Congenital Myopathies

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Congenital myopathies (CM) form a clinically, genetically, and morphologically heterogeneous group of neuromuscular disorders (NMDs) [1, 2]. Years ago, with the introduction of histochemical examinations and electron microscopy (EM), some abnormal features in muscle structures were associated with certain clinical phenotypes and were named congenital non-progressive myopathies [3, 4]. Although, they generally present with hypotonia (±muscle weakness) in the neonatal/infantile period, in some cases, the first symptoms occur in the juvenile or adult period [1, 2, 4, 5]. Studies report the prevalence of all CMs as 0.96–3.62/100,000, but in the <15–19 age group as 0.52–5.01/100,000 [6]. Core myopathies (*RYR1* gene variants that are autosomal recessive/dominant) are the most common type of CMs, followed by nemaline myopathies [7]. The most common congenital myopathies and the responsible gene loci are summarized in Table 13.1.

CMs occur due to structural defects within muscle fibers, affecting the contractile matrix, excitation-contraction coupling, T-tubules, and sarcoplasmic reticulum, while muscle membrane stability is spared. They are also a type of NMD, pathologically characterized by myopathic changes, such as differences in myofiber sizes, internalized nuclei, and the presence of degenerating fibers [1, 2, 8]. Muscle fibers might also be undifferentiated, and small/ hypotrophic type-1 fiber predominance is an important finding [1, 5]. When there is a disproportion between the sizes of type-1 and type-2 muscle fibers in a biopsy, this is considered a fiber-type disproportion (FTD) [1]. Fibrosis and replacement with fat tissue are observed in all severely affected muscles [8]. Necrosis, inflammation, and dystrophic changes due to sarcoplasmic membrane protein defects in congenital muscular dystrophies (CMD) do not occur in CMs [1, 2, 8]. Structural abnor-

Table 13.1 Most common CMs and loci of responsible genes

Disease	Protein	Gene	Chromosome
Core diseases (AD or AR)	Ryanodine receptor 1	*RYR1*	19q13
	Selenoprotein N1	*SEPN1*	1p36
Nemaline myopathies (AD or AR)	Sarcomeric thin filaments	*ACTA1, CFL2, KBTBD13, NEB, TNNT1,TPM2, TPM3*	1q42.13,14q13.1,15q22.31,2q23.3,19q13.42,9p13.3,1q21.3
Congenital fiber-type disproportion (AD or AR)		*ACTA1, MYH7, RYR1, SEPN1, TPM3*	1q42.13,14q11.2,19q13,1q36,1q21.3
Centronuclear myopathies (X-linked, AD or AR)	Myotubularin	*MTM1*	Xq28
Centronuclear myopathies (AR)	Amphiphysin	*BIN1*	2q14
Centronuclear myopathies (AD)	Dynamin 2	*DNM2*	19p13

AD autosomal dominant, *AR* autosomal recessive

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malities in a muscle biopsy are classified according to abnormally located organelles and intracellular bodies. These classifications include nemaline myopathy (NM), central core disease (CCD), multi-mini core myopathy (MmCM), centronuclear myopathy (CNM), congenital fiber-type disproportion (CFTD), and others [1, 4]. NM is known as the accumulation of Z-line proteins. Cap disease, zebra body myopathy, intranuclear rod myopathy, and myosin storage myopathy are other CMs with protein storage pathology. Cores seen in CCD and MmCMs are areas devoid of oxidative activity [9]. In some cases, different structural pathologies such as cores and rods, and rods and caps could be seen together [5]. A centrally located nucleus is the typical pathological finding in CNM and X-linked myotubular myopathy. Selective atrophy of type-1 fibers without any other structural abnormality indicates CFTD, which clinically and genetically overlaps with CM [9].

With developments in the field of molecular genetics, these diseases have been associated with more and more genes. In terms of phenotypes, clinical signs, histopathological features, and genetic features, there is overlap between CM, CMD, congenital myasthenic syndromes (CMS), and even mitochondrial myopathies [8]. Variant-specific clinical features are reported for some genes. Every gene that causes CM generally acts as a member of a clinicopathologic spectrum instead of acting individually [4]. Until now, almost 60 genes have been defined in CM cases [10]. Pathological classification and a classification based on molecular genetics are used together [1, 2, 6].

Clinical recognition of CM is rather easy, but difficult to diagnose genetically (genomic era). There is histopathological overlap between CMs themselves, and boundaries between subgroups are not always clear. Inheritance can be autosomal recessive (AR), autosomal dominant (AD), or X-linked [4, 5]. There may also be different mutations (deletion, duplication, frameshift, nonsense, missense, splice site, etc.) on the same gene, or, the variant in the same gene, which can cause both AD and AR inheritance [5]. De novo mutations are also frequently seen [4]. The variants in different genes can lead to similar histopathological changes [4]. A variant in a gene may also lead to different clinics by interacting with various gene products [4]. Additionally, the same gene variant within the same family can cause varying disease severity due to somatic mosaicism or epigenetic and environmental modifiers [1, 5]. CMs are rather rare among all NMDs but they are a heterogeneous group. Therefore, gene panels, whole-exome sequencing (WES), and whole-genome sequencing (WGS) have advantages and disadvantages compared to each other for determining a definitive diagnosis. Large genes like *RYR1, TTN,* and *NEB* are frequently found in CMs. Variants of uncertain significance (VUS) have been detected and even new mutations and genes have been discovered [2]. Thus, muscle biopsy materials are needed for functional studies, and molecular diagnostic methods may not be sufficient for determining a definitive diagnosis in some patients [2].Histopathological features, data from molecular genetic analysis, and muscle imaging should be evaluated as part of diagnostic process, and supported with omics studies, if necessary [2].

Common Clinical Features

CMs are one of the causes that lead to various degrees of clinical hypotonia and 'floppy infant' inthe neonatal/infantile period [4]. They are responsible for 14% of all newborn hypotonia cases [11]. (Table 13.2) Muscle weakness and motor retardation are other common findings [11]. Respiratory insufficiency disproportionate to muscle weakness is another typical finding [5]. Intelligence is usually normal [11]. They are a type of primary myopathy because they do not involve the central nervous system or peripheral nerves [4]. Generalized weakness that is predominantly proximal is usually evident in the neonatal/infantile period [4, 8]. Proximal involvement is more prominent in the lower extremities. Rarely, some cases display findings in the adult

Table 13.2 Classical clinical features of some common CMs

NM	CCD	MmCM	X-MTM	CNM
Early-onset	Usually, AD	Usually, AR	Severe IU onset	Diffuse weakness
Generalized hypotonia	Hypotonia	Early onset	Polyhydramnios	Facial weakness
Proximal-axial weakness	Motor delay	Scoliosis	Neonatal hypotonia	Ptosis
Respiratory insufficiency	Proximal-axial-hip-girdle weakness (LL > UL)	Spinal rigidity	Ophthalmoplegia	Ophthalmoplegia
Facial-bulbar involvement	Orthopedic complications	Respiratory involvement	Respiratory insufficiency	
		Preserved ambulation	Feeding problems	

NM nemalin myopathy, *CCD* central core disease, *MmCM* multiminicore myopathy, *X-MTM* X-linked myotubular myopathy, *CNM* centronuclear myopathy, *AD* autosomal dominant, *AR* autosomal recessive, *IU* intrauterine, *LL* lower limb, *UL* upper limb, *IU* Intrauterine

period [1, 8]. There is usually a static or slow progressive course of the disorder [4]. In some cases, axial and facial muscle weakness, ophthalmoplegia, and ptosis are seen. Some patients also present with the signs of distal arthrogryposis [4, 8]. Facial weakness is a discriminative characteristic of CM from CMD and spinal muscular atrophy (SMA). The lower half of the face is particularly involved, an open mouth, a tented upper lip, and drooling may be present [9]. Axial weakness is more prominent in AR *RYR*-related CM and *SELENON*-related CM, and distal weakness in *NEB* and *MYH7, TPM3,* and *DNM2*-related CM [7, 9, 11]. There might be decreased fetal intrauterine movements and polyhydramnios due to prenatal onset [8]. Polyhydramnios and contracture in major joints are more frequently seen in X-linked myotubular myopathies [1]. There is also a risk of dysmorphic features associated with arthrogryposis, micrognathia, high arched palate, and dolichocephaly due to decreased fetal movements [8]. In severe NM and CCD cases (*MTM1*, severe *DMN2*, severe *RYR1*), these dysmorphic findings appear more often [4]. Long typical myopathic facies are more specific for NM [4].

Ptosis and ophthalmoplegia are commonly seen in NM and CNM cases (*RYR1, DNM2, MTM1*), especially in the neonatal period, and are distinctive from CMSs [8, 9]. Eye involvement is relatively less in AD inherited core myopathy [12]. Diaphragmatic involvement is very severe together with generalized weakness in NM and MmCMs[4]. A weak, hoarse cry, and difficulty in sucking and swallowing are findings of bulbar involvement. Respiratory insufficiency and feeding problems are life-threatening in the neonatal period [1]. In studies, the need for respiratory support at birth is reported as 14–30% [7, 13]. For all CM cases at every age, 64% of patients have respiratory insufficiency, and almost half require respiratory support [7]. In the first 2 months of life, mortality is reported as 8% [14]. In AR inherited core myopathy cases, respiratory insufficiency is more prominent [12]. Particularly in NM, CNM (*MTM1)*, and severe *RYR1* cases, severe respiratory insufficiency, the need for nasogastric tube (NG tube) feeding in the neonatal period and prominent bulbar involvement are common [7, 11]. The spine is often hyperlordotic, and spinal rigidity occurs in some cases [1]. Although scoliosis is seen in the early period, 13.6% of patients need surgical intervention [7]. Scoliosis becomes prominent in the period of transition during puberty and the phase of rapid growth [1, 8]. Hip dislocation is commonly seen in CCD [4]. While cardiac involvement is the most important reason for morbidity and mortality in NMD, respiratory complications are the leading cause of morbidity and mortality in CMs and determine the prognosis [1, 5, 8, 13]. However, cardiac involvement should be considered especially in *TTN* and *MYH7* gene relatedCMs [8, 11]. In *ACTA1, RYR1, TPM2,* and *SPEG* gene-relatedCM cases, cardiac involvement is rarely reported [11, 13, 15–17] (Table 13.3).

Table 13.3 Clinical clues in congenital myopathies and related genes

Some common clinical features	Related genes
Premature birth	*MTM1, ACTA1, RYR1* (AR)
Fetal akinesia, arthrogryposis, polyhydramnios	NM (*ACTA1, NEB*), *MTM1, RYR1, KLHL40*
Respiratory insufficiency at birth	*MTM1, ACTA1, RYR1* (AR)
Neonatal respiratory/bulbar insufficiency/NG tube feeding	NM (*ACTA1, KLHL40, NEB*), *MTM1, RYR1* (AR)
Increased mortality in the first year of life	*MTM1, ACTA1, KLHL40*
Facial weakness	NM, CNM (*MTM1, RYR1, DNM2*)
Ptosis/ophthalmoplegia	CNM (*MTM1, RYR1, DNM2*), MmD-CCD, NM (*KLHL40, LMOD3, CFL2, NEB*)
Predominant axial hypotonia	*RYR1, SEPN1*
Acquisition of ambulation	*NEB, SEPN1, RYR1*
Club feet	NM, *RYR1*
Scoliosis	NM, *RYR1, SEPN1*
Rigid spine	*RYR1, SEPN1*
Cardiomyopathy	*TTN, MYH7, ACTA1, RYR1, TPM2 FLCN, SPEG, MYPN, TNNT1*
Foot drop, distal involvement	*DNM2, MYH7, NEB, TPM2, TPM3*

Investigations

Differential diagnoses of CM are CMDs, CMSs, congenital myotonic dystrophy, Pompe disease, metabolic myopathies, and SMA. Investigations are planned according to these differential diagnoses [11]. On the suspicion of NMD, the first test to be used is the level of serum creatine kinase (CK). In CM cases, serum CK levels and electrophysiological studies are not helpful for diagnosis [4]. In CM cases, serum CK is usually normal or minimally increased however, if it increases more than five times or if CK > 1000 IU/L, this finding is suggestive of muscular dystrophy [7, 8]. In electrophysiological examinations, there may be polyphasic, small amplitude motor unit potentials, myogenic features, and early recruitment or the test may be normal [1]. In patients that present with severe neonatal involvement, neurogenic changes can occur [1]. During muscle ultrasonography, increased echogenicity could be detected in the affected muscle, and it is also useful to select, which muscle to use for a biopsy sample [4]. Muscle pseudohypertrophy suggests CMD or Pompe disease [11]. In the thigh and lower leg, muscle involvements can be associated with certain gene mutations [18]. Muscle biopsy supported with immunohistochemical studies and electron microscopy is very effective for CM diagnosis and for referral to molecular genetic tests [4]. On the other hand, molecular genetic analyses are preferred over invasive tests in some centers [8]. However, pathological studies and molecular genetic analyses are generally used together to reach a definitive diagnosis.

CMs comprise genetically, clinically, and pathologically heterogeneous groups. They show overlapping features with each other and with other NMDs. Despite next-generation sequencing techniques, such as gene-targeted exome sequencing or WES, only 50% of cases could be definitively diagnosed.Clinical studies (muscle MRI and muscle biopsy) and preclinical research data may be helpful to understand pathogenicity of variants [11]. As the genetic causes of CM cases are determined, and the pathophysiology and mechanisms of the disease are clarified, we will have the opportunity to develop new treatment options.

Congenital Myopathies and Malignant Hyperthermia

Malignant hyperthermia is an emergency clinical picture in which a life-threatening, hypermetabolic catabolic state develops due to triggering agents such as depolarising muscle relaxants including succinylcholine, and inhaled volatile anesthetics like isoflurane causing hyperthermia and muscle rigidity. Factors increasing the risk of malignant hyperthermia include a family history of malignant hyperthermia susceptibility (MHS), serious problems during anesthesia, previous history of cardiac arrest, and a diagnosis of *RYR1, STAC3, and CACN1S* mutations are at risk. Dantrolene is the mainstay treatment, which suppresses the release of intracellular calcium sustained by *RYR1*[11].

Subgroups of Congenital Myopathies

Nemaline Myopathy (NM)

NM ranges from very severe forms to mild forms with a likelihood of survival until late adulthood [9]. The prevalence of NM is 0.08–0.56/100,000 in children [6]. Clinical subtypes of NM include fetal akinesia syndrome with congenital contractures, severe congenital neonatal onset, and severe respiratory insufficiency at birth and intermediate congenital with severe hypotonia. Furthermore, there is the typical congenital subtype, which presents with spontaneous respiration at birth, and predominantly facial and axial muscle weakness, motor delay and hypotonia, which develop in early childhood. Additional subtypes include, mild childhood onset with a milder clinical course, gammopathy related adult onset and distal arthrogryposis [4, 19, 20]. In subtypes presenting with neonatal and infantile hypotonia, common findings include, symmetrical proximal, neck flexor, axial, and facial muscle weaknesses along with muscle atrophy, respiratory insufficiency, and feeding difficulties [4, 19, 21]. The extraocular muscles are often spared [4]. In some cases, distal muscle involvement is experienced and foot drop may develop as a result of peroneal muscle weakness [19, 20]. Signs of respiratory insufficiency and reduced vital capacity should be closely monitored, because hypoventilation develops slowly or suddenly in cases without any previous findings, and if left untreated it may cause cor pulmonale [19, 20]. Due to weak genotype-phenotype correlation, and overlap between subgroups despite pathological and genetic classification, Sewry C.A. et al. proposed a new NM subgroup classification as follows [21];

1. Severe nemaline myopathy (ACTA1, *NEB, KLHL40, KLHL41, LMOD3, TPM2, TPM3,TNNT*),
2. Congenital nemaline myopathy (*NEB, ACTA1, CFL2, TPM2*),
3. Childhood-juvenile onset nemaline myopathy (*ACTA1, NEB, TPM2, TPM3, KBTBD13,MYPN,*AD-*TNNT1*),
4. AR-*TNNT1* (Amish) NM,
5. Childhood onset NM with slowness of movements and core-rod histology (*KBTBD13*)

In NMs, the typical feature is rod-like structures, stained red with Gomori trichrome dye in skeletal muscle fibers [19]. If only a few fibers are affected or if they are small fibers, the rod-like structures can only be detected with electron microscopy or semithin sections stained with toluidine blue[19]. Rods derived from the Z-line, and include similar proteins, such as α-actinin, actin, and Z-band filaments [4, 11]. Rods cluster at the periphery of the fibers or in areas close to nuclei [4]. There is no correlation between the number of rods and the clinical severity [4]. Rarely, based on histopathologic findings the associated defective genes can be predicted. Nuclear rods, accumulation of thin actin filaments, and expression of the cardiac actin isoform are all seen in *ACTA1*-relatedNM. Secondary nebulin deficiency might also accompany the clinical picture [4, 21]. The accumulation of thin filaments in *CFL2*-relatedNM and nuclear rods in *MYPN*-related myofibrillar myopathy, have also been reported [21]. Rods are found in both type-1 and type-2 fibers. If rods are restricted to type-1 fibers this suggests *TPM3*-relatedNM, and if restricted to type-2 fibers it suggests *TNNT3*-related NM [21]. Another typical characteristic of NM is the predominance of type-1 fibers. A variation of fiber size and small type-1 fibers may be found [4]. Although not frequently found, fibrosis may accompany *TNNT1*-gene-related NM cases [21]. Immunohistochemical investigations rarely aid in the diagnosis of CMs. A total absence of nebulin protein is not expected in *NEB*-related NM, in only severe neonatal cases with antibodies against the SH3 domain, nebulin protein might be absent [5]. Additionally, very small fibers can be found with fetal myosin antibodies, but this is not a typical finding for NM [5].

There are four basic structures in a sarcomere including Z-discs, thick filaments, thin filaments, and titin (Fig. 13.1).

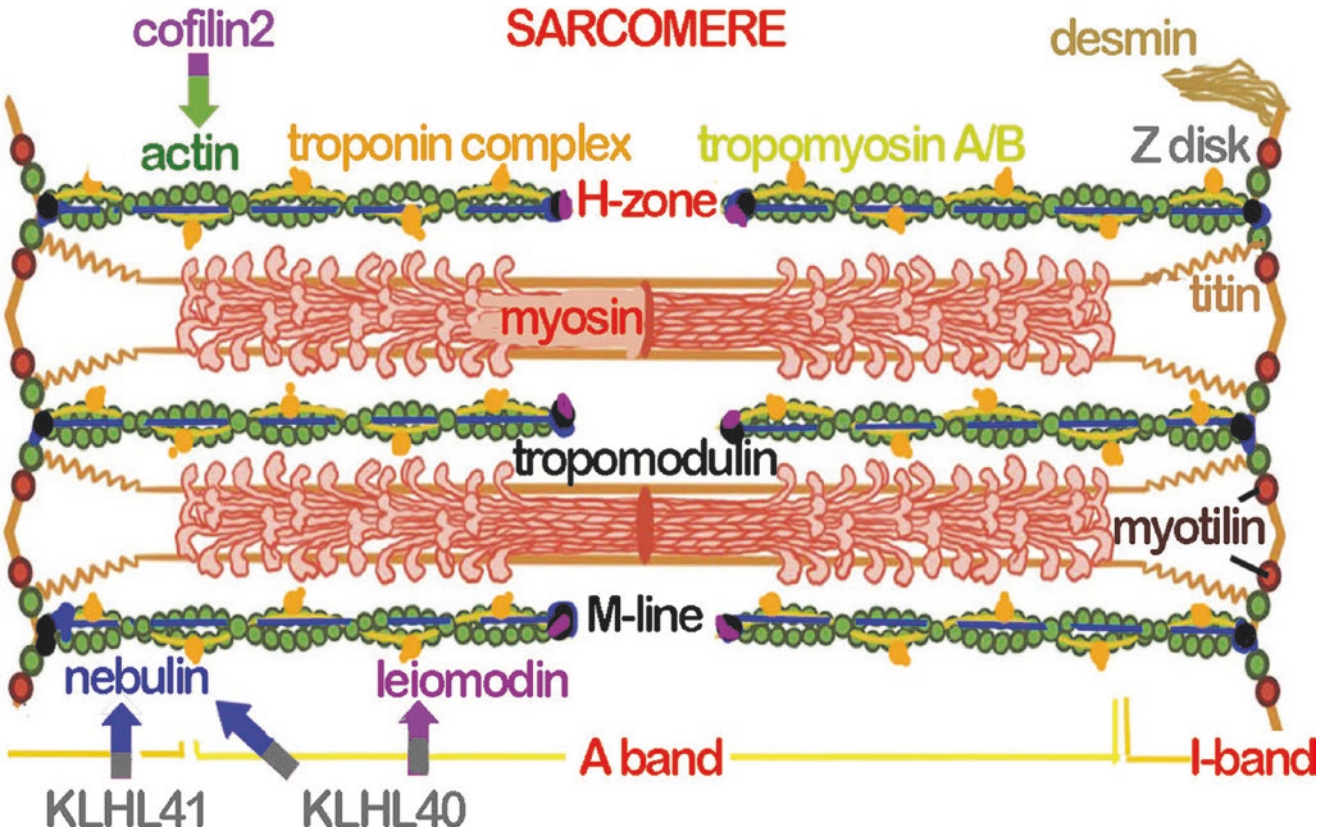

Fig. 13.1 Sarcomeric structures those are associated with the congenital myopathies

The dysfunction of these sarcomeric structures causes structural abnormalities like nemaline rods, Z-disc integrity loss, abnormal mitochondrial organization, and muscle atrophy [22]. Thin filament is formed by proteins that play a role in the actin-based backbone, actin length regulation and the actin-myosin interaction. So far, more than ten genes have been associated with NM [10, 22]. Nebulin (*NEB*), skeletal muscle actin alpha-1 (*ACTA1*), tropomyosin-beta (*TPM2*), tropomyosin-alpha (*TPM3*), and myopallidin (*MYPN*) are integral structural proteins that form the thin filament [10, 20, 22]. Nebulin anchors to Z-disc with its C-terminus, and interacts with thin filament, tropomodulin, and leiomoidin with its N-terminus. Skeletal muscle actin alpha 1 protein forms the backbone of the thin filament [22]. The AR inherited *NEB*mutation is found in 50% of NM cases and the AD inherited *ACTA1* mutation in about 25% [23]. *TPM2* and *TPM3* defects are seen in less than 10% [20]. Tropomyosin beta and alpha modulate the interaction of myosin-binding sites with actin [22]. During muscle contraction, troponin complex proteins (troponin T1, a slow skeletal protein and troponin T3, a fast skeletal protein) are necessary for thin filament function [20]. Troponin T1 modulates tropomyosins[22]. Other proteins play a dynamic role in the regulation and function of structural proteins. Kelch repeat and BTB domain containing 3 (*KBTBD13*), Kelch like family member 40 (*KLHL40*), and Kelch like family member 41 (*KLHL41*) stabilise thin filament proteins [20, 22]. Cofilin (*CFL2*) regulates actin polymerizations[22]. Leiomodin3 (*LMOD3*) is an actin-nucleation protein and organizes the length of thin filaments [20, 22]. Gene variants of these proteins cause muscle weakness through length dysregulation, cross-bridge cycling kinetic disruption, and alteration of calcium-sensitivity to force generation [20, 22–24].

Nebulin (*NEB*)is an actin-binding protein that is localized to the thin filament of sarcomeres in skeletal muscle. It is a very large protein coded by the *NEB* gene and binds nearly 200 actin monomers. Nebulin is thought to function as a regulator of thin filament during sarcomere construction, as it serves as a template for the actin filament by extending along the course of the thin filament determining its length. AR inherited *NEB*-related mutations are the most common cause of NMs, and they are identified in about 50% of cases. They usually cause the typical subtype in which proximal axial involvement is prominent and distal involvementmay develop later. In addition, they may also cause severe, intermediate, mild, or rode-core forms. Distal only involvement—distal nemaline myopathy is discriminated by the absence of nemaline rods in the biopsy. Patients with the typical form can't raise their head while lying supine, and knee flexors are more affected than knee extensors. Additional

signs include facial/bulbar weakness, a nasal voice, and palatal reflexes may be absent [19]. Muscle magnetic resonance imaging (MRI) may show sparing of the thigh muscles with selective involvement of the tibialis anterior and soleus muscles [24]. Missense variants are the most frequent type of mutation, but if this genetic variant lies outside the coding regions for actin or tropomyosin binding sites, it could be welltolerated [20]. Therefore, the most frequent pathogenic variants are splice-site, frameshift, or nonsense mutations. Additionally, although not causing frameshift, exon 55 deletion is also frequently seen in patients because it impairs the modular super-repeated structure of nebulin [20]. Findings from muscle MRI also suggest that the rectus femoris is selectively involved, and at the level of the calf, the tibialis anterior is involved in the early period [18]. These findings help discriminate NM from CCD. Unfortunately, there is no curative treatment and supportive care is needed [24].

Case 13.1

A 7-year-old boy was admitted to hospital with muscle weakness and mild intellectual disability. His weakness developed during infancy, and he was able to walk after the age of 5. He also had delayed speech development. On examination, axial and predominantly proximal muscle weakness, with generalized muscle atrophy and areflexia were noted. The CK levels were normal (104 IU/L). Needle EMG showed myopathic motor unit potentials. WES analysis revealed a pathogenic homozygous variant in the *NEB* gene, c.736dup, p.Leu246ProfsTer6 and a heterozygous VUS variant in the *NEB* gene, c.19993C > T, p.Pro6665Ser. He was diagnosed with Nemaline Myopathy 2.

ACTA1 (Skeletal muscle alpha-actin) defects are found in about 25% of cases, and most are dominant mutations [8, 19, 25]. De novo missense mutations are present in about 90% of pathogenic variants and with its dominant-negative effect it causes severe disease [20]. In 50% of severe onset neonatal cases, *ACTA1* gene variants were found [8, 19]. Rarely, it also causes typical, intermediate, and mild forms. Knee extensors become weaker than knee flexors and ankle dorsiflexion is preserved. Recessive mutations often cause null mutations and disease severity depends on the expression of cardiac actin isoform in skeletal muscles in the postnatal period [19]. NM-related *ACTA1* mutations occur on six coding exons. The hydrophobic core structure of subdomain 1 is the only area where NM related mutations are not seen [19, 25]. Nemaline rods are not seen in every patient with *ACTA1* mutations. In some cases, cores or FTD may not be detected in biopsy [19]. Types with nuclear-located rods have a poor prognosis. There is no curative therapy [24].

Defects of the *TPM2* and *TPM3* (β-tropomyosin and α-tropomyosin) genes because inherited NMs. Dominant missense mutations in both genes, and in-frame mutations that cause an amino acid deletion, are frequently seen [20]. *TPM2* dominant mutations are common causes of typical and mild NM and distal arthrogryposis. Recessive mutations cause a NM-related Escobar sequence with a severe course and fetal akinesis [8, 19]. In *TPM3* mutations, the disease is more severe. A homozygous mutation, which causes deletions in the *TPM3* gene promoter and first 2 exons, leads to severe disease [20]. FTD is seen in biopsies in *TPM3* related NM, and nemaline rods are commonly seen in small type 1 fibers [8, 19].

Defects of the slow skeletal muscle troponin T-1 (*TNNT-1*) gene cause NMs.Recessive mutations in *TNNT-1*are characterized by tremor, progressive contractures, muscle stiffness, progressive restrictive lung disease, and the findings of respiratory insufficiency [21]. In the Amish community, a homozygous nonsense mutation causes a stop codon in exon 11 and mutant protein breaks down [20]. AD inherited *TNNT1* presents with a mild course. In the heterozygous missense mutation, the mutant protein is not degraded, which causes adominant-negative effect [20]. Defects of the fast skeletal muscle troponin T-3 (*TNNT3*) gene cause respiratory insufficiency, continuous ventilator dependency, contractures, and hip dislocation different from other NMs, which are the typical features [21]. Nemaline rods are limited to type 2 fibers [21]. Recessive loss of function mutations in the Myopalladin (*MYPN*) gene are seen in cases with a mild NM disease and slowly progressive cap myopathy [20, 26]. Cofilin-2 (*CFL2*) gene-related NM is rare and cases with *CFL2* mutations have a typical presentation [20]. Facial weakness and foot drop may not develop in homozygous mutations [19]. Recessive missense mutations and deletions are reported and in the absence of cofilin-2, growth control of actin filaments is impaired, and the structure of the sarcomere is affected [20].

The recessive nonsense and frameshift variants of the Leiomodin-3 (*LMOD3*) gene are often seen in some cases with NM [20]. Protein deficiency may lead to short and disorganized actin filaments. Kelch proteins play a role in the stability, turnover, and regulation of thin filament proteins [20]. Defects of the Kelch repeat and BTB/POZ domain containing protein 13 (*KBTBD13*) cause NMs. AD inheritance is detected in *KBTBD13*-related NM [19]. Both nemaline rods and core-like structures are seen [19]. With its defect, regulation in thin filament relaxation is impaired [20]. Apart from that, patients can't adjust their body position to avoid falling, and their muscle movements are slow [19]. The Kelch-like 40 (*KLHL40*) protein also regulates nebulin and leiomodin-3, and prevents degradation. *KLHL40* dysfunction causes several clinical phenotypes. The recessive mutations of *KLHL41* cause mild, typical, and severe NM phenotypes [20].

Case 13.2
An 18-month-old boy who was born to consanguineous parents was admitted to our clinic with motor delay. Reduced in-utero movements, birth with a femur fracture, and hip dislocation were noted in the prenatal and perinatal history. His examination revealed generalized weakness with contractures of the knees, interphalangeal joints, elbows, and mild laxity of the ankles. He had mild facial weakness, kyphoscoliosis, and absent deep tendon reflexes. His CK levels were normal. A targeted gene panel revealed a likely pathogenic variant in the KLHL40 gene, c.1607 + 3A > T. He was diagnosed with nemaline myopathy 8.

Myosin XVIIIB (*MYO18B*) gene defects may cause an unusual phenotype with a Klippel-Feil abnormality and dysmorphic features, as well as muscle weakness and nemaline rods in biopsy [20]. A compound heterozygous mutation of the ryanodine receptor (*RYR3*) gene was reported in a patient with childhood-onset proximal weakness with an elongated face. Muscle biopsy showed fiber size variation, and type 1 fiber predominancy with nemaline bodies that were in perinuclear regions, the subsarcolemmal area and within the cytoplasm [27]. Severe forms, commonly caused by *ACTA1* and *KLHL40* defects, are first seen in the first 2 years [7]. Typical forms have a static/slow progressive course, and even with milestones development, some clinical improvement may be seen [21]. In the childhood or juvenile period, patients with a new onset may have mild findings. However, AR *TNNT1* mutations have a rather progressive course with progressive thorax stiffness, tremor, progressive contractures, muscle stiffness, restrictive pulmonary disease, and findings of progressive respiratory insufficiency in the early period [21].

Nemaline rods in biopsy are not pathognomonic for NM. Adenylosuccinate synthase-like (*ADSSL1*) gene defects may cause distal myopathy with nemaline rods and lipid bodies in biopsy. Similarly, filamin C (*FLNC*) may cause a distal myopathy with cardiomyopathy, as well as nemaline rods and ring fibers in biopsy [20]. Although rods, cores, and cap-like structures are reported in *RYR1* defects, *TTN* gene defect-related CM cases, and the findings are not compatible with NM [11, 20].

Additionally, nemaline rods are not universally seen in all cases with known NM gene mutations [20]. In some cases, rods and cores coexist. This is reported with *RYR1, ACTA1, NEB, CFL2, TRIP4, TNNT,* and *KBTBD13* gene defects [4, 28]. Zebra body myopathy is also a NM variant. It is related to the *ACTA1* gene [1]. Cap myopathy is a NM variant with well-demarcated cap-like structures, disorganized thin filaments, and Z-disc structures in the periphery of muscle fibers [1]. There may be decreased ATPase activity in cap structures, and pale eosin staining with H&E stain [5]. In *TPM2* related cap myopathy, immunolabeling with weak slow myosin can be detected [5]. Cap structures may be present in 4–100% of the fibers. The proportion of affected fibers is related with clinical severity. It is related to the *TPM2, TPM3, ACTA1, NEB,* and *MYPN* genes [11].

Case 13.3
A newborn baby, who was born from the first pregnancy of a 29-year-old mother by vaginal delivery on the 36th gestational week, was admitted to hospital due to severe respiratory failure soon after birth. His family history did not reveal any significance in terms of NMDs and there was no consanguinity between his parents. His antenatal screening tests were normal. However, fetal movements in the third trimester of pregnancy, felt by his mother and observed by the obstetrician on fetal ultrasonography, were decreased and marked polyhydramnios had emerged after the 32nd week of pregnancy. At 36 weeks, vaginal delivery had to be induced because of the onset of membrane rupture and fetal distress. Due to his ongoing severe hypotonia, weak cry, and lack of effort to breathe, muscle enzymes, and screening tests for congenital metabolic disorders were performed and they were found to be completely normal. EMG and cerebrospinal magnetic resonance imaging (MRI) could not be performed because of his dependency on mechanical ventilation. Eventually, to exclude congenital neuromuscular disorders, a muscle biopsy was taken from the gastrocnemius at age 8 weeks, and the diagnosis of NM was established. The rods were not visible with H&E staining but appeared as red or purple structures against the blue-green myofibrillar background with the modified Gomori trichrome stain (Fig. 13.2). The distribution of rods within myofibers showed a tendency to cluster around nuclei. Furthermore, increased oxidative enzyme activity with cytochrome oxidase enzyme stain was also demonstrated. Immunohistochemical stains were per-

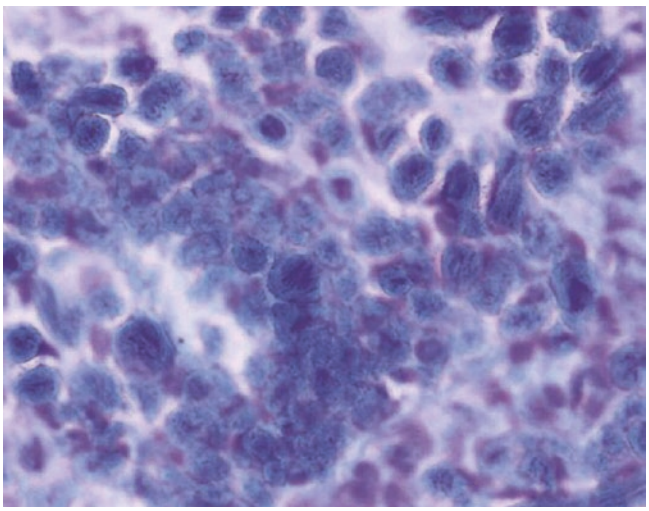

Fig. 13.2 Note the characteristic, purple-colored rods in the perinuclear region of most of the myofibers (Modified trichrome ×400)

formed using antibody against sarcomeric actin, smooth muscle actin, desmin, and vimentin. Only focal desmin positivity could be demonstrated on these rods. Genetic analysis for specific gene mutations could not be performed. The diagnosis was based on clinical findings and the presence of the characteristic, thread-like or rod-shaped structures (nemaline bodies) in muscle biopsy [29].

Case 13.4

A male infant who was 34-gestational-weeks old, and had a birth weight of 2100 grams, was born to a 22-year-old woman by normal spontaneous vaginal delivery. He was referred to the neonatal intensive care unit after delivery due to hypotonicity. His prenatal history was unremarkable. The parents were first cousins, and his 5-year old brother was healthy. There was no family history of neuromuscular disease. On admission, he was hypotonic and had little spontaneous activity. There was bilateral chorioretinal atrophy. Serum CK levels and the cerebrospinal fluid analyses were normal. Cranial magnetic resonance imaging revealed the presence of corpus callosum agenesis. The results of metabolic screening tests and electroneuromyography (EMG) were within normal limits. Muscle biopsy was performed at the age of 142 days. Microscopic examination revealed the presence of red-black colored, short, rod-like structures which were condensed predominantly on small-sized myofibrils and compatible with nemaline myopathy (Fig. 13.3). The patient was fed via an orogastric tube because of swallowing problems. He died at the age of 10 months due to a severe respiratory tract infection [30].

There is no current curative treatment. Monitoring and management of findings, preservation of muscle power/mobility/joint range of motion, maintenance of daily activities, and monitoring of respiratory and orthopedic complications are the basis of the multidisciplinary approach [21].

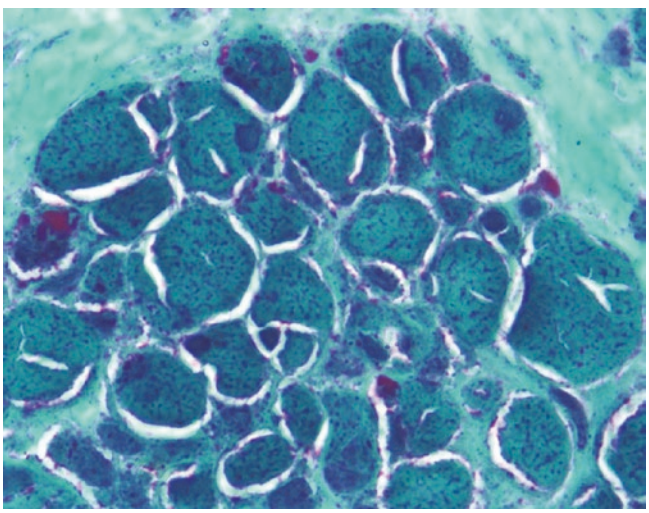

Fig. 13.3 Note the characteristic, purple-colored rods in most of the myofibres (Modified trichrome ×400)

Core Myopathies

The central core, multiminicore, dusty-core, and core-rod myopathies are clinically, pathologically, and genetically heterogeneous groups that are characterized by cores/minicores in muscle fibers [6, 24, 26]. In the pediatric period, its prevalence is 0.08–0.23/100,000 [6]. Cores are well-demarcated areas devoid of mitochondria and oxidative enzyme activity [11]. The absence of oxidative enzyme activity can be illustrated with nicotinamide adenine dinucleotide (NADH) and cytochrome c oxidase or succinate dehydrogenase dyes [4, 8]. Electron microscopic investigations prove the absence of mitochondria within cores [12]. Cores may be located centrally or peripherally, and the number of cores may be one or many [11]. There may be a large central core in CCD, and multiple cores in MmCM[11]. In some cases, cross sections in light microscopy may not be enough to distinguish central and minicores[12]. In CCD, cores extend along the whole longitudinal section within type 1 fibers.However, minicores reveal shorter areas and are seen in both type 1 and type 2 fibers [11, 12]. Other myopathic biopsy findings including variation in fiber size, increased internal nuclei, and rarely endomysial fibrosis, and fatty infiltration may be seen in biopsy specimens [4, 12]. Immunocytochemical investigations may show abnormal desmin reactivity in cores, but it is not helpful in distinguishing the myopathy subgroup [12]. Myotilin, filamin C, triadin, and αβ-crystallin can accumulate within the cores [4, 26].

Central core myopathy (CCM) or central core disease (CCD) is the most frequent CM and more than 90% of patients have dominant RYR1 gene mutations. Recessive SELENON causes multiminicore myopathy (MmCM) and is the second most common form of core myopathies[6, 12]. In contrast to CCDs, MmCMs are known to be mostly autosomal recessive [4]. *SELENON, RYR1, MYH2, MYH7, TTN, CCDC78, ACTN2, MEGF10, and UNC45B* are the genes that are reported to be causative for MmCMs[26, 28]. Core-like structures are seen in not only CCD, but also in neurogenic atrophies, myasthenias, and other CMs [4, 5]. Bi-allelic mutations of *RYR1* can cause dusty-core myopathies [28]. The coexistence of core and central nuclei is reported in CCDC78 gene-related CMs [4]. Furthermore, *RYR1, NEB, ACTA1, CFL2, TRIP4, TNNT1, and KBTBD13* gene-related myopathies may demonstrate core-rod structures in muscle biopsy [28].

CCM is the most common form of CMs and more than 90% of cases have a *RYR1* mutation [1, 8]. Basic clinical features include hypotonia and a delay in motor milestones.

It has a static, non-progressive course with prominent proximal/axial muscle involvement, and involvement of the lower extremities more so than the upper extremities, which are typical findings[4]. In pathology, core structures are more often found in type 1 fibers, but there may also be type 1 fiber predominance[4]. Fiber size variation is partially seen[4]. An absence of mitochondria and sarcoplasmic reticulum in core areas, and an irregularity in Z-lines can be visualized with electron microscopy [4]. There may also be small fibers stained with fetal myosin [4]. There may be peripheral ring-shaped PAS staining in core boundaries, but cores are stained with glycogen and phosphorylase [4]. Core structures are structures that develop secondarily and do not cause direct weakness. Its underlying mechanism is impairment in calcium homeostasis that alters excitability in muscle cells [1, 4]. In recessive cases, like *SELENON*-related MmCM, there may be multiple cores in a few sarcomeres instead of only one central core [4, 5]. In immunohistochemical examinations, cores are stained with desmin[4]. Additionally, αβ-crystallin, filamin C, and myotilin accumulate in the cores [4]. Core structures are not found in every genetically proven case, and type 1 fiber predominance alone may be present [4]. Although internal nuclei, increased connective tissue, fat between fascicles, and increased fibrous tissue are not classical findings, they are rarely seen and can be confused with muscular dystrophies [4, 5]. Central nuclei can be seen and may be confused with CNM [5]. Core is age-related, and in young cases only type 1 fibers are predominant and core is absent [4, 5]. In conclusion, central nuclei, core, and type 1 fiber predominant seen in pathological examination suggests *RYR1*-related CCD. There may be type 1 fiber predominance without core and additionally, core and rod may coexist [4, 5].

Case 13.5

A 9-year-old girl who was born to consanguineous parents was admitted to our clinic with muscle weakness. Her CK levels were normal. Muscle biopsy revealed that type 1 fiber predominance was present, and there were central cores in some type 1 fibers (Fig. 13.4). Nuclei internalization was

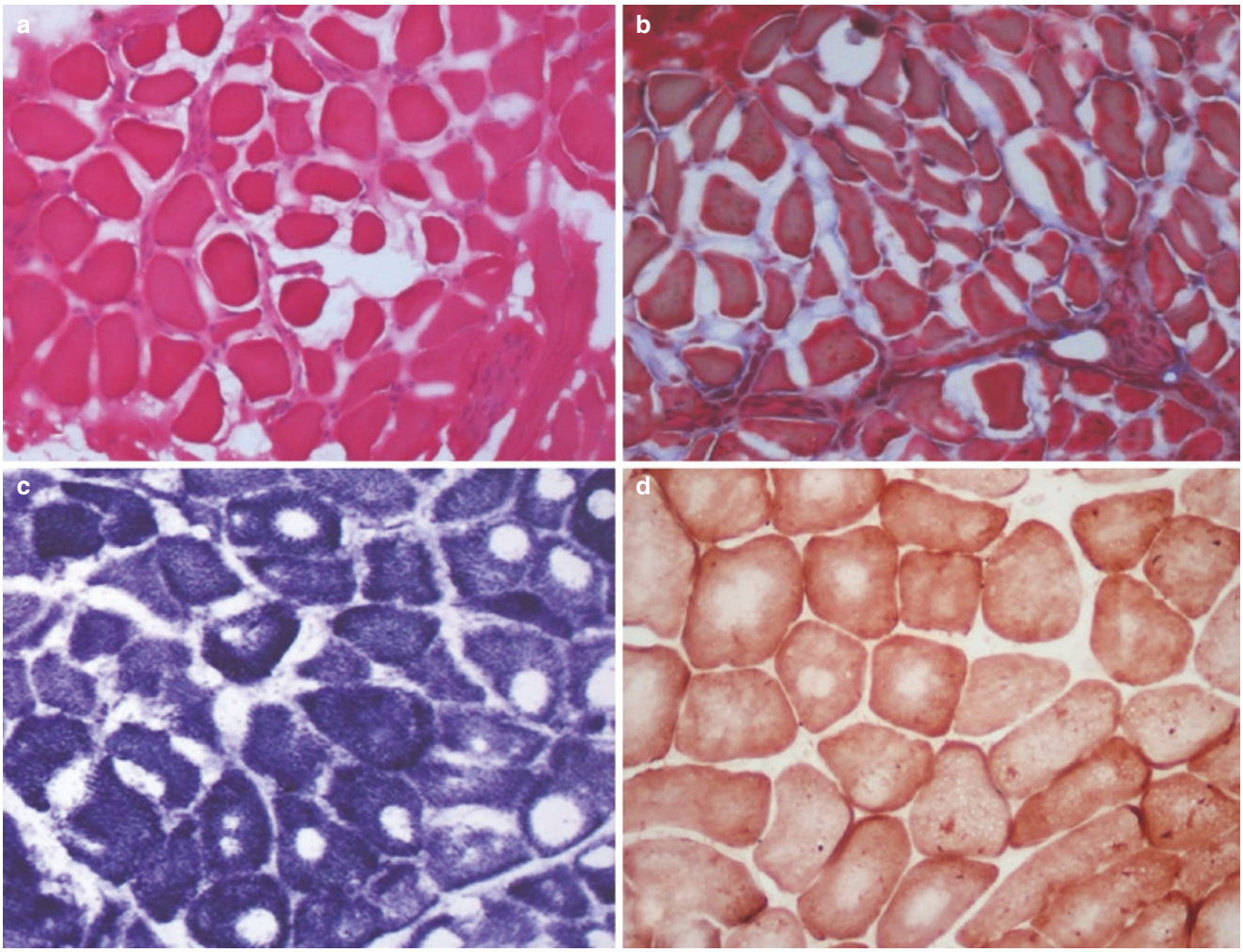

Fig. 13.4 Muscle biopsy of Case 13.5 diagnosed with CCD: (**a**) Muscle biopsy shows variation in fiber size and multiple internalized nuclei (HE ×100), (**b**) Mildly increased fibrous connective tissue (Masson trichrome ×100). Central cores are present in most type 1 fibers, (**c**) NADH-TR ×200, (**d**) COX ×200

mildly increased in both fiber types. A diagnosis of CCD was based on clinical and histopathological findings, and genetic analyses could not be performed.

The *RYR1* gene encodes the skeletal muscle ryanodine receptor protein, and it is a large gene with 106 exons [4]. The protein is a transmembrane calcium-channel protein within the sarcoplasmic reticulum that maintains cytosolic calcium homeostasis and plays a role in excitation-contraction coupling [4, 8]. Dihydropyridine receptors (DHPR) sense depolarizations induced by action potentials and then undergoconformational changes, which lead to interactions with RYR1 receptors. The C-terminal of RYR1 protein forms the calcium channel. The direct interaction between DHPR and RYR1 proteins precipitates the opening of calcium channels, and the release of calcium from the sarcoplasmic reticulum is the hallmark of excitation-contraction coupling [31–33]. *RYR1* mutations are mostly located in the transmembrane domain [11]. Dominant *RYR1* variants are clustered in three regions [32]. Mutations in cytoplasmic N-terminal and central domain often cause susceptibility to malignant hyperthermia [4]. Mutations in C-terminal exons are related to CCD [4, 5]. Mutations, especially in C-terminal 95–102 exons cause classic type CCD and are found in 66% of CCD cases. Non-C terminal *RYR1*mutations cause milder cases with atypical cores [4, 28, 31–33]. Recessive mutations can be seen in any region of the gene and result in more severe clinical presentations like fetal akinesia deformation sequence [4, 28, 31–33]. Heterozygous missense mutations are often identified [1]. Atypical cores can be found in patients with non-C terminal *RYR1* mutations. Some young patients with a family history of confirmed CCD may present with a predominance of type 1 fiber without detectable cores. This condition is defined as a congenital neuromuscular disease with uniform type 1 fibers (CNMDU1). Here, fiber type conversion occurs before core formation [12, 28]. Cores are initially found at the peripheral boundaries of fibers, and with aging, they begin to displace longitudinally toward the central area of the fiber. Therefore, the placement of the cores correlates with the stage of the disease in CNMDU1 cases [28].

Dusty core myopathies overlap with CCD, MmCM, and CNM. Core structures in dusty core myopathies have characteristic features, including unclear borders, irregular size, and non-ovoidal shape [28]. Recessive *RYR1* mutations are linked to dusty core myopathies and have an earlier onset and more severe phenotype than other CCDs [28]. As *RYR1* is a large gene, variants of uncertain significance are often found [4]. The demonstration of decreased protein content by immunoblot analyses of RYR1 proteins can support diagnoses in suspicious cases [4]. *RYR1* variants are common and digenic pathologies should be kept in mind [4]. CCD presents with a wide spectrum of clinical manifestations, ranging from mild to severe fetal akinesis [8]. Typical signs include hypotonia, motor delay, skeletal malformations (e.g., congenital hip dislocation), ankle contractures, hyperlaxity in other joints, pes cavus, pes planus, club foot, scoliosis, pain on exertion, and weakness typically of the proximal and hip-girdle muscles [1, 4, 23]. Contractures are rare except for Achilles tendon stiffness. Due to the presence of hyperlaxity, CCD may be confused with Ullrich muscular dystrophies [1, 4]. In dominantly inherited cases, the disease is mild beginning in childhood, and motor delay and weakness benefit from physical activity [7, 8]. Furthermore, facial and bulbar weakness is mild but patients may not be able to fully close their eyelids. In rare *RYR1* recessively inherited cases, the disease is more severe with signs including neonatal hypotonia, axial weakness, and ophthalmoplegia. These cases can beconfused with NM [4, 8, 25]. About 80% of both AD *RYR1* and *AR RYR1* patients can become ambulatory over time [7]. In cases presenting with severe signs such as fetal akinesis sequence, ventilation dependence and death may occur at an early age [4]. In AR *RYR1* cases, signs including decreased intrauterine movements, premature birth, respiratory insufficiency and bulbar insufficiency are seen more often than in AD *RYR1*cases [7]. In AR *RYR1* cases, the need for respiratory support and NG tube feeding develops in the neonatal-infantile period[7]. In contrast to *ACTA1, MTM1, NEB*-related CMs that cause severe neonatal symptoms, the need for support decreases as the age increases. Except in severe neonatal cases, respiratory insufficiency is milder than in other CMs, and primary cardiac involvement is rare [4, 7].

Typical muscle involvement is demonstrated with MRI, and differs from other CMs. Quadricep muscle involvement and sparing of the rectus femoris are typical characteristics [4]. The vastus, sartorius and adductor magnus muscles are involved in the upper leg, and the rectus femoris, adductor longus, and hamstring muscles are spared. At the calf level, the soleus, head of the gastrocnemius and peroneal muscles are involved, and the medial head of the gastrocnemius and anterior muscles are spared [18].

RYR1 mutations may be related to a susceptibility to developing malignant hyperthermia, exertion-induced rhabdomyolysis, King-Denborough syndrome, Escobar syndrome, fetal akinesia, distal myopathies, and late-onset axial myopathy [4, 5, 28, 31–33]. Histopathological studies may show central cores, multiminicores, central nuclei, core-rod structures, fiber type 1 predominant, dusty cores, and uniformity of type 1 fibers [31–33]. Presentations show similar findings, and the mild finding may be present in biopsy, but core-like structures may not be found in all cases [5]. Diagnosing *RYR1* gene defects is important because they cause susceptibility to malignant hypertension [1]. In cases with malignant hyperthermia, biopsies may be normal, and in less than 30%, there are central cores present [12]. In human and mouse experimental studies, an antioxidant

N-acetylcysteine proved to be efficient [24]. However, more research studies are required.

Multiminicore myopathies (MmCMs) are characterized by multiple minicores which are regions of disrupted sarcomeres that lack mitochondria [1]. These minicores differ from the cores that are seen in CCM. They are not restricted to type 1 fiber and they don't extend along the whole longitudinal section of the fiber. They are also smaller in size, and multiple cores are seen within the muscle fiber [34]. In contrast to *RYR1* related CMs, triadin, SERCA, DHPR, and calsequestrin are not found in cores in *SELENON* related MmCMs with immunocytochemical investigations [28]. MmCMs are clinically and genetically heterogeneous disorders. Multiple cores can be seen in different CM subgroups linked to several genes. Therefore, clinical correlation is required [4]. It has been classified into four categories including a classical form, moderate form, ophthalmoplegic form, and in-utero-onset severe form [34]. The most common phenotype, which constitutes almost 75% of MmCM cases, is the classical form. This form has a distinctive clinical feature and is associated with recessive *SELENON* mutations [12, 31]. Recessive *RYR1* variants are commonly seen in the other forms and these remaining three subgroups have overlapping clinical features [31].

Classical MmCM is characterized by predominant axial/neck weakness, an early onset rigid spine, scoliosis, bracket-like thighs with severe atrophy of the inner thigh muscles, and respiratory insufficiency which is disproportionate to muscle weakness [1, 4]. Poor head control and generalized hypotonia with motor delay are the common features seen in the first 2 years of age [12]. An asthenic phenotype with muscle atrophy, short stature, and joint hyperlaxity are the other common findings [12]. Recessive *SELENON* mutations compose almost 50% of classical MmCM patients [12]. *SELENON* related MmCM and *LMNA* related muscular dystrophy are the main causes of severe neck muscle weakness with poor head control [9]. Rigid spine and scoliosis cause the development of rapid-onset respiratory dysfunction, while the patients can still walk [12]. Respiratory muscle weakness, disproportionate to muscle weakness, can also present in patients with *NEB, ACTA1, or TPM3* related NMs [9]. Muscle MRI of patients with *SELENON* related MmCM shows selective sartorius involvement [18]. The S*ELENON* gene encodes an endoplasmic reticulum glycoprotein; selenoprotein N, which is predominantly found in fetal muscle [4, 12]. It is also associated with RYR1 protein [26]. It plays a role in myogenesis and sarcomere organisation. Mutations of *SELENON* can be found throughout the entire gene [1]. Recessive loss of function mutations in *SELENON* cause a group of NMDs known as *SELENON*-related myopathies, including core myopathies, rigid spine muscular dystrophies, CFTD, and desmin related myopathy with Mallory body-like inclusions [26].

Fewer than 10% of MmCMs are of the moderate form [12]. Distal upper limb weakness with hand muscle atrophy, hip girdle weakness, and joint hyperlaxity are the common features [1, 12]. The respiratory, bulbar, and paraspinal muscles are spared. It is mostly caused by recessive *RYR1* gene mutations [1]. Additionally, fewer than 10% of MmCMs are of the ophthalmoplegic form [12]. These group of patients have a similar phenotype to the classical form, but they exhibit ophthalmoplegia over time [1]. Extraocular muscle involvement is abundant with abduction and upward gaze impairment. Respiratory involvement is milder compared to the classical form [31]. The severe form of MmCM is characterized by an antenatal onset, arthrogyriposis, dysmorphism, and respiratory involvement [1].

Currently MmCMs have a wide phenotypic variability with newly found genes. For example, the multiple EGF-like domain 10 (*MEGF10*) gene, which encodes transmembrane protein of the multiple epidermal growth factor family. Bi-allelic truncating mutations of the *MEGF10* gene are characterized by early onset myopathy, areflexia, respiratory distress, and dysphagia (EAMRDD) [12, 28]. Infantile-onset weakness, adult-onset respiratory insufficiency, and distal involvement are the reported clinical presentations in patients with compound heterozygous mutations [12, 28].

Titin (*TTN*) gene mutations may cause different clinical phenotypes including familial hypertrophic cardiomyopathy (AD), dilated cardiomyopathy (AD), early-onset myopathy with fatal cardiomyopathy (AR-EOMFC), Salih myopathy, tibial muscular dystrophy (AD), CNM related to *TTN* (AR), and MmCM-related to *TTN* (AR) [10, 28]. Homozygous mutations of *TTN* cause MmCM with central nuclei and a secondary calpain deficiency on muscle biopsy [1]. Congenital-onset forms reveal early onset hypotonia with contractures, respiratory insufficiency, and cardiac involvement [8].

Heterozygous mutations of the beta-myosin heavy chain protein gene cause Laing distal myopathy with weakness of the ankle dorsiflexors and great toe [12]. In addition to multiminicores, non-specific myopathic changes including fiber size variation, central nuclei, moth-eaten like fibers, ring fibers, and fiber splitting are detected pathologically [28]. Except for cor pulmonale, cardiac involvement has not been reported in MmCMs[8]. Myosin heavy chain-7 (*MYH7*) and *TTN* mutations should be considered first in patients with cardiac involvement and multiminicores on muscle biopsy [8]. *MYH7* mutations are more commonly found to be related to hypertrophic cardiomyopathies [15]. Mutations regarding the globular head of *MYH7* protein have been found to be associated with dilated cardiomyopathy [15]. Dominantly inherited myosin heavy chain-2 (*MYH2*) gene mutations cause congenital contractures and hip dislocation. However, recessive *MYH2* mutation-related myopathies have a wide range of disease onset, ranging from childhood to adulthood.

Proximal/neck muscle weakness, facial muscle weakness, scoliosis, and ophthalmoplegia, but not ptosis, are the common clinical features. Recessive mutations usually cause truncated proteins, and in muscle biopsies, type 1 fiber uniformity with an absence of type 2A fibers is found. In rare cases with recessive missense mutations, type 2 fibers without multiminicores can be seen. In contrast to biopsy findings in cases with recessive mutations, multiminicores are present predominantly in type 2A fibers [28].

Coiled-coil domain containing 78 (*CCDC78*) gene mutations cause myopathy with the predominancy of type 1 fibers, and both central nuclei and core-like structures are common biopsy findings. Cases present with neonatal hypotonia, distal weakness, fatigue, myalgia, and mild cognitive decline [28]. Bi-allelic mutations in the Unc-45 myosin chaperone B (*UNC45B*) gene were reported in patients with childhood-onset proximal muscle weakness, calf hypertrophy, and eccentric cores on muscle biopsy [28]. Muscle biopsies showed multiple subsarcolemmal cores in cases with dominant mutations in the alpha-actinin 2 (*ACTN2*) gene, which encodes alpha-actinin-2. Patients, either neonates or adults, may exhibit asymmetrical distal weakness, ptosis, ophthalmoplegia, and cardiac/respiratory involvement [28].

The calcium voltage gated channel subunit alpha 1S (*CACNA1S*) gene encodes the DHPR receptor alpha-1 subunit. Homozygous mutations are implicated in congenital myopathy with ophthalmoplegia [12, 28]. The thyroid hormone receptor interactor 4 (*TRIP4*) gene encodes a transcriptional coactivator ASC-1 protein [35].*TRIP4* mutations lead to the depletion of ASC-1 protein which directly binds to transcription factors[12, 35]. Cases with *TRIP4* variants may present with neonatal-onset axial/proximal weakness, a rigid spine with scoliosis, and respiratory involvement, or dilated cardiomyopathy during adult life [35]. Multiminicores and other structural features such as rods, caps, and central nuclei can be seen on muscle biopsies [35].

Myotubular Myopathy (MTM)/Centronuclear Myopathy (CNM)

CNM is characterized by vesicular, large nuclei localized in the center of muscle fibers and a high proportion of small myofibers [11, 36]. This is easily shown with H&E, NADH, and electron microscopy. With NADH, the area around the nucleus is seen as a peripheral halo devoid of myofibrils [1, 5]. While *MTM1* gene mutations may cause X-linked inherited myotubular myopathy (XLMTM), the other CNMs are AR or AD [11]. XLMTM's clinical picture is typical and severe with global hypotonia and respiratory insufficiency, while autosomal CNM's clinical picture is more variable. While AR CNMs often develop in the infantile and early childhood period, AD CNMs are more frequently seen in adulthood with a milder and slowly progressive course [36]. Its incidence is reported with a rate of 0.25–0.66/100,000 in pediatric cases [6]. In XLMTM/CNM suspect cases, *RYR1*and myotonic dystrophy should be excluded because clinical overlap could occur and both central nuclei and core may be coexistent in the biopsy [4].

There are underlying mutations which may affect complex membrane systems such as membrane traffic, endocytosis, triads (junctions between two sarcoplasmic reticula and one T-tubule), T tubule formation, and excitation-contraction coupling [4, 8]. XLMTM, which is caused by *MTM1* gene defects, is one of the most severe types of CM [8]. Severe generalized hypotonia at birth, ophthalmoplegia with marked facial and bulbar weakness, and respiratory insufficiency are the most typical findings[1, 8, 23]. CNMs are found to be associated with AR or AD *DNM2, BIN1, RYR1, TTN*, *MYF6, CCDC78, MTMR14, SPEG,* and *ZAK* genes [1, 4, 8, 10, 11]. *DNM2* mutation are less frequent, followed by *BIN1, CCDC78, SPEG,* and *TTN* with increasing frequency[5]. *MTM1, DNM2*, and *BIN1* play a role in intracellular membrane pathways, endosome traffic, and T tubule formation. The dysfunction of excitation-contraction coupling is the cause of weakness [1, 8, 37]. Although single and central nuclei are typical findings in *MTM1* and *DNM2*, multiple internal nuclei are seen in *BIN1* and *RYR1*-associated CNM [38].

MTM is the most severe CM with utero-onset. In utero fetal movements are reduced. Polyhydramnios, miscarriages, and preterm deliveries are more common with MTM compared to other CMs [7]. It is an X-linked disorder. The most common clinical features include: great height and large head circumference by the week of gestation in affected male cases, hip/knee contractures, severe neonatal hypotonia, ophthalmoplegia, respiratory insufficiency, feeding problems, andundescended testicles[1, 4, 5]. If patients do not receive enough respiratory and nutritional support, they may die in the first year, and this mortality is reported to be between 25 and 50% [7, 8, 39]. Approximately, 80% of survivors live with ventilatory support and require NG tube feeding [40]. Some patients with mild forms due to missense mutations may reach adulthood [1, 8]. About 20% of cases are mild, with patients maintaining the ability to walk, but the need for bilevel positive airway pressure (BiPAP) against nocturnal hypoventilation and feeding support may need continuing [40]. The severity of the disease is defined as severe, intermediate, or mild according to the need for ventilatory support[36]. Familial intrahepatic cholestasis-like liver disease and some hepatic vascular abnormalities were reported [40]. Female carriers may either be asymptomatic or display various clinical severities [4, 40]. In carrier females, mild facial weakness can be seen similar to mild adult forms, as well as early hypotonia, and a loss of ambula-

tion. This clinical diversity can be explained by skewed X-inactivation. In addition, asymmetrical weakness may be prominent in these cases, and this may be a finding of asymmetrical X-inactivation [1, 4, 8].

XLMTM gets its name from recognisable pathological features [40]. Severe fiber size variation with very small fibers and fibers with large central nuclei are common. Disruption of T-tubules, sarcoplasmic reticulum, triad structures, and excitation-contraction coupling are the reasons for severe weakness [40]. Like other CMs, type 1 fiber predominance is present, but most of the fibers are small due to insufficient myofibrillary growth [5, 40]. Necrosis and fibrosis are not expected to be present [1, 4, 5]. During pathological examinations, the number of large central nuclei is variable. Central nuclei can be seen in both fiber types [4, 5]. Although both small and large fibers are seen in the early stages of the disease, large fibers atrophy with age and clinical progress, and small-fiber uniformity is observed [40]. Contrary to the chain-like arrangement of central nuclei in regenerating fibrils, XLMTM appears to be located at regular intervals on longitudinal sections [4, 5]. The atypical location of mitochondria, sarcotubular structures, and other organelles are other typical features [40]. Darkly stained areas, with oxidative enzyme and PAS, are the areas where mitochondria aggregate, and glycogen accumulates. Areas around nuclei, appearing as holes with H&E and ATPase staining, are areas where organelles are absent. In addition, the area around the darkly stained area, with oxidative enzyme, also shows a pale subsarcolemmal halo, which is also present in some autosomal CNMs. Unlike female carriers, basophilic loops, known as "necklace fibers", are associated with internal nuclei and strongly stain with oxidative enzyme and PAS, drawing attention to just below the sarcolemma [4, 5]. Congenital muscular dystrophy should be considered in the differential diagnosis of a hypotonic newborn with central nuclei on muscle biopsy. The pathological distinction is difficult, and so it must be supported with molecular diagnosis [4].

The *MTM1* gene encodes myotubularin which acts as an endosomal phosphatase and plays a role in signaling pathways, membrane traffic, and endocytosis [4, 8]. Various mutations are distributed throughout the entire gene without any hot spots [5, 40]. These mutations include nonsense mutations (more frequent), frameshift mutations causing premature stop codons, and splice site mutations causing loss of protein expression [5, 40]. Some missense mutations do not completely inhibit protein function and have been associated with more mild clinical symptoms [40]. Some missense variants have also been reported in the phosphatase domain, reducing protein function [40]. Increased dynamin 2 (*DNM2*) expressions in muscle tissue has been demonstrated in an XLMTM case [26]. Therefore, *MTM1* is thought to be a negative regulator of *DNM2* expression, and some secondary therapeutic options are being studied [26]. Tamoxifen, as well as Dynamin 2 modulation, are other potential therapeutic options, in addition to gene therapy, enzyme replacement, and PIK3C2B inhibition [24, 36].

Case 13.6

An 18-month-old girl who was born to consanguineous parents was admitted to our clinic with motor delays and muscle weakness. Her CK levels were increased (up to 500 U/L). Muscle biopsy revealed that type 1 fiber predominance is present, but most of the fibers are small. Nuclear internalization was mildly increased in both fiber types. There were darkly stained areas, with oxidative enzyme and PAS, and in the middle of these areas were unstained areas. Areas around nuclei appeared as holes with enzyme stains. In addition, there were necklace fibers, seen as areas around the darkly stained area, with oxidative enzyme, also seen as a pale subsarcolemmal halo (Fig. 13.5). A MTM diagnosis was based on clinical and histopathological findings. Genetic analyses could not be performed.

Case 13.7

A 10-month-old boy was admitted to the hospital with motor delays and muscle weakness. He was a hypotonic infant and had respiratory distress. There was no consanguinity between his parents. In the histopathological examination of the first biopsy material, the presence of an increased number of central nuclei and myofibers of various sizes were detected. The patient was evaluated as a suspected case of CM, such as CNM, CFTD, and so on. Two years later, a repeated muscle biopsy was performed. In the histopathologic examination of the second biopsy specimens, greater differences were seen in the sizes of fibers. Small fibers were type 1, and all fibers had internal nuclei. Muscle injury had deteriorated to muscular dystrophy (Fig. 13.6). The second biopsy was consulted by Professor Caroline Sewry, PhD, FRCpath, and genetic analysis was performed at her suggestion, covering the MTM1, TTN, and RYR1 gene regions. During genetic analyses, a homozygous MTM1 gene mutation (c.731C > T p. R241C) was identified. This variant was previously reported as pathogenic. Finally, the patient was diagnosed with CNM caused by the mutation of the MTM1 gene on Xq28 [41].

CNM is clinically characterized by diffuse weakness that can be predominantly proximal or distal. Serum CK levels are often normal and electrophysiological examinations may be normal or myopathic [42]. AD CNM begins in the adult period, has a slower clinical course, and proximal muscle weakness is likely to occur. There are two subgroups. These are AD CNMs with diffuse muscle hypertrophy, and AD CNMs without diffuse muscle hypertrophy. Those with diffuse muscle hypertrophy have a younger age of onset and a more rapid course. Although ptosis can be

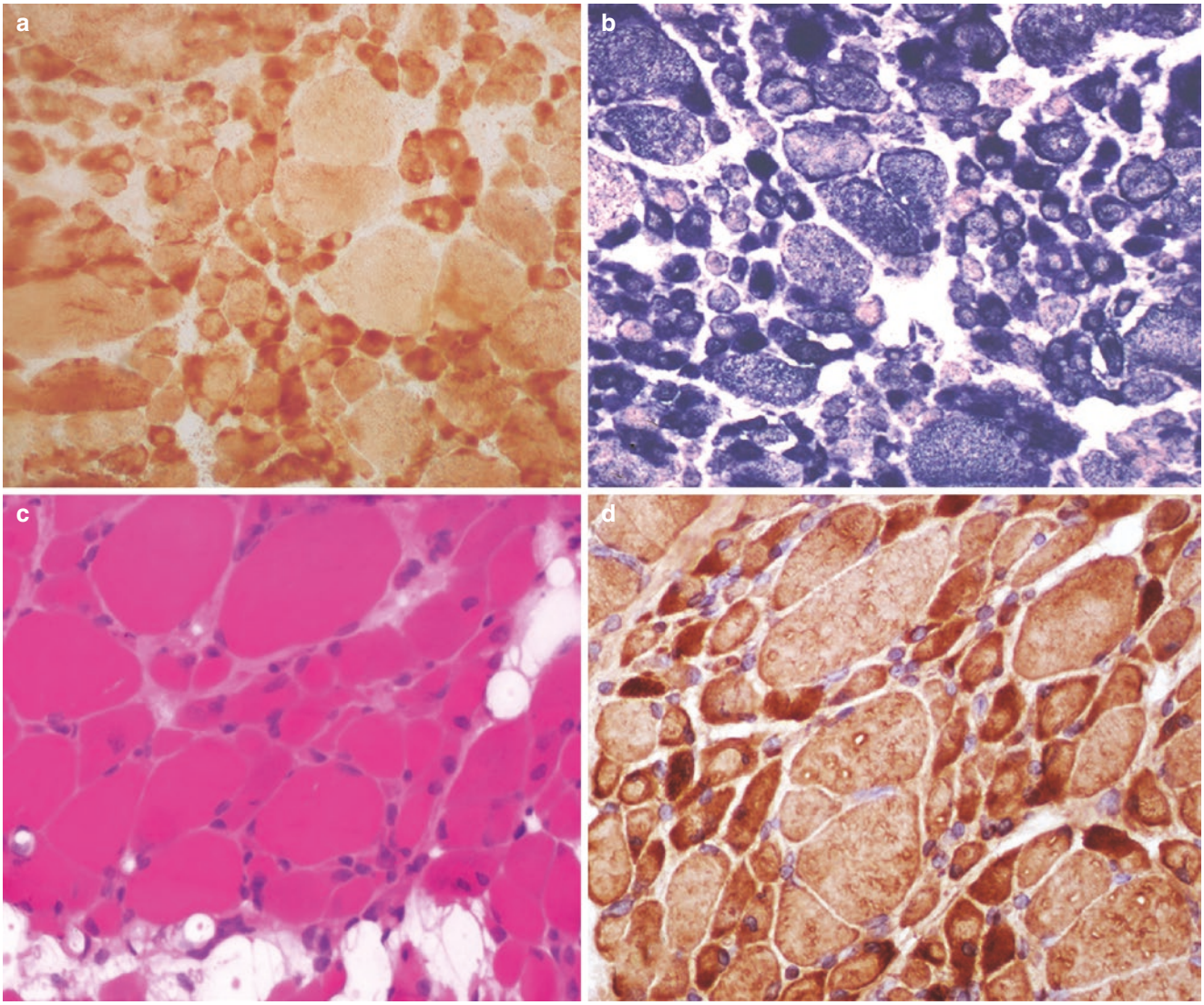

Fig. 13.5 Muscle biopsy of Case 13.6 diagnosed with MTM: (**a**) Necklace fiber appearance in most hypotrophic type 1 fibers (COX ×200), (**b**) Necklace fiber (NADH-TR ×200), (**c**) Muscle biopsy shows variation in fiber size, multiple internalized nuclei, and mildly increased fibrous connective tissue (HE ×200), (**d**) There are prominent central myotilin-unstained areas in the smaller fibers (DAB ×200)

seen, ophthalmoplegia is rare or occurs in the form of restriction in the upward gaze [42]. AR CNMs are divided into three subgroups according to ophthalmoplegia status and the age of onset. These are early-onset with ophthalmoplegia, early-onset without ophthalmoplegia, and late-onset without ophthalmoplegia.Those with ophthalmoplegia are the most severely affected. Dysmorphic facial features of other CMs, such as an elongated face and high palate, may accompany. Late-onset ones are clinically similar to AD CNMs [42].

There is significant fiber size variation with type 1 predominancy in H&E staining, and unlike XLMTM, the fibers are less rounded and more polygonal in shape. The central nuclei are also smaller and more hyperchromatic [4, 5, 42]. It has been reported that a chain appearance occurs in longitudinal sections, especially in *BIN1* mutations [38, 42]. The central dark area contains not only nuclei but also mitochondria and mislocalized organelles. Central reactivity is seen, secondary to perinuclear mitochondrial aggregation and an absence of oxidative enzymes. Poles of central nuclei contain glycogen as shown by PAS staining. The peripheral zone around the central nuclei draws attention [5, 42]. In the biopsies of *DNM2* mutation-associated, late-onset cases, intense stain lines extending radially from the centre to the periphery of fibers can be seen, and they have been rarely reported in *BIN1* mutations [4]. In almost all cases, at least 20% of myofibers have central nuclei. The distribution of centrally located nuclei is variable. In half of AR CNM, and approximately 40% of AD CNM cases, more than 80% of myofibers have central nuclei [40].

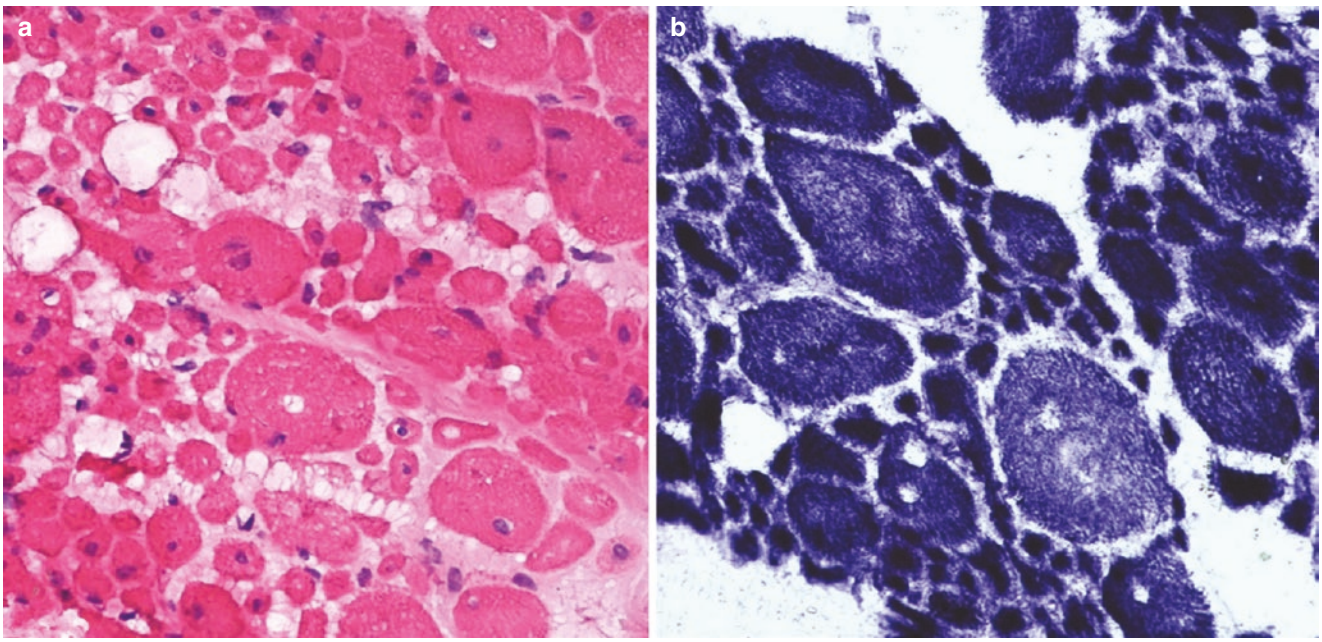

Fig. 13.6 Muscle biopsy of Case 13.7 diagnosed with MTM: (**a**) Muscle biopsy shows a huge variation in fiber size, multiple internalized nuclei, and mildly increased fibrous connective tissue (HE ×200). (**b**) The most hypotrophic are the type 1 fibers (NADH-TR ×200)

The dynamin 2 (DNM2) gene encodes GTPase, which plays a role in many intracellular pathways. AD DNM2 mutations lead to the formation of proteins with higher GTPase activity [24]. It presents with clinical findings ranging from a mild form in adults to a severe form in infants [37]. Clinically predominant distal weakness, distal contractures, ptosis, and involvement of extraocular muscles are seen in some cases. Achilles contracture, pes cavus, atrophy of calf/thenar muscles, and scoliosis may be added [1, 37]. Unlike other CMs, pain is observed during exercise [1]. In adolescents and young adults with a history of motor delay in childhood, difficulty in walking, running, and climbing stairs begins in later ages. Mild ptosis and ophthalmoplegia may accompany [37]. Infants are more severely affected, with generalized weakness, hypotonia, facial weakness, ptosis, and ophthalmoplegia [37]. Muscle biopsies show a triad of the predominance of hypotrophic type 1 fibers, radial strands with oxidative enzyme reactions, and central nuclei [37]. In muscle imaging, distal lower extremity muscles such as the gastrocnemius, soleus, and tibialis anterior are selectively involved in the early period, and the posterior thigh muscles are affected in later stages [1, 37]. Treatment studies with antisense oligonucleotides against DNM2 pre-mRNA are ongoing [24].

In some patients with CNM, homozygous missense mutations of the bridging integrator 1 (*BIN1*) gene were reported [1]. Clinical severity ranges from moderate to severe. There is motor delay, diffuse weakness with muscle atrophy, facial weakness, and ptosis [37]. Some dominant mutations were reported in mild forms with adult-onset [26]. The *BIN1* gene encodes amphiphysin2. It plays a role in membrane tubulationwith *MTM1*[26]. *MTM1* also acts as a desmin binding protein [37]. In immunocytochemical examinations, labeling with *DHPR, RYR*, and desmin are increased in *MTM1* related CNMs, especially in the central area. DNM2 related CNM has more homogenous and direct labeling, while BIN1 related CNM has a significant increase in the central cytoplasm with intense labeling. While labeling with caveolin-3 has a normal distribution in *MTM1* and *DNM2* related CNM, there is increased labeling in the central regions of *BIN1* related CNM [37]. In some cases presenting with proximal/axial predominant weakness, facial weakness, ptosis, ophthalmoplegia, contractures, and restrictive respiratory involvement in the neonatal period, central nuclei and core structures were observed, and AR *RYR1* mutations were reported [1, 37, 38].

Cases 13.8 and 13.9

Two first-degree cousins belonging to a consanguineous family from Turkey, without any ancestral history of NMDs, were admitted to hospital with muscle weakness, developmental delay, and CK elevations (up to 450 U/L). Case 13.8 was an 13-year-old girl and Case 13.9 was a 14-year-old boy. Both were slightly mentally retarded. Case 13.9 was diagnosed with CNM before because 2 years ago, a mild elevation of CK was detected, during preoperative tests due to an undescended testis, and a muscle biopsy, which was performed (Fig. 13.7).*BIN1* sequencing revealed a homozygous

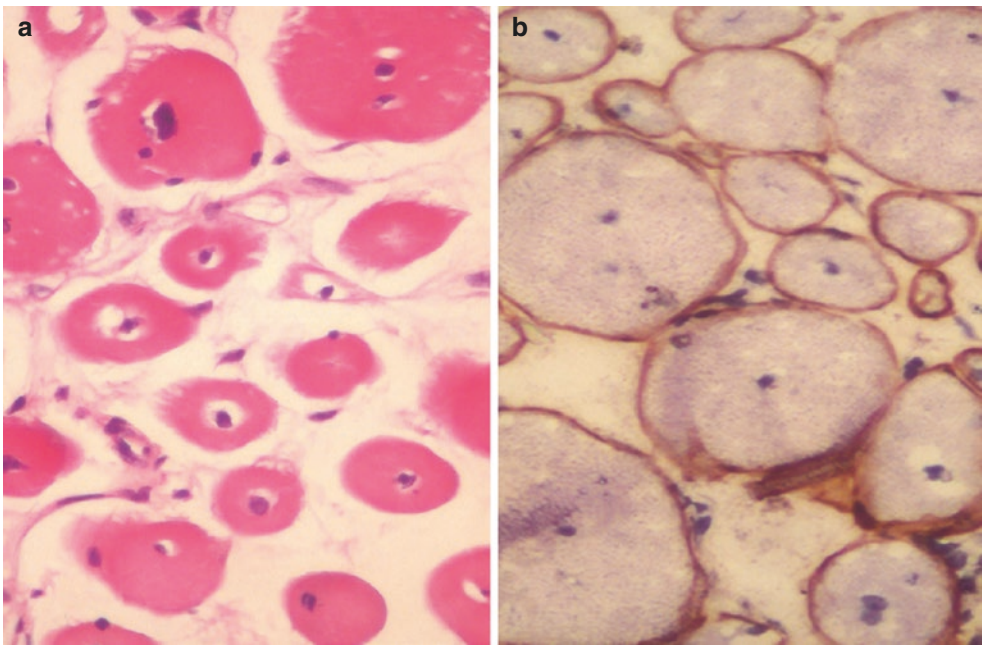

Fig. 13.7 (**a**) Muscle biopsy of Case 13.9 shows a huge variation in fiber size, plenty of internalized nuclei, and increased fibrous connective tissue (HE ×200), (**b**) Sarcolemmal dystrophin expression is normal (DAB ×200)

nonsense mutation in exon 20 in both patients (c.1717C > T; p.Gln573stop). Both patients have healthy parents who are heterozygous for this mutation [38].

In the dominant mutations of the coiled-coil domain-containing protein 78 (*CCDC78*) gene, both core structures and central nuclei were detected during biopsy [26, 28]. The truncating protein, formed with compound heterozygous *TTN* mutations, impairs excitation-contraction coupling by disrupting the triad structure and function of most genes involved in the pathogenesis of CNM, which has been reported in clinically and pathologically diagnosed cases. Titin, on the other hand, provides sarcomere assembly and binding sites for many proteins involved in excitation-contraction coupling. Clinical findings of patients with *TTN*-related CNMs include cardiomyopathy, hypotonia, proximal/distal weakness, a rigid spine, respiratory involvement, and contractures [5, 39].

The striated preferentially expressed gene (*SPEG*) plays a role in the formation of the muscle cell skeleton. It interacts with *MTM1*, and they both regulate calcium homeostasis and play a role in the correct positioning of the nucleus within the cell during the muscle maturation period [43]. In almost all cases with *SPEG* mutations, neonatal/infantile onset generalized weakness with muscle atrophy and motor delay have been reported, in some cases with ptosis, ophthalmoplegia, and respiratory weakness[43]. Dilated cardiomyopathy and tricuspid/mitral valve insufficiency are the signs of cardiac involvement [17, 43].

Myotubularin-related protein 14 (*MTMR14*) has high homology to Drosophila egg-derived tyrosine phosphatase (EDTP). This protein is also called "human JUMPY", because disruption of EDTP protein function in Drosophila causes a phenotype termed "JUMPY". It is characterized by a progressive loss of muscle control together with shaky and slower movements. *MTMR14* (JUMPY) gene expression has been shown to increase during the differentiation of myoblasts to myotubules. It also plays a role in the hydorlysis of membrane inositol rings. Heterozygous mutations have been reported in neonates with neonatal diffuse weakness and ophthalmoplegia [44].

Myogenic factors are members of the basic helix-loop-helix transcription factor family. Myogenic factor 6 (*MYF6*) plays a role in the terminal differentiation of myotubules. The heterozygous mutation was identified in a patient with mild myopathic findings and exercise-induced muscle cramps, with pathology compatible with CNM [45]. Leucine zipper and sterile alpha motif-containing kinase (*ZAK*) gene mutations present with neonatal/infantile-onset generalized weakness, motor delay, and scoliosis. Facial weakness is not expected, but joint hyperlaxity may be present. There are overlapping clinical findings with CM and LGMD, and pathologically with CNM and fiber type 1 dominance. Histopathological examinations showed fiber size variation, type 1 fiber predominancy, central nuclei, and recessive *ZAK* gene mutations, which were reported in cases where electron microscopy found that central nuclei were not surrounded by a halo of membrane and organelles, but by myofibrils, which is a typical finding for CNM. Rimmed vacuoles and an accumulation of subsarcolemmal mitochondria may be a clue. *ZAK* is a serine-threonine kinase, which plays a role in many pathways such as muscle regeneration and myogenesis [46].

Congenital Fiber Type Disproportion (CFTD)

For fiber-type disproportion (FTD), the main diagnostic finding is that the diameters of type 1 fibers are smaller than type 2 fibers [1, 4]. However, since these findings can be seen in other CMs, they should be found alone and with no other histopathological findings[4, 47]. It has been debated for many years whether CFTD is only a pathological finding or a separate disorder. However, since only pure FTD findings were detected in *ACTA1, SELENON, RYR1, TPM2, TPM3, MYH7, MYL2* gene-related CMs, they were grouped under CFTD [4, 47, 48]. It should be kept in mind that concomitant central nuclei, core, rod, or cap structures in the same gene-related CMs, may be detected in the future with age [4, 47]. CFTD is reported at a rate of 0.08–0.56/100,000 in the pediatric age [6]. Pathologically, the term FTD was first defined as the diameter of the type 1 fibers being at least 12% lower than type 2 fibers, but this finding is nonspecific as it can also be seen in other conditions such as myotonic dystrophy, and SMA [47]. Therefore, this ratio was later changed to be at least 25% [4]. As a CM subgroup, there is no pathognomonic finding for CFTD, and it is a diagnosis of exclusion. Firstly, cases should be compatible with typical CM clinical findings. These findings include a static/slowly progressive course, proximal/axial predominant weakness, facial weakness, ophthalmoplegia, dysphagia, and CK levels not five times higher than normal. Respiratory insufficiency has been reported in 30% of cases [49]. It has also been reported that the FTD rate is at least 35–40% in the majority of CFTD cases; the lesser differences are usually encountered in the non-congenital myopathy [47]. In CFTD cases, type 1 fiber predominancy (>55%) is also used in the definition [47].

ACTA1 mutations are detected in up to 5% of CFTD cases [47]. A static course, generalized and severe weakness with respiratory insufficiency are seen. Severe CFTD cases are often associated with the *ACTA1* and *RYR1* genes [47]. Although ophthalmoplegia is seen in half of CFTD cases, the extraocular muscles are spared in *ACTA1*-related patients [47, 50]. With *ACTA1* Asp294Val mutation, the replacement of a negatively charged polar amino acid with a nonpolar-neutral amino acid causes weakness by disrupting the actin-myosin interaction at the protein level. As there is no sarcomericdisorganisation, the rods are not seen in *ACTA1*-related NM [47]. Most missense mutations clustered in hotspot regions from *RYR1* mutations are associated with AD CCD, while other recessive variants have been reported with MmCD, CNM, and CFTD [47]. *RYR1* variants are detected in 20% of CFTD cases [32]. *RYR1*-related CFTD occurs over a broad clinical spectrum. Proximal and axial weakness is predominant. Facial weakness, ophthalmoplegia, and respiratory insufficiency is accompanied by generalized weakness. Unlike other CFTDs, scoliosis is more common [47].

The genetic variants most frequently detected in CFTD cases are related to the *TPM3* gene. AD mutations are rarely detected, and in 25–50% of cases AR mutations are present. CFTD may present with variable symptoms such as severe neonatal hypotonia, motor delay or proximal weakness, and difficulty with exercise [47, 51]. Axial weakness, weakness in ankle dorsiflexion, and mild ptosis with sparing of the extraocular muscles are common [24, 47]. Care should be taken with nocturnal hypoventilation that develops without a loss of ambulation, cases should be monitored for respiratory and sleep problems. Type 1 fiber hypotrophy is very prominent, and diameters can be detected which are 50–77% smaller than type 2 fibers [24, 52]. Most of the mutations are clustered in the fifth of seventh nucleotide-repeat motifs [49]. Most of the pathogenic variants are heterozygous missense variants, and rarely, recessive mutations causing a loss of function have also been reported [49]. In one case, a dropped head with marked axial weakness was reported [49]. Although some improvement with L-carnitine was reported in zebrafish models, studies are still ongoing[24]. Dominant mutation of the *TPM2* gene has been reported in one case [47, 53]. In another case, a diagnosis of *TPM2*-related cap myopathy was made upon detection of cap structures in electron microscopy, although no other pathological abnormality was observed in light microscopy [53].

A heterozygous mutation in the distal rod region of *MYH7* was reported in a patient with infantile-onset proximal weakness, waddling gait, and anterior distal leg involvement, but without cardiac or extraocular involvement, and with CFTD-compatible pathology [47, 54]. In the biopsy of an elderly patient of the same family, findings consistent with myosin storage myopathy suggested that *MYH-7*-related conditions may be a spectrum [54].

Different findings can be detected in the histopathology of *SELENON*-related myopathy. Dystrophic variants, multiminicores, Mallory bodies, and type 1 fiber hypotrophy with type 1 fiber predominancy have been reported. Those whose main pathological findings were small type 1 fibers were included in the CFTD subgroup[47].

Case 13.10

A female patient was born at 35-weeks-gestation and was the second child of consanguineous parents. She had one healthy brother, and the family history was noncontributory. She was hospitalized at 12 hours of age (postnatal) due to bilateral corneal opacity. At her physical examination, she had fair skin and brown hair; a pigment distinctly lighter compared to other family members. Dysmorphic features included a high-arched palate and micrognathia.A neurological examination revealed generalized and truncal hypotonia, with an absence of deep tendon reflexes. An ophthalmologic examination indicated bilateral anterior

sub-capsular cataracts. Her CK levels were elevated (433 U/L). Cranial ultrasonography and MRI revealed agenesis of the corpus callosum. Abdominal ultrasonography found left renal agenesis, but hydronephrosis was not present. Due to the physical and laboratory findings, Vici syndrome was suspected and whole exome sequence analysis was performed, which demonstrated a novel homozygous mutation, p.A1925Vfs*3 (c.5774_5774delC), of the *EPG5* gene. Her muscle enzymes gradually increased during the follow-up. A muscle biopsy of the vastus lateralis muscle was performed. Myofiber shape-size difference, degeneration, and atrophy-like muscle injury were identified. Slightly increased interstitial connective tissue was detected with Gomori trichrome. It was observed that the type 2 fiber ratio decreased with fast myosin and most type 1 myofibers were extremely small (Fig. 13.8). With the histopathological findings, CFTD was considered. She had continuous feeding problems, which necessitated feeding with an orogastric tube. The patient was discharged from the hospital at 5 months of age on room air and with a permanent orogastric tube for enteral feeding. She died from bronchopneumonia at the age of 8 months.

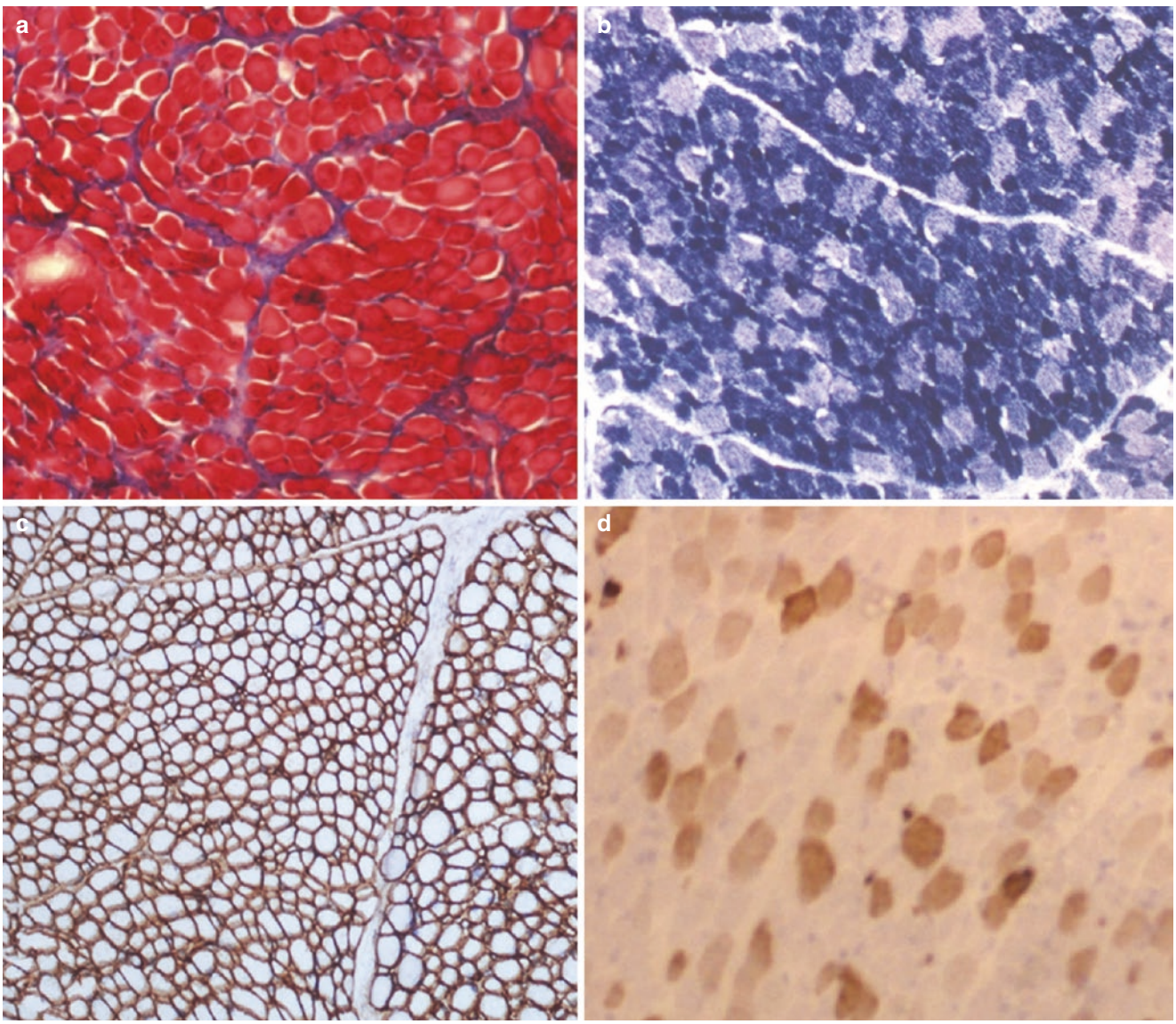

Fig. 13.8 (**a**) Muscle biopsy of Case 13.10 shows a variation in fiber size and increased fibrous connective tissue (Gomori trichrome ×100), (**b**) Numerous small sized type 1 fibers (NADH-TR ×100), (**c**) Normal sarcolemmal merosin expression, (**d**) Presence of pathological fibers with neonatal myosin (DAB ×200)

Other Congenital Myopathies

CMs have a large heterogeneous disease spectrum, and every day, newly identified genes/variants in families or cases associated with histopathological findings are reported. There are many examples of CM reported in the literature as case reports that cannot be classified into a specific CM group:

A case with findings of lamin-A *(LMNA)*gene-related FTD in the pathology was reported, but type 2 fiber hypertrophy was prominent, unlike type 1 fiber hypotrophy typical in CFTD [55]. A nonsense mutation in the neurofilament light polypeptide(*NEFL*) gene was reported in family members with nemaline rods in the biopsy, and with clinically overlapping myopathic and neuropathic findings [56]. Plectin (*PLEC*) is a protein that binds desmin with to outer nuclear membranes, Z discs, and sarcolemma. Recessive loss of function mutations causes epidermolysis bullosa with muscular dystrophy. Cases, in which progressive muscle weakness, ptosis, and ophthalmoplegia are prominent, with mild skin findings, and are classified as limb girdle muscular dystrophies [57]. A case with prominent proximal weakness, FTD, and central nuclei on biopsy was also reported [58]. Honeycomb myonuclei were reported in a case ofspectrin repeat-containing nuclear envelope protein 1 (*SYNE1*) gene-related myopathy [48]. Triadin (*TRDN*) is part of the calcium release complex in cardiac and skeletal muscles. In some cases, cardiac involvement is predominant, and muscle weakness is variable [59]. Adenylosuccinate synthetase like 1 (*ADDSL1*) gene defects causes a myopathy with childhood-to-adulthood onset. It presents with complaints such as easy fatigability, distal weakness, and falling behind in sporting activities from their peers. Nemaline rods can be seen at a rate of <10% less compared to NM in the biopsy. Thin filament structures are not affected, and lipid droplets are visible [24]. However, these cases have not yet been categorized into congenital myopathy subgroups.

Tripartite motif-containing 32(*TRIM32*) gene defects are classified in the group of sarcotubular myopathy and limb girdle muscular dystrophy [10, 11]. Recessive variants of the myosin light chain 2 (*MYL2*) gene have been rarely reported in the case of CFTD, in which both skeletal and cardiac muscles are involved [60]. The heterogeneous nuclear ribonucleoprotein A1 (*HNRNPA1*) gene is associated with AD inheritance, late adult-onset, and slowly progressive proximal weakness. There is no cognitive deficit, or neuron/ bone involvement in these cases. Histopathology showed rimmed vacuoles in atrophic fibers and isolated inclusion body myopathy-like findings [61]. Myosin binding protein C1–2 (*MYBPC1–2*) is expressed in skeletal muscle and *MYBPC3* in cardiac muscle. A homozygous truncating mutation of the MYBPC3 gene was reported in an infant with skeletal myopathy and cardiomyopathy. However, it has been shown that muscle pathology occurs with the dominant-negative effect of ectopic expression of mutant MYBPC3 [62].

Homozygous frameshift mutations of contactin-1 (*CNTN1*) gene have been associated with severe lethal congenital myopathy with fetal akinesia and nonspecific myopathic changes in muscle [63]. 3-hydroxy acyl-CoA dehydratase 1 (*HACD1*) plays a role in the biosynthesis of very long-chain fatty acids in skeletal and cardiac muscles. Homozygous nonsense mutations and homozygous long interspersed nuclear element insertions have been reported in a small number of patients with CMs. The common feature of these cases is that it presents with severe neonatal weakness, which improves over time, and biopsy shows type 1 fiber hypotrophy, type 1 fiber predominancy, and rare internal nuclei [64]. A homozygous nonsense mutation of beta-IV-spectrin (*SPTBN4*) was reported in a rare form of CM with neuropathy and deafness [65]. In GTPase (*HRAS*) defects, CM with excess muscle spindles was reported. It has a severe clinical picture with severe neonatal hypotonia, contractures, hyperlaxity of the distal joints, respiratory insufficiency, and feeding problems [66].

AR mutations of the myomaker (*MYMK*) gene cause Carey-Fineman-Ziter syndrome with non-progressive CM and facial weakness, additional dysmorphic features, and growth retardation. While the number of muscle fibers decreases due to defective myoblast fusion, compensatory hypertrophy is observed in both types [67]. SH3 and cysteine-rich domain 3 (*STAC3*) are components of excitation-contraction coupling. Recessive mutations cause variable phenotypes from severe neonatal hypotonia to slowly progressive and mild hypotonia. They also cause MHS [68]. Recessive mutations of the fragile X related 1 (*FXR1*) gene in exon 15 have been reported with various clinical presentations from neonatal hypotonia to milder presentations. Muscle biopsies have shown atrophy, type 1 fiber predominancy, multicores, and central nuclei [69].Bi-allelic variants of the transcription factor *PAX7* have been reported as a new genetic cause of myopathy with a different severity, presenting with weakness, ptosis, muscle atrophy, and scoliosis. Muscle atrophy and fibroadipose tissue replacement are detected, but necrosis and muscle fiber instability are absent. Progressive muscle wasting occurs as the survival and regeneration of satellite cells are affected [70]. Homozygous or compound heterozygous mutations in the *SCN4A* gene, causing a loss of function of skeletal muscle voltage-gated sodium channel α-subunits $Na_v1.4$, have been defined as the cause of fetal hypokinesia, severe neonatal hypotonia, and

CM, showing improvement in symptoms over time [71]. Mutations of pyridine nucleotide-disulfide oxidoreductase domain 1 (*PYROXD1*) were reported in cases with clinical early-onset myopathy. During biopsy, fiber size variation, internalized nuclei, fibrosis, core/central nuclei, and myofibrillar irregularities were noted [72]. A frameshift mutation affecting the stop codon in the transportin3 (*TNPO3*) gene has been identified in cases with myopathic abnormalities in biopsy, and slowly progressive proximal axial weakness. As a result of a transcript with an elongated C-terminal component, an accumulation of the protein is observed in the subsarcolemmal and perinuclear areas [73]. Although mucolipidosis type IV, caused by mucolipin1(*MCOLN1*) defects,is a disorder with corneal involvement, and central nervous system involvement, a diagnosis of lysosomal storage disease should be considered in cases with unidentified CK elevation and myopathy [74]. In fast skeletal troponin C (*TNNC2*) gene defects, severe congenital weakness, and respiratory insufficiency occur, and facial weakness, ptosis, and contractures may accompany them. Unlike severe CMs, patients can regain the ability to walk over time and the need for respiratory support may decrease. Myofiber atrophy or ultra-structural variants are not causes of weakness. The calcium sensitivity required for power production is decreased in sarcomeres [75]. Recessive mutations of the myosin light chain 1 (*MYL1*) gene have been reported in cases with severe CM. There is a different form of type 2 fiber atrophy/hypotrophy in biopsy [76]. A variant of the calcium voltage-gated channel subunit alpha1 H (*CACNA1H*) has been reported in a case of infantile-onset severe amyoplasia. Loop mutations, which connect intracellular I–II and II–III domains, cause congenital amyotrophy by affecting the differentiation of myoblasts at an early stage [77].

In addition, although CMs cause muscle-limited pictures, sometimes muscle involvement of various multisystem diseases may demonstrate symptoms and histopathology features similar to those of CMs. Marinesco-Sjögren syndrome (*SIL1* mutations; ataxia, cataracts, microcephaly, myopathy), King-Denborough syndrome (*RYR1* mutations; MHS, dysmorphic features, myopathy, central nuclei/cores on muscle biopsy), Stormorken syndrome (*STIM1* mutations; ichthyosis, thrombocytopathy, myopathy, tubular aggregates on muscle biopsy), Snyder-Robinson syndrome (*SMS* mutations; osteoporosis, skeletal abnormalities, myopathy), Shwachman-Bodian-Diamond syndrome (bone marrow dysfunction, pancreatic insufficiency, hypotonia), and Freeman-Sheldon syndrome (*MYH3* mutations; dysmorphic features, contractures, FTD on muscle biopsy) can be counted among the multisystemic diseases in which myopathy is also present[48].

Case 13.11

An 8-year-old boy who was born to consanguineous parents presented with frequent falls and difficulty in walking/climbing. Neurological examination showed myopathic facies (facial weakness and long-narrow face), nasal speech, dysphagia, axial/proximal muscle weakness, distal laxity, kyphoscoliosis, and reduced muscle bulk (Fig. 13.9). His CK levels were normal (41 IU/L). Nerve conduction studies revealed axonal neuropathy. Muscle biopsy shows severe fatty infiltration of the perimysium, variations in fiber size with a predominance of atrophic fibers, and internal nuclei in most fibers. WES analysis showed two PYROXD1 variants: c.464A > G and c.329_332delTCTG.

Case 13.12

A 12-year-old girl who was born to consanguineous parents was admitted to hospital with newly onset, slowly progressive muscle weakness, and reduced muscle bulk. On examination, generalized muscle atrophy, symmetrical proximal/axial muscle weakness, scapular winging, mild facial weakness, nasal speech, and absent deep tendon reflexes were found. Her CK levels were normal (75 IU/L). Nerve conduction studies revealed normal findings. However, needle EMG identifed myopathic changes. Muscle biopsy showed non-inflammatory myopathic features (Fig. 13.10). WES analysis revealed a pathogenic homozygous mutation in the *PYROXD1* gene, c.464A > G, p.Asn155Ser.

These two patients (Cases 13.11 and 13.12) were diagnosed with CM related to *PYROXD1*. Both cases were reported in the literature [68].

Case 13.13

A male infant, born at 38-weeks-gestation via cesarean section from consanguineous parents, was taken to the neonatal intensive care unit because of severe generalized weakness and respiratory insufficiency. On examination, severe hypotonia, poor sucking, ophthalmoplegia, and contractures were noted (Fig. 13.11). He died at the age of 3 months due to respiratory problems. During histopathological evaluation of the muscle biopsy, mild dystrophic changes such as contraction, regeneration, degeneration, nuclear internalization, and fibrosis were visible. In addition, many pathological immature myofibers were visualized using neonatal myosin staining. Based on immunostaining, dystrophin, merosin, and sarcoglycans were present at normal levels. Interestingly, there were several huge type 1 fibers, which are specific for infantile denervation (Fig. 13.12). WES analysis revealed a homozygous missense variant in the *CACNA1S* gene, c.2366G > A, p.Arg789His.

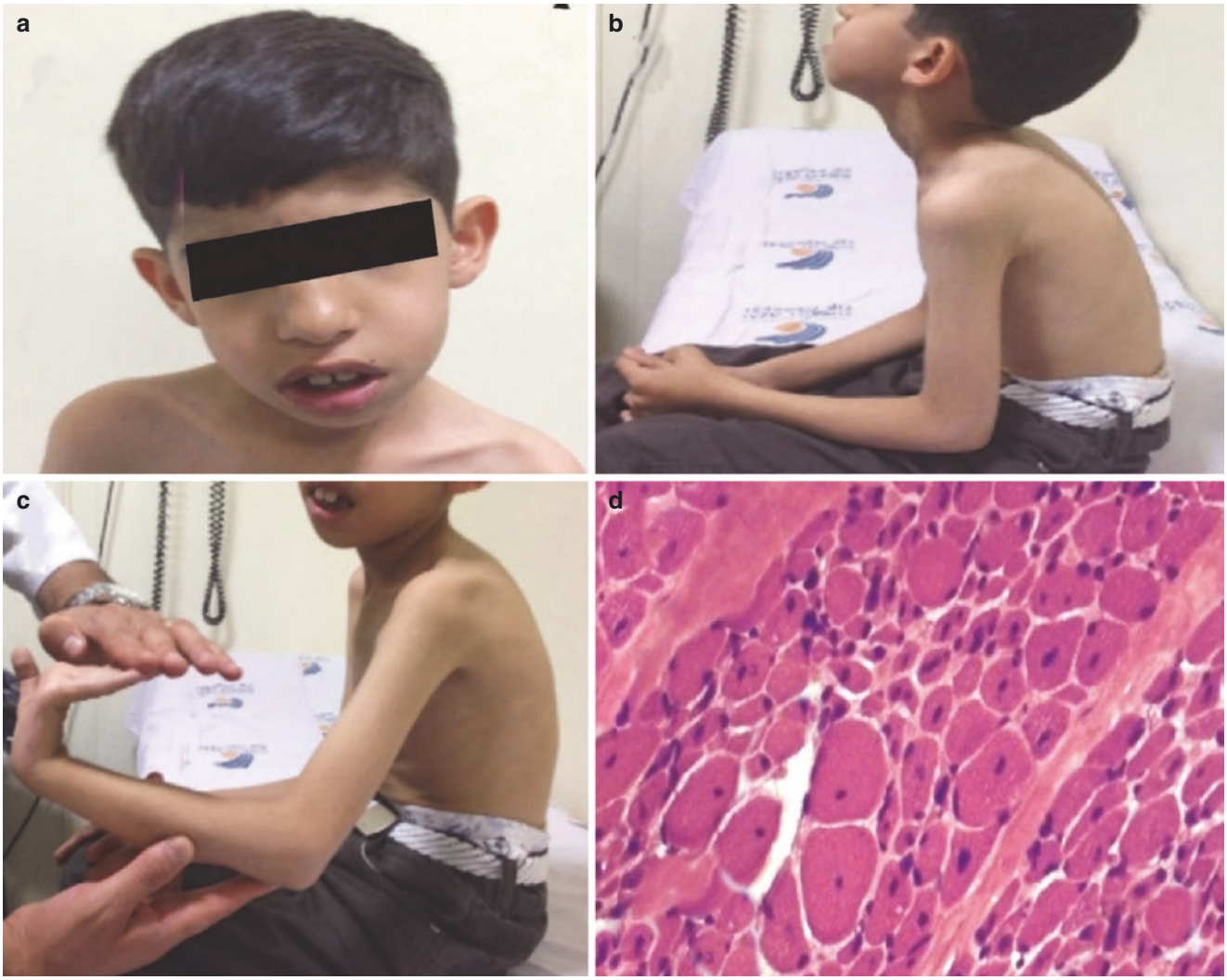

Fig. 13.9 Phenotypic features of Case 11 with *PYROXD1* mutation: (**a**) Long myopathic face and facial weakness, (**b**) Muscle atrophy of shoulder and neck muscles, (**c**) Distal joint laxity, (**d**) Note the huge differences of myofiber sizes (HE ×100)

Case 13.14

A female infant was taken to the neonatal intensive care unit because of severe hypotonia and the need of mechanical ventilation. Her examination revealed severe generalized muscle weakness, distal contractures, absent sucking, and absent deep tendon reflexes. Nerve conduction studies were normal and needle EMG showed myopathic motor unit potentials. Serum CK levels were normal. She was discharged with a tracheostomy and NG feeding tube (Fig. 13.13).During histopathological evaluation of the muscle biopsy, there was a marked variation in fiber size and shape. There was also increased nuclear internalization. There were no myofibrillary irregularities identified with modified trichrome or NADH-TR enzyme staining. Based on immunostaining, dystrophin, merosin, and sarcoglycans were present at normal levels. There were also grouping fascicules of large and small myofibers, which are specific for neuropathies (Fig. 13.14). Neurological examination revealed motor delay with generalized weakness, facial weakness with ophthalmoplegia, scoliosis, and pes equinus deformity at the age of 5. WES analysis revealed a homozygous missense variant in the *CACNA1S* gene, c.2366G > A, p.Arg789His. These two patients (Cases 13.5 and 13.6) were diagnosed with CM with ophthalmoplegia related to *CACNA1S*. These patients were reported in the literature[78].

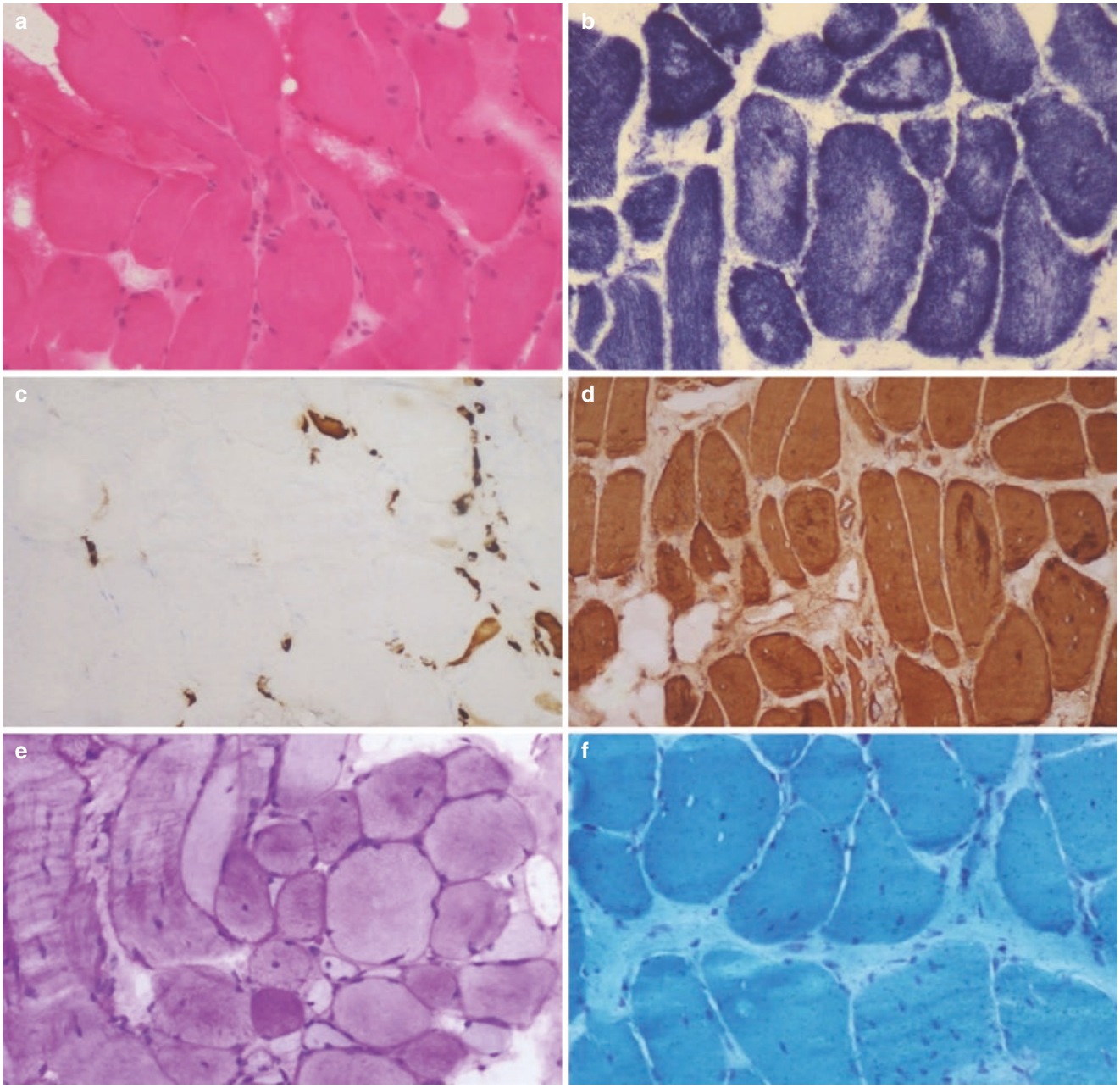

Fig. 13.10 Muscle biopsy of Case 13.12 with *PYROXD1* mutation: (**a**) Muscle biopsy shows variation in fiber size, multiple internalized nuclei, and mildly increased fibrous connective tissue (HE ×200), (**b**) Conspicuous central core formations and myofibrillar irregularity (NADH-TR ×400), (**c**) There are a lot of immature atrophic fibers expressed with neonatal myosin (DAB ×200), (**d**) There are a few central inclusions highly immunoreactive to myotilin (DAB ×200), (**e**) Increased numbers of central nuclei (PAS ×200), (**f**) Note the increased connective tissue. (Modified Trichrome ×200)

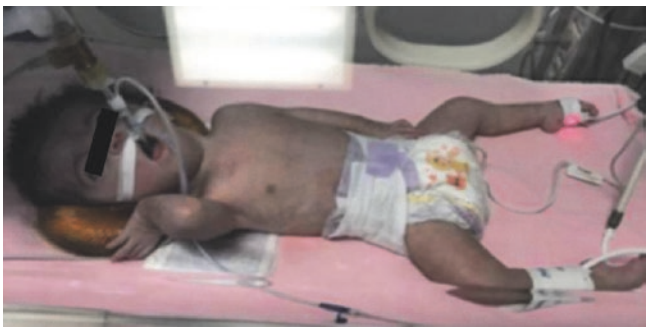

Fig. 13.11 Phenotypic features of Case 13.13 with *CACNA1S* mutation

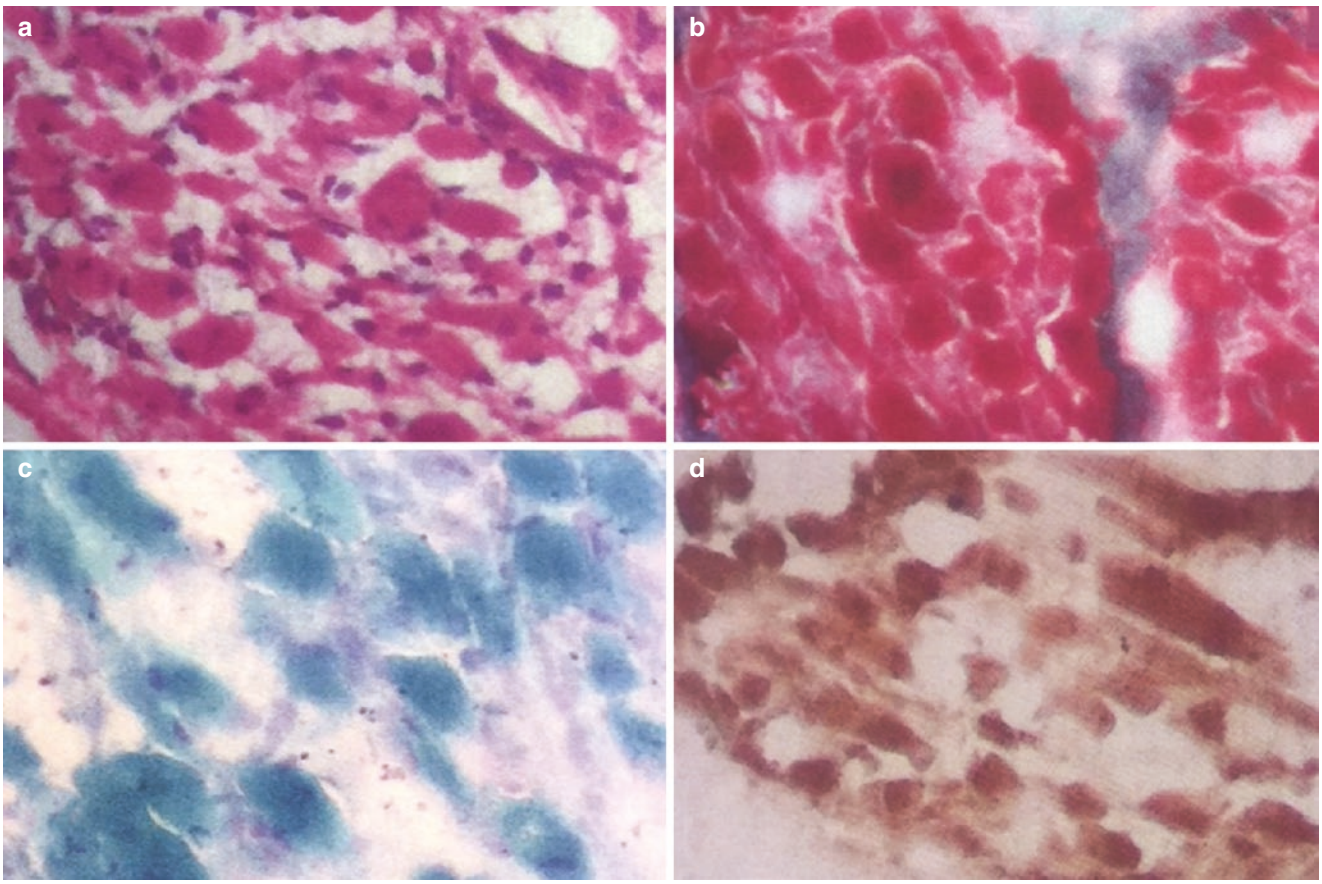

Fig. 13.12 Muscle biopsy of Case 13.13: (**a**) Note the marked variation in fiber size and shape (HE ×400), (**b**) There is marked fibrosis (Gomori Trichrome ×400), (**c**) There are no myofibrillary irregularities or rods (Modified Trichrome ×400), (**d**) Normal mitochondrial function (Combined COX- SDH ×400)

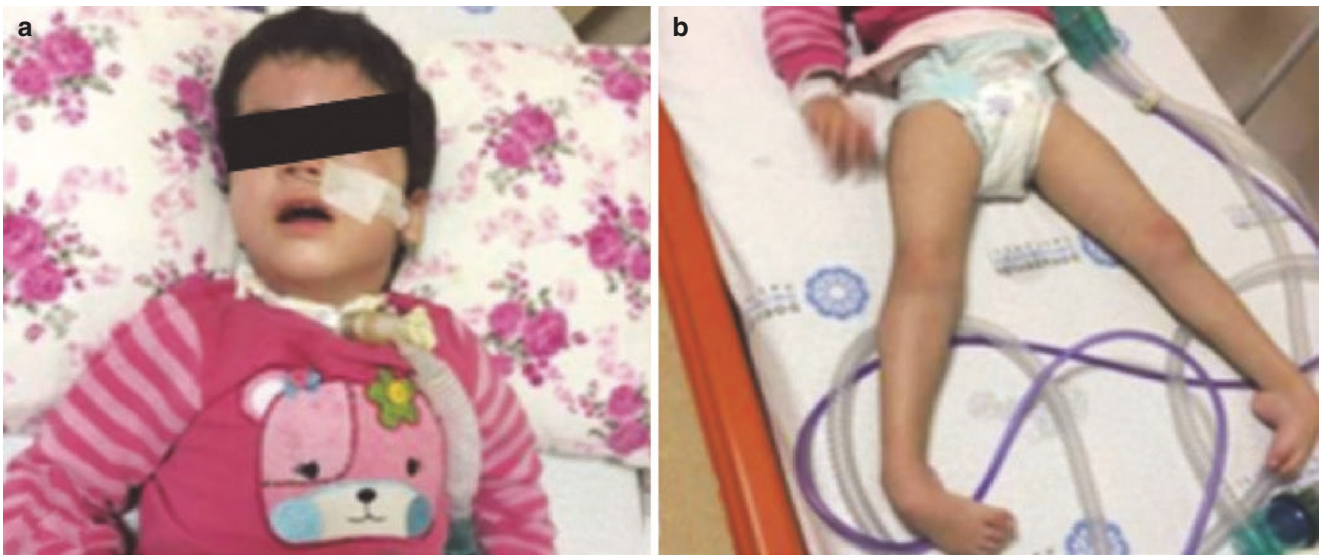

Fig. 13.13 Phenotypic features of Case 13.14 with CACNA1S mutation. (**a**) The patient need tracheostomy for ventialtion and nasogastric tube for feeding (**b**) Muscle weakness and distal contractures

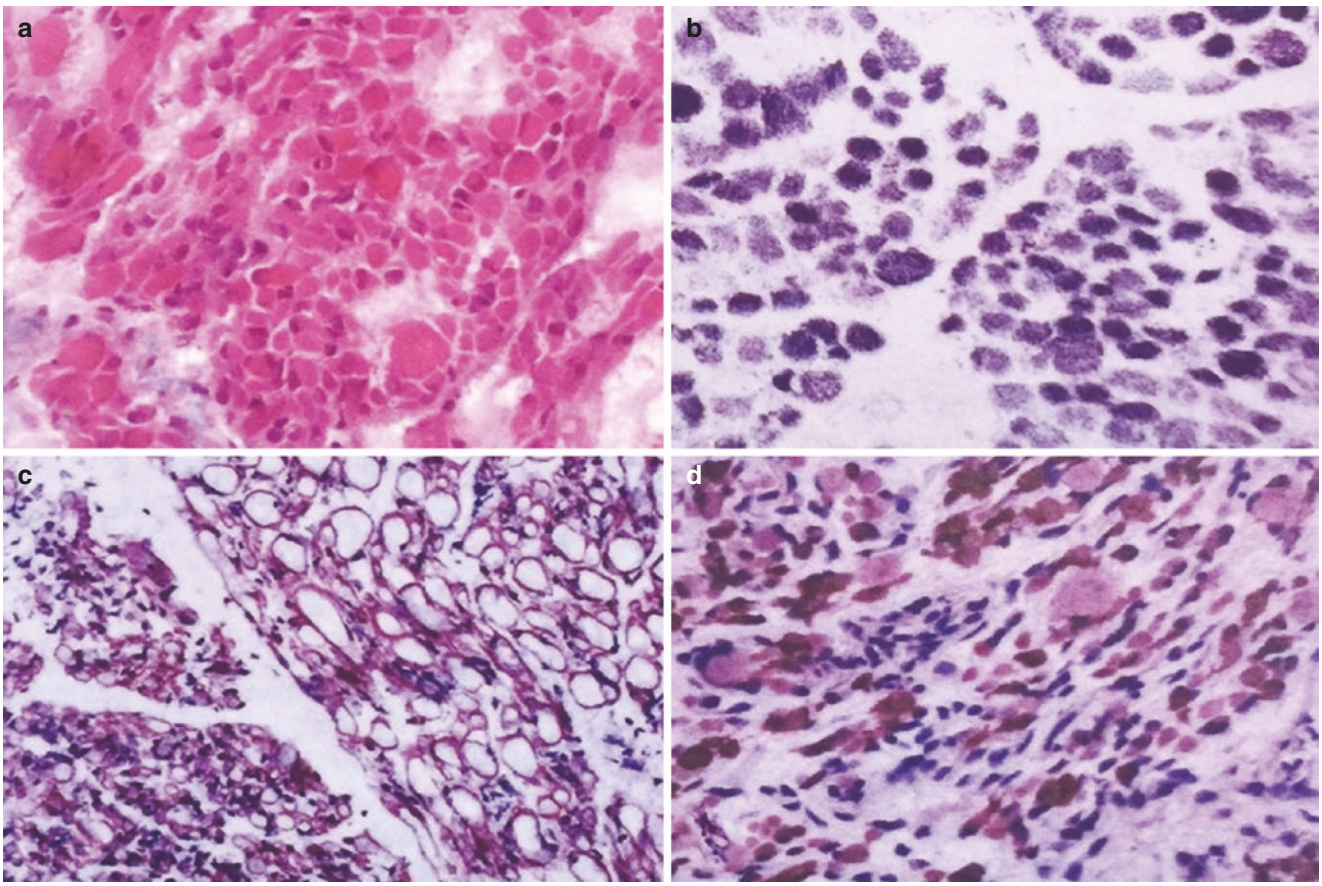

Fig. 13.14 (**a**) Note the marked variation in fiber size and shape (HE ×200), (**b**) There is no marked myofibrillary irregularity (NADH-TR ×200), (**c**) Normal sarcolemmal expression of merosin as well as grouping of large and small myofiber fascicles (DAB ×200), (**d**) Note the presence of huge type 1 fibers with fast myosin antibody (DAB ×100)

All About the Pathology of Congenital Myopathies

CMs are a group of hereditary muscle diseases that typically present at birth or in early infancy. There are multiple modes of inheritance and degrees of severity, which range from a lethal course in the newborn period to milder diseases in older children. Classically, they are defined by skeletal muscle dysfunction, which leads to hypotonia and skeletal muscle weakness. In addition, patients with CMs can have dysmorphic features, a typical facial appearance, impaired growth, and mental retardation, while in other NMDs such as muscular dystrophies or hereditary peripheral neuropathies, patients are generally of a normal phenotype at birth. Histopathologically, CMs are characterized a non-dystrophic muscle appearance with the presence of one or more pathognomonic histological features. Mutations in multiple different genes can cause the same pathology, while mutations in the same gene can cause multiple different pathologies. This has become ever more apparent now with the increasing use of NGS, and so a genetic diagnosis is achieved for a greater number of patients.Thus, the pathophysiological mechanisms underlying CMs are now better understood [79]. However, at the same time, considerable genetic and pathological overlap has emerged, blurring the classically established boundaries. Therefore, histopathological evaluation may become more necessary. For example, a 38-year-old woman was admitted to the hospital with muscle weakness. Her CK levels were normal. Muscle biopsy revealed that type 1 fiber predominance was present, and almost all of the type 1 fibers were small in size (Fig. 13.15). The presence of internal nuclei was mildly increased in both fiber types. A CMFTD diagnosis was based on histopathological findings, but during genetic analyses, two heterozygous variants were detected. One of them was a c.1941 + 8 T > C variant on the *SCN9A* gene, and the other was a c.737C > T p.Ser246Leu variant on the *TPM3* gene. Both variants have been associated with NMs. However, nemaline rods were not seen in the muscle biopsy.

The most important histopathological feature of CMs is the absence of regenerated fibers, which is the hallmark of dystrophies. This is because in the etiopathogenesis of MDs, there is a cycle of cell degeneration, necrosis, and regeneration after severe sarcolemmal damage. If repeated biopsies are performed at intervals of several months or years, the observed picture demonstrates an eventual loss of muscle fibers and their replacement by fibroadipose tissue. Finally, the biopsy will show the appearance of end-stage muscle. However, defects in different components of the sarcomere, which lead the loss of function in CMs, do not cause necrosis of myofibers. Although there are signs of muscle damage such as size and shape differences of myofibers, atrophic/hypotrophic fibers, nuclear internalization, and myofiber type changes, necrosis and regeneration are not observed.

The basic histopathologic findings in CMs are prominent size differences in mostly circular-shaped fibers (Fig. 13.16). There is often an increase in the rate of nuclear internalization, which is a marker of muscle injury. However, in CNMs, almost all myofibers have internal nuclei (Fig. 13.17). Another histopathological feature of CMs is fiber-type disproportion (FTD). This term is used to describe the myofiber hypotrophy, which alters myofiber type (Fig. 13.18). In CFTD, the main diagnostic finding is that the diameters of type 1 fibers are smaller than type 2 fibers [1, 4] However, since these findings can be seen in other CMs, they should be detected without any other histopathological findings to diagnose CFTD. In addition, this feature is also important in

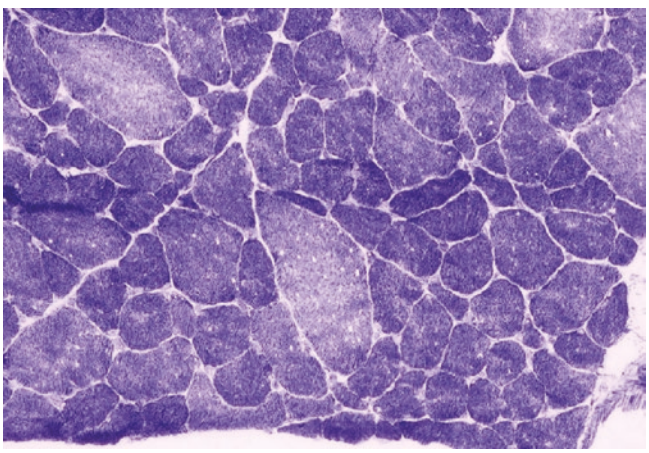

Fig. 13.15 Note the prominence of smaller type 1 fibers (NADH-TR ×200)

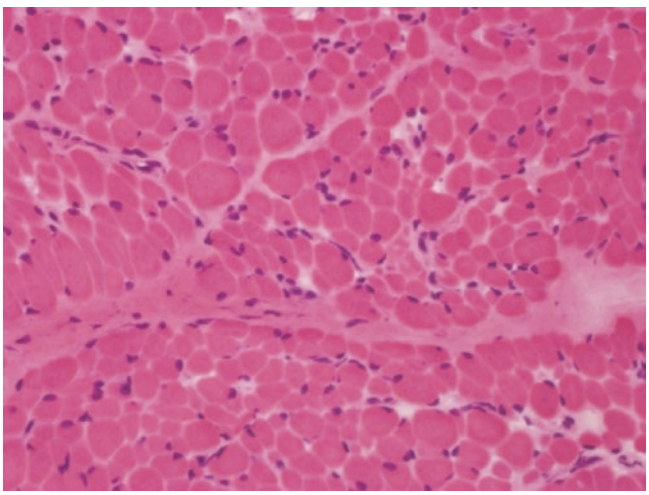

Fig. 13.16 Note the prominent variation of myofiber size in a CM (HE ×100)

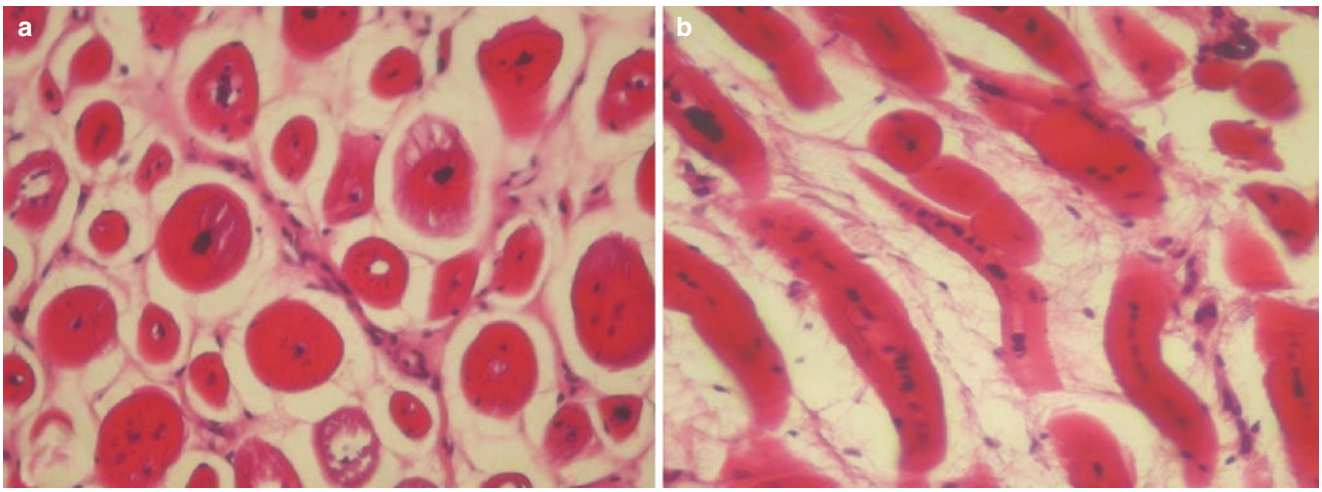

Fig. 13.17 (**a**) In the transverse section, there are centrally placed nuclei with some vacuoles, (**b**) In the longitudinal section, many internal/central nuclei are noticed (HE ×100)

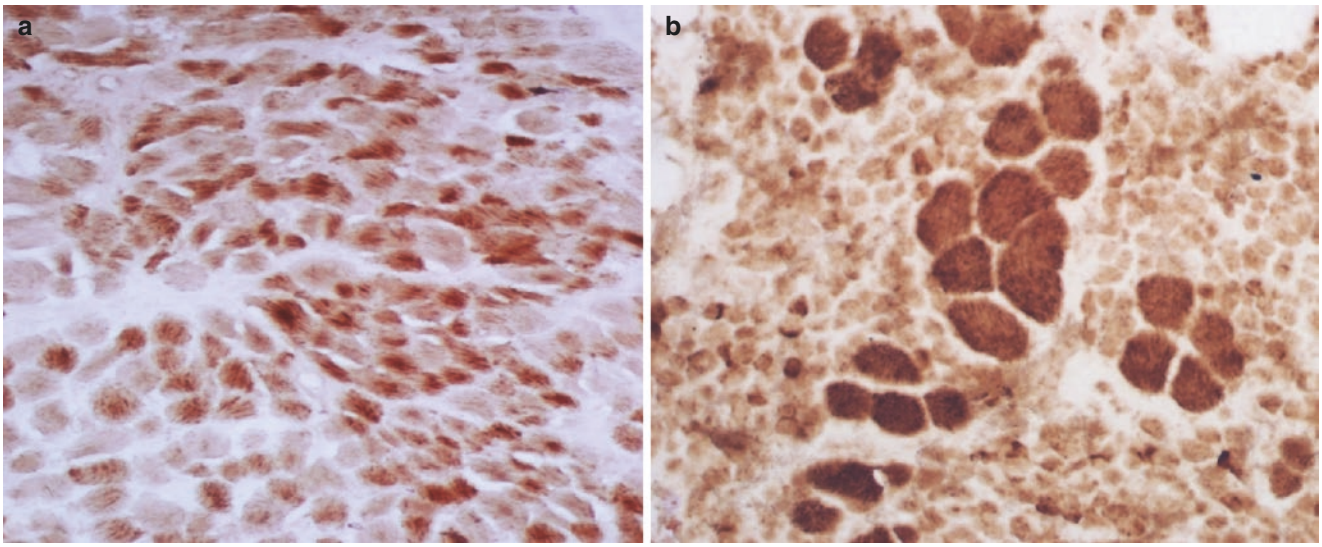

Fig. 13.18 (**a**) Type 1 fibers are smaller in size (COX ×200), (**b**) Type 1 fibers are larger in size (COX ×200)

distinguishing CFTD from infantile spinal muscular atrophy (SMA), which can be clinically confused with CMs, where hypertrophic type 1 fibers are usually observed [4, 43].

Interstitial tissue is mildly increased in CMs, and especially in the later phases, very prominent fibrosis occurs in muscular dystrophies, including the CMDs (Fig. 13.19). In the biopsies of patients with CNM, there can be peculiar sarcoplasmic alterations, which resemble a necklace. These myofibers are called "necklace fibers", which have internalized nuclei aligned in a basophilic ring (necklace) at 3 microns beneath the sarcolemma (Fig. 13.20). Ultrastructurally, it has been demonstrated that the necklace consists of myofibrils of a smaller diameter, in an oblique orientation, surrounded by mitochondria, sarcoplasmic reticulum, and glycogen granules [80].

In addition, the histopathological features that appear in CMs are in most cases indistinguishable with sharp boundaries and they may develop into different patterns over time (Fig. 13.21). For example, two muscle biopsy features were very different in a patient with genetically confirmed MTM. Especially in CNMs, the muscle's nuclei are located peripherally during the infantile period, as they are in normal skeletal muscle, and they shift towards the center during the course of myopathy. In fact, the average age of diagnosis in CNM cases is reported to be around 4 years in the literature because biopsies performed at an earlier age are generally not diagnostic. For this reason, a definitive diagnosis of CMs, especially CNMs, can sometimes be determined by examining biopsy material repeatedly in the following years [41].

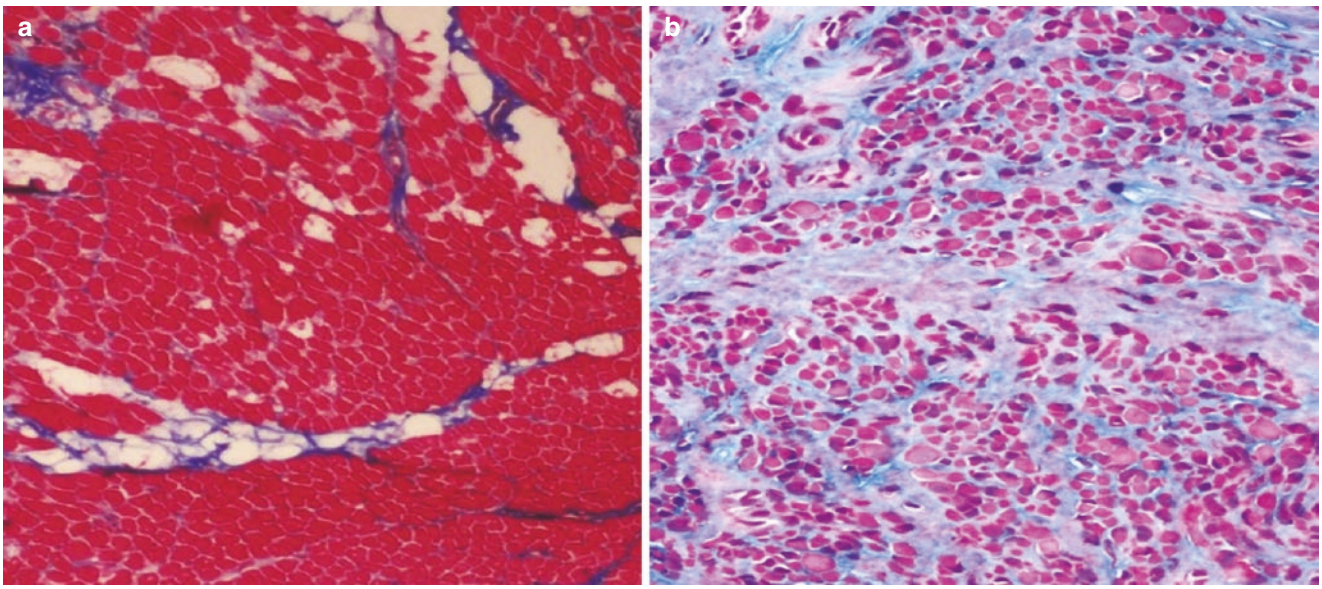

Fig. 13.19 (**a**) Mildly increased interstitial fibrosis (Masson trichrome ×100), (**b**) Prominent interstitial fibrosis in a patient with CMD (Masson trichrome ×100)

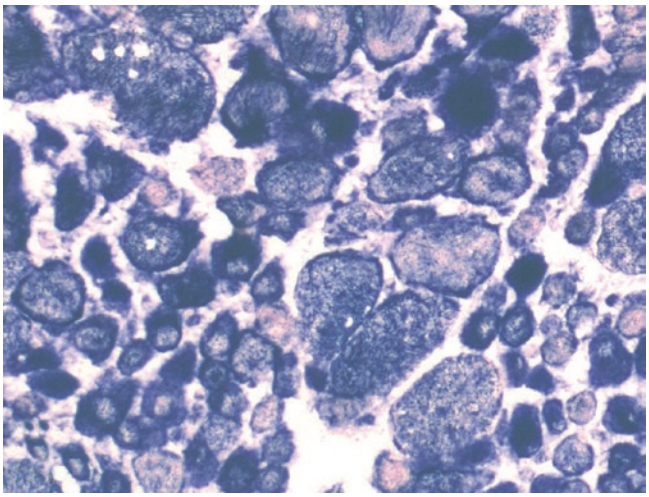

Fig. 13.20 Necklace fibers (NADH-TR ×200)

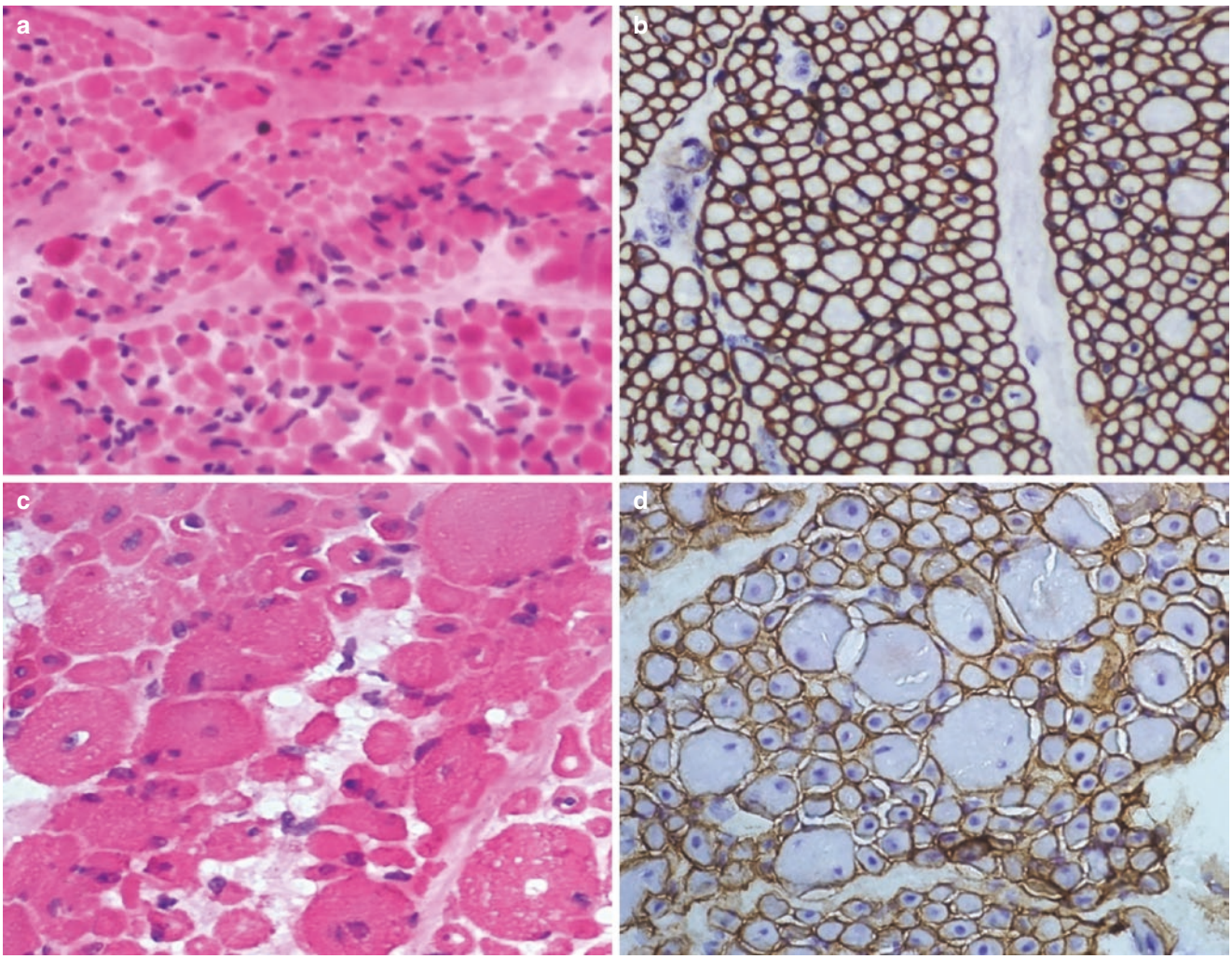

Fig. 13.21 The first biopsy of the patient shows myopathic features such as myofiber shape and size differences and mildly increased amounts of internal nuclei: (**a**) HE ×200, (**b**) Merosin positivity (DAB ×200). The second biopsy performed 2 years later shows huge size differences and prominent increased internal nuclei: (**c**) HE ×200, (**d**) DAB ×200

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