were unavailable. Rows are labeled with muscle names, and columns are labeled with patient identifiers. Row annotations (left side) indicate the "Age at onset of symptom" (color gradient from red to blue), "Time since disease onset" (light to dark blue), "Vignos Scale" (palette from green to red), and "Gene variant" (cyan, red, pink). Muscles are divided in different regions (gluteal region, thigh, leg) and hierarchical clustering is applied to both rows and columns based on the Manhattan distance using the Ward.D2 clustering method. The heatmap was generated using R programming

language, version 4.3.0, using the Complex Heatmap library to generate the heatmap and handle clustering and annotations, with the help of Frederic Fer.

FIGURE 3: Characteristic lower limb muscle MRI involvement in VMA21-linked XMEA patients. T1-weighted MRI sequences of three patients with pathogenic variants in the *VMA21* gene at differing stages of muscle fat transformation. Muscles with yellow arrows had marked fat replacement; muscles with yellow stars were completely or partially spared. **Patient 8** (P8: aged 12 years at onset of symptoms; 24 years old and Vignos 6 at MRI) shows general end stage muscle involvement with relative sparing of rectus femoris and tibialis anterior muscles. **Patient 2** (P2: aged 5 years at onset of symptoms; 23 years old and Vignos 5 at MRI) shows the described VMA21-linked XMEA muscle MRI phenotype with predominant vastus medius, intermedius and lateralis, and adductor magnus muscle involvement and sparing of rectus femoris, sartorious, gracilis and adductor longus muscles in the thigh. In the distal lower limb, we observed soleus, gastrocnemius medialis and lateralis muscle involvement with preserved tibialis anterior muscles. Finally, **patient 11** (P11: aged 25 years at onset of symptoms; 44 years old and Vignos 6 at MRI) shows similar muscle involvement on MRI to that of patient P2, with early vastus medius, intermedius and lateralis, and adductor magnus muscle fat transformation in the thigh as well as gastrocnemius medialis and lateralis muscle involvement in the distal lower limb. AL: adductor longus; AM: adductor magnus; BF: biceps femoris; Gmax: gluteus maximus; GM: gastrocnemius medialis; Gmed: gluteus medius; GL: gastrocnemius lateralis; Gr: gracillis; RF: rectus femoris; S: Soleus; Sa: sartorious; SM: semimembranosus; TA: tibialis anterior; VI: vastus intermedius; VL: vastus lateralis; VM: vastus medialis.

FIGURE 4: Genetics in VMA21-linked XMEA patients and distinct progression curves of motor function with time. A.1) Median age at onset of symptoms was significantly different between patients carrying the two most common variants (*p=0.03*). A.2) No patient carrying the c.164-7T>G variant lost ambulation (Kaplan-Meier curve) however the Log-Rank test was non-significant when compared to patients carrying the c.163+4A>G (*p=0.182*); in turn, although 6/9

patients with the c.163+4A>G variant did not loss ambulation at 16, 17 (n=2), 21 (n=2) and 27 years of disease duration, three patients did so at 35, 37 and 42 years of disease duration respectively as reflected in the Kaplan-Meier curve. Scatter plots showing A.3) Brooke and A.4) Vignos scores and their progression with time; B) pathogenic variants in the *VMA21* gene are frequently localized in intron 2, in the splicing regions flacking exons 2 and 3. Colored variants are those found in the French series.

TABLES

TABLE 1. Clinical Features and Ancillary Examinations of VMA21-linked XMEA patients

Characteristics	Our study (n=18)	Pooled (n=26)
Age at onset, y, mean±SD	9.4±9.9	10.2±12.8
Age at first consultation, y, mean±SD	23.8±17.5	24.6±18.3
Proximal lower limb weakness at onset, n (%)	18 (100)	25 (96.1)
Facial weakness, n (%)	0	0
Ptosis, n (%)	0	0
Axial weakness, n (%)	10 (55.5)	12 (46.1)
Scapula alata, n (%)	4 (22.2)	4 (15.3)
Contractures		
Scapular, n (%)	1 (5.5)	1 (3.8)
Elbow, n (%)	1 (5.5)	1 (3.8)
Finger flexors, n (%)	3 (16.6)	3 (11.5)
Hip, n (%)	2 (11.1)	2 (7.6)
Knee, n (%)	1 (5.5)	1 (3.8)
Ankles, n (%)	9 (50.0)	10 (38.4)
Pes cavus, n (%)	1 (5.5)	1 (3.8)
Cognitive impairment, n (%)	2 (11.1)	2*
Respiratory insufficiency at onset, n (%)	0	0
Cardiac involvement at onset, n (%)	0	0
Respiratory insufficiency at last examination, n (%)	8 (57.1)	9*
Cardiac involvement at last examination, n (%)	2 (12.5)	2*
CK, U/L, mean±SD	1287.9±757.8	1440.7±977.4
Myotonic discharges on EMG, n (%)	2 (33.3)	3*

Clinical and paraclinical presentation of XMEA patients with confirmed *VMA21* pathogenic variants from our French series (Our Study) and the French series + published case reports (Pooled). The percentage is calculated according to the number of observations.*Scarce data available.
CK = creatine kinase; EMG = electromyography; NA = not available; y = years.

TABLE 2. *VMA21* Gene Variants in the Pooled Analysis

Genetic Variant	Type of variant	Putative effect	ACMG Variant Annotation	Patients (n=26)	Age at Onset (Mean±SD where appropriate)
c.*13_*104del	Deletion in the 3'UTR	Reduced level of VMA21 mRNA quantity [11]	5	1	10 months
c.163+4A>G	Intronic nucleotide substitution	Splicing alteration at donor site of intron 2 with reduced level of VMA21 mRNA quantity and of V-ATPase activity [1]	5	10	5.0±4.8 years
c.164-20T>A	Intronic nucleotide substitution	Splicing alteration located in the polyT site of intron 2 (upstream of the acceptor site in the intron), may result in altered use of the acceptor site of intron 2, confirmed reduced level of VMA21 mRNA quantity [17]	5	1	11 years
c.164-7T>G	Intronic nucleotide substitution	Splicing alteration at acceptor site of intron 2 with reduced level of VMA21 mRNA quantity and of V-ATPase activity [1]	5	9	17.3±18.6 years
c.272G>C	Missense (p.Gly91Ala)	Change in amino acid and disruption of a predicted splice enhancer site with reduced level of VMA21 mRNA quantity and of V-ATPase activity [1]	5	3	14.6±6.8 years
c.54-16_54-8del	Intronic nucleotide substitution	Deletion affecting the branch point with reduced level of VMA21 mRNA quantity [11]	5	1	1 year
c.54-27A>C	Intronic nucleotide substitution	Splicing alteration at acceptor site of intron 1 with reduced level of VMA21 mRNA quantity and of V-ATPase activity [1]	5	1	3 years

Reported *VMA21* pathogenic variants, type of variant, putative effect, and age at onset of patients carrying each variant.

ACMG = American College of Medical Genetics; SD = standard deviation.