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Clinical, histological and genetic characterisation of patients with tubular aggregate myopathy caused by mutations in *STIM1*

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ABSTRACT

Background Tubular aggregate myopathies (TAMs) are muscle disorders characterised by abnormal accumulations of densely packed single-walled or double-walled membrane tubules in muscle fibres. Recently, *STIM1*, encoding a major calcium sensor of the endoplasmic reticulum, was identified as a TAM gene. **Methods** The present study aims to define the clinical, histological and ultrastructural phenotype of tubular aggregate myopathy and to assess the *STIM1* mutation spectrum.

Results We describe six new TAM families harbouring one known and four novel *STIM1* mutations. All identified mutations are heterozygous missense mutations affecting highly conserved amino acids in the calcium-binding EF-hand domains, demonstrating the presence of a mutation hot spot for TAM. We show that the mutations induce constitutive *STIM1* clustering, strongly suggesting that calcium sensing and consequently calcium homeostasis is impaired. Histological and ultrastructural analyses define a common picture with tubular aggregates labelled with Gomori trichrome and Nicotinamide adenine dinucleotide (NADH) tetrazolium reductase, substantiating their endoplasmic reticulum origin. The aggregates were observed in both fibre types and were often accompanied by nuclear internalisation and fibre size variability. The phenotypical spectrum ranged from childhood onset progressive muscle weakness and elevated creatine kinase levels to adult-onset myalgia without muscle weakness and normal CK levels.

Conclusions The present study expands the phenotypical spectrum of *STIM1*-related tubular aggregate myopathy. *STIM1* should therefore be considered for patients with tubular aggregate myopathies involving either muscle weakness or myalgia as the first and predominant clinical sign.

INTRODUCTION

Calcium (Ca^{2+}) is a physiological key factor and plays a pivotal role in a plethora of cellular functions. In skeletal muscle, Ca^{2+} triggers contraction and acts as a second messenger controlling growth and differentiation. Therefore, normal muscle function strongly depends on the tight regulation of Ca^{2+} storage and release. Store-operated calcium-entry (SOCE)

efficiently couples sensing of stored Ca^{2+} with influx of extracellular Ca^{2+} and is the primary mechanism controlling intracellular Ca^{2+} homeostasis in many cell types, including skeletal muscle. Store-operated calcium-entry is regulated by *STIM1*, a multidomain and single-pass transmembrane protein of the endoplasmic/sarcoplasmic reticulum. The luminal part of *STIM1* consists of two EF hands and a sterile α -motif (SAM), and the cytosolic C-terminus encompasses three coiled-coil domains, a serine/proline-rich segment and a lysine-rich segment.^{1–3} The stored Ca^{2+} is sensed by the EF hand domains and its binding maintains *STIM1* in a closed conformation.³ At this resting state, *STIM1* is evenly distributed at the endoplasmic reticulum/sarcoplasmic reticulum (ER/SR) membrane. Upon Ca^{2+} store depletion, the hydrophobic interaction between the EF and the SAM domains is weakened, allowing *STIM1* to unfold and oligomerize.^{3,4} The *STIM1* oligomers translocate into punctae at contact sites between the ER/SR and the plasma membrane, where they activate the calcium release-activated calcium channels, resulting in extracellular Ca^{2+} entry.^{5–10}

Mutations in *STIM1* have been associated with three different disorders. Homozygous loss-of-function mutations were shown to cause immunodeficiency (MIM #612783),^{11–13} whereas heterozygous gain-of-function mutations were found in patients with the multisystemic Stormorken syndrome (MIM #185070) or with tubular aggregate myopathy (TAM, MIM #160565).^{14–17} TAM is a progressive muscle disorder reported to be clinically heterogeneous, and either involving proximal muscle weakness, muscle cramps and pain, or myasthenic features,¹⁸ rendering its clinical entity elusive. Biopsies from patients with TAM typically show regular arrays of membrane tubules. These aggregates appear as irregular red sub-sarcolemmal depositions on modified Gomori trichrome, and consist of densely packed single-walled or double-walled membrane tubules. Tubular aggregates are the only histopathological hallmark in tubular aggregate myopathy and were most often detected in type II muscle fibres. They also appear in muscle disorders as hypokalemic periodic paralysis¹⁹ or congenital limb-girdle myasthenia,^{20,21} and strikingly accumulate in normal muscle with age in mammals.²²

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In total, seven TAM families with *STIM1* mutations have been reported to date.^{16 17} Both studies focused on the genetic basis of the disease, while the clinical, histopathological and mutational spectra of *STIM1*-related TAM remain to be defined. Here we analyse and describe seven patients from six new and unrelated TAM families harbouring one known and four novel *STIM1* mutations. All are heterozygous missense mutations affecting highly conserved amino acids in the EF hand domains and we demonstrate that they induce constitutive *STIM1* clustering. Our study provides functional evidence for the pathogenesis of the identified *STIM1* mutations and expands the clinical spectrum of *STIM1*-related tubular aggregate myopathy by describing a phenotypical TAM spectrum ranging from childhood-onset muscle weakness to adult-onset myalgia.

MATERIALS AND METHODS

Patients

Sample collection was performed with informed consent from the patients according to the declaration of Helsinki and experimentation was performed following institutional review board (IRB)-accepted protocols. Patients were from France (Families 1 and 6), Italy (Families 2, 3 and 4) and Germany (Family 5). Classification of tubular aggregate myopathy was based on histological and ultrastructural findings on muscle biopsies.

Histology

Transverse sections (10 µm) of the muscle biopsies (deltoid for Families 1, 4 and 6, quadriceps for Families 2 and 3, and biceps brachii for Family 5) were stained with modified Gomori trichrome, NADH tetrazolium reductase (NADH-TR) and succinate dehydrogenase and assessed for fibre morphology, fibre type distribution and accumulation/infiltrations.

Electron microscopy

Muscle sections were fixed in 2.5% paraformaldehyde, 2.5% glutaraldehyde and 50 mM CaCl₂ in 0.1 M cacodylate buffer (pH 7.4). Samples were postfixed with 2% OsO₄, 0.8% K₃Fe(CN)₆ in 0.1 M cacodylate buffer (pH 7.4) for 2 h at 4°C and incubated with 5% uranyl acetate for 2 h at 4°C. Muscles were dehydrated in a graded series of ethanol and embedded in epon resin. Thin sections were examined with an electron microscope (Philips CM120).

Sequencing and segregation analysis

Genomic DNA was prepared from peripheral blood by routine procedures and Sanger-sequenced for all coding exons of *STIM1* and the adjacent splice-relevant regions.¹⁶ The mutations were numbered according to GenBank NM_001277961.1 and NP_001264890.1. Nucleotide position reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon. For the patient from Family 1, mutations in the Duchenne muscular dystrophy (DMD), COL6A1, COL6A and COL6A3 collagen genes were excluded. For the patient from Family 3, mutations in DMD and lamin A/C (LMNA) were excluded. Patient IL3 from Family 4 was screened for 98 muscle disease genes by high-throughput sequencing, and the patient from Family 5 was excluded for mutations in the phosphoglycerate mutase (PGAM) gene.

Constructs

The human *STIM1*-YFP (yellow fluorescent protein) construct was a kind gift from Nicolas Demarex (University of Geneva, Switzerland). Point mutations (c.239A>C, c.286C>G, c.322T>A, c.322T>C and c.325C>A) were introduced by site-directed mutagenesis using the *Pfu* DNA polymerase (Stratagene, La Jolla, USA).

Cell culture and imaging

Murine C2C12 myoblasts were transfected with the *STIM1*-YFP constructs using Lipofectamine 2000 (Invitrogen, Carlsbad, USA) following the manufacturer's instructions, incubated with or without thapsigargin (1 µM; Sigma-Aldrich, St. Louis, USA) in Dulbecco's modified Eagle medium (DMEM) medium with 20% fetal calf serum (FCS), fixed and analysed by confocal microscopy (Leica SP2). For quantification, >100 cells/transfection were counted in three independent experiments and assessed for *STIM1* clustering. Following antibodies were used for immunofluorescence: mouse Anti-p62 Lck ligand (BD Biosciences, San Jose, USA), mouse anti mono- and poly-ubiquitinated conjugates (Enzo Life Sciences, Villeurbanne, France), rabbit anti-calnexin (Sigma-Aldrich, St. Louis, USA).

RESULTS

Sequencing identifies four novel and one known *STIM1* mutation

Our TAM cohort encompassed patients from 38 unrelated families. All were suggestive of tubular aggregate myopathy, as tubular aggregates appearing in red on Gomori staining were the only histopathological hallmark. Sanger sequencing of all coding exons and adjacent splice-relevant regions of *STIM1* revealed heterozygous mutations in six TAM families (figure 1A).

The patients from Families 1 and 2 harbour the same novel *STIM1* c.239A>C (p.Asn80Thr) mutation in exon 2 (figure 1A). They are unrelated and originate from different countries. Detection of a known *STIM1* polymorphism in exon 8 (rs2304891) in the heterozygous state only in the patient from Family 2 confirmed DNA individuality. The patient from Family 3 carries the novel *STIM1* c.286C>G (p.Leu96Val) mutation in exon 2. The patients from Family 4 harbour the novel *STIM1* c.322T>A (p.Phe108Ile) mutation in exon 3, affecting the same nucleotide as the novel mutation found in the patient from Family 5 (c.322T>C; p.Phe108Leu). Genetic screening of the patient from Family 6 revealed the *STIM1* c.325C>A (p.His109Asn) mutation in exon 3. This mutation has previously been described in another unrelated patient with TAM.¹⁶

Evidence of pathogenicity of the detected mutations comes from segregation analysis, as all affected, but none of the available unaffected family members carried the respective *STIM1* mutations. For the sporadic cases in Families 1 and 3, DNA samples from both healthy parents were available and demonstrated *de novo* appearance of the mutations in the index patients. For the sporadic case in Family 2, DNA samples of all three living unaffected siblings were available. For Family 4, DNA samples were available for unaffected for the unaffected sibling and the unaffected father. For Family 5, DNA from the unaffected sister was available and for Family 6, only the index case was sequenced. However, the fact that the same mutation has been reported in an unrelated TAM family¹⁶ is a strong proof of pathogenicity. None of the mutations was listed in the single nucleotide polymorphism (SNP) databases as dbSNP, Exome Variant Server or the 1000 genomes project, or in our inhouse database comprising 416 exomes of individuals of similar ethnic origin as our patients.

In line with previous studies reporting *STIM1* mutations in TAM,^{16 17} all identified mutations were heterozygous missense mutations in exons 2 and 3. All published and new *STIM1* mutations cluster in the Ca²⁺-binding EF hands and affect highly conserved amino acids (figure 1B, C). On the resolved protein structure of the EF-SAM domain,³ mutations reside either in proximity to the Ca²⁺-binding site, or in the hydrophobic cleft implicated in the EF-SAM interaction to maintain the closed *STIM1* conformation (figure 1D).

Genotype-phenotype correlations

Figure 1 Identification of new *STIM1* mutations in tubular aggregate myopathy. (A) Pedigrees of the six families with tubular aggregate myopathy and electropherograms showing the heterozygous *STIM1* mutations. Healthy individuals without mutation in *STIM1* are labelled as wild type (WT). DNAs from other family members were not available. (B) Schematic representation of the *STIM1* protein domains. The previously reported *STIM1* mutations are shown in black, the new *STIM1* mutations in red. All mutations cluster in the intraluminal EF hands. TM, transmembrane domain; CC, coiled-coil; SP, serine/proline-rich domain; K, polylysines. (C) Protein sequence alignment of the *STIM1* EF-hands of different species demonstrates high conservation of the mutated amino acids. (D) The resolved protein structure of the EF hands shows close proximity of the affected amino acids to the Ca^{2+} binding domain, especially for mutations involving muscle weakness as first clinical sign (left). The other mutations are depicted on the right picture. The EF1 appears in blue, the EF2 domain in brown and the sterile α -motif (SAM) domain in green.

Mutations induce STIM1 clustering independent of intraluminal Ca^{2+} level

Missense mutations in the EF hands were previously shown to induce constitutive STIM1 oligomerisation, probably as a result of decreased Ca^{2+} sensing.¹⁶ In order to assess the impact of the novel *STIM1* mutations, we transfected C2C12 myoblasts with the different STIM1-YFP constructs. Addition of the SERCA1 inhibitor thapsigargin induced sarcoplasmic reticulum Ca^{2+} depletion and resulted in clustering of wild type STIM1 (figure 2). In line with the previously published *STIM1* mutations, the myoblasts transfected with any of the new mutant constructs displayed statistically significant STIM1 clustering independently of thapsigargin, evidencing the pathogenicity of the novel *STIM1* mutations described here. Of note, the Phe108Leu mutation involved less prominent (but still significant) STIM1 clustering compared with the other mutants. We next analysed the subcellular localisation of the constitutive STIM1 clusters and their potential association with autophagy. Monitoring the reticulum marker calnexin, and the autophagic markers p62 and ubiquitin did not reveal major differences between cells expressing wild type or mutant STIM1 (figure 3). The STIM1 clusters were found to reside within the sarcoplasmic reticulum and did not involve autophagy. These results strongly suggest that the constitutive STIM1 clusters preserve their functional activity.

STIM1 mutations are associated with a large phenotypical spectrum

The patients from Families 1 and 2 carry the same *STIM1* mutation (p.Asn80Thr) and both had a similar course of disease (table 1). The patient from Family 1 presented with a childhood-onset postexercise myalgia and fatigability, associated with elevated creatine kinase (CK) levels. Mild proximal muscle weakness of the lower limbs appeared at age 35 years. The patient from Family 2 presented with adult-onset myalgia and elevated CK levels, and progressively developed muscle weakness, heel contractures, and reduced lateral and upward gaze. Owing to the age of both patients at the last medical examination (35 years vs 70 years), it is conceivable that the novel *STIM1* p.Asn80Thr mutation first involves myalgia and high CK, while additional TAM features as muscle weakness and eye movement defects appear at later stages of the disease. Similarly, the patient from Family 3 (p.Leu96Val) presented with childhood-onset myalgia and elevated CK as first symptoms and then developed muscle weakness predominantly affecting the lower limbs.

The *STIM1* mutations found in Families 4 and 5 affect the same nucleotide (c.322T>A and c.322T>C), and result in the integration of the chemically similar amino acids isoleucine and leucine (p.Phe108Ile and p.Phe108Leu). The clinical presentation is however different. The patients from Family 4 had childhood-onset limb-girdle muscle weakness. Myalgia appeared as secondary feature and was more pronounced in patient II.3. In contrast, the patient from Family 5 had adult-onset myalgia without muscle weakness and was the only patient from our cohort with normal CK levels.

The patient from Family 6, carrying the p.His109Asn mutation, presented with exercise intolerance, fatigability and high CK levels, but myalgia was not reported. His mother was referred to have an adult-onset muscle weakness of the lower limbs, but maternal DNA was not available for *STIM1* sequencing. Noteworthy, the same p.His109Asn mutation has previously been described in an unrelated TAM family with muscle weakness as predominant clinical sign.¹⁶

In summary, the novel *STIM1* p.Asn80Thr, p.Leu96Val and p.Phe108Leu mutations appear to be associated with myalgia as a first and major clinical sign. In contrast, the novel p.Phe108Ile and all previously reported *STIM1* mutations (p.Asp84Gly, p.His109Asn, p.His109Arg and p.Ile115Phe) mainly involve proximal muscle weakness.^{16 17} Interestingly, on the resolved protein structure of the STIM1 EF-SAM domain, the mutations inducing muscle weakness cluster in the Ca^{2+} -binding site (figure 1D). However, the novel p.Phe108Ile, involving myalgia without muscle weakness, also affects an amino acid in the Ca^{2+} -binding site. A genotype-phenotype correlation can therefore only partially be deduced from the three-dimensional model.

Histopathological characteristics

Muscle biopsies from all patients described in this study displayed tubular aggregates appearing in red on Gomori trichrome and in dark blue on NADH-TR staining (figure 4 and table 1). The aggregates were not detected with succinate dehydrogenase staining, indicating a reticulum and not a mitochondrial origin, as previously suggested.^{23 24} Aggregates were observed in both fibre types in all patients, although they were predominantly detected in type II fibres in the patient from family 1, and in type I fibres in the patient from Family 3. Fibre size variability and internalised nuclei were seen in the patients from families 1, 2, 3 and 4 and type II fibre atrophy was noted in patients from families 5 and 6.

Ultrastructural analysis demonstrated that the tubular aggregates are of variable size and consist of single-walled or double-walled membranes of different diameters (figure 5). We found vesicular membrane collection-type tubular aggregates in Families 1 and 6, Ic-type aggregates in Families 2, 3, 4 and 6, as well as Ib-type aggregates in Family 4. There was no obvious correlation between the tubular aggregate types and mutation, age of onset, or disease severity.

DISCUSSION

This study provides a clinical, histological, genetic and functional characterisation of six new families with tubular aggregate myopathy and mutations in *STIM1*, and their comparison with all previously published cases. We discovered four novel and one known *STIM1* mutation. Three novel *STIM1* mutations were associated with myalgia as a first and predominant clinical sign, and one involved a mixed muscle weakness/myalgia phenotype. At the molecular level, all *STIM1* mutations found in our patients induced constitutive STIM1 clustering. This study provides functional evidence for the pathogenesis of the identified *STIM1* mutations, and significantly expands the clinical spectrum of STIM1-related tubular aggregate myopathy.

Clinical spectrum

TAM has been reported with dominant and recessive inheritance and is therefore believed to be genetically heterogeneous. This is strengthened by the clinical heterogeneity of TAM, encompassing three major and distinct phenotypes.¹⁸ The first phenotype is characterised by a slowly progressive weakness predominantly affecting the proximal muscles, the second phenotype primarily involves myalgia with or without cramps, and the third phenotype associates a myopathic pattern with myasthenic features and is referred to as limb-girdle myasthenia.

With the exception of one family without apparent clinical phenotype (p.His72Gln), all previously reported patients with TAM with *STIM1* mutations (p.Asp84Gly, p.His109Asn/Arg and p.Ile115Phe) presented with progressive muscle weakness and therefore belong to the first phenotypical TAM group.^{16 17}

Genotype-phenotype correlations

Figure 2 STIM1 clustering in muscle cells in dependence of thapsigargin (TG) and quantification. C2C12 myoblasts were transfected with the different STIM1-YFP constructs. Wild type STIM1 was evenly distributed in the ER and clustered upon store depletion induced by thapsigargin. All mutant constructs clustered independently of TG. Significant STIM1 clustering without TG was seen for all mutants and to a lesser extent for the construct harbouring the Phe108Leu mutation. Total number of counted cells ($n > 100$) was set to 100%. Dark green bars correspond to the percentage of cells with major clusters, medium green bars represent the percentage of cells with minor clusters, and light green bars represent the percentage of cells without clusters. Error bars represent SD.

Figure 3 The STIM1 clusters observed in myoblasts overexpressing mutant STIM1 constructs were negative for ubiquitin or the autophagic marker p62, and overall autophagic activity was similar in cells expressing wild type or mutant STIM1. Monitoring the reticulum using calnexin did not reveal significant differences in the SR structure in transfected or non-transfected cells. The other mutations N80T, L96V and F108I/L gave the same results (not shown).

This study now describes seven new patients with TAM from six families harbouring known and novel *STIM1* mutations. The phenotype of the patient with the known p.His109Asn mutation involved exercise intolerance and fatigability, and the patient's mother was reported with muscle weakness. Similarly, patients from Family 4 with p.Phe108Ile mutations first displayed muscle weakness before presenting a secondary myalgia. In contrast, the patients from the other four families harbour new *STIM1* mutations (p.Asn80Thr, p.Leu96Val, Phe108Leu), and all presented with myalgia as the first symptom. Three of the patients developed muscle weakness and other secondary clinical features such as contractures, reduced tendon reflexes or eye movement disabilities at later disease stages. These data indicate that specific *STIM1* mutations may cause distinct TAM phenotypes with different course of disease.

There is no domain-specific clustering of *STIM1* mutations involving either muscle weakness or myalgia and these different phenotypes can even result from mutations in the same amino acid as Phe108. On the resolved protein structure,³ Asp84 belongs to the negatively charged calcium-binding site and mutation of this amino acid most probably interferes with Ca^{2+} sensing and thereby triggers STIM1 oligomerisation. Similarly, Phe108, His109 and Ile115 are on a loop in the Ca^{2+} binding region. In contrast, Leu96 resides within the hydrophobic cleft interacting with the SAM domain. Upon Ca^{2+} store depletion, the hydrophobic cleft becomes solvent-exposed, which destabilises the EF-SAM interaction. In this model, the Leu96 mutation is expected to impact on the intramolecular EF-SAM interaction irrespective of Ca^{2+} sensing, mediating constitutive activation of STIM1. The different intramolecular localisation of the *STIM1*

Table 1 Molecular, phenotypical and histopathological data of patients with TAM with *STIM1* mutations

	Family 1	Family 2	Family 3	Family 4		Family 5	Family 6
Individual	II.1	II.4	II.1	II.2	II.3	II.2	II.1
Gender	M	M	F	M	F	F	M
Mutation	c.239A>C p.Asn80Thr	c.239A>C p.Asn80Thr	c.286C>G p.Leu96Val	c.322T>A p.Phe108Ile	c.322T>A p.Phe108Ile	c.322T>C p.Phe108Leu	c.325C>A p.His109Asn
Onset	Childhood	Adulthood	Childhood	Childhood	Childhood	Adulthood	Childhood
Age at last examination	34	70	13	51	46	52	35
Muscle weakness	Proximal, lower limbs	Proximal, predominantly lower limbs	Lower limbs	Predominantly proximal	Predominantly proximal	No	No
Walking	Normal	Foot drop, unable to walk on heels	Normal	With support, unable to walk on heels	With support, unable to walk on heels	Normal	Normal
Eye movement defects	No	Reduced lateral and upward gaze	No	No	No	No	No
Contractures	No	Heels	No	Heels	Heels	No	No
CK level	10 x	6 x	8 x	4 x	n.a.	Normal	15 x
Histology	TAs (mainly type II) fibre size variability, internal nuclei, fibrosis	TAs, fibre size variability, internal nuclei, fibrosis	TAs mainly (type II) fibre size variability, internal nuclei	TAs, fibre size variability, type I fibre predominance, internal nuclei, fibrosis	TAs, fibre size variability, type I fibre predominance, internal nuclei, fibrosis	TAs, type II fibre atrophy	TAs, type I fibre predominance, type II fibre atrophy
Tubular aggregate classification	VMC	Ic	Ic	n.a.	Ib and Ic	n.a.	VMC and Ic
Additional features	Postexercise myalgia and fatigability, calf hypertrophy	Myalgia	Myalgia, reduced tendon reflexes	Myalgia	Myalgia	Myalgia	Postexercise fatigability, calf hypertrophy, episodic diplopia

TAs, tubular aggregates (Classification: Ib=tubular aggregates with central tubule; Ic=tubular aggregates with empty or moderately dense flocculent material); VMC=vesicular membrane collection; n.a., not assessed; TAM, tubular aggregate myopathy.

Figure 4 Histology of muscle biopsies from the patients with tubular aggregate myopathy. Histological analysis revealed tubular aggregates on modified Gomori trichrome and NADH tetrazolium reductase (NADH-TR) staining, but not on succinate dehydrogenase (SDH) staining. Internal nuclei are often seen.

mutations potentially indicates mutation-dependent pathomechanism underlying the muscle disease.

Of note, the novel *STIM1* p.Phe108Leu mutation was found to have a reduced effect at the molecular level, as constitutive *STIM1* clustering was moderate in comparison with all other mutants. The patient is the only one with a normal CK level, had a disease onset in the 40 s and presents myalgia as the only clinical sign at age 52 years. This mild muscle phenotype might reflect the less pronounced impact of the mutation on *STIM1* clustering.

Mutation spectrum

Including this study, *STIM1* mutations have been found in 13 unrelated TAM families, supporting *STIM1* as a major gene implicated in TAM. In total, nine different missense mutations affecting seven residues in the highly conserved *STIM1* EF hands have been found in patients with TAM. The residues Phe108 and especially His109 appear to be prone to mutations and potentially represent a TAM hotspot, as they were mutated in seven families. The fact that all mutations reside in *STIM1* exons 2 and 3 might direct and ease molecular diagnosis for patients with tubular aggregate myopathy and muscle weakness or myalgia. Importantly, our cohort encompassed 32 TAM

families without mutations in *STIM1*, strongly suggesting the implication of yet unknown genes in the development of the muscle disorder. There is no obvious clinical and histological difference compared with the TAM families with *STIM1* mutations.

Homozygous loss-of-function mutations in *STIM1* were previously associated with immunodeficiency and neonatal hypotonia.^{11–13} Very recently, two groups reported in parallel the identification of the heterozygous p.Arg304Trp missense mutation in the cytosolic domain of *STIM1* as the genetic cause of Stormorken syndrome, encompassing muscle weakness with tubular aggregates, miosis, thrombocytopenia, ichthyosis, mild intellectual disabilities, short stature and asplenia.^{14 15} Further functional analysis and the characterisation of animal models will be needed to understand how different mutations in *STIM1* cause such a phenotypical variability and a tissue-specific impact.

Histological and ultrastructural characterisation

Tubular aggregates were mainly reported in men from recessive or sporadic families and most often found only in type II fibres.²⁵ However, the present and previous studies on *STIM1*-related tubular aggregate myopathy involved a

Genotype-phenotype correlations

Figure 5 Electron microscopy of muscle biopsies from the patients with tubular aggregate myopathy. Ultrastructural analysis demonstrated prominent tubular aggregation with single-walled or double-walled membranes on transversal and longitudinal sections.

comparable number of male and female patients with equal distribution of aggregates in fibre types. It is possible that the TAM forms predominantly affecting type II fibres in men are caused by mutations in other genes.

Pathological mechanisms

All TAM-related *STIM1* mutations identified to date affect highly conserved amino acids in the intraluminal Ca^{2+} -sensing domain. According to previous studies,^{5 9 16 26 27} we demonstrated that all novel *STIM1* mutations induce *STIM1* clustering independently of drug-mediated Ca^{2+} store depletion. These data suggest that the mutants are unable to sense the calcium in the SR lumen, and constitutively cluster at the SR-plasma membrane junctions. The clusters are presumably functionally active, as they reside within normally structured sarcoplasmic reticulum without being positive for ubiquitin or p62. *STIM1* clustering has been shown to constitutively activate the calcium entry channels and to strongly alter cellular Ca^{2+} homeostasis. These findings strengthen the concept that the gain-of-function

mutations disrupt the Ca^{2+} -dependent activation/inactivation of *STIM1*, resulting in aberrant calcium homeostasis, and ultimately in myalgia or muscle weakness.

Tubular aggregates originate from the SR,^{18 23 24} and it has been proposed that sequential formation of aggregates in ageing skeletal muscle fibres is initiated by swelling of the SR cisternae and their extension into longitudinally oriented tubules.²² Different proteins involved in the uptake and the storage of Ca^{2+} such as *STIM1*, *SERCA1*, *RYR1*, sarcalumenin or triadin were previously shown to be components of the aggregates.^{16 18} The swelling of the SR in tubular aggregate myopathy potentially results from the excess of intraluminal and/or cytosolic Ca^{2+} as measured in myoblasts from a patient with TAM.¹⁶ Alternatively, tubular aggregate formation could be triggered by excessive *STIM1* oligomerisation.

CONCLUSION

In conclusion, *STIM1* mutations can cause a wide phenotypical spectrum ranging from childhood-onset muscle weakness to

adult-onset myalgia. This is of major importance for genetic diagnosis and counselling as *STIM1* mutations should be considered for patients with TAM presenting progressive muscle weakness, and for patients with myalgia as the first and predominant clinical sign. This study significantly broadens the phenotypical spectrum of muscle disorders caused by *STIM1* mutations.

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Contributors JB and JL designed and coordinated the study. JB, FC, CK, GAP and FF performed the experiments. MM, LM, BP, IM, GT, ER, IP-B, AN-P, MF, PRJ, MD, NBR and BE contributed clinical samples, histological and patient data. JB and JL wrote the manuscript.

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