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Original research

Expanding the phenotypic spectrum of *TRAPPC11*-related muscular dystrophy: 25 Roma individuals carrying a founder variant

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ABSTRACT

Background Limb-girdle muscular dystrophies (LGMD) are a heterogeneous group of genetically determined muscle disorders. TRAPPC11-related LGMD is an autosomal-recessive condition characterised by muscle weakness and intellectual disability.

Methods A clinical and histopathological characterisation of 25 Roma individuals with LGMD R18 caused by the homozygous *TRAPPC11* c.1287+5G>A variant is reported. Functional effects of the variant on mitochondrial function were investigated.

Results The c.1287+5G>A variant leads to a phenotype characterised by early onset muscle weakness, movement disorder, intellectual disability and elevated serum creatine kinase, which is similar to other series. As novel clinical findings, we found that microcephaly is almost universal and that infections in the first years of life seem to act as triggers for a psychomotor regression and onset of seizures in several individuals with *TRAPPC11* variants, who showed pseudometabolic crises triggered by infections. Our functional studies expanded the role of TRAPPC11 deficiency in mitochondrial function, as a decreased mitochondrial ATP production capacity and alterations in the mitochondrial network architecture were detected.

Conclusion We provide a comprehensive phenotypic characterisation of the pathogenic variant *TRAPPC11* c.1287+5G>A, which is founder in the Roma population. Our observations indicate that some typical features of golgipathies, such as microcephaly and clinical decompensation associated with infections, are prevalent in individuals with LGMD R18.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ *TRAPPC11*-related limb-girdle muscular dystrophies (LGMD), which has been reported so far in no more than 20 individuals, is clinically characterised by muscle weakness, intellectual disability and hyperkinetic movements.

WHAT THIS STUDY ADDS

⇒ Our phenotypic characterisation of the pathogenic variant *TRAPPC11* c.1287+5G>A, which is founder in the Roma population, indicates that some typical features of golgipathies, such as microcephaly and clinical decompensation associated with infections, are prevalent in individuals with *TRAPPC11*-related LGMD.

⇒ Our functional studies expanded the role of TRAPPC11 deficiency in mitochondrial function.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Our findings allow us to refine and expand the emerging concept of golgipathies through a better understanding of the phenotype associated with *TRAPPC11*-related LGMD.

INTRODUCTION

Limb-girdle muscular dystrophies (LGMD) are a heterogeneous group of genetically determined muscle disorders with predominant proximal weakness.[1] The age of onset, severity and clinical course may vary among LGMD subtypes, ranging from early onset myopathy with rapid progression to adult-onset with long-time preserved ambulation and normal life span.[2 3]

TRAPPC11-related LGMD R18, previously known as LGMD2S,[4] is an autosomal-recessive congenital disorder of glycosylation that is clinically

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characterised by muscle weakness, intellectual disability and hyperkinetic movements.[5–7] *TRAPPC11* encodes subunit 11 of the multiprotein TRAPP complex, a regulator of membrane trafficking and autophagy whose best characterised biochemical function is to act as a guanine nucleotide exchange factor to activate Ypt/Rab GTPases.[8–10] Pathogenic variants or deletions of TRAPP subunits are involved in several neurodevelopmental disorders, known as TRAPPopathies.[11 12] TRAPPopathies belong to the rapidly growing category of disorders of cell trafficking and in particular to membrane trafficking defects. The recent report of the cryo-EM structure of the *Drosophila* TRAPPIII complex has revealed how the TRAPP subunits assemble and the TRAPPC11 structure, shedding light on how disease-causing variants affect the structure and function of TRAPPC11.[13]

No more than 20 individuals and 12 variants in *TRAPPC11* have been reported so far.[5–7 14–19] Here, we report a founder pathogenic variant (*TRAPPC11* c.1287+5G>A; p.Ala372_Ser429del) in the Roma population and provide a comprehensive phenotypic characterisation based on the description of 25 previously unreported Spanish Roma individuals who harbour the variant in homozygosis. There are no previous reports of a direct link between *TRAPPC11* and mitochondrial function, but it is not uncommon to find associated mitochondrial defects in membrane trafficking disorders.[20 21] We have therefore studied mitochondrial energy metabolism in individuals with the variant *TRAPPC11* c.1287+5G>A to better understand the possible involvement of mitochondria in the pathophysiology of *TRAPPC11*-related muscular dystrophy.

METHODS

Recruitment of patients, clinical examinations and molecular genetic analyses

Data of individuals with a genetically confirmed LGMD R18 were collected at 12 Spanish hospitals after sending a request for collaboration to all the members of the Spanish Paediatric Neurology Society. Demographic, clinical, genetic, electroencephalographic, neuroradiological and muscle biopsy data were collected in accordance with the ethics guidelines of each of the institutions involved. The degree of intellectual disability was classified according to the impression of the neurologist who followed each individual, but a neuropsychological assessment was not carried out systematically in all individuals.

Genomic DNA samples were extracted from peripheral blood of affected individuals. Different next-generation sequencing methodologies were used to prepare and capture genomic DNA libraries, from customised panels for selected genes to exome sequencing. The same single nucleotide variant c.1287+5G>A in *TRAPPC11*, classified as pathogenic according to the American College of Medical Genetics and Genomics guidelines for germline variant classification,[22] was found in homozygosis in all the individuals. Segregation was performed on the asymptomatic parents of 13 individuals.

In silico analysis of variant *TRAPPC11* c.1287+5G>A

A homology molecular model for *human* TRAPPC11 (positions 1–574) and TRAPPC2L (positions 1–138) was constructed based on *Drosophila* TRAPPIII complex structures (7B6H and 7B6R),[13] using Modeller.[23] Positions 61–75 and 378–405, with no determined structure in the template structure, were also modelled. Sequence identity was 42.1% and 52.9% for TRAPPC11 and TRAPPC2L, respectively. *Human* TRAPPC11 structural model was visualised using Pymol[24] (http://www.pymol.org/pymol).

Muscle and skin biopsies, histological, immunohistochemical, immunofluorescence, ultrastructural studies and western blot analysis

Muscle biopsy samples were obtained for diagnostic purposes and processed following standard histological, histochemical and immunohistochemical protocols as previously described.[25] Skin biopsy samples were obtained and fibroblasts were cultered according to Hospital Sant Joan de Déu standard clinical procedures. Specific antibodies used for immunohistochemical or immunofluorescence studies are listed in online supplemental table 1. Electron microscopy studies were performed on muscle samples from individual 5. Semi-quantitative western blot analysis on fibroblasts and muscle lysates using antibodies against TRAPPC11 and alpha-dystroglycan (IIH6 and VIA4) was performed as previously described.[26] All the muscle biopsies and western blot analysis studies were expressly reviewed for this study by a pathologist (CJ), a clinical scientist (ACo) and a paediatric neurologist (DN-dB), all three specialists in neuromuscular disorders. Control muscle samples were taken from two children aged 5 and 10 years who did not have neuromuscular disease, from quadriceps and from an unknown muscle. Control fibroblasts were obtained from a skin biopsy of a child aged 10 years with no neuromuscular disease.

Characterisation of epilepsy

Classification of seizures, epilepsy course and available EEGs were reviewed by an epileptologist specialised in children (JD-C). Seizures and epilepsy were described and classified according to the International League Against Epilepsy classification.[27 28] Bilateral tonic-clonic seizures in which the onset of the seizure was not observed and which could be either ‘generalised seizures’ or ‘focal seizures that evolve into bilateral tonic-clonic seizures’ were referred to as ‘bilateral tonic-clonic seizures’. Epilepsy course was classified as (a) ‘early seizure freedom’ (seizure-free within 6 months of starting treatment), (b) ‘delayed seizure freedom’ (seizures not immediately controlled by medication, but became seizure-free at some point after 6 months), (c) ‘fluctuating course’ (periods of seizure freedom of >12 months, interspersed with relapses) or (d) ‘refractory’ (never seizure-free for a continuous 12-month period) following the method defined by Brodie *et al*.[29]

Functional analysis of the variant on mitochondrial function

Functional studies have included the analysis of non-glycolytic ATP production and mitochondrial network visualisation. Mitochondrial (as in non-glycolytic) ATP concentration was measured as explained in the study by Oyarzabal *et al*.[30] Briefly, cells from individuals with TRAPPC11-related muscular dystrophy and cells from controls were incubated with 2-deoxy-D-glucose for 2 hours to discriminate between total and non-glycolytic ATP. Finally, ATP concentration was assayed by bioluminescence using a luciferin-luciferase system (ATP Bioluminescence Assay Kit CLS II, Roche) according to the manufacturer’s instructions, corrected by protein concentration and expressed as folds over controls.

The mitochondrial network was visualised through immunofluorescence against TOMM20 (1:250, ab186735) following standard protocols. Fluorescence was imaged using Zeiss LSM 880 microscope (Zeiss, Germany) with a 63× magnification. Images were analysed using Fiji software and parametrised by Mitochondrial Analyzer plugin.[31] All experiments are done at least three times with triplicates.

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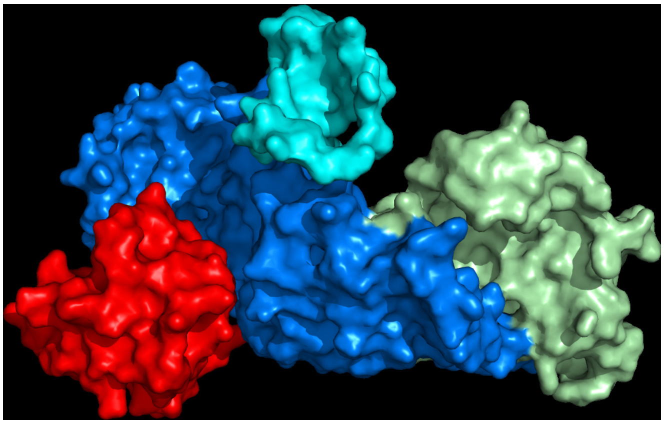

Figure 1 Molecular model of *human* TRAPPC11 (residues 1–574, in blue and green) interacting with *human* TRAPPC2L (residues 1–136, in red), in the TRAPPIII complex. TRAPPC11 foie gras domain (residues 181–566, in cyan blue) is essential for TRAPPC2L interaction. This domain contains residues 372–429 (in dark blue), which are lacking in *TRAPPC11* c.1287+5G>A splice-site variant. The N-terminal domain of TRAPPC11 is shown in green.

At the time the fibroblasts were biopsied, one of the individuals with TRAPPC11-related muscular dystrophy was 4 years old and the other was 12 years old. All controls, which included both males and females, were within this age range. All cells used were between passages 7 and 12.

RESULTS

Demographics and molecular genetics

A total of 25 individuals with TRAPPC11-related muscular dystrophy (13 males, 12 females) from 18 families were collected. All of them were Spanish with a Roma origin and 22 of 23 cases (96%) were born from consanguineous parents. The mean age at the last examination was 11.8 years old (range 2–23). The previously reported homozygous splice-site variant *TRAPPC11* (NM_021942.6) c.1287+5G>A was found in all the individuals. Segregation studies were performed on the parents of 13 individuals, confirming that the pathogenic variants were inherited each from one of the unaffected parents.

Structure consequence of *TRAPPC11* c.1287+5G>A variant

The splice-site variant *TRAPPC11* c.1287+5G>A results in an in-frame deletion of 58 amino acids (p.Ala372_Ser429del).[5] Figure 1 shows *human* TRAPPC11 and TRAPPC2L structural model interaction. The deletion of residues 372–429 in TRAPPC11 is located within the conserved foie gras domain (residues 181–566), which is responsible for the surface interaction with TRAPPC2L.[13] The lacking of this region has been recently described as resulting in defects in membrane trafficking,[5] autophagy and N-linked glycosylation.[17]

Clinical features

A history of late premature birth was reported in 3/24 individuals (12.5%) and low birth weight in 3/23 (13%). Progressive and highly significant microcephaly was almost universally found (21/23; 91%), being detected at birth in 38% of individuals and acquired during the first years of life in 52%. The mean head circumference SD score was −3.45, ranging from −1.55 to −7.2. Individuals tended to be short, with a mean height percentile of p12 compared with the Spanish population (range p<1–p35).[19] A height percentile below p2 was observed in 27% (4/15). Three individuals had neonatal hypotonia and none of them had congenital cataracts.

The most common initial presentation was a global psychomotor delay (21/25; 84%), while the presenting symptom was seizures in the four individuals who had seizures before the age of 7 months. A global psychomotor delay was observed at some point in 96% of individuals (24/25), and a subsequent intellectual disability was described in all individuals who were older than 4 years at the last follow-up (23/23). The intellectual disability was severe in eight individuals, moderate-to-severe in five, moderate in seven, mild in one and of an unknown degree in two. It is noteworthy that four individuals (16%) experienced a psychomotor regression, with loss of developmental milestones previously attained, triggered by infections. These triggering infections were even initially classified as encephalitis in these individuals, as there was drowsiness, febrile seizures and a slowed EEG. No causative microorganism was identified in the cerebrospinal fluid. These infections occurred at a median age of 13 months old (range: 4–36).

A severe language impairment was detected in 21/25 individuals (84%). Movement disorders, including hyperkinesia and choreiform movements, were reported in 79% of cases (19/24). None of the individuals with TRAPPC11-related muscular dystrophy showed signs of spasticity. The main clinical features are summarised in online supplemental table 2.

All individuals had elevated creatine kinase (CK) in serum, ranging from 300 to 5000 IU/L (mean: 1440 IU/L, SD: 1009 IU/L). Independent ambulation was acquired by 23 from 24 individuals (96%) at a mean age of 22 months, with a delay in the acquisition of autonomous ambulation—understood as above 18 months of age—observed in 40% (8/20). An early onset proximal weakness was detected in 71% of individuals (17/24), associated with myalgia and cramps in six of them (6/23; 26%). Rhabdomyolysis was not identified in any of the individuals.

Epilepsy, defined as recurrent seizures, was diagnosed in 12/25 individuals (48%), and the mean age at first seizure was 21 months (range: 4 months–8 years; median 12 months). All 12 individuals with epilepsy were treated with antiepileptic drugs. The most common type of seizure was bilateral tonic-clonic seizures, either with focal onset or with unknown onset, being experienced by 92% of individuals with epilepsy (11/12). Focal-onset seizures with impaired awareness were observed in 33% of individuals with epilepsy (4/12). Remarkably, half of individuals with epilepsy (6/12; 50%) had a history of status epilepticus, four of them in the context of fever. Seizures triggered by infections were observed in 83% of individuals (10/12). Seizure characteristics, as well as main electroclinical and neuroradiological features, are summarised in online supplemental table 2.

EEG reports were available in all 11 individuals with epilepsy. Selected illustrative EEG studies are shown in figure 2. Eight individuals (67%) showed interictal focal or multifocal epileptiform abnormalities. A high degree of variability was observed in the origin of the epileptiform abnormalities (online supplemental table 2). Although the temporal origin was slightly more frequent than in other locations, the difference was not significant and is not different from that observed in other individuals with epilepsy, given that temporal lobe epilepsy is the most common form of focal epilepsy.[32 33] Background activity was slow in 42% of individuals with epilepsy (5/12). Photosensitivity was not present in any of the individuals.

Long-term seizure control was assessed in the 12 individuals with epilepsy based on the Brodie classification system.[29] Six individuals (50%) were seizure-free within 6 months of starting treatment (a), two individuals (17%) were seizure-free after

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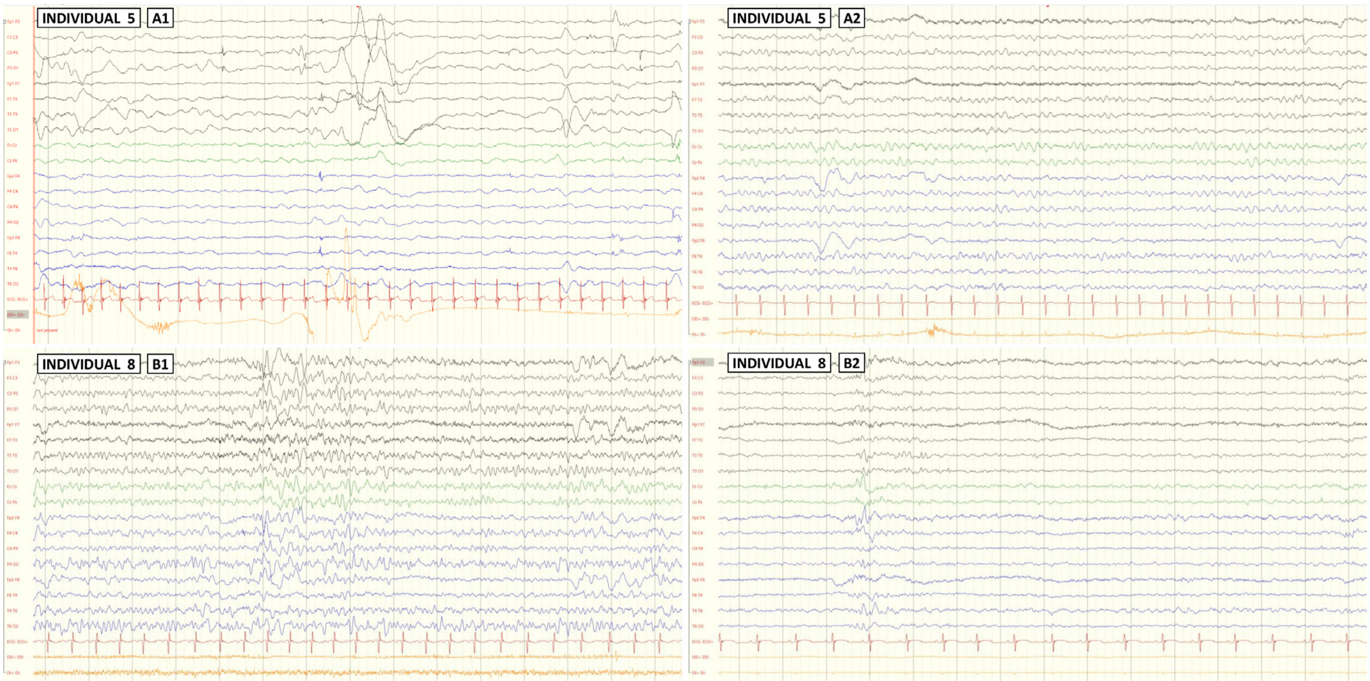

Figure 2 Awake EEG studies of individuals 5 and 8. Longitudinal bipolar montage at sensitivity 70 µV/cm is shown in all the cases. Individual 5: while EEG at 14 months old during his first seizures showed a slow and low voltage background activity without epileptic abnormalities (A1), the EEG at the age of 2 years, during wakefulness with eyes closed, showed a slow background activity without epileptiform abnormalities (A2). Individual 8: EEGs at the age of 13 years, during wakefulness with eyes closed (B1) and eyes open (B2) showed normal background activity, with epileptic abnormalities (sharp waves and spikes) in bilateral frontocentrotemporal region.

6 months of starting treatment (b) and 4 (33%) had a fluctuating course (c; periods of at least 12 months seizure-free, interspersed with relapses). A favourable response to monotherapy treatment was observed in 7/12 individuals (58%). A summary of medications used in each individual at the last follow-up is provided in online supplemental table 2. At the last visit, four individuals were seizure-free with levetiracetam monotherapy and three remained seizure-free after antiepileptic drug withdrawal. Non-pharmacological treatments, including the ketogenic diet, vagus nerve stimulation and epilepsy surgery, were not tried on any individual.

Brain MRI was performed in 84% of individuals (21/25). A generalised decrease in cortico-subcortical volume, including white matter, was found in 29% of them (6/21), with no other associated structural abnormalities. No hypomyelination was observed in the brain MRI of any individual. A prominence of the cerebellar folia was observed in individual 10 but obvious cerebellar atrophy or hypoplasia was not observed in any individual (figure 3 and online supplemental table 2).

Scoliosis was identified in 50% of individuals (5/10) over 13 years of age. No cardiac involvement was observed in the 15 individuals in whom cardiac evaluation with ECG and echocardiography was performed, except in one individual with mild dilatation of the aortic root and ascending aorta.

Morphological findings in muscle biopsies

Muscle biopsies were performed on seven individuals, taken from either the quadriceps (five individuals), the deltoids (one individual) or an unknown location (one individual), at a mean age of 5.5 years (SD: 4.5; median: 3). The main features of muscle biopsies are shown in figure 4 and in online supplemental table 3. Muscle histology showed myopathic changes with variability in fibre size and some fibres with internalised nuclei. Intermyofibrillar pattern disruptions, with areas devoid of staining for oxidative enzymes (Nicotinadmide adenine dinucleotide and Succinate dehydrogenase), were observed in all the individuals. Occasional myonecrosis and regeneration (displayed as neonatal myosin-positive fibres) were observed, while increased endomysial or perimysial connective tissue with fibrosis or fatty infiltration were not observed in any of the cases. Fibre-type distribution was normal, with a mosaic pattern, in five cases while atrophy of type II fibres was observed in two individuals.

Immunofluorescence labelling at the sarcolemma with two antibodies to alpha-dystroglycan (IIH6 and VIA4) showed a patchy or mosaic reduction of alpha-dystroglycan in 5/6 individuals, similar to four individuals with TRAPPC11-related muscular dystrophy recently described by Munot *et al*.[18] Immunohistochemical studies for spectrin, dystrophin, sarcoglycans,

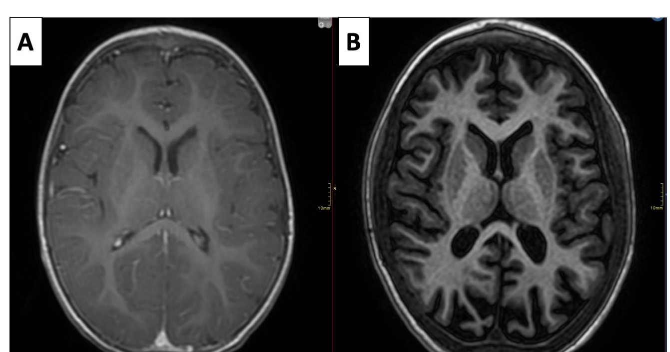

Figure 3 A progressive cortical and subcortical atrophy is observed in individual 5. MRIs at the age of 14 months (A) and 5 years (B) are shown, axial T1.

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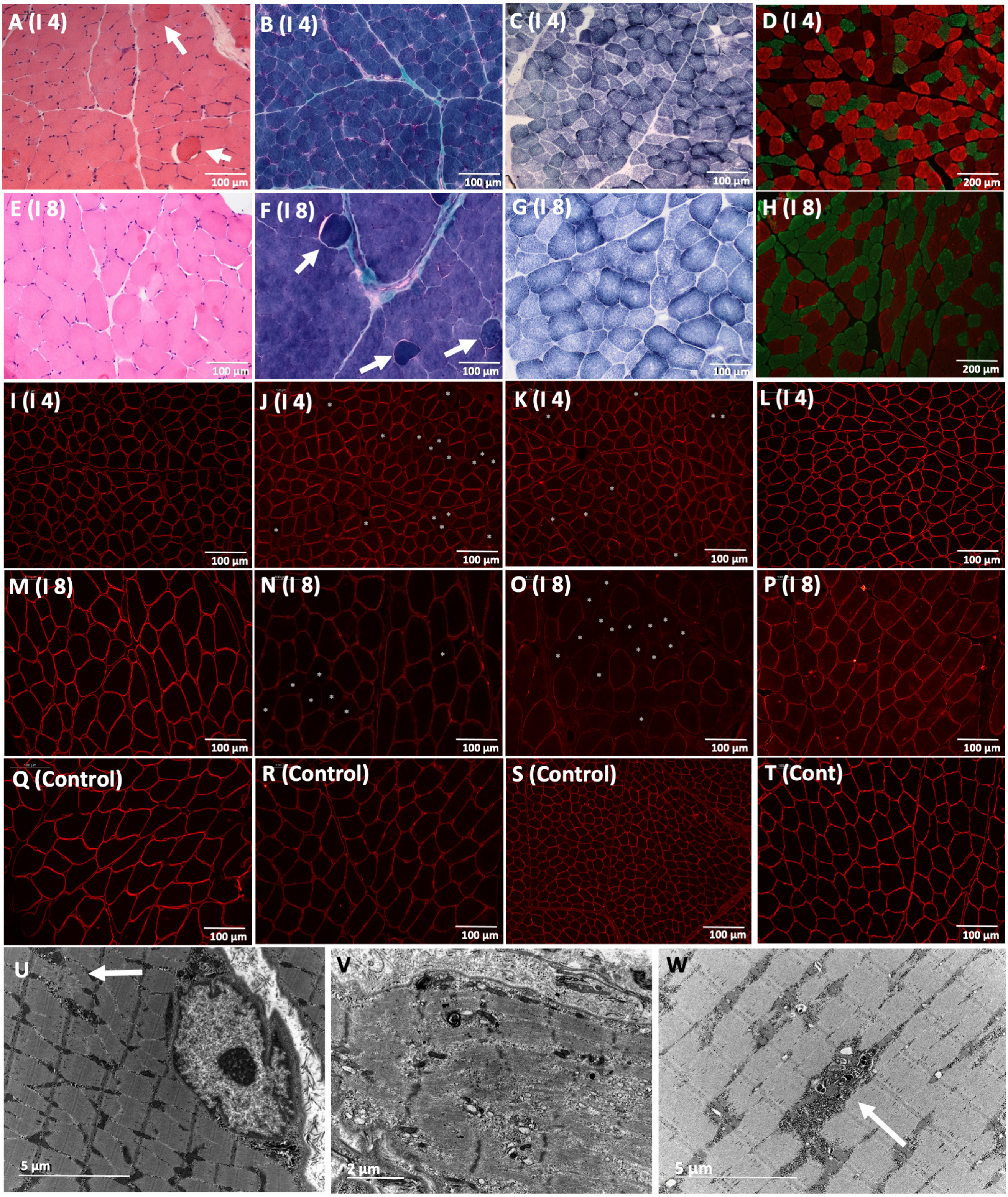

Figure 4 Myopathic muscle pathology from quadriceps and deltoid biopsies. Biopsy from Individual 4 (A–D, I–L and U–W) was taken from quadriceps at 3 years and biopsy from individual 8 (E–H and M–P) was taken from deltoid at 12 years. Variability in fibre size and fibres with internalised nuclei were observed with H&E stain in individuals 4 and 8 (A, E), as well as hypercontracted fibres (arrow A, F). Increased connective tissue with fibrosis or fatty infiltration were not observed (B, F). Blurred unstained areas in some fibres were noted with SDH (C, G). Selective atrophy of fast fibres is noted with double immunostaining of myosins. Slow-type fibres stained red and fast-type fibres stained green (D, H). Sarcolemmal beta-dystroglycan was normal (I, M) compared with control (Q), which implies that the integrity of the sarcolemma is not altered. A reduction of sarcolemmal alpha-dystroglycan with a mosaic pattern (asterisks in fibres with reduced alpha-dystroglycan) was noted in individuals 4 and 8 with IIH6 (J and N) and VIA 4 (K and O) antibodies, compared with control (R and S). Merosin immunolabelling was normal in individuals 4 (L) and 8 (P) compared with control (T). Electron microscopy (U, V and W) performed in individual 4 showed focal Z-band streamings (arrow in U), a necrotic fibre with a structural disruption of the sarcomere (V) and electron-dense degraded material (arrow in W). Scale bar=100 µm in A–T. Scale bar=5 µm in U and W. Scale bar=2 µm in V.

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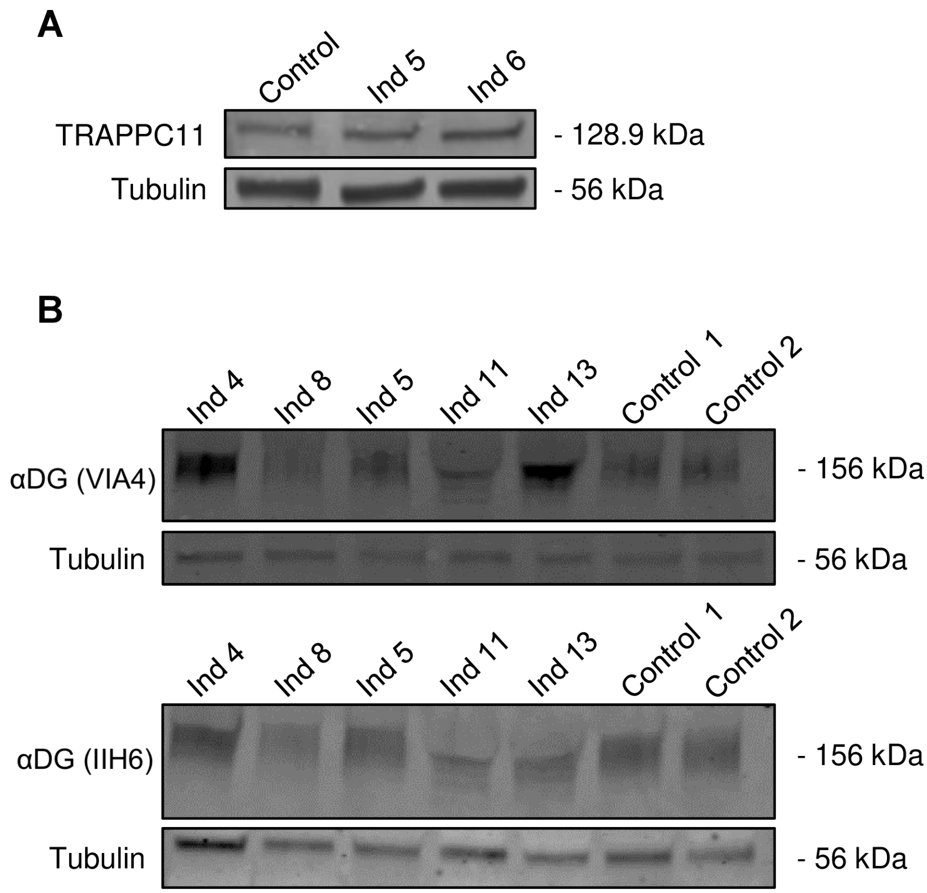

Figure 5 Western blot analyses of TRAPPC11 (in fibroblasts) and alpha-dystroglycan (αDG) glycosylation (in muscle) in some patients. TRAPPC11 expression (A) was not different in fibroblasts from individuals (Ind) 5 and 6 compared with control while αDG glycosylation in muscle was reduced in some individuals. A subtle-to-moderate reduction was noted in Ind 8 and 11 compared with controls with antibodies VIA4 and IIH6. No reductions were noted in Ind 4, 5 and 13.

beta-dystroglycan and laminin were normal. Overexpression of utrophin was not noted, except in regenerative fibres, which is physiological (figure 4).

Electron microscopy performed in individual 4 showed focal Z-band streamings, a necrotic fibre with an almost completely absent myofibrillar structure that was replaced by granular amorphous material as well as a fibre electron-dense degraded material (figure 4).

Variability in fibre size and the presence of atrophic fibres were the most prominent ultrastructural findings.

Western blot analysis studies

To study TRAPPC11 expression levels, a western blot analysis against TRAPPC11 was performed on fibroblast lysates from two individuals (individuals 5 and 6). No differences were observed compared with the control (figure 5A). An alpha-dystroglycan western blot analysis (VIA4 and IIH6 antibodies) was performed on five individuals and compared with controls to study whether glycosylation in muscle was altered. A reduction of alpha-dystroglycan expression was observed in two of the five individuals compared with the healthy control. The reduction in band intensity in individuals 8 and 11 was subtle-to-moderate. The intensity of the band in individuals 4, 5 and 13 was not reduced (figure 5B). No clear alteration in their mean molecular weight was observed compared with the control, except for individual 11, where the molecular weight appears slightly lower, which could be related to protein instability.

Pathophysiology of TRAPPC11 deficiency

We investigated the effect of TRAPPC11 deficiency on mitochondrial function in the fibroblasts of two individuals with TRAPPC11-related muscular dystrophy. Analysis of non-glycolytic ATP production (assumed as mitochondrial ATP) revealed a significant decrease of ~30% in the fibroblasts of individuals with TRAPPC11-related muscular dystrophy. Together with this finding, we observed some mild alterations in the mitochondrial network architecture, as individuals with TRAPPC11-related muscular dystrophy showed more mitochondria but with fewer and shorter branches, resulting in a 'less structured mesh' (figure 6).

DISCUSSION

We report a comprehensive description of the phenotype of 25 individuals with LGMD due to a homozygous c.1287+5G>A splice-site variant in *TRAPPC11*. Variant c.1287+5G>A has been previously reported in homozygosity in five individuals from two Hutterite families who had myopathy, infantile hyperkinetic movements, ataxia and intellectual disability.[5] Subsequently, it was also described in compound heterozygosity along with a frameshift variant (c.3379_3380insT) in a Spanish individual who was not Roma and showed hypotonia, spasticity, choreiform movements and remarkable cerebral atrophy.[17] To our knowledge, this is the first time such a large number of TRAPPC11-related LGMD R18 individuals with the same

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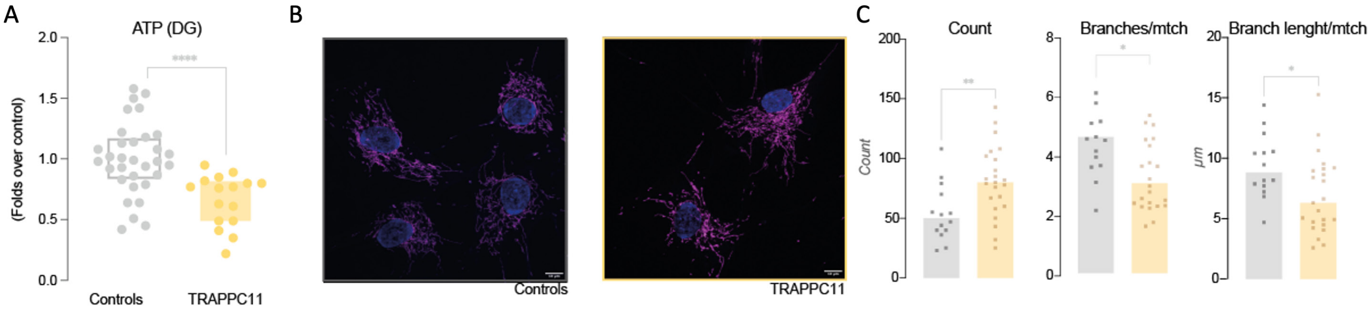

Figure 6 Analysis of the mitochondrial impact of TRAPPC11 deficiency. We explored mitochondrial function in terms of non-glycolytic ATP production (A) through a 2-hour incubation of fibroblasts from individuals with *TRAPPC11*-related muscular dystrophy and controls with 2-deoxy-D-glucose (DG) and a subsequent luciferin/luciferase assay. Results are presented as folds over control. Mitochondrial network was assayed by immunofluorescence against the mitochondrial marker TOMM20: representative images are shown in B and quantification results in C, showing an increase in mitochondrial counts, whose networks are less branched and with shorter mitochondria. Although only one representative image is shown for both control and patients, several fields of independent preparations in triplicate were analysed. The mitochondrial networks were analysed with the ImageJ plugin 'Mitochondrial Analyzer'. Nuclei are marked in blue and mitochondria in magenta. Scale bar marks 10 µm. Images have been parametrised through Mitochondrial Analyzer. Unpaired Welch's t-test was performed. *P<0.05; **p<0.01; ****p<0.0001.

homozygous variant are compiled in a single study. Furthermore, all individuals in our sample were Roma, suggesting *TRAPPC11* c.1287+5G>A is a founder pathogenic variant in Roma ethnicity.

Founder pathogenic variants are often responsible for the high prevalence of rare genetic disorders in specific populations. It is known that the Hutterite population, a communal ethnoreligious group currently settled in the USA and Canada, was first established in the 16th century in Austria and subsequently travelled through various regions of Europe.[34] In the 17th century, both Roma and Hutterite populations were present in Romania, which could be a hypothesis for the presence of the same variant in two ethnicities that are so remote from each other.

The main phenotypic characteristics of the individuals in our cohort do not differ substantially from those already described in previously reported individuals with pathogenic variants in *TRAPPC11*. Early onset muscle weakness, movement disorders, intellectual disability, epilepsy and elevation of CK in serum were almost constant findings in the individuals presented here. However, in our cohort, we have identified two additional phenotypic characteristics that are relevant and have not been previously reported. The first is a progressive and highly significant microcephaly that was almost universally found, often associated with a generalised decrease in cortico-subcortical volume. Although microcephaly has not been recognised as a feature associated with *TRAPPC11* until now, having been reported in only one individual so far,[18] postnatal microcephaly is a typical finding in most of the called 'golgipathies', a term used to describe those diseases caused by defects in Golgi-related genes.[35–40] The second novel finding is that, according to the information gathered in our cohort, infections in the first years of life seemed to act as triggers for a psychomotor regression and onset of seizures in some of the *TRAPPC11* individuals: psychomotor regression triggered by infections was observed in 16% of the individuals (4/25), and seizures triggered by infections were observed in 10/25 individuals (40%), including four who had a history of febrile status epilepticus. Even in four individuals with seizures and psychomotor regression the first diagnostic orientation was encephalitis. Indeed, other TRAPPopathies have been related to these episodes such as two novel homozygous variants in *TRAPPC2L* that associate postinfectious encephalopathy, developmental arrest, tetraplegia and rhabdomyolysis.[41 42]

These febrile illness-triggered seizures, usually presenting in the first 2 years of life, and occasionally associated with psychomotor regression, are a recognisable finding in many TRAPPC11 individuals and are similar to the phenotype showed by the few TRAPPC2L individuals reported so far.[42] In addition, individuals with pathogenic variants in *TRAPPC11* and *TRAPPC2L* share other clinical signs such as neurodevelopmental delay with speech difficulties, microcephaly and extrapyramidal symptoms. This marked phenotypic similarity leads us to hypothesise that variant *TRAPPC11* c.1287+5G>A, located in the foie gras domain, which is essential for interaction with TRAPPC2L, may alter the interaction between TRAPPC2L and TRAPPC11 (figure 1). In an analogous manner, it is possible that the variants described in *TRAPPC2L* weaken the interaction between the two proteins, thus explaining this unique phenotypic similarity. The reason why pathogenic variants of several subunits of the TRAPP complex result in microcephaly (TRAPPC2L, TRAPPC6, TRAPPC9, TRAPPC11) while others do not is unclear but may be related to the function of these proteins within the TRAPP complex and their role in membrane traffic.

On the other hand, the acute episodes observed in TRAPPC11 individuals partially resemble the acute pseudometabolic crises triggered by infections that are well-described in individuals with pathogenic variants in *TANGO2*[43] and other cell trafficking disorders. Cell trafficking disorders, which encompass 346 genes so far, are mostly progressive diseases due to the homeostatic loss of crucial molecular functions involved in neurodegeneration such as lipid transfer and membrane remodelling.[44] Although rhabdomyolysis and sustained CK elevation are hallmarks of some TRAPPopathies, they can also be observed in other anterograde trafficking disorders such as TANGO2, in which rhabdomyolysis during acute crises is associated with hypoglycaemia, hyperlactataemia and hyperammonaemia, or LPIN1, in which recurrent hyperCKaemia occurs.[40] All these features point towards a major metabolic involvement and make it advisable that cell trafficking disorders are included in the differential diagnoses of inborn errors of metabolism of small molecules (in particular intoxication disorders) and energy defects. These findings suggest that stressful situations such as infections impact the normal functioning of the trafficking machinery between the endoplasmic reticulum (ER) and the Golgi apparatus in individuals with *TRAPPC11* pathogenic variants and other disorders.

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The precise mechanisms leading to these manifestations need to be further described.

Although epilepsy represents a significant symptom of LGMD R18 with a serious impact on quality of life, to date neither the types of seizures nor the electroencephalographic abnormalities had been studied systematically in a well-described cohort of individuals. In our cohort, epilepsy occurred in more than half of individuals with TRAPPC11-related muscular dystrophy and was typically presented with early childhood-onset long-lasting bilateral tonic-clonic seizures. EEG studies showed focal or multifocal interictal epileptiform abnormalities in most of the cases. The response to antiepileptic drugs was good in a significant proportion of individuals but not in all.

Our pathological studies on muscle biopsy from seven individuals revealed a myopathic pattern with degenerative-regenerative fibres, isolated hypercontracted fibres and alterations in the intermyofibrillar pattern. Although the fibre-type distribution pattern was normal in most individuals, selective atrophy of fast fibres was identified in two cases. We detected a patchy reduction of alpha-dystroglycan of variable intensity, both with immunofluorescence and western blot analysis, which validates similar findings recently reported by Munot *et al*.[18]

TRAPPC11 is a protein involved in membrane trafficking[10 44] and had been previously related to autophagy defects, with a role in membrane recruitment for the autophagosome formation.[45] Our results expand the role of TRAPPC11 in mitochondrial function, as we observe a decreased mitochondrial ATP production capacity and network architecture in TRAPPC11-deficient fibroblasts. This sets the base for further research on the potential role of TRAPPC11 within mitochondria-Golgi-ER triad and its specific role on mitochondrial functions, which should be delimited with further experiments that are beyond the scope of this work, analysing the involvement of other mitochondrial functions in *TRAPPC11*-related muscular dystrophies such as the activity and expression of electron transport chain complexes, mitochondrial ultrastructure, reactive oxygen species production and metabolism, mitophagy and mitochondrial signalling. These findings may also help to develop targeted therapies.

In summary, we provide a comprehensive phenotypic characterisation of the pathogenic variant *TRAPPC11* c.1287+5G>A, which is founder in the Roma population. Our observations indicate that glycosylation of alpha-dystroglycan is reduced in the skeletal muscle of individuals with LGMD R18 and we emphasise some features that are typical of golgipathies, such as microcephaly and clinical decompensation associated with infections, are probably more prevalent than previously thought.

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Contributors MJ had a major role in the acquisition of the data, analysed the data, interpreted the data and drafted the manuscript for intellectual content. ÀG-C and DN-dB designed and conceptualised the study, had major role in the acquisition of the data, analysed the data, interpreted the data and drafted the manuscript for intellectual content. DN-dB is responsible for the overall content as the guarantor. The rest of the authors collected the data, performed the analysis and revised the manuscript for intellectual content.

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Data availability statement Data are available on reasonable request.

Biobank The HSJD Biobank is officially registered in the Spanish Institute of Health (Instituto de Salud Carlos III) under the number B.0000599. It is also authorised by the autonomic health system of Catalonia. All the samples of HSJD Biobank used in research are collected, managed and stored under strict quality measures (the Biobank is accredited by ISO 9001:2015 standard) and all donors or their relatives have signed an informed consent form. The HSJD Biobank website is: https://www.irsjd.org/es/servicios-cientificotecnicos/biobanco-pediatrico-para-la-investigacion/.

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Supplementary Table 1. List of antibodies used in studies for immunohistochemistry, immunofluorescence and western blot.

PRIMARY ANTIBODY	CLONALITY	HOST	DILUTION	REFERENCE	TECHNIQUE
Dystrophin (Dys1) Rod domain	MAb	Mouse	1/100	NCL-DYS1, Novocastra, Leica Biosystems, Newcastle, UK	Immunohistochemistry Immunofluorescence
Dystrophin 2 (Dys2) C terminal	MAb	Mouse	1/10	NCL-DYS2, Novocastra, Leica Biosystems, Newcastle, UK	Immunohistochemistry Immunofluorescence
Dystrophin 3 (dys3) N terminal	MAb	Mouse	1/10	NCL-DYS3, Novocastra, Leica Biosystems, Newcastle, UK	Immunohistochemistry Immunofluorescence
Beta Spectrin	MAb	Mouse	1/50	NCL- SEPC1, Novocastra, Leica Biosystems, Newcastle, UK	Immunohistochemistry Immunofluorescence
Utrophin	MAb	Mouse	1/500	Novocastra, NCL-DRP2, Leica Biosystems, Newcastle, UK	Immunohistochemistry
Alpha sarcoglycan	MAb	Mouse	1/50	Novocastra, NCL-L-a-SARC, Leica Biosystems, Newcastle, UK	Immunofluorescence
Beta Sarcoglycan	MAb	Mouse	1/20	Novocastra, NCL-L-b-SARC Leica Biosystems, Newcastle, UK	Immunofluorescence
Delta Sarcoglycan	MAb	Mouse	1/20	Novocastra, NCL-L-d-SARC Leica Biosystems, Newcastle, UK	Immunofluorescence
Gamma Sarcoglycan	MAb	Mouse	1/100	Novocastra, NCL-L-g-SARC Leica Biosystems, Newcastle, UK	Immunofluorescence
Alpha dystroglycan (VIA4)	MAb	Mouse	1/10	Merck Millipore 05-298, Burlington, MA, USA	Immunofluorescence/ Western Blot
Alpha dystroglycan (2H6)	MAb	Mouse	1/10	Merck Millipore 05-593, Burlington, MA, USA	Immunofluorescence/ Western Blot
Beta dystroglycan	MAb	Mouse	1/200	Novocastra, NCL-b-DG, Leica Biosystems, Newcastle, UK	Immunofluorescence
Alpha tubulin	Mab	Mouse	1/1000	Sigma Aldrich, T6199, USA	Western Blot
Myosin Heavy Chain fast	MAb	Mouse	1/50	Novocastra, Leica Biosystems, Newcastle, UK	Immunohistochemistry immunofluorescence
Myosin Heavy Chain slow	MAb	Mouse	1/50	Novocastra, Leica Biosystems, Newcastle, UK	Immunohistochemistry
Myosin Heavy Chain neonatal	MAb	Mouse	1/20	Novocastra, Leica Biosystems, Newcastle, UK	Immunohistochemistry
Myosin Heavy Chain slow	PAb	Rabbit	1/200	Invitrogen, PA5-34340	Immunofluorescence
TRAPPC11 (C4ORF41)	PAb	Rabbit	1/200	Invitrogen, PA5-71454	Western Blot
SECONDARY ANTIBODY	**CLONALITY**	**HOST**	**DILUTION**	**REFERENCE**	**TECHNIQUE**
Donkey anti mouse 549	PAb	Donkey	1/500	Invitrogen, A21203	Immunofluorescence
Donkey anti mouse 549	PAb	Goat	1/500	Invitrogen, SA5-10152	Immunofluorescence
Donkey anti rabbit 549	PAb	Goat	1/500	Invitrogen, A21207	Immunofluorescence

Supplementary Table 2. Clinical features of individuals with homozygous variant c.1287+5G>A in *TRAPPC11*.

Abbreviations: M = male; F = female; y= years; IU= International Units; FM-IA= Focal motor with impaired awareness seizure; FNM-IA= Focal non-motor with impaired awareness seizure; FBTC= Focal onset to bilateral tonic-clonic seizure; GM= Generalized onset myoclonic seizures; GT= Generalized onset tonic seizures; BTC= Bilateral tonic-clonic seizures; SW= Spike-waves; NA/NP= Not available/Not performed.

Ind	*Fam*	*Sex*	*Age last exam*	*Intellectual disability*	*CK (IU)*	*Muscle weakness*	*Muscle biopsy*	*Movement disorder*	*Seizure onset*	*Infection-related seizures*	*Seizure types*	*Status epilepticus*	*Epilepsy course (Brodie class)*	*Current antiseizure medication*	*EEG*	*Brain MRI*
1	1	F	13-16y	Yes (severe)	300-600	Yes	Not performed	No	6 m	Yes	BTC + GT	No	A	LEV	Normal background. Multifocal spikes, sharp, slow and SW, mainly left centro-parieto-temporal.	Normal
2	1	F	9-12y	Yes (severe)	300-1100	Yes	Not performed	Yes	6 m	Yes	FM-IA + FNM-IA + FBTC	Yes (several status with fever)	C	LEV	Normal background. Focal sharp waves in right fronto-temporal.	Generalized decrease in cortico-subcortical volume.
3	2	M	17-20y	Yes (moderate)	500-1400	Yes	Not performed	Yes	4 m	Yes	BTC + FBTC + FM-IA	Yes (febrile status epilepticus)	C	LEV+ OXC	Normal background. Multifocal SW, mainly left parieto-temporal.	Normal
4	3	M	5-8y	Yes (moderate)	500-800	Yes	Unspecific myopathic pattern	Yes	No epilepsy						Normal (2x)	Normal
5	3	M	5-8y	Yes (moderate-severe)	300-500	Yes	Unspecific myopathic pattern	Yes	14 m	Yes	FBTC + BTC	Yes (Febrile status epilepticus)	B	LTG + VPA + CLB	Slow background. Multifocal SWs, mainly left parieto-occipital and right parieto-temporal.	Generalized decrease in cortico-subcortical volume.

6	4	F	13-16y	Yes (moderate-severe)	450	Yes	Not performed	Yes					No epilepsy			Generalized decrease in cortico-subcortical volume.
7	4	M	9-12y	Yes (mild)	NA/NP	Yes	Not performed	Yes					No epilepsy			NA/NP
8	5	F	13-16y	Yes (moderate)	800-3000	Yes	Unspecific myopathic pattern	Yes	6 m	Yes	BTC	Yes (Febrile status epilepticus)	A	LEV	Normal background. Multifocal SWs, mainly frontal. No photosensitivity.	Generalized decrease in cortico-subcortical volume.
9	6	F	9-12y	Yes (severe)	350-750	Yes	Not performed	Yes	15 m	No	BTC	No	C	LEV	Normal background. Multifocal discharges.	Periventricular leukomalacia (history of prematurity)
10	6	M	21-24y	Yes (severe)	3500	No	Not performed	Yes	7y	NA	BTC	No	B	No	Slow background. Some EEG with multifocal activity: spikes in the right centrotemporal region and left centrotemporal region.	Normal
11	6	M	5-8y	Yes (moderate)	700-1000	No	Unspecific myopathic pattern	Yes					No epilepsy			Normal
12	7	F	5-8y	Yes (moderate)	1700-4500	Yes	Performed but not revised	Yes					No epilepsy			Normal
13	8	F	9-12y	Yes (severe)	820-1500	Yes	Unspecific myopathic pattern	Yes	4m	Yes	GM + FM-IA	No	C	VPA + OXC	Focal discharges (left centro-temporal)	Generalized decrease in cortico-subcortical volume.
14	9	F	17-20y	Yes (moderate)	600-5000	No	Unspecific myopathic pattern	No					No epilepsy			Normal
15	10	M	5-8y	Yes (moderate)	1950	Yes	Not performed	Yes					No epilepsy			Bilateral parieto-occipital corticosubcortical volume decrease with

																prominence of cerebellar folia and thin corpus callosum.
16	10	M	13-16y	Yes (severe)	2970	Yes	Not performed	Yes				No epilepsy			Slow background activity	NA/NP
17	11	M	21-24y	Yes (severe)	1660	No	Unspecific myopathic pattern	Yes	13 m	Yes	BTC	Yes	A	No	Slow background activity. Epileptic abnormalities not detected	Normal
18	12	F	9-12y	Yes (moderate-severe)	260-780	No	Not performed	Yes	12m	Yes	BTC	Yes	A	No	Normal background activity. Epileptic abnormalities not detected	Normal
19	13	M	5-8y	Yes (moderate-severe)	370	No	Not performed	Yes					No epilepsy			Normal
20	14	F	13-16y	Yes (severe)	2450	Yes	Not performed	No					No epilepsy			Normal
21	15	M	2-4y	≤ 4 years old	1035	No	Not performed	Yes	14m	Yes	BTC	No	A	LEV	Slow background activity. Multifocal discharges.	Generalized decrease in cortico-subcortical volume.
22	16	M	13-16y	Yes (grade unknown)	1200-2400	Yes	Not performed	Yes					No epilepsy			Megacisterna magna
23	17	M	2-4y	≤ 4 years old	550	Yes	Not performed	No					No epilepsy			Not performed
24	17	F	9-12y	Yes (grade unknown)	330-1720	Yes	Not performed	No					No epilepsy			Not performed
25	18	F	17-20y	Yes (moderate-severe)	370	Yes	Not performed	Yes	8y	Yes	FM-IA + BTC	No	A	LEV	Slow background activity. Epileptic abnormalities not detected	Normal

Supplementary Table 3. Main features of muscle biopsies of individuals with homozygous variant c.1287+5G>A in *TRAPPC11*.

	Age at biopsy	Location	Fibrosis	Adipose tissue	Fibre size	Internal nuclei	Basophilic fibres	Regenerative fibres	Hpercontracted fibres	Necrosis	Intermyofibrillar pattern	Cox negative fibres	Lipids	Fibre type distribution	Sarcolemma protein staining	ADG (IIH6)	ADG (Via4)	Beta-DG
Individual 4	3	Left quadriceps	No	No	Moderate variability. Diameter 15-55 µm	<1%	5% (some in groups)	3%	1%	Yes	Intermyofibrillar pattern disruptions	No	No lipid increase	Normal mosaic pattern. Selective fast fibres atrophy.	Normal positive sarcolemma staining	Mosaic pattern with hyperintensity and hypointensity in some fibres	Mosaic pattern with hyperintensity and hypointensity in some fibres	Normal
Individual 5	3	Left quadriceps	No	No	Mild variability. Diameter 21-42 µm	<1%	No	<1%	5%	No	Intermyofibrillar pattern disruptions	No	No lipid increase	Normal mosaic distribution.	Normal positive sarcolemma staining	Normal	Normal	Normal
Individual 8	12	Left deltoids	No	No	Mild variability. Diameter 50-95 µm	5%	2%	<2%	5%	No	Intermyofibrillar pattern disruptions	No	No lipid increase	Normal mosaic distribution.	Normal positive sarcolemma staining	Mosaic pattern with hypointensity globally	Mosaic pattern with hypointensity globally	Normal
Individual 11	3	Right quadriceps	No	No	Moderate variability. Diameter 30-40 µm	2%	1%	<1%	5%	No	Intermyofibrillar pattern disruptions	No	No lipid increase	Normal mosaic distribution.	Normal positive sarcolemma staining	Mosaic pattern with hypointensity globally	Mosaic pattern with hypointensity globally	Normal
Individual 13	1,5	Quadriceps	No	No	Mild variability. Diameter 15-34 µm	1%	1%	<1%	7%	No	Intermyofibrillar pattern disruptions	No	No lipid increase	Normal mosaic distribution. Slow fibre type prevalence	Normal positive sarcolemma staining	Mosaic pattern with hyperintensity and hypointensity in some fibres	Mosaic pattern with hyperintensity and hypointensity in some fibres	Normal
Individual 14	12	Unknown	No	No	No variability	2%	No	No	No	No	Intermyofibrillar pattern disruptions	No	No lipid increase	Not assessable	Normal positive sarcolemma staining	Not assessable	Not assessable	Not assessable
Individual 17	4	Right quadriceps	No	No	Mild variability. Diameter 25-90 µm	<1%	No	1%	2%	No	Intermyofibrillar pattern disruptions	No	No lipid increase	Normal mosaic distribution. Selective fast fibres atrophy.	Normal positive sarcolemma staining	Mosaic pattern with hyperintensity and hypointensity in some fibres	Mosaic pattern with hyperintensity and hypointensity in some fibres	Normal
Control individual without a neuromuscular disorder	5	Right quadriceps	No	No	No variability	<1%	No	No	No	No	Normal	No	No lipid increase	Normal mosaic distribution.	Normal positive sarcolemma staining	Normal	Normal	Normal