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Update on the Genetics of Congenital Myopathies

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The congenital myopathies form a large clinically and genetically heterogeneous group of disorders. Currently mutations in at least 27 different genes have been reported to cause a congenital myopathy, but the number is expected to increase due to the accelerated use of next-generation sequencing methods. There is substantial overlap between the causative genes and the clinical and histopathologic features of the congenital myopathies. The mode of inheritance can be autosomal recessive, autosomal dominant or X-linked. Both dominant and recessive mutations in the same gene can cause a similar disease phenotype, and the same clinical phenotype can also be caused by mutations in different genes. Clear genotype-phenotype correlations are few and far between.
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Introduction

The application of next-generation sequencing methods, such as whole-exome sequencing, targeted gene panels, and whole-genome sequencing has resulted in an accelerated discovery of novel disease genes and disease-causing variants underlying the various types of congenital myopathies. Furthermore, the use of custom high-density oligonucleotide arrays for comparative genomic hybridization has enabled the discovery of large copy number variations (CNVs) causing, for example, nemaline myopathy and centronuclear myopathy.[1–3]

The inheritance of congenital myopathies can be autosomal dominant, autosomal recessive or X-linked. De novo dominant disease-causing variants are common in some genes, for example, *ACTA1* and *TPM2*.[4,5] Both dominant and recessive variants have been described in several genes, for example, *ACTA1, TPM2, TPM3, RYR1, MYH2*, and *TTN*.[4–8] Interestingly, epigenetic silencing of a wild-type allele can result in monoallelic expression of a mutant allele causing a congenital myopathy. This has been described for *RYR1* and core myopathies.[9] Furthermore, it has been suggested that a common pathophysiological pathway caused by epigenetic changes is activated in some forms of congenital myopathies.[10]

Mutations in the same gene can result in more than 1 clinical phenotype, and the same clinical phenotype can result from mutations in several different genes.[11] There is also substantial variation in the severity of the clinical phenotype, even within 1 genetic entity, seldom with any discernible genotype-phenotype correlations.[12]

Nemaline Myopathies Including Cap Myopathy and Fiber-Type Disproportion

The clinical spectrum of nemaline myopathies (NM) is wide, ranging from severe congenital forms, sometimes already detectable in utero, through the typical form to milder childhood-onset and even adult-onset forms. Nemaline rods, derived from sarcomeric Z discs, and often type 1 fiber predominance, are characteristic pathological features of NM. Cap myopathy is pathologically characterised by cap-like structures of disorganised myofibrils and thickened Z discs, but usually no large rods.[11] Following the description of families and patients with variable presence of nemaline rods and/or caps,[13,14] NM and cap myopathy are considered to be overlapping entities. Fiber-type disproportion (FTD), that is, type 1 hypotrophy in the

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presence of larger type 2 fibers, but in the absence of specific pathological features, may be caused by the same genes as NM and cap myopathy.[11] FTD and type 1 fiber predominance are common features in the other congenital myopathies also, caused by mutations in other genes.

Eleven NM-causing genes have been described to date[15–25] (Fig., Table). Seven of these genes, that is, *ACTA1, NEB, TPM2, TPM3, TNNT1, LMOD3,* and *MYPN*, encode structural proteins of the skeletal muscle sarcomere, *CFL2* regulates actin filament dynamics and is essential for muscle maintenance, whereas three of the genes, that is, *KBTBD13, KLHL40,* and *KLHL41*, encode proteins involved in the maintenance of sarcomeric integrity by regulating turnover of sarcomeric proteins.

The Nebulin Gene (*NEB*)

Disease-causing variants in the nebulin gene (*NEB*) are the most common cause of autosomal recessive NM, accounting for approximately 50% of all NM cases, and the most common cause of the typical form. The majority of the patients are compound heterozygous for 2 different *NEB* mutations. Point mutations causing aberrant splicing, small indels causing frameshifts, and nonsense mutations are the most common mutation types in *NEB*.[12] A custom high-density oligonucleotide array, the NM-CGH array, has revealed several large, 1-143 kb, CNVs in *NEB*, including recurrent CNVs in the triplicate region spanning exons 82-105.[1,26] Eight exons are repeated 3 times in the 32-kb triplicate (TRI) region of *NEB*, and the normal copy number is 6 (3 copies in each allele). Deletion or duplication of one TRI copy is nonpathogenic, but gains of 2 or more TRI copies segregate with NM in 4% of the families studied, and are, thus, interpreted to be pathogenic. The CNVs in the TRI region of *NEB* can currently be detected only using the NM-CGH-array.[26] We have estimated that a large pathogenic CNV in *NEB* is present in 10%-15% of NM patients.

Missense variants are very common in *NEB*. In the current release of the ExAC Browser (http://exac.broadinstitute.org), 63% of the variants in the coding region (including splice sites and UTRs) of *NEB* are missense, 24% are synonymous changes, and 4% are apparent pathogenic variants (nonsense, splice site, frameshift, indels). Most of the missense variants are rare, 76% of the variants being present in only 1-3 heterozygous carriers (allele frequencies well below 0.01). This makes the interpretation of the pathogenicity of missense variants extremely difficult. Our current recommendation is that only variants affecting conserved actin- and tropomyosin-binding sites in *NEB* can readily be considered as pathogenic, but all the others require functional studies for assessment of their pathogenicity. Actin- and tropomyosin-binding experiments may be used for this purpose.[27]

In addition to the "classical" forms of NM, recessive disease-causing variants in *NEB* may cause distal nebulin myopathy without nemaline rods,[28] core-rod myopathy,[29] distal forms of NM,[30] and lethal multiple pterygium syndrome.[31]

To date, only 1 clearly dominant *NEB* variant has been found. It is a ~100 kb in-frame deletion spanning *NEB* exons 14-89 resulting in the expression of substantially smaller nebulin proteins, expected to have a dominant-negative effect. This variant segregates with a distal form of NM in a 3-generation Finnish family.

The Skeletal Muscle Alpha-Actin Gene (*ACTA1*)

According to our estimate, 23% of NM cases are caused by mutations in *ACTA1*. Most of the pathogenic variants in *ACTA1* are dominant (90%) missense variants, most often causing severe NM. Of the sporadic cases with *ACTA1* variants, approximately 85% have been shown to be caused by de novo missense variants. Autosomal recessive variants are rarer (10%), and result in null alleles (splice site, nonsense, frameshift, and some missense variants).[32] Dominant variants inherited across

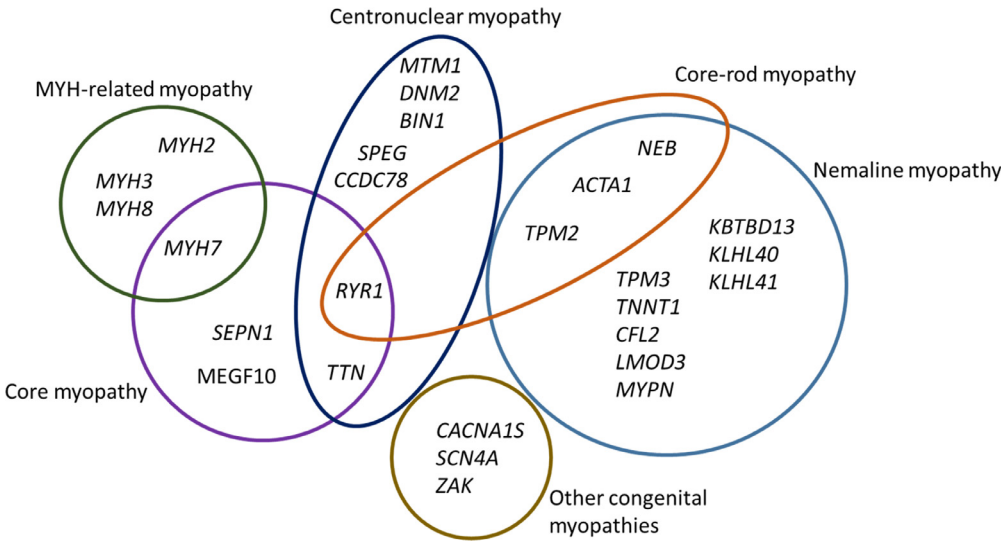

Figure Congenital myopathy-causing genes. The diagram shows 27 genes implicated in various forms of the congenital myopathies, and the overlap between different entities. Core-rod myopathy was included to illustrate the overlap between nemaline myopathy and core myopathy.

Table Genes Causing Congenital Myopathies

Disorder	Gene	Inheritance	Other Entities Caused by Mutations in the Gene
Nemaline myopathy	*NEB*	AR, AD*	Distal nebulin myopathy, distal nemaline myopathy, core-rod myopathy, lethal multiple pterygium syndrome
	ACTA1	de novo, AD, AR	Actin-accumulation myopathy, core-rod myopathy, intranuclear rod myopathy, zebra body myopathy, CFTD, progressive scapuloperoneal myopathy, distal nemaline myopathy
	TPM3	AD, de novo, AR	CFTD
	TPM2	AD, de novo, AR†	CFTD, core-rod myopathy, distal arthrogryposis, Escobar syndrome (AR)
	TNNT1	AR	
	CFL2	AR	
	LMOD3	AR	
	MYPN	AR	Cardiomyopathy
	KBTBD13	AD	
	KLHL40	AR	
	KLHL41	AR	
Core myopathy	*RYR1*	AD, AR	Core-rod myopathy, CFTD, malignant hyperthermia, multi-minicore disease with ophthalmoplegia, arthrogryposis multiplex congenita
	SEPN1	AR	Rigid spine muscular dystrophy, CFTD, desmin-related myopathy with Mallory body-like inclusions, myofibrillar myopathy
	TTN	AR	TMD, LGMD2J, HMERF, adult-onset recessive proximal muscular dystrophy, Emery-Dreifuss-like phenotype without cardiomyopathy, cardiomyopathy
	MYH7	AD	Laing distal myopathy, CFTD, myosin storage myopathy (hyaline body myopathy), cardiomyopathy
	MEGF10	AR	
Centronuclear myopathy	*MTM1*	X-linked	
	DNM2	AD	CMTDIB, CMT2M
	RYR1	AR	See above
	BIN1	AR, AD	
	TTN	AR	See above
	SPEG	AR	
	CCDC78	AD	
MYH-related myopathy	*MYH2*	AD, AR	
	MYH7	AD	See above
	MYH3	AD	Distal arthrogryposis
	MYH8	AD	Distal arthrogryposis
Other congenital myopathies	*CACNA1S*	AD, AR	Hypokalemic periodic paralysis type 1, malignant hyperthermia
	SCN4A	AR	Hypokalemic periodic paralysis type 2, congenital myasthenic syndrome 16, myotonia congenita, paramyotonia congenita
	ZAK	AR	Split-foot malformation with mesoaxial polydactyly

AD, autosomal dominant; AR, autosomal recessive; CFTD, congenital fiber type disproportion; CMT, Charcot-Marie-Tooth neuropathy; HMERF, hereditary myopathy with early respiratory failure; LGMD, limb-girdle muscular dystrophy; TMD, tibial muscular dystrophy.

*Only 1 dominant *NEB* mutation has been identified to date.

†Only 1 recessive *TPM2* mutation has been identified to date.

2 or more generations have been identified in less than 5% of *ACTA1* families, while mosaicism has been observed in a few families.[32-34] We have recently described a dominant *ACTA1* missense variant segregating in a 3-generation family with clinically variable NM, illustrating the clinical and histological variability of NM between patients sharing the same mutation.

In addition to NM, dominant, mostly de novo, disease-causing variants in *ACTA1* can cause actin-accumulation myopathy,[35] cap myopathy,[36] congenital fiber type disproportion,[37] core-rod myopathy,[38] intranuclear rod myopathy,[39,40] zebra body myopathy,[41] progressive scapuloperoneal myopathy,[42] and distal myopathy with nemaline rods.[43]

The Alpha- and Beta-Tropomyosin Genes (*TPM3* and *TPM2*)

Mutations in *TPM3* and *TPM2* are relatively rare causes of NM, accounting for less than 10% of the cases. In addition to NM, mutations in *TPM3* and *TPM2* can cause cap myopathy, core-rod myopathy, congenital fiber type disproportion, distal

arthrogryposes, and Escobar syndrome.[5] The majority of the mutations in both *TPM3* and *TPM2* are dominant missense variants or in-frame deletions removing one amino acid. A few recurrent mutations have been described in both genes; p.Lys7del and p.Glu139del in *TPM2*, and p.Arg168His, p.Arg168Cys, and p.Arg168Gly in *TPM3*. The mutations alter the conserved coiled-coil structure of the tropomyosins, resulting in aberrant tropomyosin-actin-binding.[5,44]

Recessive mutations are more common in *TPM3* than in *TPM2*. In *TPM2* only 1 recessive homozygous nonsense mutation has been described, causing Escobar syndrome associated with NM.[45] In *TPM3*, a few recessive mutations, including nonsense, frameshift, and stop-lost mutations have been described.[46–49] NMs caused by mutations in *TPM2* usually have milder presentations than NMs caused by mutations in *TPM3*.[5] Recessive disease caused by mutations in these genes appears mostly to be severe. No clear correlation was found between the type of mutation and the clinical phenotype. Cap formation in the muscle biopsy may be seen in disorders caused by mutations in either gene, and type 1 fiber hypotrophy and predominance is common in both groups.[5] Furthermore, we have identified a large, 17-21 kb homozygous deletion that removes the promoter and the first 2 exons of *TPM3*, causing a severe form of NM.[2]

The Troponin T1 Gene (*TNNT1*)

The first mutation in *TNNT1*, a recessive nonsense mutation causing a severe form of NM with tremor in the first months of life and contractures in the Old Order Amish, was described almost 20 years ago.[18] Not until recently have a few other NM-causing mutations in *TNNT1* been identified, all showing recessive inheritance. Compound heterozygosity for a splice site mutation resulting in skipping of *TNNT1* exon 8, and an exon 14 deletion was identified in a Dutch patient with a similar clinical phenotype as in Amish NM.[50] A clinical phenotype similar to Amish NM was also observed in a Hispanic patient homozygous for a nonsense mutation (different from the Amish one) in *TNNT1*.[51] Homozygosity for a complex indel mutation in *TNNT1* causing premature truncation of the protein has been described in 9 unrelated Palestinian patients with a severe form of NM.[52]

The Cofilin-2 Gene (*CFL2*)

Recessive mutations in the *CFL2* gene are rare causes of NM. The first *CFL2* mutation was described in 2007. The homozygous missense mutation, p.Ala35Thr, was found to cause NM with some minicores in a large consanguineous family of Middle Eastern origin showing congenital onset, delayed milestones and no facial weakness or foot drop.[20] The second *CFL2* mutation was published in 2012. Again a homozygous missense mutation, in this case p.Val7Met, was found in 2 sisters of Iraqi Kurdish origin with axial and limb girdle weakness who were born to consanguineous parents. The sisters had NM with features of myofibrillar myopathy.[53] A third mutation described in *CFL2* is a homozygous 4 base pair deletion causing a frameshift, p.Lys34Glnfs*6. The mutation had caused a severe form of NM in a Saudi Arabian consanguineous family.[54]

The Leiomodin-3 Gene (*LMOD3*)

Recessive mutations in *LMOD3* have hitherto been described in 15 families with severe, often lethal forms of NM, which in some cases were associated with perinatal fractures.[24,55] Most of the mutations are nonsense or frameshift variants causing loss of leiomodin-3 protein expression. The patients were homozygous or compound heterozygous for the mutations.[24]

The Myopalladin Gene (*MYPN*)

Recessive mutations in *MYPN* have been described in four families with childhood or adult-onset mild NM, and in 2 families with congenital slowly progressive cap myopathy.[25,56] All *MYPN* mutations described to date are loss-of-function variants, either nonsense, frameshift or splice site variants, leading to no or very low expression of myopalladin in skeletal muscle. The patients are either homozygous or compound heterozygous for the mutations. Intranuclear rods, previously only associated with *ACTA1* mutations, were observed in the muscle biopsies of 2 of the patients with mild NM.[25] Interestingly, dominant *MYPN* mutations have been reported to cause dilated, familial hypertrophic or familial restrictive cardiomyopathy.[57–59] Contrary to the NM- and cap myopathy-causing mutations, the cardiomyopathy-causing *MYPN* mutations lead to the expression of mutant proteins with dominant-negative effects in cardiomyocytes.[58]

The Kelch Repeat- and BTB/POZ Domain-Containing Protein 13, the Kelch-Like 40 and the Kelch-Like 41 Genes (*KBTBD13, KLHL40*, and *KLHL41*)

KBTBD13, KLHL40, and *KLHL41* encode proteins of the Kelch superfamily including altogether 66 genes and 63 protein members.[60] KBTBD13 interacts with Cullin 3 ubiquitin ligase, and this interaction is required for the formation of a functional Cul3 RING ubiquitin ligase complex, which is involved in the ubiquitination of proteins destined for degradation.[61] Three different missense variants, p.Arg248Ser, p.Lys390Asn, and p.Arg408Cys in *KBTBD13* have been found to cause autosomal dominant NM with cores, and unusual clinical presentations including a characteristic slowness of movement.[21]

KLHL40 has been shown to bind and stabilize nebulin and LMOD3 in the sarcomere, as well as prevent ubiquitination of LMOD3.[62] Recessive mutations in *KLHL40* are a fairly common cause of severe NM, often with fetal akinesia or hypokinesia and contractures, fractures, respiratory failure, and swallowing difficulties at birth. Mutations in *KLHL40* account for up to 28% of severe cases of NM in the Japanese population due to a founder mutation, p.Glu528Lys.[22] One patient with a mild form of NM has been reported to be homozygous for a missense mutation, p.Arg500Cys, in *KLHL40*.[63] This mutation has not been found in the severe cases published to date.[22,64,65] Furthermore, one patient with severe NM due to compound heterozygous mutations in *KLHL40* showed prolonged beneficial response to treatment with high-dose acetylcholinesterase inhibitors (pyridostigmine).[66] Such a response

has also been observed in other congenital myopathies, for example, myotubular/centronuclear myopathy.[67]

KLHL41 shows high homology to KLHL40, but KLHL41 preferentially stabilizes nebulin rather than LMOD3.[68] Five families with clinically different forms of NM have been found to have recessive mutations in *KLHL41*. Frameshift mutations correlated with severe phenotypes with neonatal death, whereas missense variants resulted in impaired motor function with survival into late childhood and/or early adulthood compatible with mild, typical or intermediate NM.[23]

Core Myopathies

Central core disease, minicore myopathy, and multiminicore disease are historical definitions of congenital myopathies with cores, that is, areas devoid of mitochondria and, thus, lack of oxidative enzyme activity in muscle biopsies. There is pathologic, clinical and genetic overlap in congenital myopathies with cores, and thus the term "core myopathy" is nowadays preferentially used.[11] Five genes have been reported to cause core myopathies. The ryanodine receptor 1 encoding gene, *RYR1*, was the first one to be discovered,[69,70] and is now known as the major core myopathy-causing gene.[71] The second most common core myopathy-causing gene, *SEPN1* encodes selenoprotein N.[72] Occasional mutations causing core myopathies have also been described in the satellite cell gene *MEGF10*,[73] the titin gene *TTN*,[74] and the myosin heavy chain encoding gene *MYH7*[75] (Fig., Table).

The Ryanodine Receptor 1 Gene (*RYR1*)

RYR1 encodes the skeletal muscle specific ryanodine receptor RYR1, which is a calcium release channel involved in excitation-contraction coupling activating muscle contraction. Both dominant and recessive mutations in *RYR1* have been found to cause core myopathies, but also related disorders such as core-rod myopathy, congenital FTD and centronuclear myopathy, as well as malignant hyperthermia susceptibility.[6,76]

RYR1 is a large gene with 106 exons encoding a polypeptide of 5037 amino acids, which forms the subunits of the tetramer calcium release channel. More than 200 *RYR1* mutations have been reported.[77,78] Most mutations causing core myopathies and malignant hyperthermia are dominant missense variants changing conserved amino acids, many of them clustered in specific hotspot regions in the N-terminus, central region and in the C-terminal transmembrane region of RYR1.[71,76] De novo dominant *RYR1* variants have been reported to cause core-rod myopathy.[79-81]

Recessive *RYR1* mutations are widespread throughout the gene and patients with such mutations are generally more severely affected than those with a dominant mutation.[82,83] The recessive mutations include null mutations, but also combinations of missense variants. Among the recessive variants, one recurrent allele carrying 3 different missense variants (p.Ile1571Val, p.Arg3366His, and p.Tyr3933Cys) has been reported in the Dutch population, but it is unclear whether one of the variants is causative, or if a combination of 2 or all 3 variants cause disease. Furthermore, this 3 missense variant-carrying allele, as well as some other missense variants are associated with the malignant hyperthermia trait in heterozygous individuals, but cause recessive *RYR1*-related myopathies in homozygous or compound heterozygous individuals.[83]

In addition to variants affecting a single or a few base pairs, 2 recessive large-scale *RYR1* deletions associated with myopathies have been published.[84,85] The first one, an in-frame deletion of 54 out of 106 *RYR1* exons, was identified in a child with a congenital myopathy with lethal neonatal weakness and atypical histopathologic features. The child was compound heterozygous for the deletion and a single amino acid duplication.[84] The second large deletion starts in *RYR1* exon 91 and ends within exon 98, causing a frameshift. The deletion was detected in a family with recessive late-onset core myopathy, the patients being compound heterozygous for the deletion and a missense variant.[85] In addition, a recessive deletion of *RYR1* exons 70-71 has been described in a family with severe arthrogryposis multiplex congenita.[86]

Tissue-specific epigenetic silencing of the maternal *RYR1* allele has been documented in a cohort of patients with recessive core myopathies. Silencing of the maternal allele in skeletal muscle tissue unmasked the recessive paternal allele causing the disease.[9]

The Selenoprotein N Gene (*SEPN1*)

SEPN1 encodes selenoprotein N, which is an integral membrane glycoprotein of the endoplasmic reticulum. SEPN1 is expressed at high levels in several human fetal tissues, and is thought to have a role in early muscle development.[87] SEPN1 is physically associated with ryanodine receptors and modifies RYR channel activity.[88] Furthermore, it has recently been shown that SEPN1 is a key component of redox-regulated calcium metabolism in the endoplasmic reticulum, through its interaction with the SERCA2 calcium pump.[89]

Recessive loss-of-function mutations in *SEPN1* have caused entities termed rigid spine muscular dystrophy, core myopathy, congenital fiber type disproportion, and desmin-related myopathy with Mallory body-like inclusions.[72,90,91] Due to the overlap of clinical and histopathologic features these disorders are now collectively referred to as SEPN1-related myopathies. All types of mutations have been identified in *SEPN1*, many being truncating nonsense or frameshift variants, but missense variants affecting conserved amino acids are also common. Homozygous mutations seem to be surprisingly prevalent, also in affected children born to non-consanguineous parents.[92,93]

The Multiple EGF-Like Domain 10 Gene (*MEGF10*)

A recessive congenital myopathy with minicores has been described, caused by missense variants in *MEGF10*. Three siblings were compound heterozygous for 2 different missense variants, p.Cys326Arg and p.Cys774Arg in *MEGF10*.[73] MEGF10 regulates myoblast function via the NOTCH signalling pathway,

and the interaction between MEGF10 and NOTCH1 is impaired by the p.Cys774Arg variant.[94]

The Titin Gene (*TTN*)

The huge *TTN* gene with 363 exons encodes titin, the largest polypeptide in nature. One titin molecule reaches from the Z disc to the M line in the skeletal and cardiac muscle sarcomeres.[95,96] Given the size of titin, it is not surprising that several clinically distinct disorders affecting skeletal and/or cardiac muscle are caused by dominant or recessive mutations in *TTN*.[8] Most of these disorders have adult onset. However, five patients from 2 families with congenital muscle weakness, minicore-like lesions and abundant centrally located nuclei, and severe childhood-onset dilated cardiomyopathy were found to be homozygous for truncating mutations in the C-terminus of TTN. The parents were consanguineous in both families.[97] Furthermore, in a cohort of 31 patients with congenital core myopathy combined with primary heart disease, 7 pathogenic *TTN* variants were identified in 5 patients from 4 families. The variants included missense and truncating mutations. The patients were homozygous or compound heterozygous for the mutations.[74]

The Myosin Heavy Chain 7 Gene (*MYH7*)

The majority of the more than 500 missense mutations identified in the slow skeletal muscle fiber myosin heavy chain encoding gene *MYH7* cause cardiomyopathy.[98] A subset of the mutations cause skeletal muscle disease, including Laing distal myopathy and myosin storage myopathy.[99] More recently mutations in *MYH7* have been reported in dominant core myopathies.[75,100] Cullup al. described 4 patients from 2 families affected by multiminicore disease caused by novel dominant missense mutations in *MYH7*.[75] Romero et al described four patients in a 3-generation family with autosomal dominant central core disease. They identified a novel missense mutation in *MYH7* that segregated with the disease in the family.[100] The mutations identified in these families are located in the MYH7 tail region, close to previously described mutations causing Laing distal myopathy.

Centronuclear Myopathies

Centrally located nuclei in the muscle fibers are hallmarks of centronuclear (myotubular) myopathies (CNM), but some muscle biopsies may also show additional pathological features such as type 1 fiber predominance, type 1 fiber hypotrophy, and cores. The most common genes causing centronuclear myopathies are *MTM1, DNM2, RYR1,* and *TTN*. Minor causative genes are *BIN1, CCDC78,* and *SPEG*[11] (Fig., Table). *RYR1* and *TTN* variants identified in CNM will be discussed briefly below. The other CNM genes will be the focus of separate paragraphs.

Mutations in *RYR1* have turned out to be a fairly common cause of autosomal recessive CNM (ARCNM). The patients are usually compound heterozygous for 2 mutations, often one truncating mutation on one allele and a missense one on the other allele. The mutations are spread all across the *RYR1* gene. Some of the *RYR1* mutations found in ARCNM patients have previously been reported in core myopathy or malignant hyperthermia susceptibility.[101–104]

Compound heterozygous truncating mutations causing ARCNM have also been identified in the *TTN* gene. To date, 7 unrelated patients with ARCNM due to mutations in *TTN* have been described.[103,105,106] The CNM-causing mutations are spread all along the *TTN* gene. One of the mutations has previously been reported to cause tibial muscular dystrophy, and another caused adult-onset cardiomyopathy in the heterozygous state.[105]

The Myotubularin Gene (*MTM1*)

Mutations in *MTM1*, encoding myotubularin, a ubiquitously expressed lipid phosphatase, cause X-linked myotubular myopathy (XLMTM).[107] Myotubularin colocalizes with RYR1 at the junctional sarcoplasmic reticulum in skeletal muscle, and it is a key regulator of sarcoplasmic reticulum remodelling together with its lipid substrate phosphatidylinositol 3-monophosphate (PtdIns3*P*). Lack of MTM1 activity leads to disorganisation of the sarcoplasmic reticulum, which is considered to be the primary cause of most of the organelle positioning defects observed in muscles biopsies from XLMTM patients.[108]

The XLMTM-causing mutations in *MTM1* are loss-of-function mutations spread across the 15 exons of the gene. The majority of the patients are neonatally severely affected boys. Most mutations are truncating, but missense variants affecting conserved amino acids essential for MTM1 activity are common also.[109,110] A few large deletions removing one or more *MTM1* exons, as well as whole-gene deletions of *MTM1* including neighbouring genes have been reported. The latter causes contiguous gene syndromes.[3,109] Several different types of *MTM1* pre-mRNA splicing affecting mutations have also been described.[109,111,112] Germ line mosaicism for de novo *MTM1* mutations has been documented in a few families, in some cases manifesting as paternal transmission of the X-linked pathogenic variant.[109,113]

Evidence is accumulating that there is a higher number of females manifesting XLMTM than previously anticipated.[3,114] Females with XLMTM are usually less severely affected than males, but the clinical phenotype is highly variable in age of onset and severity. The most severely affected females can show a similar clinical course as a severely affected XLMTM male. In general, those *MTM1* mutations that cause a severe phenotype in males, cause a milder phenotype in females, probably due to the normal pattern of approximately 50-50 X-chromosome inactivation in females. However, there is an increased prevalence of highly skewed X-chromosome inactivation in females affected by XLMTM, although it has not been possible to determine which of the X chromosomes is preferentially inactivated.[114] Not all manifesting females show any skew, even in muscle tissue.

Interestingly, dynamin 2 (DNM2) expression levels are increased in the muscles of XLMTM patients, as well as in *MTM1* knock-out mice, indicating that MTM1 may be a

negative regulator of DNM2 expression.[115] This finding has led to the development of a potential therapeutic approach aiming towards reducing DNM2 levels in the muscles of XLMTM patients. Proof-of-principle has been achieved with antisense oligonucleotide-mediated *DNM2* knockdown in a mouse model for XLMTM.[116]

The Dynamin 2 Gene (*DNM2*)

DNM2 encodes dynamin 2, a large GTPase involved in diverse cellular processes, among others endocytosis, cytokinesis, phagocytosis, and cell migration. Mutations in *DNM2* cause autosomal dominant ADCNM with onset usually in adolescence or early childhood, with ptosis, distal weakness and contractures, and often radial strands in muscle fibers on biopsy, and Charcot-Marie-Tooth (CMT) peripheral neuropathy (CMTDIB and CMT2M).[117–119] However, cases with earlier onset have been reported due to de novo mutations in the pleckstrin homology domain of DNM2.[120] The ADCNM-causing mutations in *DNM2* are gain-of -function mutations, predominantly missense variants. One in-frame deletion of one amino acid, as well as one splice site mutation causing an in-frame deletion of three amino acids in addition to an in-frame insertion of 23 new amino acids have been identified. Many of the missense variants are recurrent and present in several unrelated families. The mutations causing ADCNM are distinct from the ones causing CMT.[119]

Functional studies of common ADCNM *DNM2* mutations show abnormal self-assembly of mutant DNM2 resulting in abnormally high GTPase activity of the protein, which in turn leads to T-tubule fragmentation.[121] The hyperactive mutant DNM2 protein is a potential therapeutic target in ADCNM, that is, downregulation of DNM2 activity should have a similar beneficial effect in ADCNM muscle as in XLMTM muscle.[116,121]

The Bridging Integrator 1 Gene (*BIN1*)

BIN1 encodes for amphiphysin 2, a protein involved in membrane tubulation. The membrane tubulation activity of BIN1 is enhanced by its interaction with MTM1.[122] Nicot et al described the first disease-causing variants in *BIN1* 10 years ago. Two missense variants and 1 nonsense variant were shown to cause ARCNM with congenital or childhood onset in 3 consanguineous families. The patients were homozygous for the mutations.[123] Subsequently, 1 novel homozygous missense mutation and 1 novel homozygous nonsense mutation have been published as causative for ARCNM.[124,125] Furthermore, a homozygous acceptor splice site mutation in intron 10 causing abnormal splicing of the skeletal muscle-specific *BIN1* exon 11 was identified in patients with rapidly progressive ARCNM in 1 consanguineous family. The corresponding splice site was found to be mutated in canine Inherited Myopathy of Great Danes, which, thus, represents a mammalian model for *BIN1*-related CNM.[126]

Dominant mutations in *BIN1* have been reported to cause mild and adult-onset forms of CNM.[127,128] Three of the mutations are single base pair deletions in the stop codon of *BIN1*, causing read-through and extension of the protein with 52 novel amino acids. Two other dominant mutations were 1 in-frame deletion of 1 amino acid, and 1 missense mutation, located in the N-terminus of BIN1.[127] A second dominant missense mutation, also in the N-terminus, was recently published.[128] The dominant *BIN1* mutations are distinct from the recessive ones, with different impacts on protein function, suggesting different pathomechanisms for dominant and recessive *BIN1*-related CNM.[127]

The SPEG Complex Locus Gene (*SPEG*)

SPEG interacts with myotubularin at the junctional sarcoplasmic reticulum in skeletal muscle. SPEG is also expressed in cardiac muscle. The first *SPEG* mutations were described in 6 CNM patients from 3 families. In addition to CNM, 2 unrelated patients had dilated cardiomyopathy. The mutations were recessive loss-of-function mutations (nonsense or frameshifts), and the patients were compound heterozygous or homozygous for the mutations.[129] Two novel *SPEG* mutations were recently reported in 2 unrelated CNM patients. One of the patients had dilated cardiomyopathy also. The patient with CNM and cardiomyopathy was homozygous for a nonsense mutation, and the patient with CNM without cardiac involvement was homozygous for a frameshift mutation in *SPEG*.[130]

The Coiled-Coil Domain-Containing Protein 78 Gene (*CCDC78*)

Only 1 dominant mutation in *CCDC78* causing CNM with atypical cores has been described in 1 family with patients in 3 generations. The mutation changes the acceptor splice site of intron 1 in *CCDC78*, causing retention of the intron, which is in-frame with the coding sequence. This is predicted to result in the addition of 74 amino acids to the protein.[131]

Myosin-Related Myopathies

Myosin heavy-chain genes, especially *MYH7, MYH2, MYH3,* and *MYH8* are implicated in various myopathies affecting skeletal and/or cardiac muscle (Fig., Table). Some of these myopathies are congenital. *MYH7* was already discussed in the context of the core myopathies, but dominant mutations in *MYH7* can also cause, for example, congenital fiber type disproportion without any other specific histological features. One such case was recently reported to be due to a de novo heterozygous splice site mutation causing skipping of *MYH7* exon 38.[132] A few cases of *MYH7*-related congenital myopathy were also found in a cohort of Italian patients. These patients had dominant missense mutations in *MYH7*.[133]

Both dominant and recessive mutations in *MYH2* can cause a usually mild congenital myopathy with external ophthalmoplegia. The dominant cases are caused by missense mutations, and the recessive ones usually by truncating mutations in *MYH2*.[7] A homozygous splice site mutation causing skipping of *MYH2* exon 12, leading to a frameshift,

was recently reported in a consanguineous family where 4 patients had a congenital myopathy with ophthalmoplegia.[134] A novel homozygous frameshift mutation in *MYH2* has also recently been described to cause a congenital myopathy with chronic aspiration pneumonia in infancy.[135]

MYH3 and *MYH8* encode embryonic and fetal myosin heavy-chain isoforms. Dominant missense mutations in *MYH3* and *MYH8* cause distal arthrogryposis syndromes, probably as the result of a severe muscle weakness already during fetal development.[7,136,137]

Other Genes Causing Congenital Myopathies

Two genes, *CACNA1S* and *SCN4A*, previously known channelopathy-causing genes, have now been implicate in congenital myopathies as well.[138–141] A third gene, *ZAK*, has also recently been identified as a novel congenital myopathy-causing gene[142] (Fig., Table).

A dihydropyridine receptor (DHPR) congenital myopathy caused by dominant or recessive mutations in the *CACNA1S* gene was recently described in 11 patients from 7 families. The muscle biopsies showed features of centralised nuclei, focal zones of sarcomeric disorganisation, and cores. DHPR directly regulates the RYR1 calcium release channel. Both the dominant and recessive mutations identified in *CACNA1S* are hypothesised to cause a decrease in overall DHPR function in skeletal muscle.[138]

Recessive loss-of-function mutations in the *SCN4A* gene encoding the alpha-subunit of the skeletal muscle voltage-gated sodium channel ($Na_v1.4$) have been identified in patients from 8 families with a congenital myopathy of variable severity, severe or "classical." Histological features were unspecific; abnormal fiber size variability, in some with type 1 predominance, and no pathognomic findings.[139–141] Partial loss-of-function mutations were associated with a milder disease phenotype.[140]

A congenital myopathy with fiber type disproportion caused by recessive loss-of-function mutations in the mitogen-activated protein triple kinase encoding gene, *ZAK*, was recently reported in six patients from three families. The patients were homozygous for frameshift or nonsense mutations in *ZAK*. The parents were consanguineous in all families. All mutations are located in the kinase domain of ZAK.[142] Interestingly, in 2 families recessive mutations in the SAM domain of ZAK have been associated with split-foot malformation with mesoaxial polydactyly.[143]

Conclusions

Here we have described 27 different genes implicated in various forms of the congenital myopathies. It is clear that the number of genes will increase due to the accelerated use of next-generation sequencing methods. Moreover, large CNVs and rearrangements are likely to be discovered as causative mutation types in many more disorders than those currently known.

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