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ORIGINAL COMMUNICATION

The first Italian family with tibial muscular dystrophy caused by a novel titin mutation

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Abstract Tibial muscular dystrophy (TMD) or Udd myopathy is an autosomal dominant distal myopathy with late onset, at first described in the Finnish population. We report here the first Italian cases of *TTN* mutated titinopathy. The proband, a 60 year-old female, had the first muscular signs at the age of 59 years, with difficulty in walking and right foot drop. Muscle imaging showed selective fatty degenerative change in the anterior compartment of leg muscles. Her 67 year-old brother, started to show muscle weakness, pain at lower limbs and hypertrophy of calf muscles at the age of 66 years. Their mother began to show foot drop and impaired walking from the age of 60 years. Other relatives are reported to be affected in a similar way. Because the phenotype appeared compatible with TMD, we analyzed the *TTN* gene in the DNA of the proband and we identified a heterozygous mutation 293326A>C. This mutation is also present in the brother and in the other affected individuals of the same family. The mutation predicts a His33378Pro change located next to the previously known Belgian TMD mutation. The mutation was not found in 100 Italian control DNA samples. Then, since the introduction of a proline in the last domain of titin was previously known to cause TMD in French families, we can conclude that this missense mutation is the obvious pathogenetic mutation in the affected patients. No other disease causing mutations in the *TTN* gene have so far been reported in the Italian population.

Keywords Tibial muscular dystrophy · Distal myopathy · *TTN* gene · Italian patients

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Introduction

Tibial muscular dystrophy (TMD), or Udd myopathy, is an autosomal dominant distal myopathy with late onset, described for the first time in the Finnish population [1, 2]. Afterwards, it was described also in French [3] and Belgian families [4]. Recently Hackman et al. have identified three new mutations in families from Spain and France [5]. TMD is caused by heterozygous mutations in the last two exons of the *TTN* gene, which encodes the giant skeletal muscle protein titin (Table 1). Homozygous occurrence of the TMD mutations in the titin gene causes the more severe limb–girdle muscular dystrophy type 2J (LGMD2J) [6, 7]. Homozygous mutations N-terminal of the *TTN* gene cause lethal cardiac and skeletal myopathy [8]. Heterozygous

Table 1 *TTN* mutations previously published comprising the new mutation reported in this manuscript

Origin	Location of mutation in *TTN* gene	Type of mutation	Consequence on protein level
Finnish founder FINmaj	Exon 363 g.293269_293279del AAGTAACATGG insTGAAAGAAAAA	Insertion/deletion, missense	p.E33359_ W33362delinsVKEK
French A	Exon 363 g.293356T>C	Point mutation missense	p.L33388P
Belgian	Exon 363 g.293329T>A	Point mutation missense	p.I33379 N
Spanish	Exon 363 g.293378delA	Deletion, frameshift	p.K33395NfsX9
French B	Exon 363 g.293379C>T	Point mutation nonsense	p.Q33396 > X
French C	Exon 362 g.292998delT	Deletion, frameshift	p.S33315QfsX10
Italian	Exon 363 g.293326A>C	Point mutation missense	p. H33378P

The *first column* indicates the population in which the mutation has been found

Mutations have been listed in order of discovery

mutations in other parts of the same gene have been associated with hypertrophic [9], dilatative cardiomyopathy [10] and hereditary myopathy with early respiratory failure (HMERF) [11].

Here we report a family with clinical signs of tibial muscular dystrophy and a new heterozygous mutation in *TTN* gene. This is the first report of *TTN* mutated TMD in Italy.

Case description

The proband (II-8), a 60 year-old female, came to our attention because she had muscular deficit in the lower limbs since she was 59 years old (Fig. 1.). Medical history was unremarkable until that age. She had gait disturbances due to right sciatalgia and right foot drop. Serum creatine kinase (CK) and serum lactic acid were normal. A neurologic examination showed severe loss of ankle and big-toe dorsiflexion, more on the right side, bilateral hypotrophy of anterior compartment of the leg, hypertrophy of the calves, steppage gait and reduced right Achilles tendon reflex. Electromyogram (EMG) showed myopathic and neurogenic changes only in the muscles of the anterolateral loggia of both legs, without signs in the quadriceps and gastrocnemii. In the right anterior tibialis a reduced size of compound motor response was recorded. Nerve conduction

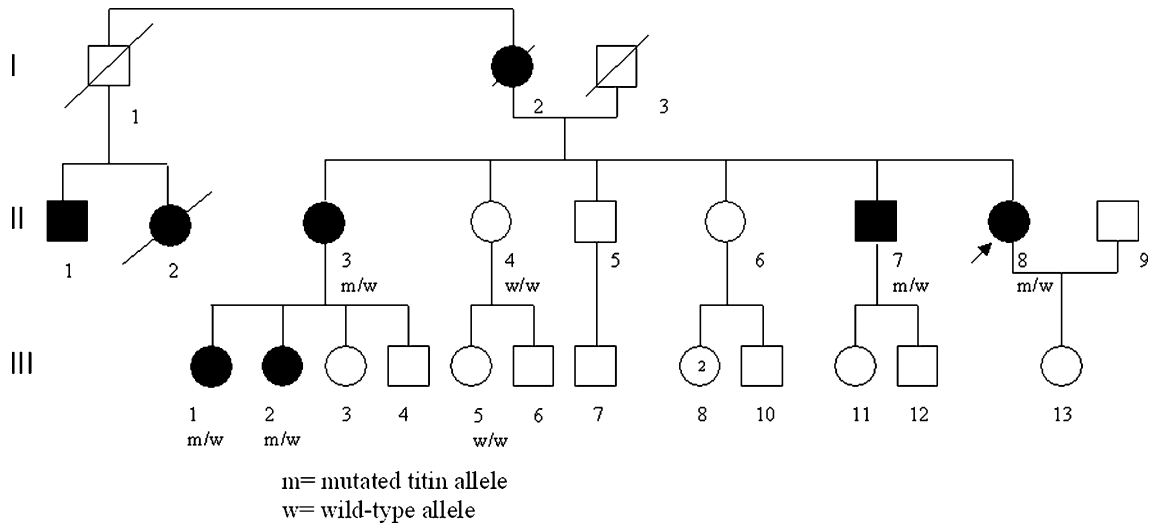

Fig. 1 Pedigree of the described family with TMD. An *arrow* indicates the proband who first came to our attention. *Black squares* and *circles* indicates the affected individuals. Mutant (*m*) and normal (*wt*) alleles are marked in the analyzed individuals

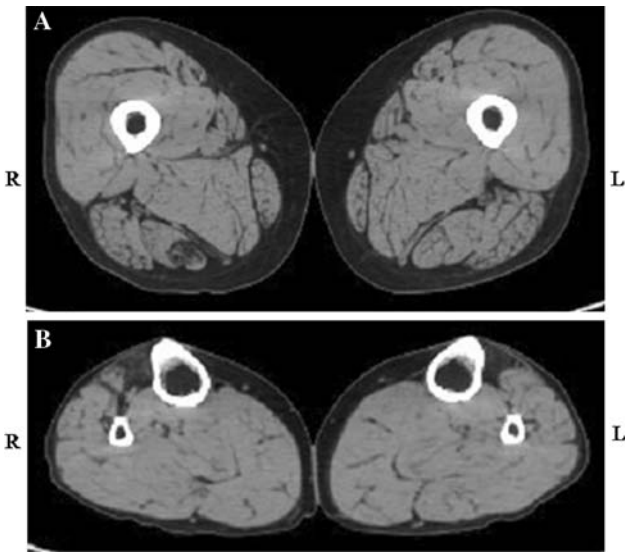

Fig. 2 CT imaging sections of the mid thigh (**a**) show early fatty degenerative changes in the hamstring muscles and rectus femoris bilaterally. On the lower legs (**b**) there is the typical selective total replacement of both anterior tibialis muscles and marked change in extensor hallucis longus muscle on the right side. Other muscles are within normal range

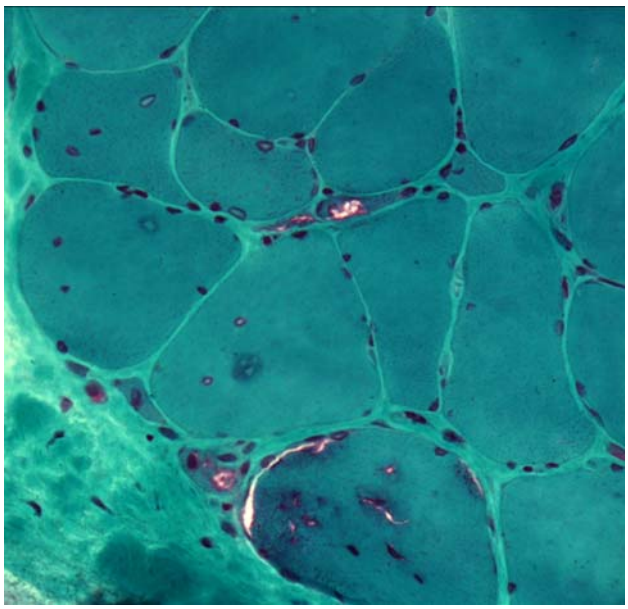

Fig. 3 Image of the Gomori trichrome stained muscle biopsy of patient II-7 obtained from the anterior tibial muscle showing variation in fiber size, numerous internal nuclei and several rimmed vacuoles

velocities were normal. CT scan showed the typical selective replacement of both anterior tibialis muscles and extensor hallucis on the right side (Fig. 2). Muscle biopsy from the right anterior tibial muscle showed an almost complete substitution of fibroadipose tissue. Only rare and sparse muscle cells of variable size were observed (Fig. 3). Using antibodies against Caveolin3, Dysferlin, Dystrophin 1,2 and 3, Emerin, α, β, γ and δ sarcoglycans, and Telethonin immunohistochemistry was normal.

The 67 year-old brother of the proband (II-7) had difficulties running and muscular cramps in the lower limbs since the age of 64 years. He had a mild anserine-steppage gait, hypotrophic lower legs prominent in the proximal portions of the right side, pseudohypertrophy of the calves, pes cavus and bilateral foot drop. Serum creatine kinase (CK) in the serum was normal. EMG showed myopathic changes of the distal muscles of the lower limb. Compared with the CT muscle imaging in his younger sister, the patient has a much more marked fatty degenerative involvement of the hamstring muscles on the thigh and also early changes in the vastus lateralis (data not shown). Muscle biopsy from right anterior tibialis showed variation in fiber size, several rimmed vacuoles (mostly in hypotrophic fibers), 15% nuclear internalization, a small group of basophilic fibers and slight increase of endomysial connective tissue. The eldest sister of the proband (II-3), who is 78 years old, had gait disturbances since she was 57, and she has been in a wheelchair since the age of 76 years. Her 46 year-old daughter (III-1) had foot drop since the age of 41 years. A neurologic examination showed steppage gait and markedly reduced ankle dorsiflexion with inability to stay on heels. The neurological examination has shown her 50 year-old daughter (III-2) to have marked weakness of dorsiflexors of feet bilaterally. The deceased mother (I-2) had gait disturbances and bilateral foot drop since the age of 60 years. II-1 is reported to have severe gait disturbances but he was not examined by a neurologist. The remaining family members were reported as normal.

Materials and methods

Direct sequencing of the two last *TTN* exons 362–363 (Mex5-Mex6) was performed on DNA extracted from blood samples of subjects. Sequencing primers were designed to include exons and exon–intron borders. Primer sequences are available on request. PCR products were sequenced on an ABI 3130 Genetic Analyzer (Applied Biosystems, Forster City, CA), using the Big-Dye Terminator kit and they were analyzed with the multiple alignment program MACAW 2.0.5 (National Center for Biotechnology Information, Bethesda, MD). After finding the mutation in the proband, DNA samples from affected and unaffected relatives were collected and analyzed for the identified mutation in the proband. We also analyzed 100 Italian control DNA. Samples from the subjects were obtained after informed consent in agreement with local ethic approval.

Results

The sequence analysis of the *TTN* gene revealed two heterozygous variations in the proband. One of them is a silent g.293189A>G change, which leads to no amino acid substitution. The other is the point mutation g.293326A>C, which causes a His33378Pro change (Fig. 4). This second mutation is located in the last exon 363 (Mex6) of the gene and introduces a proline residue on the protein. The variation g.293189A>G was found in one of the 200 control DNA whereas g.293326A>C was not seen in any of them. In addition, we identified a heterozygous intronic variation g.293124T>A in an unaffected relative (III-5). This variation has been previously reported and it is regarded as a normal polymorphism.

Discussion

Here we report the first family with TMD and the disease causing *TTN* mutation in the Italian population. TMD, or Udd myopathy, is an autosomal dominant distal myopathy with late-onset and benign evolution [1, 2]. The age of onset is variable, but it is usually between 35 and 60 years in patients with mutations in the last exon of titin. However, in the recently reported French family with mutation in the second-to-last exon, the onset was earlier, after the age of 20 years, and the disease evolution was much more severe [5]. Patients have rather selective weakness of ankle dorsiflexion and inability to walk on the heels. Muscular weakness is slowly progressive but usually it remains confined to the anterior lower leg and hamstring muscles even at an advanced age [7]. The long-toe extensors become involved later (from 10 to 20 years after the onset), causing foot drop and clumsiness during walking. In some cases Udd myopathy is so mild that it can remain clinically unnoticed even in the elderly.

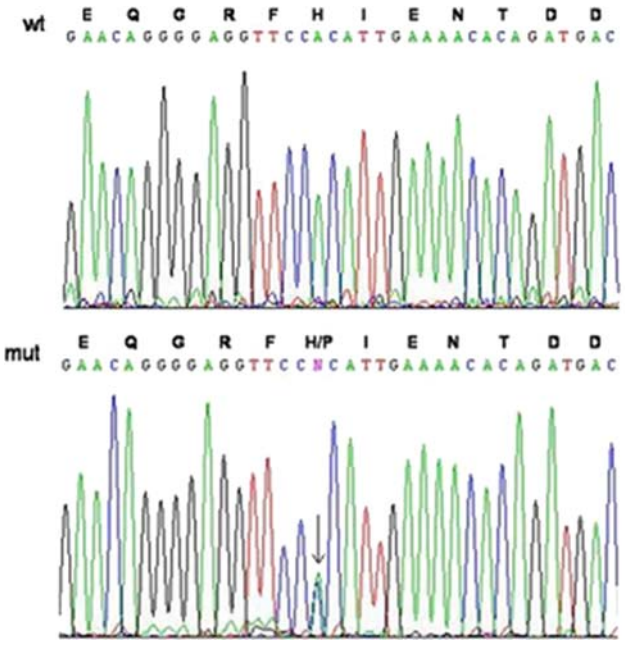

Fig. 4 Chromatograms of *TTN* sequence. In the *upper panel* wild-type sequence is shown; arrow indicates the site of mutation. The mutated sequence is reported in the *lower panel*

In the Italian family reported here, the older generation patients did not complain of leg muscle weakness before they were nearly 60 years old. However, the extensive loss of ankle dorsiflexion and the severe fatty degenerative changes in the anterior lower leg and hamstring muscles indicate that the disease process had started at least 20 years earlier also in these patients. This is also compatible with the findings on neurological examination and age of onset in the next generation.

In TMD there is no cardiomyopathy and serum CK levels are normal or mildly elevated. Electromyography shows myopathic changes in the affected muscles. However, similar to the interpretation of EMG findings in other TMD families, the Italian patients findings also were reported as to some extent neurogenic, which may be confusing for further diagnostic accuracy. Imaging of muscles, such as muscular CT and NMR, is very helpful and confirms the dystrophic replacement and selective involvement of anterior tibial muscles and possible lesions in the proximal hamstring muscles of the lower limbs. In the family we are considering, the imaging findings were directly suggestive of a titinopathy. Muscle biopsy of the target muscle, anterior tibialis, usually shows rimmed vacuoles in combination with severe general dystrophic changes.

TMD is caused by mutations in the *TTN* gene, which encodes the giant skeletal muscle protein titin (GenBank accession number AJ277892). This gene is located in 2q24.3 and it has 363 exons. In all affected Finnish families a common mutation is present in Mex6, the last exon of *TTN*. This founder mutation is called FINmaj and it consists of an 11-bp deletion/insertion mutation that changes four amino acid residues [12]. Other mutations were later identified in families from different European populations, such as in French [3] and Belgian populations [4], and recently two new mutations in different parts of France and one new mutation in two families from Spain [5] (Table 1). Homozygous TMD causing mutations in the titin gene cause the more severe limb–girdle muscular dystrophy type 2J (LGMD2J) [6]. Heterozygous mutations in the same gene were found in hypertrophic [9], dilatative cardiomyopathy [10] and hereditary myopathy with early respiratory failure (HMERF) [11].

No mutations in the *TTN* gene have been reported in the Italian population until today. Here we report the first

mutation we found in an Italian family with tibial muscular dystrophy. The point mutation 293326A>C segregates in the reported family, with the presence of the mutation in the affected individuals, and its absence in the unaffected individuals of the same family. This mutation causes an introduction of a proline in position 33378. This marked amino acid change in the protein and the fact that the mutation is next to a known Belgian mutation associated with TMD (g.293329T>A), lead us to consider this missense mutation an obviously pathogenic mutation. The other mutation identified, g.293189A>G, was found in one of the control DNA samples screened. In addition, the mutation was only identified in individuals II-3, II-7 and II-8 and it was not inherited by III-1 and III-2. These findings, and the fact that the protein sequence is unchanged, bring us to conclude that g.293189A>G has no pathogenic effect and it can be regarded a normal polymorphism. The biological function of the last domain of titin has not been identified. The Finnish TMD causing titin mutation, FINmaj, in the same domain, does not cause any disintegration of the sarcomeric structure, suggesting that the harmful effects of the mutated C-terminal titin may be mediated by aberrant signaling functions.

Other Italian families can now be examined in order to find the same or new mutations in the *TTN* gene to be responsible for tibial muscular dystrophy.

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