Review

Hereditary myopathy with early respiratory failure (HMERF): Still rare, but common enough

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Abstract

Phenotypic and genetic/allelic heterogeneity is a feature of many neuromuscular disorders, titinopathies being one of them. Hereditary Myopathy with Early Respiratory Failure (HMERF) has been considered an extremely rare disease with definite clinicopathologic hallmarks, and geographically restricted to the Northern European population with one single titin gene defect identified in previous years. The recent availability of massive parallel sequencing techniques, allowing the screening of all coding regions of the genome in undiagnosed patients, together with a growing awareness of the main muscle MRI features of the disease, has led to the discovery of a number of HMERF families and new titin mutations in the last five years. We reviewed the clinical, pathological and muscle imaging findings that are still cornerstones for the diagnosis of this disease, as well as the most recent molecular genetic findings. HMERF is more common and geographically widespread than previously expected, and the knowledge of the whole phenotypic and molecular spectrum of HMERF can increase the number of diagnosed patients considerably.

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1. Introduction

Since its original description more than 20 years ago, only scattered sporadic cases or single families, mainly belonging to Northern European populations, have been reported as affected by Hereditary Myopathy with Early Respiratory Failure (HMERF, OMIM#603689) before 2012. HMERF was therefore thought to be an extremely rare disease. In the last five years, several papers provided insights on new molecular genetic data and detailed phenotype, pointing out that HMERF could indeed be less exceptional, and that patients can be identified on the basis of consistent muscle imaging findings, even without typical clinical phenotype or muscle pathology.

The aim of this review is to summarize the known features and the recent advances in the understanding of this more and more recognized but still likely underdiagnosed disease.

2. Historical notes

Lars Edström and coworkers described HMERF for the first time as a separate nosological entity in 1990 [1]. The authors reported 16 patients from 7 Swedish families characterized by the following clinical and laboratory features:

1) respiratory failure, often developing subacutely and frequently being the symptom at onset of the disease;
2) limb-girdle distribution of muscle weakness accompanied by foot extensor and neck flexor weakness;
3) myopathic findings on electromyography and normal or slightly elevated serum creatine kinase level;
4) absence of neuropathy and heart involvement;
5) autosomal dominant mode of inheritance of the disease, with onset between the second and the fifth decade.

On muscle biopsy, the hallmark of HMERF was the presence of circumscribed "plaques" which were eosinophilic on hematoxylin and eosin and stained red, purple or dark green on Gomori trichrome [1]. These plaques were strongly reactive with phalloidin suggesting they contained filamentous actin, while desmin was largely absent from them. At the

ultrastructural level, these plaques were described as "filamentous masses" with an electron density that was lower than the Z-disks, and the other finding was myofibrillar lesions with medium dense streaks of material arising from the Z-disk over the sarcomere [1].

Some other reported patients were classified as cytoplasmic body myopathy (CBM) with respiratory failure but no genetic data have been published on these patients. These included a family where the mother and two siblings had features reminiscent of congenital myopathy such as long narrow face, scoliosis and facial weakness [2]. The children presented at age 13 with respiratory failure, while the mother had mild proximal weakness at age 55 and CBs on muscle biopsy. Other early onset or congenital CBMs with respiratory failure were later also considered to be affected by possible HMERF [3], as well as fairly atypical sporadic patients such as one affected by myopathy and axonal neuropathy, with weakness of neck flexors since childhood, severe respiratory impairment which was the cause of death at age 31 and CBs only in type 1 fibers [4]. Importantly, a handful of families with autosomal dominant inheritance from different ethnic backgrounds were also retrospectively or prospectively identified, such as a Japanese autosomal dominant CBM with respiratory involvement and asymptomatic accumulation of CB-like structures in cardiac and smooth muscles [5].

In the group of later genetically determined HMERF titinopathies, two families need a special mention being the most extensively investigated and best characterized from a clinical and pathological point of view. In the family described by Chapon et al. [6], affected individuals had onset between 18–40 years, often with respiratory failure, and normal creatine kinase level. Pathology was rather homogeneous, with presence of CBs, which stained for phalloidin and dystrophin, less prominently using anti-actin antibodies. Desmin was present only at their periphery, in the filamentous halo, and desmin staining was increased in the cytoplasm of CB containing fibers [7]. Chinnery et al. [8] reported one family with 11 affected individuals over 2 generations with congophilic CBs and rimmed-vacuolar myopathy (RVM), variable respiratory dysfunction and predominant anterolateral lower leg muscle involvement. Disease onset was slightly later (mean 50.2 years) than previously described in HMERF. No other organ involvement (including heart) was found on autopsy in one patient [8].

On the whole, since the first description several names have been used to define this condition, such as the eponym "Edström myopathy" (OMIM database), or the clinically-oriented "autosomal dominant myopathy with proximal weakness and early respiratory muscle involvement" [9] and "autosomal dominant distal myopathy with early respiratory failure" [8], as well as the more general and pathology-oriented "familial" [6] or "inherited cytoplasmic body myopathy [5].

3. Molecular genetic findings: a historical perspective

In two of the originally described Swedish families, a genetic locus was established by linkage to the region 2q24-q31 [9]. The affected individuals from the two families shared a common haplotype, which suggested an identical mutation with the same origin. Titin was considered a strong candidate for the disease, and one single change in titin, cosegregating with the disease and associated with the identical haplotype was later found in the two families linked by Nicolao et al. [9] and in one sporadic patient [10]. This change was a C to T transition resulting in an arginine to tryptophan substitution at codon 279 of the kinase domain (R279W, or p.R34091W, predicted according to the longest theoretical titin transcript, NM 001267550), in exon 359, the first M-line exon (Mex1) of the titin gene (TTN).

Titin is actually the biggest protein in the human cells and in nature. TTN has 364 exons comprising the longest cDNA of 100 kb in the human genome: this makes it approximately 8 times bigger than DMD, the dystrophin gene. Titin is a structural protein, which spans half of the sarcomere from M-line to Z-disk, and thus can be descriptively divided into portions that correspond to the four sarcomeric regions identifiable on electron microscopy (EM): Z-disk, I-band, A-band, and M-line. Titin has indeed diverse functions segregated in different portions: mechanical functions, contributing to passive tension and determining relaxed sarcomere length, but also regulatory functions as many of the structural proteins [11]. In particular, the serine-threonine kinase domain, lying in the periphery of M-line titin close to the A-band, is a mechanical sensor that regulates transcription and other cellular functions through a complex network of direct and indirect interactions with other proteins involved in autophagy or protein degradation machineries (nbr1, p62, MURF2), as well as transcription factors such as SRF. The p.R34091W change appeared to perturb the interaction of titin with nbr1 [10]. The localization of nbr1 was abnormal in the patients' muscles and p62 was accumulated in diseased fibers, while MURF2 showed unusual nuclear signal. Given these indications coming from molecular genetic and functional studies, the p.R34091W change was considered to be disease-causing in these patients.

After 2005, the identification of patients in whom the kinase domain variant was excluded corroborated the idea that other mutations could also be responsible for the disease [12]. However, despite continuous interest in TTN being a very strong candidate as the causative gene also for non-kinase domain mutated patients, the huge size of this gene made it cumbersome to study until extensive genetic screening methods became widely available.

In 2012, two groups identified, through whole exome sequencing studies, the same causative missense mutation in TTN exon 344, g.274375T>C - p.C31712R (previously named as p.C30071R), in other HMERF families. The mutated residue is located in the myosin-binding fibronectin-III (FN3) domain 119 of A-band titin (formerly A150 domain) [13]. One group [14] identified this mutation in 8 affected individuals from three apparently unrelated Swedish families, two of them reported in the original Edström publication [1]. All the mutated patients shared a common 6.99 Mb haplotype. Another group [15] identified the same mutation in the family originally described by Chinnery et al. and in two additional UK families, classified

as affected by the same disease on the basis of clinical and muscle imaging similarities. The UK families shared a haplotype in chromosome 2 of 2.93 Mb. The clinical disease spectrum was fairly broad, and included mild phenotype at advanced age.

An international collaborative study [16] provided a comprehensive evaluation on the clinical, morphological and MRI findings of a large number of patients with very wide geographic distribution, and also reported four novel mutations, always in the same FN3 119 domain of titin A-band (exon 344), one of them behaving in a peculiar semi-recessive/dominant manner. This study also included the French family described by Chapon et al. where a linkage study had excluded titin [17], showing a novel g.274367C>G, p.P31709R mutation. Of note, a later linkage study after reclassification of the patients based on muscle imaging results allowed linkage to the titin locus and the discovery of the TTN mutation. In HMERF, MRI thus seems to be more sensitive than clinical and pathological data, which provided both false positive and false negative results in the first assessment of the members of this and other families. Other novel dominant mutations were identified in one German and one British family, besides the semi-recessive/dominant g.274436C>T, p.P31732L mutation in Southern European families [16,18]. The most common mutation in this cohort was the p.C31712R, which was found in patients of Northern European descent but also in one Italian and one Argentinian family. Interestingly, the authors were able to identify some sharing of alleles in the p.C31712R families with a haplotype of 1.3 Mb, suggesting a common founder with the p.C31712R mutation. In another study, this mutation was found to be relatively frequent in the UK and present in a proportion of patients (5.5%) previously classified as molecularly undiagnosed myofibrillar myopathies (MFMs) [19]. These authors also identified the p.P31732L mutation in one UK family with incomplete penetrance and a new p.N31786K likely pathogenic variant in one Brazilian patient [19].

Other exome sequencing studies led to the discovery of novel mutations causing HMERF: p.W31729L in the Japanese family described by Abe et al. [20] and p.G31791D in a US family [21]. The latter group also extended the study of the A-band titin region to a cohort of MFM patients from different ethnicities, finding the p.C31712R mutation in two of them, one North American with Indian ancestry and one Spanish. The Indian had a different haplotype compared to the British shared one, thus suggesting an independent origin of the two identical mutations [22]. More recently, a Chinese patient with the p.C31712R mutation not sharing the UK common haplotype has been also reported [23]. Uruha et al. also described 17 additional HMERF patients from Japan, confirming the highest prevalence of the p.C31712R mutation, as well as reporting three novel missense mutations (p.C31712Y, p.G31791R and p.G31791V) and one non-frameshift deletion (g.284913_284921delGAGGGCAGT - p. R31783_V31785del) in TTN exon 344 [24]. Clinical and pathology data of the affected individuals in these latter studies were very consistent with what was previously reported in this disease. The location of TTN mutations so far associated with HMERF is illustrated in Fig. 1.

The exact pathogenic role of the originally described kinase variant is currently questioned. Evidences following its publication suggested that this variant is present at very low frequency in normal controls (dbSNP rs140319117) [16] and therefore can be better classified as a polymorphism rather than as a pathogenic mutation, and that the originally described HMERF patients harboring the kinase variant also had the semi-recessive mutation, p.P31732L [25,26], thus challenging its role in causing HMERF. This prompted a debate [26–28] between two different views: one supporting the hypothesis that the kinase variant is totally harmless, and another one according to which the kinase variant may interplay with the A-band mutation on the same allele, which normally has an incomplete penetrance [19], thus acting as a modifier of its effect (i.e., modulating either its penetrance or expressivity).

4. Clinical features and management

The typical features of the disease are the ones already clearly reported in the original description [1]. The onset is in adulthood and respiratory failure, which gives the name to the disease, is consistently present although with different degrees of severity. It is frequently evident in the early stages of the disease and disproportionate to the degree of limb weakness. It usually presents with exertional dyspnea and/or daytime sleepiness and morning headache, which are symptoms of nocturnal hypoventilation. Nocturnal monitoring of blood gases (oximetry) is able to pick up the hypoventilation, and pulmonary function tests in both standing and supine positions can help to identify respiratory failure caused by muscle weakness. Diaphragmatic weakness is usually present and can be discovered as a significant drop (>25%) of forced vital capacity in supine position. Hypercapnia and chronic modifications of arterial blood gases may be evident as well. To treat these patients, non-invasive mechanical ventilation is often required, starting during the night hours.

Scapular winging due to both trapezius and serratus anterior weakness may be present. Clinical presentation can be however fairly variable, and even head drop with marked trapezius hypotrophy and nasal speech or rigid spine have been reported in some patients [12,23]. Some degree of asymmetry is well compatible with the disease. Surprisingly for a mutation in A-band titin, clinically relevant cardiomyopathy is not common. Electromyography findings in rare cases showed mixed neurogenic/myopathic patterns but peripheral neuropathy is not present and not expected from mutant titin [16]. A notable clinical hint often present is a striking abdominal weakness in some cases leading to constipation (Tasca and Udd, personal observations) (Fig. 2). Dysphagia has also been sporadically reported [24]. Calf hypertrophy [14] or sometimes hypotrophy [21] can be present as well (Fig. 2). Initial clinical diagnosis may be suspicion of acid maltase deficiency or facioscapulohumeral muscular dystrophy.

Management includes thorough and regular screening of respiratory function and eventual support with non-invasive ventilation, with the aim of both preventing acute respiratory failure and improving patients' quality of life. Cardiac function workup is warranted in the light of recent findings suggesting
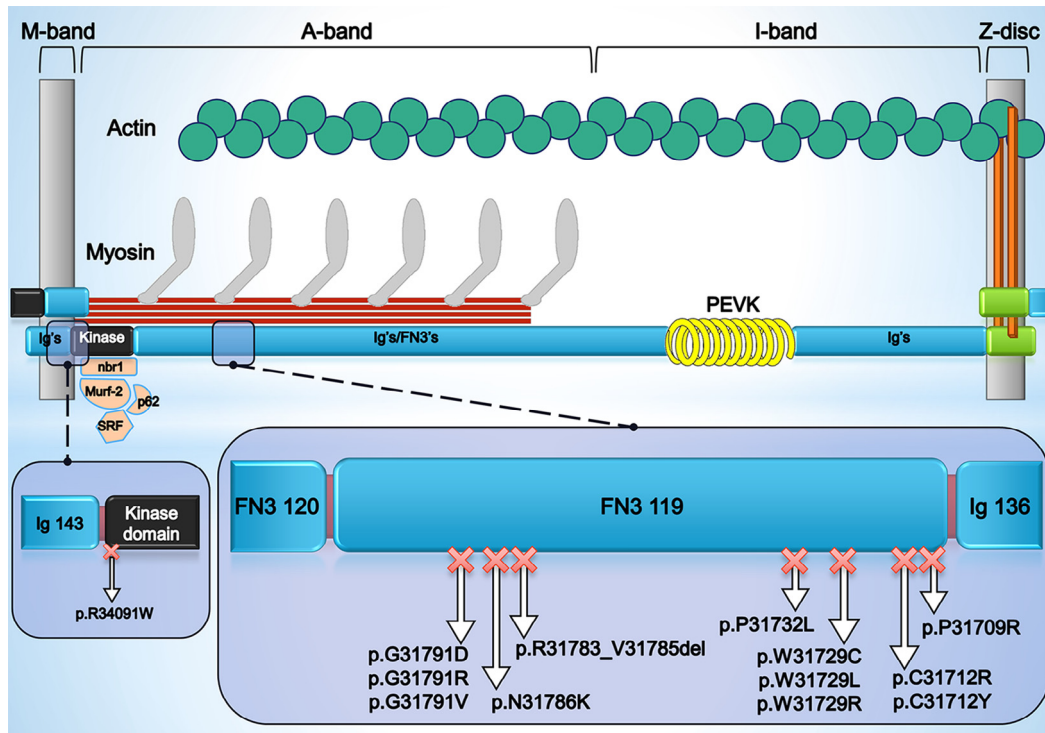
Fig. 1. Schematic representation of the titin protein with the 12 so far known mutations associated with HMERF. Reported mutations (see text for details): p.C31712Y [24]; p. G31791D [21,24]; p.G31791V [24]; p.G31791R [24]; p.N31786K (likely pathogenic) [19]; p. R31783_V31785del [24]; P31732L [16,18,19,23]; p.W31729C [16]; p.W31729L [20]; p.W31729R [16]; p.C31712R [14–16,19,21,23,24]; p.P31709R [16]. The p.R34091W variant, not pathogenic by itself but originally described as an HMERF causing mutation [10], is also represented (see text for details).

that heart involvement may be associated at least with the p.C31712R mutation [29]. Ankle-foot orthoses may be needed in case of foot drop that impairs walking. There is no standardized treatment for constipation, but patients may benefit from diet adjustments with increased fiber intake, behavioral advices and use of laxatives.

5. Muscle pathology

Patients with different mutations share common histological findings. On routine stainings, the pathological signature of HMERF is the presence of CBs, which are mostly present but may be less striking at first glance or present in rare fibers only. The location of CBs is generally subsarcolemmal, and they are often multiple and gathered in circular formations surrounding part or the whole fiber in transverse sections [24]. Some fibers also show areas of myofibrillar disintegration, often in central position, and another remarkable pathological feature is the presence of rimmed vacuoles (RVs) (Fig. 3).

CBs may differ in size, but all of them display similar immunohistochemical features. Myotilin and alpha-B crystallin antibodies usually stain the whole body, as well as phalloidin and anti-actin antibodies. Desmin is positive in a rim surrounding the CBs (Fig. 3c). The areas of myofibrillar disruption, which can be observed in fibers harboring CBs but also in other fibers apparently devoid of them in transverse sections, show affinity for myotilin, alpha-B crystallin, p62 and desmin as reported in MFMs [30]. Remarkably, the CBs do not stain with anti-titin I-band antibody, TDP43 or anti-ubiquitin

Fig. 2. Clinical features in HMERF disease. Abdominal weakness in two HMERF patients (homozygous P31732L in **a**, heterozygous p.C31712R in **b**). Calf hypotrophy (**b**) or hypertrophy (homozygous P31732L in **c**) are also sometimes present.
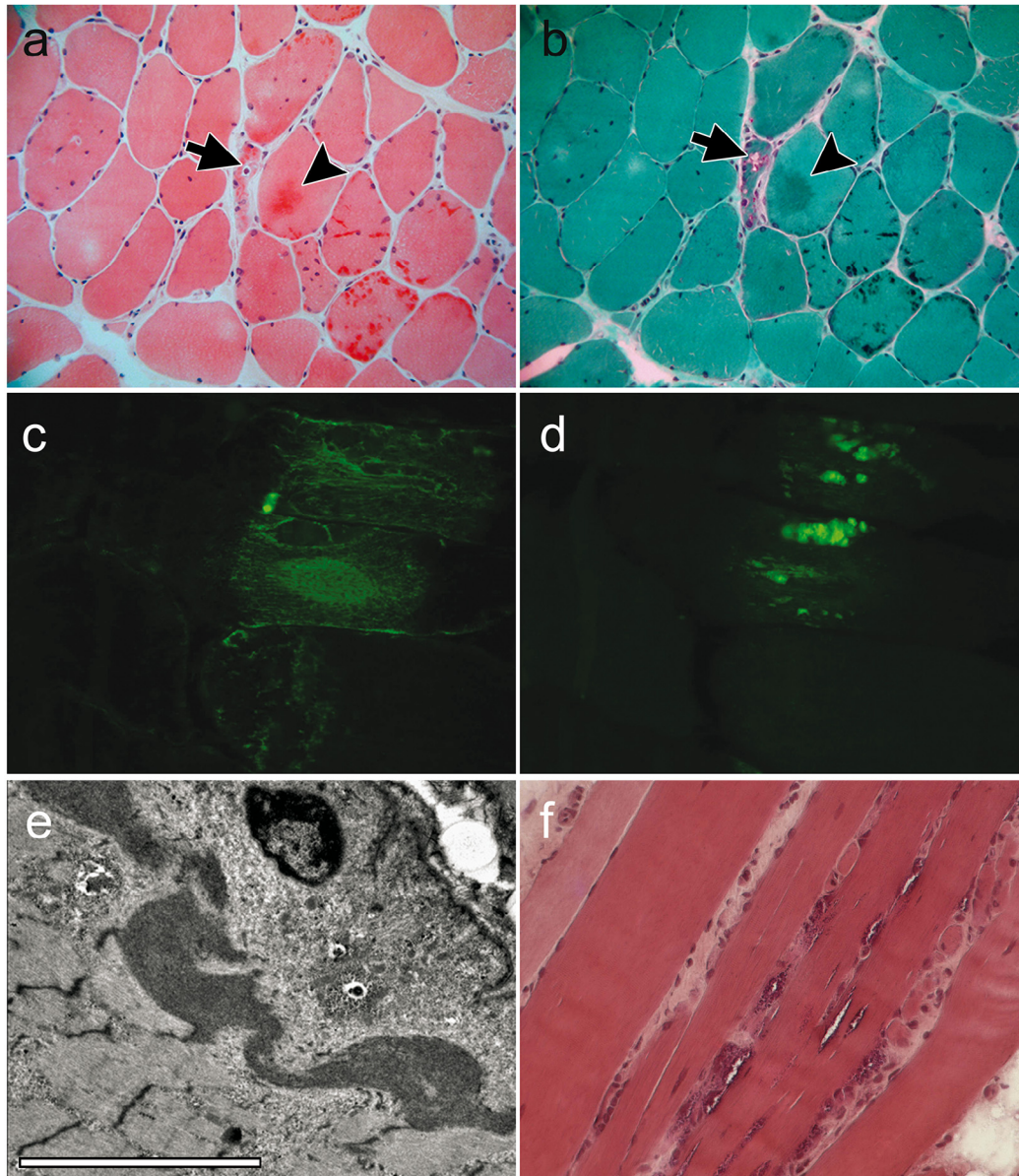
Fig. 3. Muscle pathology in HMERF disease (homozygous p.P30091L). Seriate hematoxylin and eosin (**a**) and modified Gomori Trichrome (**b**) showing the presence of round, mainly subsarcolemmal cytoplasmic bodies in a cluster of fibers. Cytoplasmic bodies appear purple or dark green on Gomori staining. One fiber harboring a rimmed vacuole (arrow) and another one with a central area of myofibrillar disruption that differs from cytoplasmic bodies (arrowhead) are also present. Seriate desmin (**c**) and myotilin (**d**) stainings showing in two longitudinally oriented myofibers the intense reaction with myotilin of the cytoplasmic bodies that are devoid of desmin except for a thin peripheral rim. An area of desmin positivity is also present in the center of the fiber. Electron micrograph (**e**) showing a typical cytoplasmic body in longitudinal sectioning (**e**, scale bar: 5 microns). (**f**) Rimmed vacuolar pathology in a different patient.

antibodies [16]. The autophagosome marker LC3 and p62/sequestosome 1 label the RVs but are largely absent from the bodies. TDP43 and p62 are sometimes present at the periphery of the CBs [21] and may show a dotted signal in atrophic fibers, as it happens in other RVMs, this being likely related to autophagic activation pathways.

EM shows the typical appearance of CBs with a dense, irregular core surrounded by a paler, filamentous halo (Fig. 3e). It is also reported that CBs can be absent due to sampling bias. Areas of completely dissolved myofibrils, or including just remnants of intact myofilaments, are also present. Some sarcomeres and myofibrils also show the peculiar semidense accumulations in the shape of stripes connecting the Z-disks and other Z-line abnormalities, such as Z-line streaming and dispersion of the osmiophilic Z-disk material.

The above-mentioned alterations are not always widespread, and frequently fascicles with groups of fibers containing the CBs are intermingled with fascicles with only normal fibers [14,19]. Thus, it is possible that the typical pathology could be missed or overlooked in the routine diagnostic setting if the biopsy is not properly targeted and the alterations are very minor or even non-specific [15].

6. Muscle imaging

The first report on muscle imaging of HMERF patients documented an early involvement of the semitendinosus and obturator externus with relative sparing of the biceps femoris and semimembranosus in 7 affected members of the family described by Chinnery et al [31]. The other most affected muscles in the lower limbs were the iliopsoas, gracilis, anterior leg muscles and, peculiarly, popliteus. Notably, in this first study the authors already hypothesized the use of MRI for the discovery of new families or sporadic cases with HMERF and the testing of oligosymptomatic individuals, with implications on respiratory follow-up. The pattern of involvement was later confirmed and found to be very consistent in other HMERF patients irrespective of the mutation [12,14–16,19–21,23,24]. Among the more than 50 patients imaged, including some presymptomatic ones, only one did not display any involvement of the semitendinosus [15]. Other helpful clues for the diagnosis are the involvement of the gluteus minimus at pelvic level, the sartorius at the thigh and the tibialis posterior at lower leg level (Fig. 4) and a proximo-distal gradient of involvement of the semitendinosus, which is evident until end-stage fatty replacement. This pattern is highly suggestive because it preferentially affects muscles that are normally spared in other myopathies (such as the semitendinosus, sartorius, gracilis and tibialis posterior). It could even be considered pathognomonic, and the only *caveat* is the significant overlap with the pattern reported in desminopathy and alpha-B crystallinopathy [32], and exceptionally in LGMD2L [33].

While several studies have analyzed in detail the involvement of lower limb muscles, just one reported on the scapular girdle muscles and only in a few HMERF patients [31]. The most affected muscles were the supraspinatus, infraspinatus and serratus anterior with relative sparing of the pectoralis major and subscapularis. In our patients we confirm, quite reliably, a severe involvement of the serratus anterior to a lesser extent followed by pectoralis minor, rhomboids, sternocleidomastoid and subscapularis, a variable involvement of supra and infraspinatus and the sparing of pectoralis major (Fig. 4) [12,34]

7. Open questions and conclusions

Mutations in *TTN* are known to cause different human skeletal and cardiac muscle diseases. Titin is a major gene responsible for dilated cardiomyopathies, mostly caused by mutations that cluster in the A-band [35]. Skeletal muscle diseases other than HMERF are caused by mutations in C-terminal titin exons and include the autosomal dominant tibial muscular dystrophy and the more severe recessive LGMD2J, due to mutations in the two last exons 363 (Mex5) and 364 (Mex 6), which are not associated with heart disease. A severe recessive muscular dystrophy with lethal cardiomyopathy also called Salih myopathy (due to mutations found in Mex 1 and 3) [36], and more lately several other myopathic phenotypes, such as recessive congenital myopathies with prominent central nuclei [37], childhood-onset disorders with or without contractures and cardiomyopathy [37], and recessive distal myopathies [38] were also reported. The exact
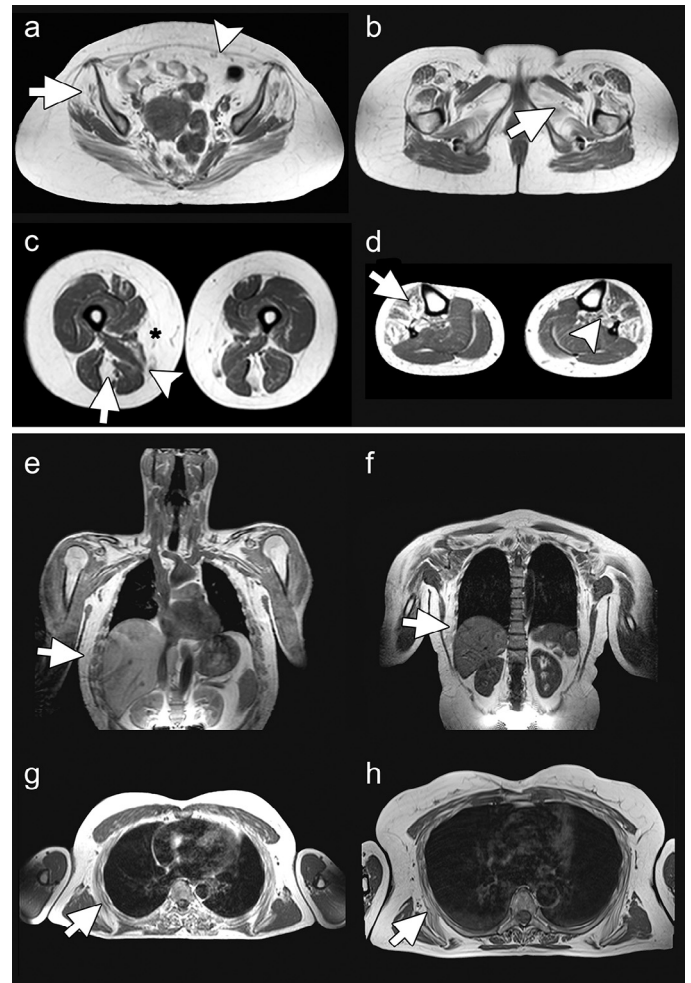

Fig. 4. Muscle imaging. Typical HMERF lower limb imaging in a patient with p.C31712R mutation (**a-d**): peculiar involvement of the gluteus minimus and medius (**a**, arrow) together with abdominal muscles (**a**, arrowhead) and obturator externus (**b**, arrow) at the pelvic level, of the semitendinosus (**c**, arrow), gracilis (**c**, arrowhead) and sartorius (**c**, asterisk) at the thigh level and anterolateral compartment (**d**, arrow) and tibialis posterior (**d**, arrowhead) at the leg level. Scapular girdle MRI scans (**e-h**) show prominent involvement of serratus anterior (arrows) in patients with different mutations and different degree of clinical severity (homozygous P31732L in **e** and **g**, heterozygous p.C31712R in **f**, asymptomatic heterozygous P31732L in **h**).

mechanisms through which the different mutations in the titin gene cause HMERF and the other diseases are still rather obscure. Before the genetic defect was known, Edström hypothesized a failure in the normal length determining mechanism of actin polymerization, because of the presence of filaments spanning between two Z-disks for lengths greater than one sarcomere [1]. Interestingly, among the other functions titin also acts as a molecular guide for sarcomere assembly [39].

At variance with the elastic I-band titin, which performs as a molecular spring, A-band is stiff and most of its residues are highly conserved [40]. This high degree of conservation is probably due to the need of keeping exact protein-protein interactions in a dynamic environment where big, structural proteins are very close to each other. One of such interactions

takes place between FN3 elements and sub-fragment 1 (S1) of myosin [41], and perturbations of these interactions may negatively influence spatial relationships between myosin heads and actin filaments. However, A-band titin is still poorly characterized from a structural and functional point of view and it is highly likely that it holds many more specific and localized important functions [13]. This could explain why the mutations cluster in the same FN3 119 domain despite the pronounced molecular similarities among all the FN3 domains.

Further insights on possible mechanisms come from pathology studies. Although experimental evidences suggest an increased tendency of FN3 domains to aggregate when the most conserved proline are mutated to alanine [42], and an *in vitro* decreased solubility of the FN3 119 domain harboring some HMERF mutations has been shown [43], one surprising finding is the absence of the mutated titin protein in the aggregates [16]. This is at variance from other diseases in which the mutations increase the probability of the protein to form aggregates, and in these cases the mutated protein is one of the main components seeding the aggregates themselves, as it happens for some *FLNC* mutations [44]. A possible hypothesis is that HMERF mutations derange the normal dynamic interactions with neighboring proteins, and eventually cause the alteration of autophagy processes needed for maintenance and turnover leading to secondary aggregation of other proteins and autophagic RV pathology. Inappropriate triggering of autophagy with a role in the process of fiber hypotrophy, as it happens in other titinopathies and RVMs, had been also postulated in the original paper by Edström, who found some fibers with an increased lysosomal acid phosphatase activity [1]. Ultrastructural pathology confirms that maintenance of sarcomeric structures is altered in HMERF leading to accumulations of proteins not correctly re-cycled in combination with an impaired autophagic processing.

TTN is a highly polymorphic gene, and each whole exome, whole genome or targeted sequencing study performed usually brings back to the clinician several variants that could be potential disease causing titin mutations. This challenge of interpretation underlines the importance of deep phenotyping in the next generation sequencing era, making it necessary to correctly suspect HMERF on clinical and pathological grounds to address the genetic testing. According to some of the most recently published guidelines [45], population data (i.e., frequency in the databases such as the Genome Aggregation Database, http://gnomad.broadinstitute.org), prediction of the impact on the protein product, together with functional and segregation data should be all considered to correctly interpret the value of the discovered genomic variants in each gene. This is particularly true for huge genes like *TTN*, for which the creation of a locus specific genomic database that includes associated phenotypic data has been encouraged [46]. In this context, we must once more stress the value of muscle imaging to correctly classify patients in segregation studies and deep phenotype affected and clinically unaffected individuals in families with suspect titinopathy.

The issue on the hypothesized pathogenic role of the kinase variant is still opened. It is now clear that the kinase domain change cannot cause HMERF by itself and should be considered, at the current state of the art, as a polymorphism. Studies are ongoing to clarify whether or not it might potentiate the effect of the mutation *in cis* and thus manifest or worsen the phenotype in a similar way to what has been already described for other titinopathies [47]. Interestingly, the components of the signaling complex interacting with the kinase domain (p62 and nbr1) have been shown to have essential roles in regulating the autophagy machinery in muscle [48,49].

Muscle imaging pattern is highly specific for HMERF, and muscle MRI has been the tool used to pick up additional families and clarify the known families for the genetic studies. The main feature of the disease is the early and constant involvement of the semitendinosus muscle. Other *TTN* mutations preferentially hit muscles that are also affected in HMERF, like the tibialis anterior [50]. Although the final reasons for the extreme susceptibility to damage of the semitendinosus, usually one of the most resistant in other myopathies, as well as that for respiratory muscles are not known, the level of titin expression or of its isoforms in different muscles may contribute to this selectivity: a recent study indeed showed an overexpression of transcripts of several titin isoforms, and in particular those including distal A-band exons, in the semitendinosus compared with other posteromedial thigh muscles [51]. Interestingly, a similar pattern of involvement is present in desminopathy and alpha-B crystallinopathy. A possible, intriguing explanation at the molecular level regards perturbed interactions between titin and alpha-B crystallin [15], and since Z-disk structures are the first to be altered in HMERF and desmin is involved in Z-disk functions a connection at the molecular level is likely. Involvement of the semitendinosus is a very sensitive imaging marker, and subclinical semitendinosus involvement could be even detected in presymptomatic patients [15,16]. This is of importance because the identification of HMERF patients before the onset of symptoms may allow the appropriate screening for respiratory involvement, since respiratory failure can be the presenting symptom in this disease.

The recent genetic data also provides significant new understandings. Some of individuals harboring the so far most common HMERF mutation (p.C31712R) share a core haplotype, suggesting a common Northern European ancestry. However, the discovery of a different haplotype in two families suggests that this site is more likely to be a mutational hotspot, with the possibility of *de novo* mutations occurring worldwide [23]. Moreover, the existence of one mutation behaving in a semi-recessive/dominant manner raises the possibility that the frequency of heterozygous individuals with this mutation could be higher than currently understood in the general population. Carriers of this mutation show either a later onset milder HMERF, or may be clinically healthy although showing signs of myopathy on muscle imaging and thus having a subclinical myopathy, or may not have any detectable sign of muscle involvement even on MRI.

It would be useful to clarify whether the earlier onset or congenital cases [2,3] and the severe CBM with onset in the second decade, rapid progression and some atypical features like

prominent ankle contractures [52] are caused by mutations in the *TTN* gene, thus further expanding the spectrum of HMERF, or if they are different diseases. Tentatively, similarly to one Italian HMERF patient [12], the most severe cases could be due to two *TTN* mutations. In particular, the disease reported by Patel et al. [2] could fit with a semi-recessive/dominant inheritance with the mother being a paucisymptomatic carrier. In other descriptions the presence of additional complicating features such as axonal neuropathy [4], could worsen the phenotype.

In terms of differential diagnosis, the main challenge regards the distinction from other MFMs and in particular desminopathies, due to the broad overlap on muscle imaging, the possible overlap of clinicopathological features and the autosomal dominant inheritance. The presence of CBs, the early respiratory involvement and the absence of cardiomyopathy should suggest a diagnosis of HMERF. CBs could represent the initial lesion and their presence is still the most useful pathological sign to address the genetic testing towards HMERF rather than other MFMs. Thus, although overlaps with MFM pathology [19] and proteomic profiles [53] are certainly present, in our opinion it would be useful to pathologically subclassify HMERF as a CBM in the context of MFMs. Other possible clinical overlaps regard myopathies that could present with or display a marked respiratory involvement in ambulant patients such as congenital myopathies and in particular late-onset nemaline myopathy, acid maltase deficiency, inflammatory myopathies (anti Jo-1 myopathy), muscular dystrophies due to lamin A/C gene mutations, and to a lesser extent sarcoglycanopathies, LGMD2A and LGMD2I [54]. Myogenic causes of respiratory failure are not always easily recognized and should also be distinguished from neurogenic and myasthenic causes. When respiratory failure is not clinically evident differential diagnosis with other adult onset LGMDs or distal myopathies can be considered.

The advisable molecular diagnostic algorithm includes at the moment at least the sequencing of *TTN* exon 344, followed by whole *TTN* gene screening, in suspect cases. HMERF and titinopathies in general are and will be increasingly recognized now that novel techniques such as next-generation sequencing allow the analysis of giant genes in a fast and cost-effective way.

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