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Autosomal recessive limb-girdle and Miyoshi muscular dystrophies in the Netherlands: The clinical and molecular spectrum of 244 patients

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Abstract

In this retrospective study, we conducted a clinico-genetic analysis of patients with autosomal recessive limb-girdle muscular dystrophy (LGMD) and Miyoshi muscular dystrophy (MMD). Patients were identified at the tertiary referral centre for DNA diagnosis in the Netherlands and included if they carried two mutations in *CAPN3*, *DYSF*, *SGCG*, *SGCA*, *SGCB*, *SGCD*, *TRIM32*, *FKRP* or *ANO5* gene. DNA was screened by direct sequencing and multiplex ligand-dependent probe amplification (MLPA) analysis. A total of 244 patients was identified; 68 LGMDR1/LGMD2A patients with *CAPN3* mutations (28%), 67 sarcoglycanopathy patients (LGMDR3-5/LGMD2C-E) (27%), 64 LGMDR12/LGMD2L and MMD3 patients with *ANO5* mutations (26%), 25 LGMDR2/LGMD2B and MMD1 with *DYSF* mutations (10%), 21 LGMDR9/LGMD2I with *FKRP* mutations (9%) and one LGMDR8/LGMD2H patient with *TRIM32* mutations (<1%). The estimated minimum prevalence of AR-LGMD and MMD in the Netherlands amounted to 14.4×10^{-6}. Thirty-three novel mutations were identified. A wide range in age of onset (0-72 years) and loss of ambulation (5-74 years) was found. Fifteen patients (6%) initially presented with asymptomatic hyperCKemia. Cardiac abnormalities were found in 35 patients (17%). Non-invasive ventilation was started in 34 patients (14%). Both cardiac and respiratory involvement occurs across all subtypes, stressing the need for screening in all included subtypes.

KEYWORDS

limb-girdle muscular dystrophy, Miyoshi muscular dystrophy, neurology, neuromuscular disorders

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1 | INTRODUCTION

The limb-girdle muscular dystrophies (LGMDs) are a heterogeneous group of genetic myopathies typically characterised by weakness of the pelvic and shoulder girdle muscles, whereas Miyoshi muscular dystrophy (MMD) is mostly characterised by initial weakness of the calf muscles. In the last 20 years, mutations in more than 20 different genes have been found to cause autosomal recessive (AR) LGMD and mutations in two genes cause MMD.[1] Previous studies in a strictly defined LGMD cohort in the Netherlands showed that sequencing of eight genes (*CAPN3*, *DYSF*, *SGCA*, *SGCB*, *SGCG*, *SGCD*, *FKRP* and *ANO5*) resulted in a diagnosis in about two-thirds of the families with AR-LGMD.[2,3]

Recently, a new classification system for LGMD subtypes was proposed which we will use in conjunction to the old system in this article.[4] MMD1 and LGMDR2/LGMD2B are allelic and caused by a defect in *DYSF*; gene defects in *ANO5* may cause MMD3 and LGMDR12/LGMD2L.[5,6] Severity of symptoms is variable between patients with the same type of AR-LGMD or MMD and within families.[7] Early diagnosis is essential for patient management and counselling as different subtypes may differ in prognosis (viz. less severe muscle weakness in LGMDR12/LGMD2L and MMD3), comorbidity (viz. cardiac involvement in LGMDR3-6/LGMD2C-F and LGMDR9/LGMD2I) and potential treatment (ie, future gene therapy).[7–10] Recently, a shift of DNA diagnostics occurred from direct sequencing performed in a single tertiary referral centre in the Netherlands to whole exome sequencing (WES) being available in multiple academic medical centres. In this retrospective study, we estimate the prevalence and aim to describe the frequencies of subtypes, mutation spectrum, and clinical symptoms of patients in the Netherlands with a DNA confirmed diagnosis of AR-LGMD and MMD identified by direct gene sequencing.

1.1 | Materials and methods

In this retrospective study, we searched the database of the tertiary referral centre for DNA diagnosis of AR-LGMD and MMD in the Netherlands (Laboratory for Diagnostic Genome Analysis, Department of Clinical Genetics, Leiden University Medical Center) for patients with mutations in genes known to cause AR-LGMD and MMD. This database contains all patients diagnosed with these disorders in the Netherlands up until the introduction of WES.

Inclusion criteria were pathogenic mutations on both alleles in *CAPN3* (LGMDR1/LGMD2A), *DYSF* (LGMDR2/LGMD2B and MMD1), *SGCG* (LGMDR5/LGMD2C), *SGCA* (LGMDR3/LGMD2D), *SGCB* (LGMDR4/LGMD2E), *SGCD* (LGMDR6/LGMD2F), *TRIM32* (LGMDR8/LGMD2H), *FKRP* (LGMDR9/LGMD2I), and *ANO5* (LGMDR12/LGMD2L and MMD3), or a pathogenic mutation on one allele and a novel mutation on the other allele plus either abnormal protein expression of calpain-3, dysferlin or sarcoglycan in muscle biopsy. All patients diagnosed from 1995 until March 2016 were included.

1.1.1 | Protein studies

Muscle biopsy specimens if available had been analysed immunohistochemically (IHC) for expression of all four subtypes of sarcoglycan[11] or by multiplex Western blotting for studying simultaneously calpain-3, dysferlin, dystrophin and laminin-alpha2 expression.[12]

1.1.2 | Genomic sequencing and MLPA

Genomic DNA was extracted from patients' whole blood or muscle after standard procedures and screened for mutations in *CAPN3*, *DYSF*, *SGCG*, *SGCA*, *SGCB*, *SGCD*, *TRIM32*, *FKRP* and *ANO5* by direct sequencing and MLPA analysis. MLPA was performed for screening whole-exon deletions/duplications of *CAPN3*, *DYSF*, *SGCG*, *SGCA*, *SGCB*, *SGCD* and *FKRP* using kits SALSA P176, P268-A1 and P116-A1 SGC from MRC Holland, respectively, and according to the manufacturer's instructions. The MLPA kit for *ANO5* (SALSA MLPA kit P436) was not implemented for regular diagnostics but only used if Sanger sequencing of *ANO5* showed a pathogenic heterozygous mutation or homozygous mutation or variant of uncertain significance. An earlier pilot study with MLPA was carried out by screening DNA of at least 30 patients with LGMD carrying a pathogenic heterozygous or homozygous mutation in *ANO5*. No gross deletions/duplications were detected in *ANO5*. Prediction of the pathogenicity of novel mutations was performed by segregation analysis in the family and "in silico" analysis using the tools provided by Alamut Visual version 2.8.1 (Interactive Biosoftware, Rouen, France). All novel mutations were submitted to the Leiden Open Variation Database.

1.1.3 | Clinical data and serum creatine kinase activity

Clinical data were obtained retrospectively from the patient files. We report age of onset of muscle weakness and specify if there is onset in childhood (<10 years), adolescence (10-18 years) or adulthood (>18 years). We report initial pattern of muscle weakness of the lower limb (predominantly proximal, proximal and distal, or distal weakness). Involvement of the proximal muscles of the upper limb and age at loss of ambulation were reconstructed from follow-up data.

Serum creatine kinase (CK) activity is expressed as international units/litre (IU/L). The upper limit of normal for serum CK activity of the Dutch laboratories was 145 IU/L for women and 171 IU/L for men. Only patients with hyperCKemia with muscle weakness were included in calculating the median age of onset.

Results from cardiac ultrasound aimed at revealing dilated cardiomyopathy (DCM), left ventricle (LV) dysfunction (LV hypertrophy and/or decreased ejection fraction) and electrocardiography showing cardiac arrhythmia were collected if available. Age at start of non-invasive ventilation was reconstructed from data obtained from the nationwide centre of home ventilation service in the Netherlands (Centrum voor Thuisbeademing).

The study was conducted in accordance with the Declaration of Helsinki and the research codes provided by the regional review board guidelines.

2 | RESULTS

A total of 244 patients from 216 different families were identified (Figure 1, Table S1). Clinical data, serum CK activity and cardiorespiratory involvement are described in Tables 1 and 2.

The prevalence was estimated on 1 March 2016. The number of inhabitants in the Netherlands in 2016 was 16 979 120. Therefore, the minimum prevalence of AR-LGMD and MMD cases amounted to 14.4×10^{-6} (244/16 979 120) individuals.

Pre-screening by IHC identified nearly all sarcoglycanopathy cases. Pre-screening by multiplex Western blotting identified most cases of dysferlinopathy, but in the case of calpain-3 analysis some false positive and negative results were found.

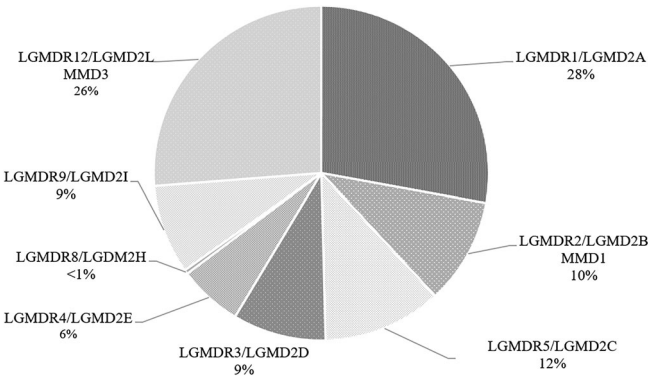

FIGURE 1 The frequencies of autosomal recessive limb-girdle muscular dystrophies and Miyoshi muscular dystrophies in the Netherlands

2.1 | Calpainopathy (LGMDR1/LGMD2A)

Calpainopathy is the largest subgroup of AR-LGMD in the Netherlands with 68 patients from 62 different families (28%). Detailed clinical data were missing from one patient. Two sporadic patients presented with asymptomatic hyperCKemia (3%). In one of them, serum CK activity was assessed at 30 years of age when she presented at the emergency room because of syncope. She did not develop muscle weakness until her last follow-up in her forties. No follow-up data were available from the other patient. Onset was in childhood in 12 patients (18%), in adolescence in 26 (40%) and in adulthood in 27 (42%). Loss of ambulation was found in 14 patients (21%). Cardiac abnormalities were found in seven patients (13%), including one with DCM, three with LV hypertrophy, two with atrial fibrillation and one with bradycardia.

Forty-nine different mutations were identified in *CAPN3*. Most mutations were distinct types of mutations, found only once, and scattered throughout the *CAPN3* gene. The most frequent mutations were c.1981del, p.Ile661* in 9, c.550del, p.Thr184Argfs*36 and c.759_761del, p.Lys254del in seven and c.640G>A, p.Gly214Ser in six patients. All other mutations were identified no more than twice in different families.

In total, five novel mutations were identified. By means of MLPA, a novel exon 20 duplication was detected next to a pathogenic nonsense mutation (c.2182C>T, p.Gln728*) on the other allele. Mutation c.2182C>T was identified homozygously in exon 20 which fits with the exon 20 duplication found in this gene. A novel acceptor splice site mutation (c.2115+4T>A) was found in a patient with a large exon 2-24 deletion on the other allele. In two patients two very probably pathogenic (class 4) novel variants (c.404T>C; p.Ile135Thr and c.2018T>C, p.Leu673Pro) were detected next to a pathogenic mutation on the other allele, in one patient a class 4 novel (homozygous) variant (c.2269C>G, p.His757Asp) was found. In all five patients calpain-3 was either reduced in expression or absent in muscle tissue.

2.2 | Dysferlinopathy (LGMDR2/LGMD2B and MMD1)

Twenty-five patients from 23 different families were diagnosed with a dysferlinopathy (10%). Missing data were age of onset and serum CK activity in two and respiratory involvement in three patients, respectively. One patient had an age of onset in childhood (4%), eight in adolescence (35%) and 14 in adulthood (61%). Loss of ambulation occurred in 11 patients (44%). The group of 25 patients included 13 patients with strictly proximal muscle weakness of the lower limb (LGMDR2/LGMD2B) (52%), three with proximodistal muscle weakness (12%) and nine with Miyoshi type muscular dystrophy (MMD1) (36%). There were no differences in sex, age of onset, age at loss of ambulation and serum CK activity between patients with proximal and proximodistal muscle weakness compared with distal muscle weakness of the lower limb. Involvement of the proximal muscles of the upper limb was found more frequently in patients with proximal

TABLE 1 Summary of clinical data of autosomal recessive limb-girdle muscular dystrophies and Miyoshi muscular dystrophies

Gene	Disease	Number of patients	Sex male/female	Median age of onset in years (range)	Median age at loss of ambulation in years (range)	Asymptomatic hyperCKemia	Involvement of proximal muscles of the upper limb	Median CK in IU/L (range)
	All	244	133/111	16 (0-72)	16 (5-74)	15/241 (6%)	175/237 (74%)	3112 (48-76 659)
CAPN3	LGMDR1/ LGMD2A	68	31/37	16 (1-59)	33 (12-48)	2/67 (3%)	63/67 (94%)	1844 (48-21 283)
DYSF	LGMDR2/ LGMD2B MMD1	25	13/12	20 (8-61)	28 (14-39)	0/25 (0%)	16/25 (64%)	6022 (210-20 000)
SGCG	LGMDR5/ LGMD2C	28	17/11	7 (1-13)	13 (9-41)	0/29 (0%)	23/26 (88%)	6000 (68-32 000)
SGCA	LGMDR3/ LGMD2D	22	12/10	6 (0-35)	10 (7-18)	1/20 (5%)	18/19 (95%)	11 000
	(1000-76 659)	*SGCB*	LGMDR4/ LGMD2E	15	5/10	4 (0-12)	12 (5-26)	1/15 (7%)
14/15	(93%)	6527			(635-33 266)	*TRIM32*	LGMDR8/LGMD2H	1
0/1	14	41	0/1 (0%)	1/1 (100%)	125			
FKRP	LGMDR9/ LGMD2I	21	10/11	6 (0-20)	14 (9-32)	2/21 (10%)	16/20 (80%)	7000 (166-19 952)
ANO5	LGMDR12/ LGMD2L MMD3	64	45/19	38 (12-72)	58 (30-74)	9/64 (14%)	24/64 (38%)	2555 (516-10 490)

Abbreviaions: LGMD, limb-girdle muscular dystrophy; MMD; Miyoshi type muscular dystrophy; ULN, upper limit of normal; CK, creatine kinase activity.

and proximodistal muscle weakness of the lower limb compared to patients with strictly distal muscle weakness of the lower limb (85% and 44%, respectively). Patients with proximal and distal muscle weakness reported a median age of onset of 19.5 years compared with 30 years in proximodistal muscle weakness.

In total, 32 different mutations were identified. Most mutations were found only once or twice. The most frequent mutation was c.4765C>T, p.Arg1586*. Various types of mutations were found of which the majority were missense mutations. Also gross deletions and duplications were identified in *DYSF*. Twelve novel mutations were detected in nine patients. Three different class 4 novel missense variants were identified by sequencing: c.1106T>C, p.Leu369Pro, c.3802G>A, p.Gly1268Arg, and c.4439A>C, p.Lys1480Thr. Besides three novel missense variants six different novel small mutations were found c.1176_1180+3dup, c.1765del, p.Arg589Glyfs*38 (in two non-related patients), c.3444T>A, p.Tyr1148*, c.3618C>G, p.Tyr1206*, c.4907dup, p.Leu1637Serfs*13 and c.5302_5328del, p.Arg1768_Pro1776del. Complete absence of dysferlin was found in muscle tissue from seven of the nine patients which confirmed the DNA results. No muscle tissue was available for multiplex Western blot analysis of two brothers in whom a novel frameshift mutation c.4907dup, p.Leu1637Serfs*13 next to a pathogenic missense mutation (c.3118C>T, p.Arg1040Trp) on the other allele was identified. Segregation analysis in the family showed that the mutations were present on different alleles. By means of MLPA analysis, three novel whole-exon deletions/duplications were detected in two patients: one patient had two gross deletions of exon 2-5 (in-frame) and a deletion of exon 25-27/28. The exact boundary of deletion 25-27/28 could not be determined because genomic DNA of the patient was not available and mutation analysis had to be performed on a small amount of DNA extracted from muscle tissue. The second patient carried a novel gross duplication of exon 1-12 in addition to a pathogenic missense mutation (c.3892A>G, p.Ile1298Val) on the other allele. Reduced amounts of dysferlin were detected in her muscle tissue.

2.3 | Sarcoglycanopathies (LGMDR3-6/LGMD2C-2F)

Sixty-seven patients were diagnosed with sarcoglycanopathy.

In 90% of the patients with a sarcoglycanopathy IHC analysis of the sarcoglycans had been performed which confirmed the genetic diagnosis. Segregation analysis was performed if a novel mutation was found and no muscle tissue was available for IHC analysis.

Twenty-eight patients from 19 different families were diagnosed with LGMDR5/LGMD2C (gamma-sarcoglycanopathy). The data on involvement of proximal muscles of the upper extremity and serum CK activity was missing in two patients. Onset was in childhood in 24 patients (86%) and in adolescence in four (14%). Loss of ambulation occurred in 17 patients (61%).

In total, seven different mutations were identified in *SGCG*. Twenty LGMDR5/LGMD2C patients carried the North African founder (frameshift) mutation (c.525del, p.Phe175Leufs20*) and originated from Morocco. The mutation was found homozygously in 18 patients. In one Moroccan family, three sibs were homozygous for c.525del, p.Phe175Leufs20*, and two carried a heterozygous out-of-frame

TABLE 2 Cardiac and respiratory dysfunction in autosomal recessive limb-girdle muscular dystrophies and Miyoshi muscular dystrophies

Gene	Disease	Cardiac analysis performed	Cardiac dysfunction	Dilated cardiomyopathy	LV dysfunction	Cardiac arrhythmia	Other	Non-invasive ventilation	Median age at start of non-invasive ventilation in years (range)
	All	202/242	35/202 (17%)	6	21	7	1	34/240 (14%)	35 (12-69)
CAPN3	LGMDR1/LGMD2A	52/68	7/52 (13%)	1	3	3		7/68 (10%)	54 (41-59)
DYSF	LGMDR2/LGMD2B MMD1	20/25	2/20 (10%)		1	1		3/22 (14%)	54 (47-69)
SGCG	LGMDR5/LGMD2C	23/28	5/23 (22%)	2	3			9/28 (32%)	35 (15-46)
SGCA	LGMDR3/LGMD2D	19/22	1/19 (5%)		1			4/22 (18%)	23.5 (14-34)
SGCB	LGMDR4/LGMD2E	15/15	5/15 (33%)	1	3	1		4/15 (27%)	26.5 (12-33)
TRIM32	LGMDR8/LGMD2H	1/1	1/1 (100%)				1	1/1 (100%)	64
FKRP	LGMDR9/LGMD2I	20/21	7/20 (35%)	2	5			5/21 (24%)	37 (19-43)
ANO5	LGMDR12/LGMD2L MMD3	52/64	7/52 (13%)		5	2		1/63 (2%)	68

Abbreviations: LGMD, limb-girdle muscular dystrophy; MMD, Miyoshi type muscular dystrophy; LV, left ventricle.

deletion of exon 7 on the other allele.[13] All five patients (two girls and three boys) from this family were severely affected. One of the girls was described previously.[11] Two mutations were novel: nonsense mutation c.496C>T, p.Arg166* and class 4 variant c.702G>T, p.Met234Ile. IHC analysis of muscle tissue of these two patients showed reduced expression of the sarcoglycan complex.

Twenty-two patients from 21 different families were diagnosed with LGMDR3/LGMD2D (alpha-sarcoglycanopathy). Missing data were age of onset and pattern of muscle weakness in one patient, involvement of proximal muscles of the upper limb in two, and serum CK in three patients. DNA analysis was performed in an asymptomatic case with hyperCKemia because of an affected sibling and showed compound heterozygosity (c.157+1G>A; c.229C>T, p.Arg77Cys) in both of them. The asymptomatic patient eventually developed severe muscle weakness. Onset was in childhood in 17 patients (85%), in adolescence in one (5%) and in adulthood in two (10%). Loss of ambulation occurred in 16 patients (73%).

Fifteen distinct mutations were found in *SGCA*. Eleven patients carried missense mutation c.229C>T, p.Arg77Cys; in three of them in a homozygous state and eight as compound heterozygotes. The most common splice-site mutation c.157+1G>A was identified in six patients either homozygously in two or compound heterozygously in four of them. Two mutations were novel. Novel frameshift mutation c.834_837del, p.Thr279Argfs*41 was found homozygously in three affected brothers. Previous analysis of muscle tissue in the eldest brother showed reduced expression of alpha-sarcoglycan. Novel variant c.671A>C, p.Gln224Pro (class 4) has been identified in three unrelated patients. IHC analysis on muscle tissue confirmed alpha-sarcoglycanopathy in two of them. No muscle tissue was available of the third patient. Segregation analysis in this family showed that pathogenic mutation c.739G>A, p.Val247Met was located on the other allele.

Fifteen patients from 14 different families with LGMDR4/LGMD2E (beta-sarcoglycanopathy) were included. Serum CK activity of two patients was missing. One sporadic male patient initially presented with elevated serum CK activity at age 18 (7%). He did eventually develop severe muscle weakness. Onset was in childhood in 13 patients (93%) and in adolescence in one (7%). Loss of ambulation occurred in 11 patients (73%).

Three different mutations in *SGCB* were identified: in 11 patients the recurrent missense mutation c.341C>T, p.Ser114Phe, in two patients, nonsense mutation c.31C>T, p.Gln11* and frameshift mutation c.214_215delTT, p.Leu72Valfs*26 in one patient.

2.4 | *TRIM32*-related muscular dystrophy (LGMDR8/LGMD2H)

A female patient was diagnosed with LGMDR8/LGMD2H. Her parents were full cousins. Loss of ambulation occurred at age 41. The patient carried a novel homozygous variant c.1184T>C, p.Ile395Thr (class 4) in *TRIM32*.

2.5 | *FKRP*-related muscular dystrophy (LGMDR9/LGMD2I)

In 21 patients from 19 families mutations were detected in *FKRP*. Data on involvement of proximal muscles of the upper limb were missing in one patient, data on serum CK activity was missing in two patients. Two sporadic patients presented with asymptomatic hyperCKemia (10%) at age 13 and 16, respectively. One of them was tested because of unexplained fatigue and she did develop severe muscle weakness over time; of the other patient no follow-up data were available. In 10 patients, the age of onset was childhood (53%), in eight, adolescence (42%) and in one, adulthood (5%). Loss of ambulation occurred in eight patients (38%). Normal serum CK activity was found in one patient with end-stage disease.

Six distinct mutations were identified. In 15 cases c.826C>A, p.Leu276Ile was found in a homozygous state and in five patients in a compound heterozygote state. Four novel mutations were found.

Novel variants c.535A>C, p.Thr179Pro (class 4) and c.1087G>A, p.Val363Met (class 4) were identified in addition to c.826C>A, p.Leu276Ile. In one patient two novel mutations were detected: a nonsense mutation c.1253G>A, p.Trp418* alongside novel variant c.854A>C, p.Glu285Ala (class 4). In muscle tissue of this patient absence of laminin-alpha2 was found.

2.6 | Anoctamin-5–related (*ANO5*) muscular dystrophy (LGMDR12/LGMD2L and MMD3)

In 64 cases from 58 families, *ANO5*-related muscular dystrophy was diagnosed. Serum CK activity and information about the respiratory function was missing in one patient. In seven cases, hyperCKemia was the initial reason for mutation analysis (11%), and in two cases, DNA analysis was performed because of hyperCKemia and an affected sibling (3%). Of the seven patients who presented with hyperCKemia, four had severe myalgia and in one patient, rhabdomyolysis occurred. No follow-up data were available of the two *ANO5*-related muscular dystrophy patients in whom DNA analysis was performed because of an affected sibling. Onset was adolescence in two (4%) and adulthood in 53 (96%). Loss of ambulation occurred in nine patients (14%). In 38 cases, proximal muscle weakness of the lower limb (LGMDR12/LGMD2L) (59%) and in nine cases proximodistal muscle weakness (14%) was observed, eight patients had a MMD3 phenotype (13%), There were no differences in clinical characteristics and serum CK activity between patients with proximal and proximodistal muscle weakness of the lower limb compared with strictly distal muscle weakness of the lower limb besides percentage of proximal muscle involvement of the upper limb, which was 49% and 13%, respectively.

Patients with proximal and distal muscle weakness reported a median age of onset of 35 years compared to 40 years in proximodistal muscle weakness.

Thirty-three different mutations were identified. The founder mutations c.191dup, p.Asn64Lysfs*15 and c.1898+1G>A were both detected homozygously in 11 patients and in a compound heterozygote state in 15 and 20 patients, respectively. Twenty-one mutations were missense mutations and mostly found only once. Seven distinct novel mutations were detected. Five of them were novel missense variants: c.173G>A, p.Arg58Gln (class 3, probably pathogenic); c.368C>T, p.Ser123Leu (class 4); c.1166T>C, p.Phe389Ser (class 4); c.1801T>G, p.Cys601Gly (class 4) and c.2170G>C, pAla724Pro (class 3). Furthermore, a novel nonsense c.1267C>T, p.Gln423* and acceptor splice-site mutation c.2521-1del were identified. Novel variant c.173G>A, p.Arg58Gln was identified in addition to pathogenic mutation c.1733T>C, p.Phe578Ser in a patient with proximal and distal muscle weakness of the lower limb. In the same codon (58) a pathogenic mutation was reported (Arg58Trp) in a patient with persistent asymptomatic hyperCKemia.[14] Novel variant c.368C>T, p.Ser123Leu was found twice in patients from different families suffering from LGMDR12/LGMD2L and MMD3, respectively. Novel variant c.1166T>C, p Phe389Ser was found in addition to a novel nonsense mutation c.1267C>T, p.Gln423* in an affected brother and sister with LGMDR12/LGMD2L. Novel variant c.1801T>G, p.Cys601Gly is in compound heterozygosity with pathogenic nonsense mutation c.1879_1880del, p.Ile627* in a patient with MMD3. Novel variant c.2170G>C, pAla724Pro was identified in heterozygosity with c.191dup, p.Asn64Lysfs*15 in a patient with proximodistal muscle weakness of the lower limb. Novel acceptor splice-site mutation c.2521-1del was found in compound heterozygosity with c.1898+1G>A in a patient with LGMDR12/LGMD2L.

3 | DISCUSSION

The minimum prevalence of AR-LGMD and MMD cases in the Netherlands amounted to 14.4×10^{-6} individuals which is similar to the prevalence in the United Kingdom and less than in Italy.[15,16] In our study, we found similar frequencies of AR-LGMD and MMD subtypes to what is reported in Eastern, Central and Southern Europe but differs from the United Kingdom and Denmark.

Similar to Italy, the Czech Republic, Brazil, and Japan, the largest proportion of the AR-LGMD that was identified in this study is LGMDR1/LGMD2A (*CAPN3*).[17,18] The second most frequent subtype includes the sarcoglycanopathies, albeit almost 30% of the sarcoglycanopathy patients were LGMDR5/LGMD2C patients who originated from Morocco. The third most frequently found subtype is the *ANO5*-related muscular dystrophies. This could be an underestimation due to the less severe phenotype, although LGMDR12/LGMD2L was not found more frequently in cohorts in which diagnosis was established with WES or next generation sequencing.[19,20] The frequency of *ANO5* related muscular dystrophies is similar to that in central European countries, but differs from Eastern and Southern Europe and, Denmark.[14,21,22] The frequency of sarcoglycanopathies and *DYSF*-related muscular dystrophies is almost the same as in other European countries.[15,23,24] In the United Kingdom and Denmark, LGMDR9/LGMD2I (*FKRP*) is the most frequent AR-LGMD (21% and 37%, respectively), whereas in the Netherlands, LGMDR9/LGMD2I is rare (9%).

Cardiac dysfunction was found in 17% of all patients and across all subtypes. LV dysfunction (LV hypertrophy and/or decreased ejection fraction) was most frequently found followed by cardiac arrhythmia (atrial fibrillation, frequent ventricular extrasystoles for which an ICD was implanted) and DCM. It was not feasible to ascertain which percentage of the cardiac abnormalities found were directly related to the muscular dystrophy as some of the abnormalities (ie, LV dysfunction and atrial fibrillation) are not uncommon in the general population.

Non-invasive ventilation was started in 14% of all patients and across all subtypes. This is the first report of a patient with *ANO5*-related muscular dystrophy who started with non-invasive ventilation. Cardiac dysfunction and respiratory insufficiency are known to be a frequent complication of the sarcoglycanopathies and LGMDR9/LGMD2I but has only recently been described in LGMDR1/LGMD2A and the dysferlinopathies.[15,25,26] There are conflicting reports on cardiac dysfunction in *ANO5*-related muscular dystrophy.[27,28] We describe a much higher percentage of cardiac abnormalities in the LGMDR1/LGMD2A patients as compared to the literature, including one patient with DCM.

Our study corroborates that cardiac dysfunction can be found in the dysferlinopathies and *ANO5*-related muscular dystrophies. The only LGMDR8/LGMD2H patient in this study underwent aortic valve replacement because of aortic valve stenosis and started non-invasive ventilation. Cardiac dysfunction has only been previously reported in two and respiratory insufficiency in one patient with LGMDR8/LGMD2H.[29,30]

Because data on age at last follow-up was not available and the duration of follow-up varied percentages of loss of ambulation, cardiac abnormalities and respiratory insufficiency should be interpreted with caution. However, based on our findings, we recommend that cardiac analysis should be conducted in all subtypes, which differs from the current guideline on the management of LGMDs.[31] Furthermore, it is important to be aware of symptoms of respiratory insufficiency in any subtype of AR-LGMD and to perform regular respiratory function testing. When respiratory impairment is present a patient should be referred to a multidisciplinary team for respiratory management.

Age of onset was predominantly in childhood in the sarcoglycanopathies (LGMDR3-5/LGMD2C-E), in childhood or adolescence in LGMDR1/LGMD2A, LGMDR9/LGMD2I and the dyferlinopathies, and in adulthood in *ANO5* related muscular dystrophies. Loss of ambulation occurs at a median of 58 years (range 30-74 years) in *ANO5* related muscular dystrophy. This corroborates earlier reports that the phenotype of *ANO5* related muscular dystrophy is relatively mild.[7] In *ANO5*-related muscular dystrophy the proximal muscle weakness is often initially confined to the lower limbs.

Nine patients with *ANO5*-related muscular dystrophy (15%) presented with hyperCKemia without muscle weakness. Asymptomatic hyperCKemia was also the initial presentation in a number of LGMDR1/LGMD2A, LGMDR4/LGMD2E and LGMDR9/LGMD2I patients in this study. In the subgroup of asymptomatic hyperCKemia patients, DNA analysis was performed in one LGMDR3/LGMD2D and two *ANO5* related muscular dystrophy patients at a young age, respectively, because of affected siblings.

When combining the AR-LGMD and MMD cases, there was no specific gender predominance, as expected in an AR disease. However, around two-thirds of the *ANO5*-related muscular dystrophy patients were male. The male predominance in this subtype has been reported before.[2,32] In our study, two-thirds of the LGMDR4/LGMD2E patients were female, which has not been reported before but could be due to the low number of LGMDR4/LGMD2E patients.

Novel mutations were found in all AR-LGMD subtypes except for LGMDR4/LGMD2E. In both *DYSF* and *FKRP*, a high number of novel mutations has been identified. Founder mutations were detected in the following genes: the North African founder in *SGCG*, and two distinct common mutations in *ANO5*.

In short, this study shows that the frequency of AR-LGMD and MMD subtypes in the Netherlands is similar to what is reported in Eastern, Central and Southern Europe, but differs from that found in the United Kingdom and Denmark. Cardiac abnormalities and respiratory insufficiency were associated with all AR-LGMD and MMD subtypes, stressing the need for cardiac and respiratory screening for all subtypes included.

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CONFLICT OF INTEREST

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS

L.t.D. contributed to design and conceptualised study, analysed the data and drafted the manuscript for intellectual content. W.S.F., W.H.J.P.L., C.S.S., E.H.N., C.G.F., J.M.F., J.B.K., E.B. I.F.d.C., N.V., A.V., J.E.H., W.L.v.d.P. and M.d.V. played a major role in the acquisition of data and revised the manuscript for intellectual content. D.W. played a major role in the acquisition of data. A.J.v.d.K. and H.B.G. played a major role in the acquisition of data and drafted the manuscript for intellectual content.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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