UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE MEDICINA
PROGRAMA DE PÓS-GRADUAÇÃO EM MEDICINA: CIÊNCIAS MÉDICAS

CARACTERIZAÇÃO CLÍNICA E MOLECULAR DAS MIOPATIAS HEREDITÁRIAS NO BRASIL

PABLO BREA WINCKLER

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PABLO BREA WINCKLER

Orientador: Profa. Dra. Márcia Lorena Fagundes Chaves
Co-orientador: Prof. Dr. Jonas Alex Morales Saute

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"O importante é não parar de questionar; a curiosidade tem sua própria razão de existir"

Albert Einstein

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RESUMO

As miopatias hereditárias (HM) são um grupo de doenças com grande heterogeneidade clínica e genética e o tempo decorrido desde o início dos sintomas até o correto diagnóstico pode ser muito longo. Diversos exames complementares de alto custo e invasivos são realizados na tentativa de um diagnóstico definitivo implicando num elevado custo pessoal, familiar e social. O recente uso das tecnologias de sequenciamento por nova geração (NGS) tem revolucionado o diagnóstico molecular dessas condições. Entretanto, são escassos os estudos que avaliam o rendimento diagnóstico dessa estratégia e faltam dados epidemiológicos sobre essas condições no Brasil e na América Latina. O objetivo da presente tese foi avaliar o rendimento de um painel de genes de NGS para o diagnóstico das HM no sul do Brasil assim como avaliar as características clínicas, genéticas e eventuais modificadores de fenótipo no subgrupo das distrofias musculares de cinturas (LGMD) no Brasil. Para isso, dois estudos foram realizados. No primeiro estudo, uma coorte histórica multicêntrica de casos índices consecutivos e seus familiares com diagnóstico genético ou patológico de LGMD recessiva (LGMD2) foi avaliada. Curvas de sobrevida para incapacidade física nas LGMD2A, LGMD2B e sarcoglicanopatias foram construídas e avaliaram-se as progressões clínicas de acordo com o sexo e genótipo. No segundo estudo, um estudo transversal de casos índices consecutivos com suspeita clínica de HM num centro único especializado em doenças neuromusculares no sul do Brasil, objetivou-se avaliar o rendimento diagnóstico de um painel NGS personalizado compreendendo 39 genes como teste de primeira linha para o diagnóstico de HM além de caracterizar os achados clínicos e moleculares de famílias com HM nesta população. O primeiro estudo avaliou 370 pacientes (305 famílias) com LGMD2, os subtipos mais frequentes foram LGMD2A e LGMD2B, cada um representando cerca de 30% das famílias. As sarcoglicanopatias foram o subtipo de início na infância mais frequente, representando 21% das famílias. Cinco por cento das famílias tinham LGMD2G, um subtipo ultrarraro em todo o mundo. Mulheres com LGMD2B tiveram progressão menos grave para deficiência que homens e pacientes com LGMD2A com variantes truncadas tiveram início da doença mais precoce e progressão mais grave para deficiência do que pacientes sem variantes truncadas. No segundo estudo 51 casos índices consecutivos foram avaliados e o rendimento diagnóstico geral do painel NGS foi de 52,9%, aumentando para 60,8% ao incluir casos de HM com variantes candidatas. O painel NGS resolveu o diagnóstico de 12/25 (48%) probandos LGMD, de 7/14 (50%) com doenças musculares congênitas (CMD) e de 7/10 (70%) com distrofia muscular com contraturas articulares proeminentes (MDJC). Os diagnósticos mais frequentes para LGMD foram LGMD2A e LGMD2B; para CMD, distúrbios relacionados ao *RYR1*; e para

MDJC, distrofia muscular de Emery Dreifuss tipo 1 e distúrbios relacionados com *COL6A1*. Dezesseis novas variantes foram relatadas. Através da presente tese foi possível definir dados epidemiológicos importantes de LGMD no Brasil, sendo os subtipos LGMD2A e LGMD2B os mais frequentes, diferentemente do que se pensava anteriormente, seguido pelas sarcoglicanopatias que foram os subtipos de início na infância mais frequentes. Conseguimos avaliar modificadores de fenótipos como a gravidade da mutação para LGMD2A e o efeito do sexo para LGMD2B. Essa é a maior série mundial de LGMD2 com dados clínicos e moleculares detalhados já descrita. Além disso o painel NGS personalizado quando aplicado na investigação inicial de HM resulta em um alto rendimento diagnóstico, reduzindo a odisseia diagnóstica do paciente e fornecendo informações importantes para aconselhamento genético e para participação em ensaios clínicos futuros.
Palavras-chave: distrofia muscular de cinturas, distrofia muscular congênita, distrofia muscular de Emery-Dreifuss, miopatia metabólica, miopatia distal, miopatia congênita, sequenciamento de nova geração, neurogenética, doenças neuromusculares.

ABSTRACT

Hereditary myopathies (HM) are a group of diseases with great clinical and genetic heterogeneity, and the time from the onset of symptoms to the correct diagnosis can be very long. Several high-cost and invasive complementary exams are performed to reach a definitive diagnosis, implying a high personal, family and social cost. The recent use of next generation sequencing (NGS) technologies has revolutionized the molecular diagnosis of these conditions. However, there are few studies that assess the diagnostic yield of this strategy and there is a lack of epidemiological data on these conditions in Brazil and Latin America. The aim of this thesis was to evaluate the performance of a panel of NGS genes for the diagnosis of MH in southern Brazil, as well as to evaluate the clinical, genetic and possible phenotype modifiers in the limb girdle muscular dystrophy (LGMD) subgroup in Brazil. For this, two studies were carried out. In the first study, a multicentric historical cohort of consecutive index cases and their families with genetic or pathological diagnosis of recessive LGMD (LGMD2) was evaluated. Survival curves for physical disability in LGMD2A, LGMD2B and sarcoglycanopathies were constructed and clinical progressions were evaluated according to sex and genotype. In the second study, a cross-sectional study of consecutive index cases with clinical suspicion of HM in a single center specializing in neuromuscular diseases in southern Brazil, aimed to evaluate the diagnostic yield of a personalized NGS panel comprising 39 genes as a first-tier test for the diagnosis of HM in addition to characterizing the clinical and molecular findings of families with HM in this population. The first study evaluated 370 patients (305 families) with LGMD2, the most frequent subtypes were LGMD2A and LGMD2B, each representing about 30% of families. Sarcoglycanopathies were the most frequent childhood onset subtype, representing 21% of families. Five percent of families had LGMD2G, an ultra-rare subtype worldwide. Women with LGMD2B had less severe progression to disability than men, and LGMD2A patients with truncated variants had earlier disease onset and more severe progression to disability than patients without truncated variants. In the second study 51 consecutive index cases were evaluated and the overall diagnostic yield of the NGS panel was 52.9%, increasing to 60.8% when including cases of HM with candidate variants. The NGS panel resolved the diagnosis of 12/25 (48%) LGMD probands, 7/14 (50%) with congenital muscle diseases (CMD) and 7/10 (70%) with muscular dystrophy with prominent joint contractures (MDJC). The most frequent diagnoses for LGMD were LGMD2A and LGMD2B; for CMD, *RYR1* related disorders; and for MDJC, Emery Dreifuss muscular dystrophy type 1 and *COL6A1* related disorders. Sixteen new variants were reported. Through this thesis, it was possible to define important epidemiological data of LGMD in Brazil, with the subtypes

LGMD2A and LGMD2B being the most frequent, differently from what was previously thought, followed by the sarcoglycanopathies that were the most frequent subtypes of childhood onset. We were able to assess phenotype modifiers such as mutation severity for LGMD2A and sex effect for LGMD2B. This is the world's largest LGMD2 series with detailed clinical and molecular data ever described. Furthermore, the customized NGS panel when applied in the initial investigation of HM results in a high diagnostic yield, reducing the patient's diagnostic odyssey and providing important information for genetic counseling and for participation in future clinical trials.
Key words: limb-girdle muscular dystrophy, congenital muscular dystrophy, Emery-Dreifuss muscular dystrophy, metabolic myopathy, distal myopathy, congenital myopathy, next generation sequencing, neurogenetics, neuromuscular diseases.

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LISTA DE ABREVIATURAS E SIGLAS

CK	Creatinofosfoquinase
CM	Miopatia Congênita
DM	Distrofia Muscular
DMD	Distrofia Muscular de Duchenne
EDMD	Distrofia Muscular de Emery-Dreifuss
FSHD	Distrofia Facioescapuloumeral
GSD	Distúrbios de Armazenamento de Glicogênio
HCPA	Hospital de Clínicas de Porto Alegre
LGMD	Distrofia Muscular de Cinturas
LGMD2	Distrofia Muscular de Cinturas Recessiva
MD	Miopatia Distal
MDC	Distrofia Muscular Congênita
MH	Miopatia Hereditária
MM	Miopatia Metabólica
NGS	Sequenciamento de Nova Geração
RM	Ressonância Magnética Nuclear
SUS	Sistema Único de Saúde
ES	Sequenciamento do Exoma

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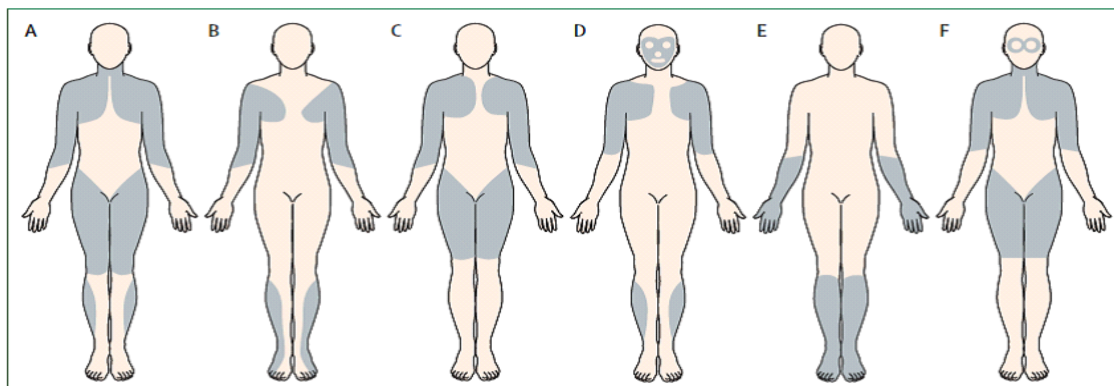
1. INTRODUÇÃO

As miopatias hereditárias (MH) são um grupo de doenças com grande heterogeneidade clínica e genética. As distrofias musculares (DM) são miopatias hereditárias, que cursam com fraqueza progressiva, em que há necrose do tecido muscular e substituição por tecidos conjuntivos e gordurosos, o que ajuda a distingui-las de outras miopatias hereditárias (Shieh, 2013). As DM/DM são caracterizadas por fraqueza muscular progressiva que pode afetar a musculatura apendicular, axial e da face em graus variados. Em formas específicas, também pode haver envolvimento da musculatura cardíaca e respiratória assim como da musculatura distal. Nas últimas décadas, com a descrição da base genética das principais formas de DM/MH, constatou-se uma levada heterogeneidade fenotípica desta condição (Pasnoor M, 2019).

1.1 Classificação das Distrofias Musculares/Miopatias Hereditárias

As DM/MH são historicamente classificadas com base no achado clínico principal e na idade de início dos sintomas. Contudo, com os avanços recentes de genética molecular a lista das doenças hereditárias associadas com doenças musculares aumentou dramaticamente levando à necessidade de revisão das classificações clínicas para que os dados moleculares pudessem ser incorporados a estas. Mesmo com esse avanço, a classificação das DM/MH segue partindo de dados clínicos (Shieh, 2013). Os principais fenótipos clínicos (Figura 1) são os de envolvimento predominante da cintura escapular e pélvica, como nas distrofias musculares de cinturas (LGMD, sigla internacional) e distrofias musculares congênitas (MDC, sigla internacional); envolvimento facial e de cintura escapular com escápulas aladas, como na distrofia facioescapuloumeral (sigla internacional, FSHD); envolvimento de cinturas e contraturas proeminentes, como na distrofia muscular de Emery-Dreifuss (DME) e em algumas formas de MDC, entre outros (Mercuri & Muntoni, 2013).

Figura 1 Padrão de distribuição de fraqueza muscular nas distrofias musculares



Legenda: (A) Distrofia muscular de Duchenne e Becker. (B) Distrofia muscular de Emery-Dreifus. (C) Distrofia muscular de padrão Limb Girdle (D) Distrofia muscular de Facioescapuloumeral. (E) Distrofia muscular distal. (F) Distrofia muscular de Óculo Faríngea. Regiões em cinza representam musculatura afetada (Mercuri & Muntoni, 2013).

Os avanços no conhecimento sobre as diferentes formas de DM/MH levaram tanto à descrição de novas pistas diagnósticas quanto à compreensão de que não é possível realizar a classificação adequada destas condições única e exclusivamente com base no fenótipo. Devido à grande heterogenidade clínica das LGMD e MDC, uma subclassificação tanto de acordo com o padrão de herança como de acordo com o gene envolvido passou a ser adotada. Por exemplo, para as LGMD as formas autossômicas dominantes são classificadas como LGMD tipo 1 e as recessivas como LGMD tipo 2. A letra de classificação após o número, ex LGMD1A indica o gene relacionado, neste caso a LGMD autossômica dominante relacionado ao gene *MYOT*. A Tabela 1 descreve as principais formas de DM/MH e sua respectiva classificação. Frequentemente mutações em um mesmo gene podem ocasionar fenótipos claramente distintos e mutações em diferentes genes podem ocasionar fenótipos clinicamente indistinguíveis, sendo desta forma fundamental tanto dados fenotípicos quanto genotípicos para a adequada classificação das DM/MH (Mercuri & Muntoni, 2013; Straub V, 2018).

Tabela 1 Principais formas de DM/MH e respectivas classificações

Doença	**Herança**	**Locus**	**Gene**	**Proteína**	**Principal Localização**
Distrofinopatias					
Distrofia Muscular de Becker ou Duchenne	Ligada X	Xq21.2	*DMD*	Distrofina	Proteína associada ao sarcolema
Distrofia Muscular de Cinturas (LGMD)					
Tipo 1A	AD	5q31	*MYOT*	Miotilina	Proteína associada ao sarcômero (disco Z)
Tipo 1B	AD	1q21.2	*LMNA*	Lamina A/C	Proteína associada a lamina nuclear
Tipo 1C	AD	3p25	*CAV3*	Caveolina-3	Proteína associada ao sarcolema
Tipo 1D	AD	7q	*DNAJB6*	Co-chaperonaLB6	Proteína associada ao sarcômero (disco Z)
Tipo 1E	AD	6q23	*DES*	Desmina	Proteína de filamento intermediário
Tipo 1F	AD	7q32	Desconhecido	Desconhecido	Desconhecido
Tipo 1G	AD	4p21	Desconhecido	Desconhecido	Desconhecido
Tipo 1H	AD	3p23-p25	Desconhecido	Desconhecido	Desconhecido

Tipo 2A	AR	15q15.1	*CAPN3*	Calpaina-3	Proteína associada a miofibrila
Tipo 2B	AR	2p13	*DYSF*	Disferina	Proteína associada ao sarcolema
Tipo 2C	AR	13q12	*SGCG*	γ-sarcoglicano	Proteína associada ao sarcolema
Tipo 2D	AR	17q12-q21.33	*SGCA*	α-sarcoglicano	Proteína associada ao sarcolema
Tipo 2E	AR	4q12	*SGCB*	β-sarcoglicano	Proteína associada ao sarcolema
Tipo 2F	AR	5q33	*SGCD*	σ-sarcoglicano	Proteína associada ao sarcolema
Tipo 2G	AR	17q12	*TCAP*	Titina (teletonina)	Proteína associada ao sarcômero (disco Z)
Tipo 2H	AR	9q31-q34	*TRIM32*	Tripartite motif-containing 32 (ubiquitina ligase)	Proteína associada ao sarcômero (disco Z)
Tipo 2I	AR	19q13.3	*FKRP*	Relacionada a Fukutina	Enzimas putativas de glicosiltransferase
Tipo 2J	AR	2q31	*TTN*	Titina	Proteína sarcomérica
Tipo 2k	AR	9q34	*POMT1*	O-manosil-transferase 1	Enzima glicosiltransferase
Tipo 2L	AR	11p14.3	*ANO5*	Anoctamina 5	Proteína transmembrana, possivelmente do retículo sarcoplasmático
Tipo 2M	AR	9q31	*FKTN*	Fukutina	Enzimas putativas de glicosiltransferase
Tipo 2N	AR	14q24	*POMT2*	O-manosil-transferase 2	Enzima glicosiltransferase
Tipo 2O	AR	1p34	*POMGNT1*	O-ligada a manose β-1,2-N-aminiltransferase 1	Enzima glicosiltransferase
Tipo 2P	AR	3p21	*DAG1*	Glicoproteína 1 associada à distrofina	Proteína associada ao sarcómero
Tipo 2Q	AR	8q24	*PLEC1*	Plectina 1	Proteína associada ao sarcolema (disco Z)
Distrofia Muscular Fascio Escapulohumeral					
Tipo 1	AD	4q35	Desconhecido	DUX4 e rearranjo de cromatina	Nuclear
Tipo 2	AD	18	*SMCHD1*	SMCHD1	Nuclear
Doença	**Herança**	**Locus**	**Gene**	**Proteína**	**Principal Localização**
Distrofia Muscular de Emery-Dreifuss					
Tipo 1 ligado ao X	Ligada X	Xq28	*EMD*	Emerina	Proteína de membrana nuclear
Tipo 2 ligado ao X	Ligada X	Xq27.2	*FHL1*	Four and a half LIM domain 1	Sarcômero e sarcolema
Autossômica dominante	AD	1q21.2	*LMNA*	Lamina A/C	Proteína de membrana nuclear
Autossômica recessiva	AR	1q21.2	*LMNA*	Lamina A/C	Proteína de membrana nuclear
Com defeito de nesprina-1	AD	6q25	*SYNE1*	Spectrin repeat containing, envelope nuclear 1 (nesprina-1)	Proteína de membrana nuclear
Com defeito de nesprina-2	AD	4q23	*SYNE2*	Spectrin repeat containing, envelope nuclear 2 (nesprina-2)	Proteína de membrana nuclear
Distrofias Musculares Congênitas					
MDC com deficiência de merosina (MDC1A)	AR	6q2	*LAMA2*	Cadeia α2 laminina da merosina	Proteína de matriz extracelular
MDC	AR	1q42	Desconhecido	Desconhecido	Desconhecido
MDC e glicosilação anormal de distroglicano (MDC1C)	AR	19q13	*FKRP*	Relacionada a Fukutina	Enzima putativa glicosiltransferase
MDC e glicosilação anormal de distroglicano (MDC1D)	AR	22q12	*LARGE*	Glicosiltransferase like	Enzima putativa glicosiltransferase
MDC de Fukuyama	AR	9q31-q33	*FCMD*	Fukutina	Enzima putativa glicosiltransferase
SWW com defeito de fukutina	AR	9q31-q33	*FCMD*	Fukutina	Enzima putativa glicosiltransferase
SWW com defeito de proteína 1-O-manosil-transferase 1	AR	9q34	*POMT1*	O-manosil-transferase 1	Enzima glicosiltransferase

SWW com defeito de proteína 1-O-manosil-transferase 2	AR	14q24	*POMT2*	O-manosil-transferase 2	Enzima glicosiltransferase
SWW com defeito de proteína-O-ligada a manose β-1,2-N-aminiltransferase 1	AR	1p34	*POMGNT1*	O-ligada a manose β-1,2-N-aminiltransferase 1	Enzima glicosiltransferase
SWW com defeito de proteína relacionada a fukutina	AR	19q13	*FKRP*	Relacionada a fukutina	
MEB com defeito de proteína-O-ligada a manose β-1,2-N-aminiltransferase 1	AR	1p34	*POMGNT1*	O-ligada a manose β-1,2-N-aminiltransferase	Enzima glicosiltransferase
MEB com defeito de proteína relacionada a fukutina	AR	19q13	*FKRP*	Relacionada a Fukutina	Enzima putativa glicosiltransferase
MEB com defeito de proteína 1-O-manosil-transferase 2	AR	14q24	*POMT2*	O-manosiltransferase 2	Enzima glicosiltransferase
MDC devido à desordem de glicosilação	AR	9q34.1	*DPM2*	Peptídeo 2 dolicol-fosfato manosiltransferase	Enzima glicosiltransferase
MDC devido à desordem de glicosilação	AR	1q21.3	*DPM3*	Peptídeo 3 dolicol-fosfato manosiltransferase	Enzima glicosiltransferase
MDC com anormalidades estruturais mitocondriais	mtDNA	22q13	*CHKB*	Colina kinase	Membrana do sarcolema e da mitocôndria
MDC com síndrome da espinha rígida	AR	1p36	*SEPN1*	Selenoproteína N1	Proteína do retículo endoplasmático
MDC com defeito no colágeno tipo VI (Ullrich), subunidade α1	AR	21q22.3	*COL6A1*	Colágeno tipo VI, subunidade α1	Proteína externa ao sarcolema
MDC com defeito no colágeno tipo VI (Ullrich), subunidade α2	AR	21q22.3	*COL6A2*	Colágeno tipo VI, subunidade α2	Proteína externa ao sarcolema
MDC com defeito no colágeno tipo VI (Ullrich), subunidade α3	AR	2q37	*COL6A3*	Colágeno tipo VI, subunidade α3	Proteína externa ao sarcolema
MDC com déficit de Integrina α7	AR	12q13	*ITGA7*	Integrina α7	Proteína externa ao sarcolema
MDC com déficit de Integrina α9	AR	3p21.3	*ITGA9*	Integrina α9	Proteína externa ao sarcolema
Outras formas de distrofias musculares					
Distrofia muscular com lipodistrofia generalizada	AR	17q21-q23	*PTRF*	Polimerase I e fator de liberação de transcrição (cavina 1)	Túbulos T e sarcolema
Distrofia muscular oculofaríngea	AD/AR	14q11.2	*PABPN1*	Proteína de ligação de poliadenilato nuclear 1	Desconhecido

Legenda **Tabela 1**: AD, autossômico dominante; AR, autossômico recessivo; MDC, distrofia muscular congênita; MEB, Muscle-Eye-Brain disease; SWW, Síndrome de Walker-Warburg. (Mercuri & Muntoni, 2013)

1.2 Aspectos epidemiológicos

A forma mais frequente de DM/MH da infância é a distrofia muscular de Duchenne, a forma mais grave de distrofinopatia e que não será abordada na presente tese, com uma prevalência estimada de 9:100.000 homens. Tal condição possui características clínicas peculiares como padrão de herança ligada ao X, fraqueza acomentendo cinturas escapular e pélvica, pseudohipertrofia de panturrilhas e níveis séricos de creatinofosfoquinase (CK) 10 vezes ou mais acima da normalidade. Há uma boa capacidade se suspeição clínica e direcionamento da investigação com base nestes achados clínico laboratoriais iniciais (Pasnoor M, 2019).

A distrofia miotônica e FSHD são as formas mais frequentes em adultos, contudo por ambas as condições apresentarem um quadro clínico e neurofisiológico característico além de bases moleculares muito distintas das demais formas de distrofias musculares, estas condições também não serão avaliadas no presente estudo (Thornton CA, 2014).

Os dados epidemiológicos sobre LGMD, MDC, EDMD, MM, MD e Miopatias Congênitas (CM, sigla internacional) são escassos, com prevalências distintas em diferentes regiões mundiais. Considerando informações internacionais as LGMD recessivas são as formas mais prevalentes de LGMD, sendo a LGMD2A, uma calpainopatia, a forma mais comum na maioria das regiões avaliadas. As LGMD2I e LGMD2L parecem ser as formas mais prevalentes nos países da Escandinávia devido a efeito fundador. Na América do Norte, tanto nos Estados unidos, quanto no México a forma mais prevalente de LGMD é LGMD2B, uma disferlinopatia. A prevalência das diferentes LGMD está representada na tabela 2 (Omar et al). As LGMD são consideradas as segundas causas mais frequentes de DM/MH na Inglaterra, México e Turquia, perdendo em prevalência apenas para as distrofinopatias (Omar A, 2014).

Entre as MDC, a forma de Fukuyama é a mais frequente no Japão enquanto a MDC de Ullrich (*COL6*) parece ser a mais frequente na maioria das regiões avaliadas, seguida pela MDC merosina negativa, relacionada ao gene *LAMA2*. (Mercuri & Muntoni, 2013). Em um estudo italiano a prevalência de MDC foi de 0,5: 100.000 habitantes, sendo as formas mais frequentes as deficiências de glicosilação do α-distroglicano (40,18%), seguido pela deficiência de laminina α2 (24,11%) e doenças do colágeno do tipo VI (20,24%), MDC relacionadas aos genes *SEPN1* e *LMNA* foram mais raras (Graziano A, 2015).

1.2.1 Epidemiologia no Brasil

A maioria dos estudos brasileiros em distrofias/miopatias hereditárias avaliou pacientes com MDC, tendo sido realizada caracterização clínica e patológica por biópsia muscular em casos de deficiências de merosina (Werneck 1997, Ferreira 2005, Rocco 2005) do colágeno VI (Reed 2005) e de outras formas de MDC (Ferreira, 2005).

Encontramos dois estudos que realizaram caracterização clínica e histológica das LGMD no Brasil. O primeiro avaliou 39 casos com suspeita de LGMD em um centro de referência de doenças neuromusculares de São Paulo e identificou as sarcoglicanopatias (LGMD2C, LGMD2D, LGMD2E e LGMD2F) como as formas mais prevalentes de LGMD (40,5% dos casos), seguida pelas LGMD2B e LGMD2A, ambas encontradas em

13,5% dos casos (Albuquerque 2014). O segundo estudo foi uma série de 56 pacientes com suspeita de LGMD em um centro de referência de doenças neuromusculares do Paraná, novamente as sarcoglicanopatias foram as condições mais frequentes, seguidas pela LGMD2B e LGMD2A (Comerlato et al, 2005).

Diversos relatos de caso de formas mais raras também foram encontrados. Nenhum dos estudos brasileiros realizou uma abordagem sistemática de avaliação molecular, sendo a prevalência e os tipos de mutações relacionadas as principais formas de DM/MH desconhecidas no Brasil.

1.3 Manifestações clínicas gerais

O início dos sintomas das distrofias musculares é variável podendo ocorrer em todas as fases da vida, desde o nascimento, infância, até a vida adulta. Enquanto nas MDC os sinais clínicos em geral já são claros ao nascimento ou ao longo dos primeiros meses de vida. Nas LGMD os sintomas podem se manifestar na infância ou idade adulta após a aquisição de marcha independente em ambos os casos (Pasnoor M, 2019).

A fraqueza da musculatura esquelética frequentemente acompanhada de atrofia da musculatura envolvida é um achado constante nas DM/MH e seu padrão de apresentação pode dar pistas para o diagnóstico etiológico, como na FSHD e na distrofia oculofaríngea (com padrões específicos de envolvimento facial). A presença de outros achados como contraturas tendinosas proeminentes de articulações (cotovelos, tornozelos e espinhais) também podem dar pistas diagnósticas importantes, sendo mais frequentemente encontradas no fenótipo Emery-Dreifuss e na MDC de Ullrich. A progressão do quadro muscular é variável e não há muitos estudos com instrumentos validados sobre a história natural destas condições. Em geral os pacientes com MDC não adquirem marcha independente. Para as LGMD, as formas de início infantil apresentam progressão mais rápida, levando a perda da marcha independente ao longo da segunda década de vida. Já nas formas de início na vida adulta, a progressão dos sintomas é mais lenta. O envolvimento da respiração é frequente, mas o início e forma de apresentação pode apresentar grande variabilidade. Na maioria das formas o envolvimento respiratório irá ocorrer apenas após a perda da deambulação como resultado de fraqueza generalizada que também envolve a musculatura ventilatória. Entretanto, em algumas formas o envolvimento respiratório pode ser precoce como resultado de fraqueza seletiva da musculatura diafragmática. O envolvimento cardíaco também é comum, mas não é um achado constante entre as distrofias musculares. Na maioria das formas com

envolvimento cardíaco o padrão de acometimento é de uma cardiomiopatia dilatada, entretanto em formas como a EDMD os defeitos de condução cardíaca são os achados mais graves, estando invariavelmente presentes (Mercuri & Muntoni, 2013).

Na MDC e raramente nas LGMD podem ocorrer alterações funcionais ou estruturais encefálicas. Os defeitos estruturais estão presentes em formas como a MDC de Walker-Warburg, Fukuyama e no fenótipo muscle-eye-brain (Amato AA, 2016).

1.4 Aspectos clínicos e genéticos específicos

A seguir serão descritos os aspectos clínicos e moleculares das principais formas de distrofias/miopatias hereditárias abordadas no presente projeto.

1.4.1 Distrofia de Cinturas

As distrofias de cinturas são um grande grupo de doenças musculares progressivas em que a fraqueza proximal da musculatura é maior do que a fraqueza distal, não havendo envolvimento facial significativo. Conforme já descrito acima, as LGMD são classificadas como LGMD1 quando o padrão de herança é autossômico dominante, podendo ainda ser classificada em LGMD1A (*MYOT*, miotilina), LGMD1B (*LMNA*, lamina A/C), LGMD1C (*CAV3*, caveolina-3), etc. de acordo com o gene causal relacionado (Tabela 1). A LGMD2 tem herança autossômica recessiva, sendo classificadas também de acordo com o gene envolvido em diferentes subtipos. Em 2018, um novo sistema de classificação foi proposto com as iniciais LGMD, seguida das letras "D" ou "R" correspondente ao padrão de herança autossômico dominante (D) ou autossômico recessivo (R), e então um número de acordo com a ordem de descoberta do gene envolvido seguido pelo nome da proteína afetada já que a quantidade de novos genes descobertos ultrapassou as letras do alfabeto, exemplos: LGMD-R1-Calpaina3 ou LGMD-R7-Teletonina (Straub V, 2018). As duas nomenclaturas têm sido utilizadas atualmente. A maioria das formas tem início na vida adulta, entretanto em algumas LGMD o início pode ser na infância como nas sarcoglicanopatias (LGMD2C-F) e em formas relacionadas a proteína fukutina (LGMD2I, *FKRP*). Apesar de haver pistas relacionadas ao padrão de envolvimento muscular e padrão de herança a diferenciação precisa entre os diferentes tipos de LGMD não é possível apenas com base clínica, sendo necessário a diferenciação por estudos moleculares ou por biópsia muscular com imunohistoquímica (Shieh, 2013).

1.4.2 Distrofia Muscular de Emery Dreifuss

A Distrofia Muscular de Emery Dreifuss (EDMD) clinicamente se apresenta com fraqueza muscular, contrações tendinosas e arritmia cardíaca, podendo qualquer uma destas manifestações ser a primeira a aparecer e com o tempo ocorrer o aparecimento das demais. O quadro clínico típico caracteriza-se por fraqueza muscular discreta, já nos primeiros anos de vida, da musculatura dos braços e das pernas, em ambas as lojas anteriores e posteriores, associados a contraturas dos tendões dos tornozelos, cotovelos e cervical posterior e deformidades nos pés. As arritmias cardíacas vão desde um bloqueio A-V de 1º grau até um bloqueio A-V completo. As manifestações cardíacas são mais tardias, mas podem também ser as manifestações iniciais. A EDMD pode apresentar uma herança genética ligada ao X, um padrão autossômico dominante (AD) ou autossômico recessivo (AR), neste trabalho abordaremos as mutações do gene *EMD* e *FHL1* que possuem herança ligada ao X. (Amato AA, 2016; Katirji B, 2014).

1.4.3 Distrofias Musculares Congênitas e Miopatias Congênitas

As MDC e as CM são um grupo heterogêneo de doenças neuromusculares genéticas que na maioria dos casos têm início no período perinatal, geralmente com hipotonia, fraqueza muscular com risco de disfunção ventilatória, deformidades ortopédicas e alterações na biópsia muscular. Historicamente, a classificação destes distúrbios baseava-se nos achados de biópsia muscular. Enquanto nas MDC as biópsias musculares apresentavam achados distróficos, nas CM havia alterações estruturais típicas observadas na histoquímica e microscopia eletrônica. Assim, as MDC foram descritas pela primeira vez no início do século XX, enquanto as CM não foram reconhecidas até que as secções criostáticas e a histoquímica das biópsias musculares entrassem em uso no final da década de 1960 (Graziano A, 2015).

A hipótese diagnóstica de MDC é aventada quando há associação de achados clínicos e exames complementares tais como disfunção cognitiva, epilepsia, alterações estruturais dos olhos, deformidades ortopédicas e neuroimagem anormal do encéfalo. Quando há fraqueza muscular facial e bulbar, ausência de envolvimento estrutural ocular além da paralisia do olhar e uma biópsia muscular não distrófica a hipótese diagnóstica de CM pode ser firmada. Entretanto, do ponto de vista nosológico, não há consenso na literatura quanto ao que distingue as CM das MDC (Amato AA, 2016). Os achados clínicos e de biópsia muscular auxiliam na caracterização das MDC e CM, mas o

diagnóstico específico só é possível com análise molecular dos genes envolvidos (Gilbreath HR, 2014).

1.4.3.1 Distrofias Musculares Congênitas

As MDC são um grupo de distrofias musculares raras que foram tradicionalmente descritas como tendo início dos sintomas no nascimento. Estudos epidemiológicos são escassos, em sua maioria sendo baseados em populações europeias. Estima-se que a prevalência de MDC seja ao redor de 1:100.000 indivíduos. Com o avanço do diagnóstico molecular, um amplo espectro de fenótipos passou a ser classificado como MDC. A idade de início dos sintomas pode ser de difícil determinação, principalmente nas formas clínicas leves. Uma criança com quadro compatível com distrofias musculares de início nos primeiros dois anos de vida, principalmente no primeiro ano, em geral terá a suspeita de uma forma de MDC. Há três categorias de divisão com fenótipos bem descritos e uma quarta nomeada “MDC não classificada” (ver Tabela 1). A tabela 2 correlaciona os fenótipos e categorias de MDC com suas principais características clínicas (Kress W, 2017).

Tabela 2 Características clínicas das distrofias musculares congênitas

Doença	Início	Fraqueza	Sintomas cardíacos	Sintomas respiratórios	Sintomas do SNC	Sintomas oculares
Colagenopatias						
DMC Ullrich	Nascimento	++	0	++	0	0
Miopatia de Bethlem	Nascimento	+	+	+	0	0
Merosinopatias						
DMC por deficiência de merosina	Nascimento	++	+	++	+ (lesões na substância branca, convulsões, envolvimento cognitivo leve)	+ (foram reportados casos de oftalmoplegia)
Distroglicanopatias						
DMC Fukuyama	Nascimento	++	++	++	+ (convulsões, envolvimento cognitivo)	+
Muscle-Eye-Brain	Nascimento	+++	0	?	++ (convulsões, envolvimento cognitivo)	+++
Síndrome de Walker-Warburg	Nascimento	+++	0	?	+++	+++
DMC Não-classificáveis						
Doença da espinha rígida	Nascimento	++	++	++	?	?
Doença Multiminicore	Nascimento	++	?	++	?	?
DMC-L	Nascimento	++	+	++	?	?

Legenda **Tabela 2** –DMC = distrofia muscular congênita; DMC-L = LMNA associada à DMC. 0 – sem comprometimento; +, leve; ++, moderado; +++, grave (Adaptada de Kang et al., 2015)

1.4.3.2 Miopatias Congênitas

O termo miopatia congênita foi inicialmente utilizado para descrever um tipo de miopatia com apresentação preferencial, mas não exclusiva, ao nascimento com características morfológicas distintas das MDC. Todavia, doenças antes consideradas MDC são hoje sabidamente alélicas a formas de CM. Por exemplo, MDC com síndrome da espinha rígida, multiminicore e alguns casos de miopatia miofibrilar são causados por mutação na selenoproteina N (gene *SELENON*). Geralmente, as CM se apresentam na infância com hipotonia e fraqueza generalizada, os marcos do desenvolvimento motor são tipicamente atrasados. Entretanto, alguns pacientes podem ter início dos sintomas mais tardiamente e até mesmo na vida adulta. As CM eram inicialmente consideradas não progressivas, embora hoje saiba-se que a fraqueza muscular pode progredir em alguns casos. As CM podem ter herança AD, AR ou ligada ao X. Além da grande heterogeneidade genética e fenotípica (Tabela 3). Dentro de uma mesma família, e consequentemente acometimento pela mesma mutação, podem ser encontrados indivíduos com apresentações clínicas bem distintas. A CK pode ser normal ou levemente elevada. A classificação das CM leva em consideração achados clínicos e análise das alterações estruturais na microscopia eletrônica e microscopia convencional em biópsia muscular (Amato AA, 2016).

Tabela 3 Miopatias congênitas

Doença	Herança	Proteína (gene)	Manifestações clínicas
Miopatia Central core	AD (raro AR) AR AD AR AD	Receptor de rianodina (*RYR1*) *Muscle slow*, cadeia β de miosina cardíaca pesada (*MYH7*) α actina 1 (*ACTA1*) Titina (*TTN*) *Coiled-coiles domain-containig* 78 (*CCDC78*)	Início: infância e ocasionalmente em adultos jovens. Fraqueza proximal de membros e fraqueza facial leve. Anormalidades do esqueleto; Risco para HM nos que apresentam mutação em *RYR1*
Miopatia Multiminicore	AR	Selenoproteína N (*SELENON*)	Início: infância; músculos proximais e faciais; raro oftalmoparesia; fraqueza de músculos respiratórios e comprometimento cardíaco; anormalidades do esqueleto; risco para HM nos que apresentam mutação *RYR1*.
	AD/AR	Receptor de Rianodina (*RYR1*)	
	AR	Titina (*TTN*)	
	AD	*Muscle slow*, cadeia β de miosina cardíaca pesada (*MYH7*)	
	AR	*Multiple EGF like domains 10* (*MEGF10*)	
Miopatia *Core-rod*	AD/AR	α actina 1 (*ACTA1*)	Início na infância. Fenótipos podem se assemelhar àqueles vistos em miopatia nemalina.
	AR	Nebulina (*NEB*)	
	AD	*Kelch repeat and* BTB (*KBTBD13*)	
Miopatia Nemalínica	AR	Nebulina (*NEB*)	Forma de início infantil: hipotonia severa generalizada, fraqueza; fraqueza respiratória; anormalidades esqueléticas; geralmente fatal no primeiro ano de vida.
	AD/AR	α actina 1 (*ACTA1*)	
	AD/AR	α tropomiosina (*TMP3*)	

	AD/AR AR AR	β-tropomiosina (*TPM2*) Troponina lenta T (*TNNT1*) Cofilina-2 (*CFL2*)	Forma leve de início precoce: subtipo mais comum; início na infância; hipotonia leve generalizada e fraqueza; músculos faciais; rara ptose; oftalmoparesia; face dismórfica e anormalidades esqueléticas
	AD	*Kelch repeat and* BTB *domain containing* 13 (*KBTBD13*)	Forma adulta: início na vida adulta; fraqueza proximal leve e, ocasionalmente, distal; ausência de anormalidades faciais ou esqueléticas
	AR	*Kelch-like family member 40 and 41 genes* (*KLHL40* e *KLHL41*)	
Miopatia miotubular/ centronuclear	Ligado ao X AD AR AR AR	Miotubularina (*MTM1*) Dinamina-2 (*DYN2*) Receptor de rianodina (*RYR1*) Anfifisina 2 (*BIN2*) Titina (*TTN*)	Hipotonia e fraqueza neonatal severa; fraqueza dos músculos respiratórios; ptose e oftalmoparesia; apresenta mau prognóstico na maioria dos casos Início na infância tardia ou primeira infância com fraqueza e hipotonia generalizada; fraqueza facial, oftalmoparesia e ptose; anormalidades faciais Início na infância tardia ou em adultos jovens com fraqueza proximal ou distal leve; ptose é comum; músculos faciais e extraoculares possuem envolvimento variável; ausência de anormalidades faciais e esqueléticas; anormalidades sensoriais médias Casos com mutações BIN podem ter fraqueza severa em extremidades inferiores
Desproporção congênita de tipos de fibras	AD	α-tropomiosina (*TMP3*)	Início na infância ou adulto jovem, fraqueza proximal ou generalizada; pode apresentar fraqueza facial e comprometimento assimétrico dos músculos respiratórios; anormalidades esqueléticas
	AD	Receptor de Rianodina (*RYR1*)	
	AR	Raramente causado por mutações em *ACTA1*, *SELENON*, *MYL2*, *TPM2* e *MHC7*	
Miopatia com corpos redutores	Ligado ao X	Four and a half LIM (*FHL1*)	Início na infância ou adulto jovem; fraqueza generalizada não-progressiva; ocasionalmente apresenta fraqueza dos músculos respiratórios; anormalidades esqueléticas e faciais
	AR	Desmina (*DES*)	
Miopatia *Fingerprint body*	Desconhecida	Desconhecida	Inicío na infância; fraqueza proximal lenta e não-progressiva
Miopatia sarcotubular (alélica à LGMD 2 H)	AR	*Tripartite motif containing protein 32*q (*TRIM* 32)	Início na infância; Fraqueza proximal lenta
Miopatia trilaminar	Desconhecida	Desconhecida	Início na infância: fraqueza generalizada; anormalidades esqueléticas
Miopatia com corpos hialinos/ Miopatia familiar com lise de miofibrilas/ Miopatia de armazenamento de miosina	AD	*Muscle slow*, cadeia β de miosina cardíaca pesada (*MYH7*)	Início na infância ou na vida adulta; Fraqueza de cinturas, escapuloperoneal ou distal
H-IBM 3/ Miopatia de armazenamento de miosina	AD	Cadeia pesada da miosina tipo IIa (*MYH2*)	Artrogripose congêntita; oftalmoparesia; no adulto inicia com mialgias e fraqueza proximal; *rimmed vacuoles* e inclusões na biópsia muscular (H-IBM tipo 3)
Miopatia de Cap	AD	β-tropomiosina (*TPM2*)	Início na infância; fraqueza generalizada; anormalidades esqueléticas
	AD	α-tropomiosina (*TPM3*)	
	AD	α-actina (*ACTA1*)	
Miopatia com corpos Zebra	AR	α-actina (*ACTA1*)	Início na infância; fraqueza generalizada – pode ser assimétrica e pior nos braços

Miopatias associada a agregados tubulares	AD AD	Molécula de interação estromal 1 (*STIM1*) Orai1 (*ORAI1*)	Início:infância ou adolescência; fraqueza de cinturas; imunodeficiência; mutações em *ORAI1* também são associadas com miose
	AR	UDP-N-acetilglicosamino dolicol fosfato N acetilglicosaminofosfotransferase 1 (*DPAGT1*)	Mutações em *GDPAGT1* e *GFPT1* são associadas com síndrome miastêmica infantil com fadigabilidade.
	AR	Glutamina-frutose-6-fosfato transaminase 1	

Legenda Tabela 3 *AD- autossômica dominante; AR- autossômica recessiva; HM- hipertermia maligna.* (Adap. de Amato AA, 2016)

1.4.4 Miopatias Metabólicas

Pacientes com miopatias metabólicas podem apresentar sintomas dinâmicos, desde mialgias ou cãibas até rabdomiólise induzida por esforço ou por outras formas de estresse metabólico. Alternativamente, o curso pode ser predominantemente estático ou lentamente progressivo, similar a uma distrofia muscular. Em pacientes com sintomas dinâmicos, identificar o gatilho dos sintomas pode ser uma pista importante, pois em geral o defeito residirá na degradação do glicogênio versus na utilização de ácidos graxos. A atividade isométrica de alta intensidade ou sustentada depende da degradação do glicogênio e da glicólise, e ocorre em condições anaeróbicas, sendo o piruvato convertido em lactato ao invés de entrar no ciclo de Krebs. Durante o exercício moderado a submáximo, como a natação ou a corrida, a quebra de glicogênio também é uma fonte importante de energia, mas ocorre sob condições aeróbicas. Assim, certas desordens da glicogenólise (distúrbios de armazenamento de glicogênio, GSD) podem manifestar-se em qualquer uma das situações acima (Darras, 2000).

Durante o jejum, doença sistêmica ou exercício de baixa intensidade prolongado (> 1 hora), os ácidos graxos são a principal fonte de energia do músculo. Distúrbios do metabolismo lipídico devem ser considerados se os sintomas forem desencadeados nessas condições. Por fim, como as mitocôndrias servem de caminho comum final para a produção de energia aeróbica, pacientes com miopatias mitocondriais podem demonstrar sintomas durante atividades aeróbicas de baixa a moderada intensidade, como caminhar ou correr (Tarnopolsky MA, 2006). A análise da CK, lactato, e piruvato podem ajudar no diagnóstico diferencial, bem como o teste de exercício do antebraço. De um modo geral, a CK estará elevada no repouso na maioria das GSD, e geralmente será normal nos transtornos mais comuns do metabolismo lipídico. As elevações de lactato ou piruvato, ou uma relação lactato/piruvato maior que 20 devem levantar a suspeita de possível defeito na cadeia de transporte de elétrons na mitocôndria. A incapacidade de usar o piruvato como substrato inicial no processo de fosforilação oxidativa leva a níveis

aumentados de piruvato e impulsiona a conversão de piruvato em lactato (Dougherty FE, 2004). A análise do perfil de acilcarnitinas sérico, juntamente com os níveis de carnitina total e livre no plasma e de ácidos orgânicos na urina são ferramentas de rastreio úteis para muitos dos distúrbios do metabolismo lipídico (Rinaldo P, 2008). Finalmente, a biópsia muscular pode auxiliar no diagnóstico (em músculo imediatamente congelado em -70 ° C para preservar a atividade enzimática), sendo possível a análise de lipídios neutros no músculo e de acúmulo de glicogênio pela coloração PAS (*period acid*- Schiff) (Adler M, 2015).

Tabela 4 Miopatias metabólicas (Gligogenoses)

Condição: deficiência enzimática	Achados	Gene
GSD II (doença de Pompe) Maltase ácida (ácido α-1,4-glicosidase)	Comprometimento respiratório EMG anormal Fraqueza axial e de cinturas	*GAA*
GSDIII (doença de Cori-Forbes) Debrancher (amylo-1,6-glicosidase)	NCS anormal: diminuição da velocidade ou prolongamento nas latências dos CMAP e/ou SNAP Fraqueza distal da perna	AGL
GSD IV (doença de Anderson) Brancher (amylo-1,4-1,6-transglicosidase)	Geralmente início na infância	*GBE*
GSD V (doença de MCArdle) Miofosforilase	*Second wind* fenômeno 1q3 com fraqueza permanente	*PYGM*
GSD VII (doença de Tarui) Fosfofrutoquinase (PFK)	Reticulocitose Hiperuricemia Hiperbilirrubinemia	*PFKM*
GSD IX: fosforilase b quinase (PHK) (GSD IXd é um subtipo relatado do músculo)	Elevação do lactato no teste do exercício CK normal ou elevada Herança ligada ao X em fenótipos musculares isolados	*PHKB*
GSD X: fosfoglicerato mutase (PGAM)	Elevação discreta ao invés de ausência de lactato no teste do exercício Agregados tubulares subsarcolemais na biópsia	*PGAM2*
GSD XI: lactato desidrogenase (LDH)	Elevação do piruvato no teste do exercício Lesões de pele	*LDHA*
GSD XII: Aldolase A	Ambos casos musculares relatados em crianças	*ALDOA*
GSD XIII: β-enolase	Único caso reportado em adulto	*ENO3*
GSD XIV: fosfoglicomutase 1 (PGM1)	Aumento exagerado de amônia no teste de exercício	*PGM1*
Fosfoglicerato quinase (PGK)	Ligado ao X	*PGK1*

Tabela 4 – *CK- creatina quinase; CMAP- potencial de ação muscular composto; EMG eletromiograma; GSG- doenças de estoque de glicogênio; NCS- estudo de condução nervosa; SNAP – potencial de ação nervoso sensitivo* (Adaptado de Adler M, 2015).

1.5 Diagnóstico

A combinação dos achados clínicos com a informação sobre possível mecanismo de herança pode levar a suspeita de formas específicas de distrofias musculares e direcionar o diagnóstico. Entretanto, devido à grande heterogeneidade clínica e genética destas condições um diagnóstico definitivo não será possível apenas com base em achados clínicos. A elevação de CK é totalmente inespecífica nas distrofias musculares e sua ausência não exclui este diagnóstico. A eletroneuromiografia em geral é de pouca

utilidade na diferenciação entre as formas hereditárias de doença muscular, com exceção dos transtornos miotônicos (Pasnoor M, 2019).

1.5.1 Biópsia muscular

Até há pouco tempo, a biópsia muscular era considerada o exame diagnóstico de escolha para as DM, permitindo a avaliação de achados morfológicos específicos e a utilização de anticorpos para avaliar a quantidade e a localização das diferentes proteínas musculares (Fanin M, 2002). Porém, nem todos os tipos de doenças musculares hereditárias tem uma assinatura de deficiência proteica. Mesmo biópsias realizadas com as melhores práticas, lançando mão das principais técnicas disponíveis para o estudo das miopatias, acabarão por servir no direcionamento da testagem genética, não substituindo o exame molecular, uma vez que não permitem a identificação da mutação familial e com isso não é possível obter informações valiosas para o aconselhamento genético (Mercuri & Muntoni, 2013).

1.5.2 Ultrassom de músculo

O ultrassom é uma técnica não invasiva que poder ser utilizada na avaliação de patologias do músculo esquelético. O ultrassom oferece algumas vantagens em comparação com outros testes auxiliares na avaliação da patologia muscular pois é indolor, tem baixo custo e permite que o examinador rastreie grandes áreas musculares rapidamente de forma complementar ao exame físico neurológico (Walker F, 2011).

1.5.3 Ressonância magnética nuclear (RM) de músculo

A RM de músculo pode auxiliar no diagnóstico dos pacientes com distrofias/miopatias hereditárias tanto ao identificar padrões de acometimento muscular específicos como para auxiliar na seleção do grupamento muscular a ser biopsiado quando houver esta indicação. Novamente os dados de RM de músculo irão dirigir o teste genético, mas não irão substituí-lo (Mercuri & Muntoni, 2013).

1.5.4 Sequenciamento de nova geração (NGS)

O NGS é um sequenciamento genético em larga escala que permite a análise simultânea de múltiplos genes e apresenta melhorias em relação ao processo original de

sequenciamento pela técnica de Sanger, em que o sequenciamento dos genes é realizado individualmete (Monies D, 2016).

1.5.4.1 Painéis de genes

A grande heterogeneidade clínica e genética das distrofias/miopatias hereditárias, associada a escassez de dados sobre quais formas e mutações são mais frequentes na população brasileira, torna extremamente laborioso e caro estudos que utilizem técnicas convencionais de sequenciamento de DNA. Com o advento da tecnologia de NGS, é possível analisar vários genes ao mesmo tempo com custo reduzido. O diagnóstico molecular preciso irá encerrar a investigação do paciente, na maioria das vezes não havendo a necessidade de exames complementares adicionais de alto custo e procedimentos invasivos. Da mesma forma, possibilita aconselhamento genético apropriado e com isso possibilita a orientação de opções reprodutivas adequadas, além da orientação para possíveis estratégias terapêuticas (Kuhn M, 2016).

1.5.4.2 Sequenciamento por exoma (ES)

Com o avanço das técnicas de NGS tornou-se possível realizar o sequenciamento completo de praticamente todos os éxons de um indivíduo (Reddy H, 2017). Considerando a grande heterogeneidade genética das miopatias/distrofias musculares e o tamanho dos genes, ao menos 2 dos maiores genes dos seres humanos codificam proteínas musculares (*DMD*, distrofina, 79 exons, 3.685 aminoácidos, 14kb; *TTN*, titina, 363 exons, 26.926 aminoácidos, 82kb) a análise de painéis de genes pode tornar-se de alto custo, ou ter custo similar ou mesmo maior do que o ES (Ghaoui R, 2017). As evidências sobre o rendimento dos painéis de NGS e ES para o diagnóstico das distrofias/miopatias hereditárias serão descritas na próxima seção.

2. REVISÃO DA LITERATURA

Para a busca dos estudos que utilizaram técnicas de NGS para o diagnóstico de distrofias/miopatias hereditárias foram consultadas as bases PubMed utilizando-se os descritores: “Muscular Dystrophies, Limb-Girdle"[Mesh] OR "Distal Myopathies"[Mesh] OR "Muscular dystrophy congenital, merosin negative" [Supplementary Concept] OR "Bethlem myopathy" [Supplementary Concept] OR "Muscular dystrophy, congenital, with central nervous system involvement" [Supplementary Concept] OR "Muscular Dystrophy, Congenital, Due To Integrin Alpha-7 Deficiency" [Supplementary Concept] OR "Muscular Dystrophy, Congenital, Lmna-Related" [Supplementary Concept] OR "Muscular Dystrophy, Congenital, due to Partial LAMA2 Deficiency" [Supplementary Concept] OR "Muscular Dystrophy, Congenital, 1C" [Supplementary Concept] OR "Muscular Dystrophy, Congenital, 1B" [Supplementary Concept] OR "Rigid spine syndrome" [Supplementary Concept] OR "Walker-Warburg Syndrome"[Mesh] OR "Myopathies, Structural, Congenital"[Mesh] OR "Glycogen Storage Disease Type V"[Mesh] OR "Glycogen Storage Disease XII" [Supplementary Concept] OR "Lysosomal-Associated Membrane Protein 2"[Mesh] OR "Adenosine monophosphate deaminase deficiency" [Supplementary Concept] OR "Myopathy, Myofibrillar, Desmin-Related" [Supplementary Concept] AND "High-Throughput Nucleotide Sequencing"[Mesh] e LILACS utilizando os termos “Muscular Dystrophies” [Categoria DeCS expandida] OR Myopathies, Structural, Congenital [Categoria DeCS expandida] AND High-Throughput Nucleotide Sequencing [Categoria DeCS expandida].

Estudos que avaliassem apenas distrofia muscular de duchenne/Becker, distrofia miotônica e distrofia facioescapuloumeral foram excluídos. Relatos de caso também foram excluídos da análise.

Foram encontrados 71 artigos, sendo que 14 destes se relacionavam ao tema pesquisado e serão revisados a seguir (Ver **Figura 2**).

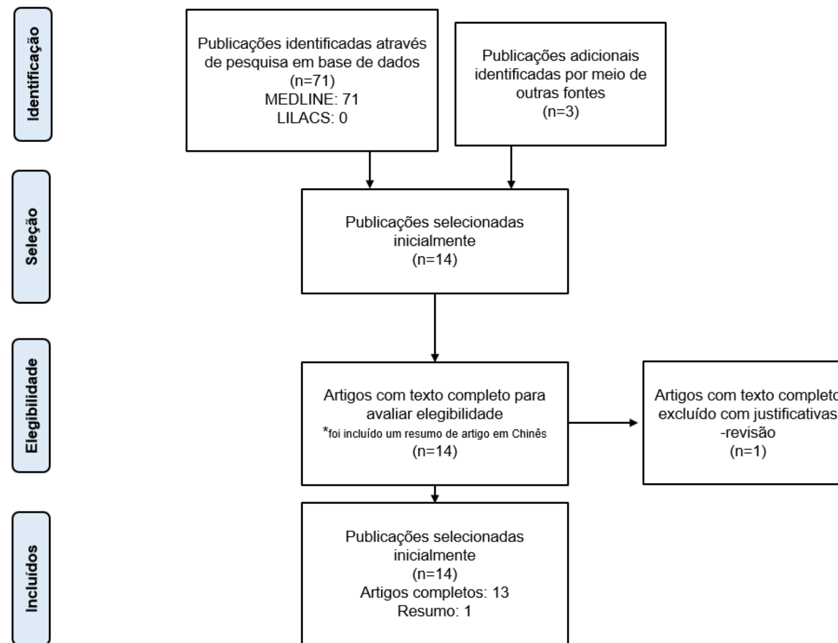


Figura 2 Fluxograma de seleção dos estudos para elaboração do estudo

2.1 Qual o rendimento do painel NGS para o diagnóstico de miopatias hereditárias em diferentes populações?

Encontramos apenas sete estudos na literatura que avaliaram esta pergunta. Três estudos chineses, o primeiro em pacientes com suspeita de miopatias hereditárias (Fu et al, 2015), o segundo em pacientes suspeita de LGMD (Yu et al, 2017) o último com suspeita de CM (Wang et al, 2020). Na Europa encontramos um estudo alemão que avaliou famílias com suspeita de LGMD (Kuhn et al, 2016) e um estudo francês que avaliou pacientes com suspeita de miopatia distal (Sevy et al, 2015), um estudo turco que avaliou pacientes com LGMD (Özyilmaz B et al, 2019) e um estudo multicêntrico envolvendo países da America Latina avaliando LGMD (Bevilacqua JA, et al 2020).

2.1.1 Miopatias hereditárias

Um estudo transversal chinês avaliou 134 pacientes com suspeita de miopatia hereditária com 2 painéis de NGS. O rendimento diagnóstico obtido foi de 55,22%. Um paciente foi diagnosticado com miopatia metabólica, 5 com CM, 22 com distrofia muscular congênita 1A (MDC1A), 11 com MDC de Ullrich, 6 com MDC de Bethlem, 12 com distrofia muscular de duchenne (DMD) causada por mutações de ponto no gene DMD, 5 com MDC relacionada ao LMNA, 1 com EDMD, 7 com alfa-distroglicanopatias

e 4 com LGMD. Este estudo foi publicado em chinês, desta forma tivemos apenas acesso ao resumo em inglês (Fu et al, 2015).

2.1.2 LGMD

Um outro estudo do mesmo centro chinês, avaliou 180 pacientes consecutivos com suspeita de LGMD, que já haviam realizado biópsia muscular, por NGS de painel de 420 genes relacionados a doenças neuromusculares. Foram encontradas uma ou mais variantes raras em 138 pacientes, sendo 113 variantes patogênicas. Em 10 pacientes esporádicos foi encontrada apenas uma variante patogênica em heterozigose relacionada a LGMD recessiva e 15 tinham variantes de significado incerto. Nenhuma mutação causadora de doença foi encontrada nos 42 pacientes restantes. Combinado com os achados miopatológicos, o rendimento diagnóstico relatado foi de 68,3% (123/180), sendo encontradas mutações em genes relacionados as LGMD em 95 casos, em outros 10 casos foi encontrada apenas 1 variante em heterozigose de LGMD2B. Entre os 105 pacientes que receberam o diagnóstico de LGMD os subtipos mais comuns foram LGMD2B em 52 (49,5%), LGMD2A em 26 (24,8%) e LGMD 2D em oito (7,6%), LGMD1B em sete (6,7%), LGMD1E em quatro (3,8%), LGMD2I em três (2,9%), e LGMD2E, 2F, 2H, 2K, 2L em um paciente (1,0%), respectivamente (Yu et al, 2017).

Um estudo alemão avaliou 58 pacientes com LGMD sem diagnóstico molecular por painel de 38 genes por NGS (23 destes especificamente relacionados às LGMD). O diagnóstico molecular foi confirmado em 19/58 (33%) pacientes, sendo a LGMD2A, seguida por LGMD2L e LGMD2I as formas mais frequentes. Em 2 destes pacientes o diagnóstico envolveu um dos genes que não era primariamente relacionado às LGMD (Kuhn et al, 2016).

O estudo realizado pelo grupo turco analisou um painel NGS baseado em captura de alvo personalizado cobrindo 31 genes associados a LGMD (*MYOT, LMNA, CAV3, DES, DNAJB6, FLNC, CAPN3, DYSF, SGCG, SGCA, SGCB, SGCD, TCAP, TRIM32, FRKP , TTN, POMT1, ANO5, FKTN, POMT2, POMGnT1, DAG1, PLEC, GAA, GMPPB, HNRNPDL, TNPO3, LIMS2, POMK, TRAPPC11, ISPD*) em 74 pacientes com suspeita de LGMD. O rendimento diagnóstico alcançado foi de 25/74 (33,8%), com uma ou mais variantes patogênicas ou provavelmente patogênicas sendo encontradas em 13 genes diferentes (Özyilmaz B et al, 2019).

Por fim, dados compilados de 2103 pacientes de 20 centros de doenças neuromusculares de países da américa latina (Brasil, Argentina, Peru, Equador, México e

Chile) avaliaram um painel de 10 genes (*ANO5, CAPN3, DYSF, FKRP, GAA, SGCA, SGCB, SGCD, SGCG, TCAP*) para investigação de LGMD com 335/2103 (16%) recebendo um diagnóstico molecular definitivo (Bevilacqua JA, et al 2020).

2.1.3 Miopatia distal

Um estudo avaliou o rendimento de painel de genes por NGS (298 genes associados a doenças neuromusculares) em pacientes franceses com suspeita de miopatia distal. Foram avaliadas 37 famílias, 20 destas já possuíam diagnóstico molecular confirmado por análises de único gene e 17 permaneciam sem diagnóstico. Todas as mutações encontradas nas 20 famílias com diagnóstico prévio foram identificadas pelo NGS, validando a plataforma, e o diagnóstico molecular foi confirmado em 8/17 (47%) das famílias sem diagnóstico prévio. Interessantemente, os diagnósticos foram relacionados a genes usualmente associados a LGMD ou a neuropatias motoras hereditárias (Sevy et al, 2015).

2.2 Qual o rendimento do uso do sequenciamento do exoma (ES) e sequenciamento com análise focal do exoma (FES) para o diagnóstico das miopatias hereditárias em diferentes populações?

Encontramos apenas 4 estudos que avaliaram esta pergunta. O primeiro estudo foi realizado em 60 famílias com suspeita de LGMD da Austrália e Nova Zelândia e utilizou o ES como abordagem molecular diagnóstica. O diagnóstico genético provável foi estabelecido em 27/60 (45%) famílias com esta abordagem. Novamente, assim como nos estudos de painéis amplos, mutações em outros genes, não primariamente associados a LGMD, foram encontradas nesta população, incluindo genes associados a MDC, miopatias metabólicas, miopatia distal e síndromes miastênicas congênitas. Em parte desta amostra foi realizado ES apenas do caso índice (solo) e em parte exomas de trios (indivíduo afetado e genitores, por exemplo), sendo o rendimento diagnóstico de 40% nos casos solo e de 60% para os trios (Ghaoui et al, 2015).

Um segundo estudo avaliou o rendimento do ES para o diagnóstico das LGMD nos Estados Unidos. Cinquenta e cinco famílias foram avaliadas e o diagnóstico foi obtido em 22 famílias (40%) (Reddy et al, 2016).

Outro estudo avaliou o FES para investigar uma coorte de 100 casos complexos de miopatia em adultos, já com extensa investigação e que permaneciam sem diagnóstico, num centro de referência em doenças neuromusculares do Reino Unido. O rendimento

diagnóstico da abordagem pelo exoma focado foi de 32%, mutações no gene *TTN* foram as mais prevalentes (4/32). Observou-se que o início dos sintomas na infância teve maior probabilidade de estar associado a um diagnóstico positivo na testagem genética. Fenótipos atípicos e novos achados clínico-patológicos foram identificados, incluindo um o novo grupo de genes associados a neuromiopatia (*HSPB1*, *BICD2*). Os dados demonstram a eficácia diagnóstica do FES quando combinada com a fenotipagem clínico-patológica detalhada numa coorte de casos neuromusculares complexos e sugere que características clínico-patológicas atípicas podem ter o processo diagnóstico atrasado se forem utilizados painéis de NGS mais restritos (Bugiardini E et al, 2019).

Finalmente, um estudo norte americano analisou uma coorte retrospectiva de 106 pacientes pediátricos com doenças neuromusculares sem diagnóstico definido num grande centro de referência terciário com uma combinação de ES, microarray cromossômico (CMA) e Sanger. Um diagnóstico molecular foi obtido em 37/79 (46%) pacientes com ES, 4/44 (9%) nos pacientes com CMA e 15/74 (20%) nos pacientes testados por Sanger, sugerindo que ES apresenta rendimento diagnóstico superior em comparação aos outros testes avaliados, e deve ser considerado no início da jornada diagnóstica para pacientes selecionados (Herman I et al, 2021).

2.3 Outros estudos de interesse

Apesar de ter uma abordagem distinta, 1 estudo avaliou os dados disponíveis em 2 bancos de dados de indivíduos que realizaram exoma, a coorte CinSeq (n=951) e *Exome Sequencing Project* (ESP, n=4.247 americanos com descendência europeia e n=2.201 afro-americanos). O objetivo do estudo foi o de avaliar a prevalência de portadores para mutações no gene *PYGM*, relacionado a doença de McArdle e com isso predizer a prevalência da doença na população. Os resultados da análise de 6 variantes patogênicas comuns no *PYGM* indicaram uma prevalência de 1/7.650 indivíduos com a doença de McArdle, números muito superiores do que os publicados previamente na literatura (De Castro et al, 2015).

Um estudo brasileiro realizou a caracterização clínica e molecular de distrofias relacionadas ao colágeno VI (COL6-RDs) em 28 pacientes, concluindo que COL6-RDs apresentam manifestações clínicas variáveis, mas achados comuns auxiliam na suspeita clínica. Não obstante, o NGS é uma abordagem valiosa para o diagnóstico, fornecendo informações úteis para o aconselhamento genético de famílias (Zanoteli E et al, 2020).

Um estudo chinês analisou sequenciamento por painel de NGS 48 pacientes com CM que tinham biópsia muscular típica para miopatia nemalínica buscando as mutações genéticas causais. Além disso, a reação em cadeia da polimerase de transcrição reversa (RT-PCR) foi usada para confirmar o efeito patogênico de uma variante de *splicing* da nebulina (NEB). Os resultados mostraram que as variantes foram encontradas em cinco genes associados à miopatia nemalínica, incluindo *NEB*, *ACTA1*, *TNNT1*, *KBTBD13*, e *CFL2*, em 34 (73,9%), 7 (15,2%), 3 (6,5%), 1 (2,2%) e 1 (2,2%) pacientes, respectivamente, em um total de 46/48 (95,8%) pacientes com NM (Wang Q et al, 2020).

2.4 Conclusões da revisão sistemática

Foram poucos os estudos publicados até o momento que avaliaram o potencial diagnóstico do NGS, seja através de painéis de múltiplos genes ou por ES, nas distrofias/miopatias hereditárias. O grupo mais estudado foi o das LGMD e o rendimento diagnóstico de painel de genes e do ES foi semelhante (ligeiramente maior para ES, em especial de trios). Dois estudos destacaram-se quando ao rendimento do painel de genes por NGS, consideravelmente maior em relação aos demais, (Yu et al, 2017; Wang Q et al, 2020). Porém, estes resultados podem ser explicados pela seleção de pacientes, que necessitavam realização prévia de biópsia muscular. Cabe ressaltar que foram encontradas mutações em genes não relacionados inicialmente ao grupo de suspeita, LGDM por exemplo, em todos os estudos com painéis amplos ou ES, o que sugere que caso a abordagem de painéis de genes seja escolhida ela deveria conter genes associados a diversas doenças musculares e não apenas à suspeita clínica inicial.

Não encontramos estudos específicos em MDC, EDMD ou miopatias metabólicas. O único estudo realizado na América Latina compilou dados centralizados num laboratório de genética e não definiu critérios para seleção dos pacientes a serem submetidos ao painel NGS para LGMD, destoando assim dos demais por apresentar um rendimento diagnóstico baixo.

3. MARCO CONCEITUAL

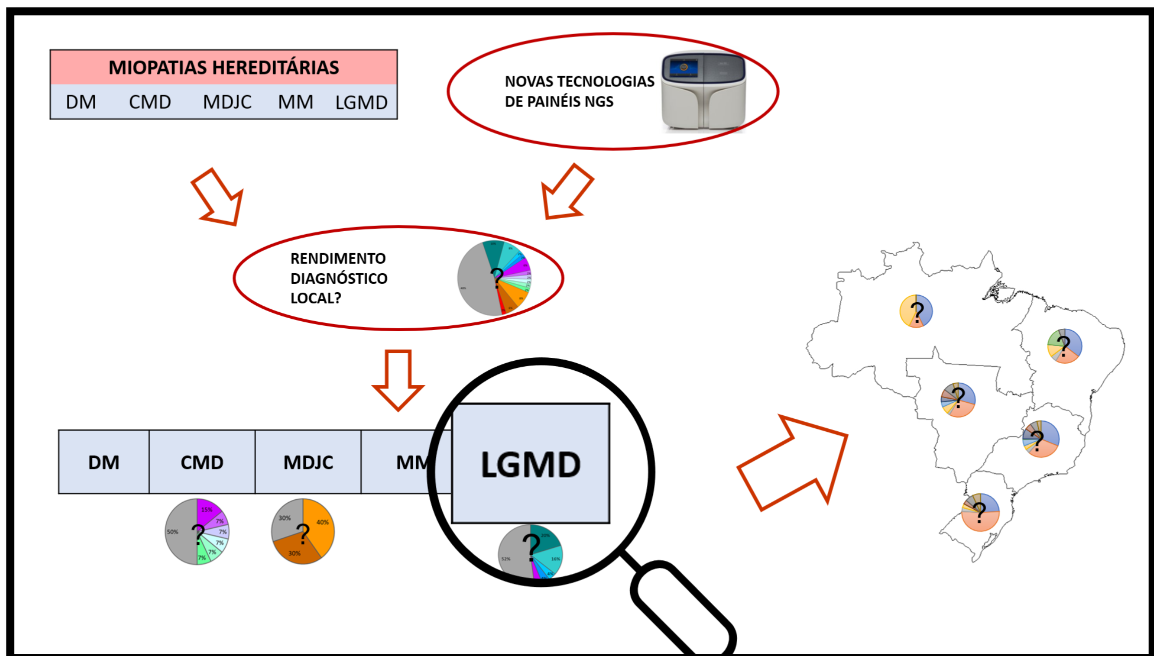


Figura 3: Marco conceitual esquemático. Fonte: Elaborado pelo autor (2021)

4. JUSTIFICATIVA

As miopatias hereditárias são um grupo de doenças com grande heterogeneidade clínica e genética e, assim como ocorrem em outras doenças neurogenéticas, o tempo decorrido desde o início dos sintomas até o correto diagnóstico pode ser muito longo. Diversos exames complementares, muitas vezes invasivos e de alto custo são realizados na tentativa de um diagnóstico definitivo e isto implica em um alto custo pessoal, familiar e social, mesmo sendo um grupo de doenças raras.

Os ambulatórios integrados de neurologia e genética das doenças neuromusculares dos serviços de Neurologia e de Genética Médica do Hospital de Clínicas de Porto Alegre acompanham cerca de 70 famílias, levantamento realizado em 2017 período do iníco deste trabalho, com suspeita clínica de LGMD, MDC, EDMD, MM, MD e CM, a maioria destas sem diagnóstico molecular definido e muitas sem uma clara definição do padrão de herança da doença.

O uso de tecnologias de NGS, por permitirem a análise simultânea de múltiplos genes de forma relativamente rápida, pode ter um papel fundamental no diagnóstico molecular preciso das distrofias/miopatias hereditárias de forma custo e tempo-efetiva.

Existem poucos estudos que avaliaram o rendimento de painel de NGS para o diagnóstico de pacientes com suspeita de distrofias/miopatias hereditárias, muitos deles apresentam limitações metodológicas com relação ao delineamento e população incluída. Não encontramos estudos específicos em miopatias congênitas, distrofias congênitas, EDMD ou miopatias metabólicas. Nenhum dos estudos encontrados foi realizado na América Latina até o ano de 2016 quando demos início a este trabalho. A frequência relativa das principais formas de distrofias/miopatias hereditárias no Brasil é desconhecida, bem como é desconhecido as características clínicas e moleculares destes indivíduos.

O esforço científico para buscar o conhecimento das causas genéticas específicas de distrofia/miopatia hereditária trará benefícios diretos aos pacientes e familiares, especialmente relacionados ao aconselhamento genético e ao evitar procedimentos invasivos como a biópsia muscular. Além disso, auxiliará na melhor compreenção entre os achados fenotípicos, especialmente para as formas mais raras, além do conhecimento epidemiológico local para o desenvolvimento de futuros projetos e definição de protocolos diagnósticos assistenciais para a avaliação deste grupo de doenças.

Dois trabalhos foram realizados e expostos em formato de artigos científicos buscando avaliar o rendimento de um painel de genes de NGS para o diagnóstico das HM no sul do Brasil assim como avaliar as características clínicas, genéticas e eventuais modificadores de fenótipo no subgrupo LGMD no Brasil. O primeiro artigo avalia uma coorte histórica multicêntrica de casos

índices consecutivos e seus familiares com diagnóstico genético ou patológico de LGMD2. Curvas de sobrevida para incapacidade física nas LGMD2A, LGMD2B e sarcoglicanopatias foram construídas e se avaliou as progressões clínicas de acordo com o sexo e genótipo. O segundo artigo, um estudo transversal de casos índices consecutivos com suspeita clínica de HM num centro único especializado em doenças neuromusculares no sul do Brasil, avalia o rendimento diagnóstico de um painel NGS personalizado compreendendo 39 genes como teste de primeira linha para o diagnóstico de HM além de caracterizar os achados clínicos e moleculares de famílias com HM nesta população. Os métodos e resultados, bem como a discussão dos mesmos serão apresentados em dois artigos que se encontram a seguir.

5. OBJETIVOS

5.1 Objetivo geral

Avaliar o rendimento diagnóstico do painel NGS para pacientes com suspeita de miopatias hereditárias e descrever o perfil clínico, molecular e epidemiológico dessas condições no Brasil.

5.2 Objetivos específicos

- Avaliar o rendimento diagnóstico da análise com painel de 39 genes por NGS nas DMC, DC, EDMD, MM, MD e CM em pacientes acompanhados no ambulatório de genética das doenças neuromusculares do HCPA;
- Descrever a caracterização clínica e molecular das famílias com LGMD2 em centros especializados em doenças neuromusculares de todas as regiões do Brasil
- Avaliar a frequência relativa das diferentes formas de miopatias hereditárias de acordo com a classificação clínica inicial e padrão de herança;
- Descrever achados que possam ajudar no diagnóstico diferencial desses distúrbios e fornecer correlação genótipo fenótipo, prognóstico e informações de história natural sobre os subtipos mais frequentes que encontrarmos.
- Descrever os achados moleculares (mutações mais frequentes, descrição de novas mutações) da avaliação por painel de múltiplos genes por NGS para o diagnóstico de pacientes com suspeita de miopatias hereditárias;
- Descrever os achados clínicos associados aos achados moleculares encontrados;
- Descrever achados função cardíaca por ecocardiograma e eletrocardiograma de acordo com os genótipos identificados;
- Descrever os achados de função pulmonar por espirometria de acordo com os genótipos identificados;

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7. ARTIGO I
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Clinicogenetic lessons from 370 patients with autosomal recessive limb-girdle muscular dystrophy

Running Title: Autosomal recessive LGMD in Brazil

Pablo Brea Winckler[1,2]
André Macedo Serafim da Silva[3]
Antônio Rodrigues Coimbra Neto[4,5]
Elmano Carvalho[6]
Eduardo Boiteux Uchôa Cavalcanti[7]
Cláudia Ferreira da Rosa Sobreira[8]
Carlo Domenico Marrone[9]
Marcela Câmara Machado-Costa[10]
Alzira Alves de Siqueira Carvalho[11]
Raimunda Helena Ferreira Feio[12]
Cleonísio Leite Rodrigues[13]
Marcus Vinicius Magno Gonçalves[14]
Renata Barreto Tenório[15]
Rodrigo de Holanda Mendonça[3]
Ana Cotta[6]
Júlia Filardi de Oliveira Paim[6]
Cynthia Costa e Silva[7]
Camila de Aquino Cruz[8]
Marjory Irineu Bená[8]
Daniel Fernando Arias Betancur[9]
Antonette Souto El Husny[12,16]
Isabel Cristina Neves de Souza[12]
Regina Célia Beltrão Duarte[17,18]
Umbertina Conti Reed[3]
Márcia Lorena Fagundes Chaves[1,2,19]
Edmar Zanoteli[3]
Marcondes Cavalcante França Junior[4,5]
Jonas Alex Morales Saute[1,2,15,19]*

1- Neurology Division, Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre, Brazil
2- Graduate Program in Medicine: Medical Sciences, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil
3- Universidade de São Paulo (USP), Faculdade de Medicina, Departamento de Neurologia, São Paulo, SP, Brazil
4- Department of Neurology, Faculdade de Ciências Médicas, Universidade Estadual de Campinas (UNICAMP), Campinas, Brazil
5- Graduate Program in Medical Physiopathology, UNICAMP, Campinas, Brazil
6- Rede SARAH de Hospitais de Reabilitação, Belo Horizonte, Brazil
7- Rede SARAH de Hospitais de Reabilitação, Brasília, Brazil
8- USP, Ribeirão Preto Medical School, Department of Neurosciences, Ribeirão Preto, Brazil
9- Physiatry Division, Hospital São Lucas da Pontifícia Universidade Católica, Porto Alegre, Brazil
10- Hospital Universitário Professor Edgard Santos, Universidade Federal da Bahia, Salvador, Brazil
11- Faculdade de Medicina do ABC, Santo André, Brazil
12- Hospital Universitário Bettina Ferro de Souza, Universidade Federal do Pará (UFPA), Belém, Brazil
13- Neurology Division, Hospital Geral de Fortaleza, Fortaleza, Brazil
14- Universidade da Região de Joinville, Joinville, Brazil

15- Medical Genetics Division, HCPA, Porto Alegre, Brazil
16- Centro Universitário do Estado do Pará, Belém, Brazil
17- Hospital Ophir Loyola, Belém, Brazil
18- Department of Neurology, UFPA, Belém, Brazil
19- Department of Internal Medicine, UFRGS, Porto Alegre, Brazil

*Corresponding author: Jonas Alex Morales Saute, Medical Genetics Divison, HCPA Ramiro Barcelos, 2350, Zip-code 90035-903, Porto Alegre, Brazil, Phone:+55-051-33598011

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Conflict of interest statement
Winckler PB, Macedo A, Coimbra-Neto AR, Carvalho E, Cavalcanti EBU, Sobreira CFR, Marrone CD, Machado-Costa MC, Carvalho AAS, Feio RHF, Rodrigues CL, Gonçalves MVM, Tenório RB, Mendonça RH, Cotta A, Paim J, Costa-e-Silva C, Cruz CA, Bená MI, Betancur DFA, El-Husny AS, Neves I, Duarte RC, Reed UC, Chaves MLF, Zanoteli E have no disclosures related to this study. França Jr. MC and Saute JA received research grants from PTC Therapeutics. Study design; collection, analysis and interpretation of data; writing of the report and the decision to submit the report for publication had no participation of the study sponsors.

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

ABSTRACT
Limb-girdle muscular dystrophies (LGMD) are a group of genetically heterogeneous disorders characterized by predominantly proximal muscle weakness. We aimed to characterize epidemiological, clinical and molecular data of patients with autosomal recessive LGMD2/LGMD-R in Brazil. A multicenter historical cohort study was per- formed at 13 centers, in which index cases and their affected relatives' data from consecutive families with genetic or pathological diagnosis of LGMD2/LGMD-R were reviewed from July 2017 to August 2018. Survival curves to major handicap for LGMD2A/LGMD-R1-calpain3-related, LGMD2B/LGMD-R2-dysferlin-related and sarcoglycanopathies were built and progressions according to sex and genotype were estimated. In 370 patients (305 families) with LGMD2/LGMD-R, most frequent sub- types were LGMD2A/LGMD-R1-calpain3-related and LGMD2B/LGMD-R2- dysferlin-related, each representing around 30% of families. Sarcoglycanopathies were the most frequent childhood-onset subtype, representing 21% of families. Five percent of families had LGMD2G/LGMD-R7-telethonin-related, an ultrarare subtype worldwide. Females with LGMD2B/LGMD-R2-dysferlin-related had less severe progression to handicap than males and LGMD2A/LGMD-R1-calpain3-related patients with truncating variants had earlier disease onset and more severe progression to handicap than patients without truncating variants. We have provided paramount epidemiological data of LGMD2/LGMD-R in Brazil that might help on differential diagnosis, better patient care and guiding future collaborative clinical trials and natural history studies in the field.
KEYWORDS disease modifier, epidemiology, limb-girdle, muscular dystrophy, natural history

1 | INTRODUCTION

Limb-girdle muscular dystrophies (LGMD) represent a heterogeneous group of genetic disorders characterized by predominantly proximal muscle weakness starting after independent ambulation is attained.[1,2] LGMDs have wide clinical spectrum varying from severe infantile forms leading to loss of ambulation early in life to milder adult forms with little handicap.[1,3]

LGMDs are classified according to the inheritance mode into LGMD1/LGMD-D, with autosomal dominant inheritance, and LGMD2/LGMD-R, with autosomal recessive inheritance. Among these groups, each specific subtype is designated by a letter or number given in the chronological order of locus mapping, respectively, in the classical [4,5] and novel nomenclature systems. [6]

A recent meta-analysis estimated LGMDs prevalence as 1.63 per 100 000 individuals, ranging from 0.56 in Italy to 6.9 per 100 000 individuals in Spain.[7] LGMD2/LGMD-R are the most common group of these disorders,[8] but relative frequencies among LGMD2/LGMD-R subtypes vary from region to region.[8-11] There is a lack of epidemiological data about LGMD in Latin America, with only few single-center studies reporting sarcoglycanopathies as the most frequent subtypes in Brazil.[12-14]

The differential diagnosis among LGMD subtypes relies upon muscle immunohistochemistry (IHC) and genetic diagnosis.[1,2] However, considering the growing number of subtypes and its clinical overlap, the lack of specific tests to identify protein defects for most recently described subtypes, and the costs and laboriousness of conventional Sanger sequencing diagnosis for genetically heterogeneous disorders; target or unbiased next-generation sequencing (NGS) is becoming widely used as both confirmatory and first-tier approaches for LGMD diagnosis. [9,15-17]

We performed a collaborative study encompassing 13 centers specialized in neuromuscular diseases from all regions of Brazil to characterize clinical and molecular information regarding LGMD2/ LGMD-R families in the country. We also aimed to provide information that might help in the differential diagnosis of these disorders, and to provide genotype-phenotype correlation, prognostic and natural history information of the most frequent subtypes that might be useful for designing of future therapeutic trials.

2 | MATERIALS AND METHODS

2.1 | Design and subjects

We performed a multicenter historical cohort study at 13 neuromuscular disorders centers in Brazil: three centers from South, five centers from Southeast, one center from Midwest, two centers from North- east, and two centers from the North region.

Index cases and affected relatives data from consecutive families with clinical suspicion of LGMD2/LGMD-R were reviewed from July 2017 to August 2018. Eligibility was clinical suspicion of LGMD[18] and the presence of at least one of the following criteria (a) presence of at least a likely pathogenic variant in an LGMD2/LGMD-R gene; or (b) LGMD2/LGMD-R subtype diagnosis based on muscle IHC or western blot (WB) analysis. Patients with GAA pathogenic variants were excluded from the study.

After considered eligible, patients were classified into three diagnostic certainty categories: (a) diagnosed, when two pathogenic variants in an LGMD2/LGMD-R gene were found or when muscle IHC/WB analysis were diagnostic; (b) likely diagnosed, when two likely pathogenic variants or one likely pathogenic and one pathogenic variant were found in an LGMD2/LGMD-R gene; and (c) possible diagnosis, when single pathogenic or likely pathogenic variants were found on an LGMD2/LGMD-R gene, accompanied or not by variants of unknown significance (VUS). The study was approved by the Ethics in Research Committees of the institutions (GPPG-HCPA/17-0340; HGF-1.347.489), which follow the Declaration of Helsinki. Informed written consent was obtained from individuals' or their guardians or data utilization con-sent was signed for reviewing medical records.

2.2 | Clinical and histological data collection

We collected data regarding sex, age, parental consanguinity, family recurrence, age at onset (first sign of weakness or respiratory/cardiac- related symptoms), age at any walking aid (AWA) and wheelchair (AWC) dependency, and highest creatine kinase (CK) levels. Such information was collected from patients and relatives and reviewed from medical records. When AWC was provided, but AWA was not, we considered the same value for both variables. When information regarding walking aid and wheelchair dependency was not provided data was censored. Muscle IHC/WB data were used either as a diagnostic standard or as functional evidence of pathogenicity for novel variants, when available.

Cardiac and respiratory functions were assessed according to each center protocol. Conduction abnormalities were categorized as significant and non-significant, and structural cardiac abnormalities were classified as related and non-related to LGMD by an internal medicine experienced physician. Respiratory function was investigated by spirometry with forced vital capacity (FVC) and forced expiratory volume (FEV1). Severity of lung restriction was classified according to FVC, where predicted FVC > 60% was considered mild, predicted FVC between 51% and 59% was considered moderate and predicted FVC ≤50% was considered severe lung involvement. Necessity of non-invasive or invasive ventilatory support was also assessed. We reported data on the last available electrocardiogram, holter, echocardiogram and spirometry.

2.3 | Molecular analysis

DNA was extracted from peripheral blood and most centers performed a commercial targeted NGS panel that includes the most common genes related to LGMD2/LGMD-R in other populations (*ANO5*, *CAPN3*, *DYSF*, *FKRP*, *SGCA*, *SGCB*, *SGCD*, *SGCG* and *TCAP*). Some centers were able to target Sanger sequencing according to the pathological characteristics when there was a specific protein defect on muscle specimen. Sequences were searched for using the National Center for Biotechnology Information (NCBI) protein database, and variants were described with reference to the following transcripts: *ANO5* (NM_213599.2), *CAPN3* (NM_000070.3), *DYSF* (NM_003494.3), *FKRP* (NM_001039885.2), *SGCA* (NM_000023.4), *SGCB* (NM_000232.4), *SGCD* (NM_000337.5), *SGCG* (NM_000231.2), *TCAP*

(NM_003673.3). Sequence variations were compared to data available in the Human- Gene-Mutation-Database (HGMD), Leiden-Muscular-Dystrophy- Database (http://www.dmd.nl) and ClinVar. Variants were classified according to 2015 American College of Medical Genetics and Geno- mics (ACMG) criteria.[19] PolyPhen-2,[20] SIFT,[21] CADD,[22] M-CAP,[23] Mutation-Taster,[24] Human-Splicing- Finder-v3.0[25] and ESEfinder v.3.0[26] were used for in silico analysis. Phylogenetic conservation was estimated with Genomic Evolutionary Rate Profiling (GERP++),[27] and allele frequencies were searched on gnomAD[28] and 1000[29] genomes browser.

For genotype/phenotype correlations in survival analysis the identified variants were classified as null/truncating (nonsense, frameshift and canonical splice site variants) and non-truncating variants (mis-sense, in-frame deletions, and other splice site variants). Patients who carried truncating and non-truncating variants were defined as with one truncating variant.

2.4 | Statistical analysis

Normal distribution was evaluated by Shapiro-Wilk test. Quantitative features were reported as mean and SD for parametric and median and interquartile range for non-parametric data. Age at onset comparisons across LGMD2/LGMD-R subtypes was performed by one-way analysis of variance (ANOVA), followed by Tukey post hoc test. Maxmum CK levels comparisons across LGMD2/LGMD-R subtypes were performed by Kruskal-Wallis followed by the Dunn post hoc test. Age at onset comparisons between sexes across subtypes were performed with independent samples Student t test. Median age and disease duration at walking aid or wheelchair dependency for the most frequent LGMD2/LGMD-R subtypes and according to sex and variants severity within subtypes were compared by Kaplan-Meier analysis with Log-Rank comparisons. A P-value of <.05 was considered statistically significant.

3 | RESULTS

We included 370 patients (347 with likely or defined diagnosis) from 305 families. Consanguinity was reported by 115/288 (40.2%) families and 188/282 (66.7%) were simplex cases. Our sample is representative of the LGMD2/LGMD-R population in Brazil, as the referral centers covered all regions of the country.

3.1 | Relative frequency of the different LGMD2/ LGMD-R forms

The relative frequency of the LGMD2/LGMD-R subtypes in Brazil and according to each country region is shown in **Figure 1**. Defined diagnosis was based only on IHC/WB analysis in 70 families: a single family with LGMD2A/LGMD-R1-calpain3-related with a single pathogenic variant in *CAPN3*, and 48 families with LGMD2B/LGMD-R2-dysferlin-related, 20 families with LGMD2C-F (sarcoglycanopathies) and one family with LGMD2G/LGMD-R7-telethonin-related, in which molecular analysis was not performed. Overall, most frequent subtypes of LGMD2/LGMD-R in Brazil were LGMD2A/LGMD-R1-calpain3-related (N = 98/305, 32.1%) and LGMD2B/LGMD-R2-dysferlin-related (N = 93/305, 30.5%).

LGMD2B/LGMD-R2-dysferlin-related was the most frequent form when only considering patients with likely or defined diagnosis (N = 89/284, 31.3%), followed by LGMD2A/LGMD-R1-calpain3-related (N = 86/ 284, 30.3%), sarcoglycanopathies as a group (63/284, 22.2%), LGMD2I/LGMD-R9-FKRP-related (N = 19/284, 6.7%), LGMD2G/LGMD-R7-telethonin-related (N = 15/284, 5.3%) and LGMD2L/ LGMD-R12-anoctamin5-related (12/284, 4.2%, **Table 1** and **Figure 1**). Within sarcoglycanopathies with available genotype (N = 43), LGM D2D/LGMD-R3-α-sarcoglycan-related represented 37.2%, LGMD2E/ LGMD-R4-β-sarcoglycan-related 30.2%, LGMD2C 23.2% and LGMD2F/LGMD-R6-δ-sarcoglycan-related 9.3% of families. Regional relative frequencies of LGMD2/LGMD-R subtypes were similar to national frequencies, except for the North region (N = 7).

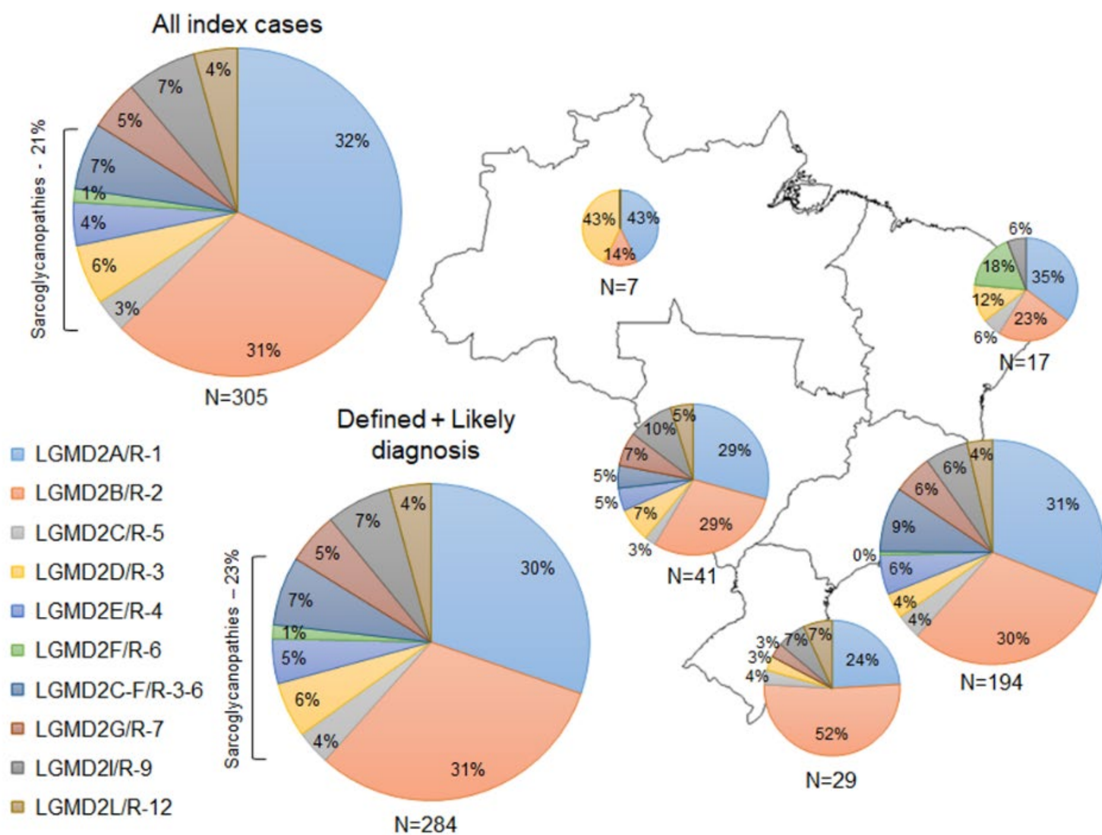

FIGURE 1 Relative frequencies of LGMD2/LGMD-R in Brazil. Relative frequency of LGMD2/LGMD-R subtypes in Brazil and according to each country region (with defined or likely diagnosis) is presented in percentages. N indicates the number of indexes cases in the country and per region. Sum of cases per region is greater than the country number, because some families were considered for more than one region. LGMD2CF/R-3-6 refers to sarcoglycanopathies diagnosis based only on immunohistochemistry analysis.

TABLE 1 Clinical characteristics of LGMD2/LGMD-R subtypes

	LGMD2A/R1	LGMD2B/R2	LGMD2C-F/R3-6	LGMD2G/R7	LGMD2I/R9	LGMD2L/R12
Affected gene	*CAPN3*	*DYSF*	*SGCA-B-G-D*	*TCAP*	*FKRP*	*ANO5*
Families/Patients	86/111	89/109	63/70	15/18	19/27	12/12
Male sex (%)	46 (41.4%)	51 (46.8%)	22 (31.4%)	9 (50%)	20 (74.1%)	10 (83.3%)
Age[a] (SD)	34.5 (13.2)	38.5 (13.6)	27.93 (12.8)	35.38 (13.2)	38.4 (9.9)	42.9 (17.7)
Age at Onset[a] (SD)	13.7 (8.1)	22.9 (8.5)	8.8 (8.0)	12.9 (8.1)	22.6 (12.9)	36.6 (17.4)
Disease Duration[a] (SD)	20.75 (10.62)	15.62 (10.8)	19 (10.2)	23.19 (12.5)	15.8 (13.8)	6.2 (4.0)
Walking aid (%)	59/106 (55.7%)	40/103 (38.8%)	53/67 (79.1%)	7/16 (43.8%)	8/25 (32%)	2/11 (18.2%)

AWA[b] (95%CI)	36 (31.2–40.7)	42 (35.5-48.4)	18 (12.7-23.2)	36 (26.1-45.8)	46 (39.5-52.5)	66 (NA)
Wheelchair bound (%)	44/106 (41.5%)	22/100 (22%)	43/65 (73.8%)	4/16 (25%)	5/25 (20%)	0/12 (0%)
AWC[b] (95%CI)	45 (37.3–52.7)	56 (NA)	18 (14-21.9)	42 (NA)	51 (43.6-58.4)	NA
Max CK IU/L[c] (IQR)	1507 (2410) range 141-25 541	4371 (5524) range 153-27 578	3340 (7757) range 46-27 300	600 (1065) range 63-3496	2000(3485) range 46-27 300	3897 (7108) range 46-27 300
Heart conduction (1%)disorder (%)	1/105	1/83 (1.2%)	0/53 (0%)	0/17 (0%)	6/20 (30%)	0/12 (0%)
Structural heart (0%)disease (%)	0/91	1/83 (1.2%)	3/51 (5.8%)	0/17 (0%)	8/20 (40%)	0/12 (0%)
Respiratory involvement (%)	22/102(21.5%)	13/80 (16.2%)	22/39 (56.4%)	3/13 (23.1%)	9/14 (64.3%)	2/10 (20%)
Lung restriction severity[d]						
Mild	16/100 (16%)	9/79 (11.4%)	9/35 (25.7%)	2/13 (15.4%)	4/11 (36.4%)	1/10 (10%)
Moderate	6/100 (6%)	2/79 (2.5%)	3/35 (8.6%)	1/13 (7.7%)	0/11 (0%)	0/10 (0%)
Severe	2/100 (2%)	1/79 (1.3%)	6/35 (17.1%)	0/13 (0%)	5/11 (45.5%)	1/10 (10%)
Ventilatory support (%)	2/104 (1.9%)	1/105 (1%)	4/38 (10.5%)	0/15 (0%)	2/22 (9.1%)	0/9 (0%)

Note: The total number of patients were information on walking aids and wheelchair necessity, and cardiac and respiratory involvement is available is givenfor each LGMD subtype. classification. Abbreviations: CI, confidence interval; IQR, interquartile range. [a]Mean Kaplan-Meyer survival analysis. [b]Median from Kaplan-Meyer survival analysis.[c]Median. [d]Presented when spirometry data was available to allow severity.

3.2 | Molecular analysis

We identified 119 different disease-related variants in the investigated genes, 37 of those were not previously described (16 in *CAPN3*, 12 in *DYSF*, four in *ANO5*, two in *FKRP* and one each *SGCA*, *SGCB*, *SGCG*, **Table 2** and **Table S2**). Twelve of these variants were classified as VUS and they were reported because in all these cases these variants were found together with a pathogenic/likely pathogenic variant. Most variants were unique, with few exceptions (**Table S1**).

3.3 | Age at onset

Mean age at onset was different across LGMD2/LGMD-R subtypes ($F = 37.060$, $P < .001$, **Table 1**, **Figure 2**). Patients with sarcoglycanopathies had the earliest mean age at onset (8.87 years; 95% confidence interval [CI], 6.9-10.7), followed by patients with LGMD2G/LGMD-R7-telethonin-related (12.3 years; 95% CI, 8.2-16.3) and LGMD2A/LGMD-R1-calpain3-related (13.75 years; 95% CI, 12.26-15.25), LGMD2I/LGMD-R9-FKRP-related (22.5 years; 95% CI, 17.4-27.7) and LGMD2B/LGMD-R2-dysferlin-related (22.9 years; 95% CI, 21.3-24.5), and LGMD2L/LGMD-R12-anoctamin5-related that had the latest age at onset (36.6 years; 95% CI, 24.9-48.3) among LGMD2/LGMD-R subtypes.

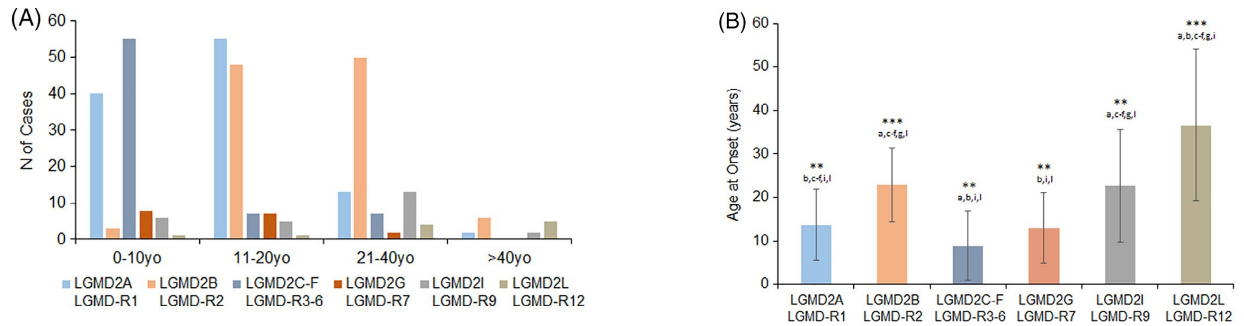

FIGURE 2 Age at onset of LGMD2. A) Number of families with LGMD according to age of onset of the index case. B) Age at onset for each LGMD subtype. Bars represent means and lines SDs. Different in Tukey post-hoc test from: a, LGMD2A/LGMDR1-calpain3-related; b, LGMD2B/LGMD-R2-dysferlin-related; c-f, sarcoglycanopathies; g, LGMD2G/LGMD-R7-telethonin-related; i, LGMD2I/LGMD-R9-FKRP-related; l, LGMD2L/LGMDR12-anoctamin5-related; **p < 0.01; ***p < 0.001

3.4 | CK levels

Maximum CK levels were different across LGMD2/LGMD-R subtypes (P < .001, **Table 1**, **Figure S1**).

3.5 | Muscle weakness progression

Sarcoglycanopathies (N = 65, 62 families) had the earliest median age of walking aid dependency (18 years; 95% CI, 12.7-23.2; P < .001 for overall comparisons, **Figure 3A**), followed by LGMD2A/LGMD-R1-calpain3-related (N = 106, 86 families; 36 years; 95% CI, 31.2-40.7) and LGMD2B/LGMD-R2-dysferlin-related (N = 100, 85 families; 42 years; 95% CI, 37.4-46.6), which were statistically different from each other (P = .012). The same happened for wheelchair dependency, in which sarcoglycanopathies had the earliest median age at dependency (18 years; 95% CI, 14.04-21.9), followed by LGMD2A/LGMD-R1-calpain3-related (45 years; 95% CI, 37.3-52.7) and LGMD2B/LGMD-R2-dysferlin-related (56 years; 95% CI, not available; P < .001 for overall and between groups comparisons, **Figure 3B**). When we have considered disease duration as the time variable, median disease duration at walking aid dependency occurred earlier for sarcoglycanopathies (9 years; 95% CI, 4.4-13.5; P < .001 for overall comparisons, **Figure 3C**), but at similar durations (P = .136) for LGMD2A/LGMD-R1-calpain3-related (21 years; 95% CI, 16.9-25) and LGMD2B/LGMD-R2-dysferlin-related (20 years; 95% CI, 15.9-24.1). The same happened for wheelchair dependency, in which sarcoglycanopathies had the earliest median disease duration at dependency (11 years; 95% CI, 7.1-14.8; P < .001 for overall comparisons, **Figure 3D**), but with similar durations (P = .343) for LGMD2A/LGMD-R1-calpain3-related (26 years; 95% CI, 22-29.9) and LGMD2B/LGMD-R2-dysferlin-related (27 years; 95% CI, 22.3-31.6). Results were similar when only analyzing index cases (data not shown).

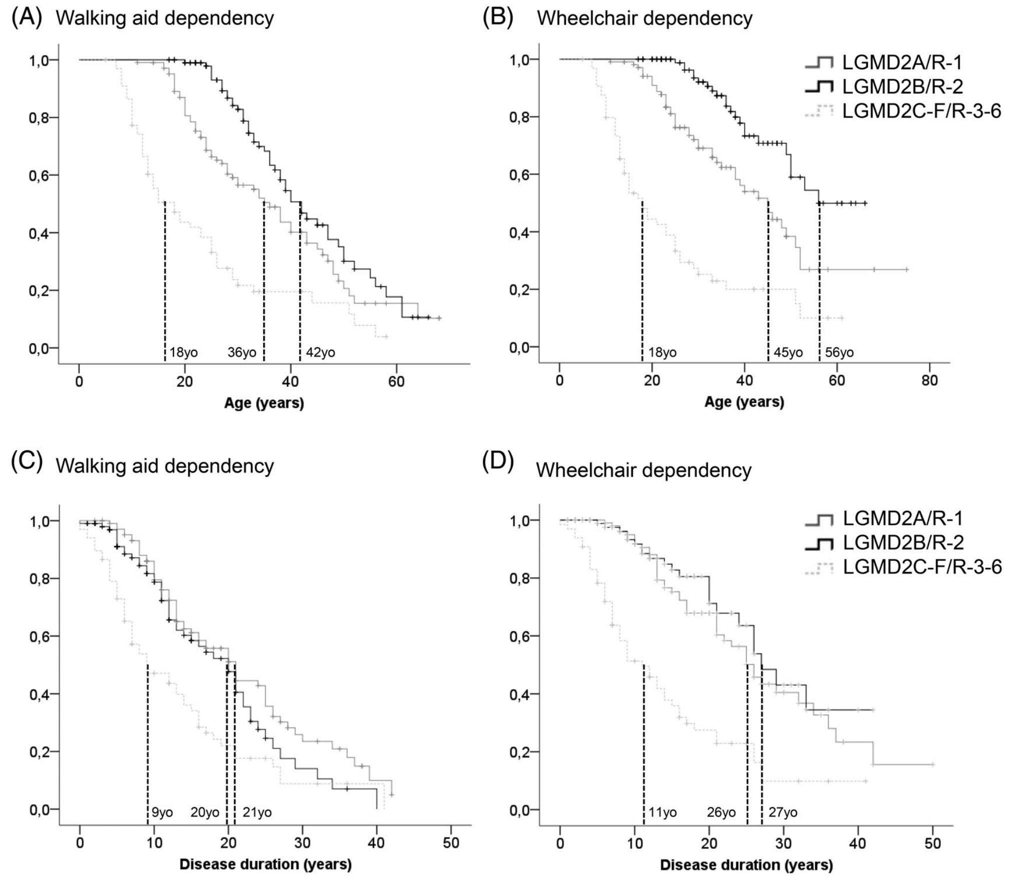

FIGURE 3 Progression to major handicap in most frequent LGMD2. The figure shows Kaplan-Meier analysis of loss of independent walking (A) and wheelchair dependency (B) according to age, and loss of independent walking (C) and of wheelchair dependency (D) according to disease duration for individuals with LGMD2A/LGMD-R1-calpain3-related, LGMD2B/LGMD-R2-dysferlin-related and sarcoglycanopathies (LGMD2C-F). Median values of progressions to major handicap are informed and highlighted by dashed lines

TABLE 2 Novel variants in LGMD2/LGMD-R genes

Gene	Nucleotide change	AA change	Mutation type	AF[a]	AF[b]	SIFT	Poly Phen2	MT	CADD	MCAP	GERP++[c]	Segregation	Functional evidence	Fam	N[d]	Leiden	ClinVar	ACMG criteria[e]	Classification
ANO5	c.689A > G	p.Asp230Gly	Missense	0.00011	0.0004	0.21	0.006	0.94	21.6	0.055	5.77	NA	NA	1	1	NA	Yes (2 VUS)	PM1,PM2	VUS
ANO5	c.1359C > G	p.Tyr453*	Nonsense	0.000004	0	NA	NA	1	25.1	NA	−3.91	NA	NA	4	4	NA	No	PVS1,PS4, PM2	Pathogenic
ANO5	c.2012A > G	p.Tyr671Cys	Missense	0.000008	0	0.03	0.992	0.99	26.7	0.086	5.71	NA	NA	1	1	NA	Yes (1 VUS)	PM2,PP3	VUS
ANO5	c.2190G > T	p.Trp730Cys	Missense	0	0	0	0.980	0.99	34	0.272	5.57	NA	NA	1	1	NA	No	PM2,PP3	VUS
CAPN3	c.390G > A	p.Try130*	Nonsense	0	0	NA	NA	1	44	NA	6.07	NA	NA	1	1	No	No	PVS1,PM2	Likely pathogenic
CAPN3	c.412dupC	p.Leu138 Profs*15	Frameshift	0	0	NA	NA	1	NA	NA	6.07	NA	NA	5	6	No	No	PVS1,PS4, PM2	Pathogenic
CAPN3	c.550A > G	p.Thr184Ala	Missense	0.00003	0	0.02	0.997	0.99	25.9	0.487	5.88	NA	NA	5	6	No	No	PS4,PM1 PM2, PM5 PP1, PP3	Pathogenic
CAPN3	c.692 T > C	p.Phe231Ser	Missense	0	0	0	1	0.99	29.8	0.673	5.41	Yes	NA	1	2	No	No	PM1,PM2 PM3, PP1	Likely pathogenic
CAPN3	c.1240 T > C	p.Cys414Arg	Missense	0	0	0	0.981	0.99	29.4	0.587	5.37	Yes	NA	1	2	No	No	PM1, PM2, PP1, PP3	Likely pathogenic
CAPN3	c.1314G > A	p.Trp438*	Nonsense	0	0	NA	NA	1	42	NA	5.37	NA	NA	1	1	No	No	PVS1,PM2	Likely pathogenic
CAPN3	c.1314G > T	p.Trp438Cys	Missense	0	0	0	0.995	1	33	0.592	5.37	Yes	NA	1	2	No	No	PM1, PM2, PP1,PP3	Likely pathogenic
CAPN3	c.1354 + 21G > A	-	Intronic	0	0	NA	NA	0.99[f]	11.33	NA	3.35	NA	NA	1	1	No	No	PM2	VUS
CAPN3	c.1456C > T	p.Gln486*	Nonsense	0	0	NA	NA	1	39	NA	4.54	NA	NA	1	1	No	No	PVS1,PM2	Likely pathogenic
CAPN3	c.1782 + 1G > T	-	Missense	0	0	NA	NA	1	32	NA	4.71	Yes	NA	1	2	No	No	PVS1,PM2, PP1	Pathogenic
CAPN3	c.2031_2032insC	p.Asn678 Glnfs*22	Frameshift	0	0	NA	NA	1	NA	NA	4.79	NA	Yes (calpain absent - WB)	1	2	No	No	PVS1,PS3, PM2, PP1	Pathogenic
CAPN3	c.2180 _2183dupGGCA	p.Lys729 Alafs*8	Frameshift	0	0	NA	NA	1	NA	NA	4.72	NA	NA	1	1	No	No	PVS1,PM2	Likely pathogenic
CAPN3	c.2263G > C	p.Gly755Arg	Missense	0	0	0	1	0.99	34	NA	4.54	NA	NA	1	1	No	No	PM2,PP3	VUS
CAPN3	c.2360 _2361insTTCA	p.Arg788 Serfs*2	Frameshift	0	0	NA	NA	1	NA	NA	4.4	NA	NA	1	1	No	No	PVS1,PM2	Likely pathogenic
CAPN3	c.2362delA	p.Arg788 Glyfs*95	Frameshift	0	0	NA	NA	1	NA	NA	3.03	NA	NA	1	1	No	No	PVS1,PM2	Likely pathogenic
CAPN3	c.2275A > T	p.Asn759Tyr	Missense	0	0	0	0.999	0.99	31	0.602	5.4	NA	NA	1	1	No	No	PM1,PM2, PP3	VUS
DYSF	c.855 + 1G > A	-	Splice Site	0	0	NA	NA	1	31	NA	4.46	Yes	Yes (dysferlin absent)	1	2	No	No	PVS1,PS3, PM2	Pathogenic
DYSF	c.1163 _1165dupCCG	p.Ala388dup	Small duplication	0	0	NA	NA	0.99	NA	NA	5.47	NA	NA	1	1	No	No	PM1,PM2, PM4	Likely pathogenic
DYSF	c.1165G > T	p.Glu389*	Nonsense	0	0	NA	NA	1	48	NA	5.47	NA	NA	1	1	No	No	PVS1,PM2	Likely pathogenic
DYSF	c.2076delC	p.His693 Thrfs*4	Frameshift	0	0	NA	NA	1	NA	NA	4.38	NA	NA	1	1	No	No	PVS1,PM2	Likely pathogenic

TABLE 2 (Continued)

Gene	Nucleotide change	AA change	Mutation type	AF[a]	AF[b]	SIFT	Poly Phen2	MT	CADD	MCAP	GERP++[c]	Segregation	Functional evidence	Fam	N[d]	Leiden	ClinVar	ACMG criteria[e]	Classification
DYSF	c.2218delC	p.Leu740 Cysfs*9	Frameshift	0	0	NA	NA	1	NA	NA	3.54	NA	NA	1	1	No	No	PVS1,PM2	Likely pathogenic
DYSF	c.2901dupC	p.Met968 Hisfs*3	Frameshift	0	0	NA	NA	1	NA	NA	3.37	NA	Yes (dysferlin reduced)	3	2	No	No	PVS1,PS3, PM2	pathogenic
DYSF	c.2996G > A	p.Trp999*	Nonsense	0	0	NA	NA	1	41	NA	4.64	Yes	Yes (dysferlin absent)	1	2	Yes	No	PVS1,PS3, PM2	Pathogenic
DYSF	c.3071C > T	p.Pro1024Leu	Missense	0.00009	0	0.01	1	0.99	24	0.231	4.14	NA	NA	1	1	No	Yes (1 VUS)	PM2,PP3	VUS
DYSF	c.3115C > T	p.Arg1039Trp	Missense	0.00002	0	0.02	1	0.99	25.5	0.472	3.27	NA	NA	1	1	Yes (dysferlin absent - IHC)	Yes (2 VUS)	PS3,PM2, PP3	Likely pathogenic
DYSF	c.3235 _3236ins AGGCGG	p.Phe1079*	In-frame insertion (nonsense)	0	0	NA	NA	1	NA	NA	5.6	NA	NA	1	1	No	No	PVS1,PM2	Likely pathogenic
DYSF	c.3280 T > C	p.Trp1094Arg	Missense	0	0	0.01	0.997	0.99	29.9	0.205	5.6	NA	NA	1	1	No	No	PM2,PP3	VUS
DYSF	c.3486 _3487delGG	p.Asp116 3Profs*11	Frameshift	0	0	NA	NA	1	NA	NA	3.37 (1.15-5-59)	NA	NA	1	1	No	No	PVS1,PM2	Likely pathogenic
FKRP	c.1180 T > A	p.Trp394Arg	Missense	0	0	0	0.997	0.99	27.2	0.844	5.05	NA	NA	1	1	No	No	PM2,PP3	VUS
FKRP	c.1403 T > C	p.Phe468Ser	Missense	0	0	0	0.879	0.99	29.1	0.730	5.44	NA	NA	1	1	NA	No	PM2,PP3	VUS
SGCA	c.502G > A	p.Gly168Arg	Missense	0.00001	0	0.09	0.755	0.99	24.1	0.551	4.58	NA	NA	1	1	No	Yes (1 VUS)	PM2	VUS
SGCB	c.753 + 5G > A	—	Intronic	0	0	NA	NA	1	21.7	NA	5.12	NA	NA	1	1	Yes (1 case)	No	PM2,PP3[g]	VUS
SGCG	c.629A > G	p.His210Arg	Missense	0.00001	0	0.13	0.012	0.99	14.29	0.079	4.44	NA	Yes (γ-sarcoglycan deficient)	1	1	Yes (2 cases)	No	PS3,PM2, PP3	Likely pathogenic

Abbreviations: AA, amino acid; AF, allele frequency; Fam, families; IHC, immunohistochemistry; MT, Mutation Taster; NA, not available; VUS, variant of unknown significance; alteration of the WT donor site (HSF 3.0); WB, Western Blot.

[a]Allele frequencies on gnomAD.

[b]Allele frequencies on 1000 genomes browsers.

[c]GERP++ data is shown as mean (SD) or raw value.

[d]Total number of individuals.

[e]American College of Medical Genetics and Genomics criteria, Richards et al, 2015.

[f]Polymorphism prediction.

[g]Alteration of the WT donor site on Human Splicing Finder 3.1 (http://www.umd.be/HSF3/).

3.6 | Sex-related effect

Distribution of sex across LGMD2/LGMD-R subgroups is shown in **Table 1**. Age at onset in males and females was similar in all subtypes (data not shown). Men and women curves for age and disease duration at walking aid or wheelchair dependency were similar in LGMD2A/LGMD-R1-calpain3-related and sarcoglycanopathies ($P > .10$ for all comparisons). Regarding LGMD2B/LGMD-R2-dysferlin-related, median age at walking aid dependency was 11 years earlier for men (36 years; 95% CI, 32.2-39.7) than for women (47 years; 95% CI, 40.4-53.6; P = .009, **Figure S2A**), and median disease duration at walking aid dependency was 10 years earlier for men (12 years; 95% CI, 9.8-14.1) than for women (22 years; 95% CI, 19.9-24.1; P = .024, **Figure S2C**). Median age at wheelchair dependency was not achieved by women and it occurred at 50 years old (95% CI, not available) for men; however, these survival curves were not statistically different (P = .274, **Figure S2B**). Median disease duration at wheelchair dependency was similar (P = .405, **Figure S2D**) between men (26 years; 95% CI, 23.4-28.5) and women (29 years; 95% CI, 21.8-36.1) with LGMD2B/LGMD-R2-dysferlin-related. Results were similar when only analyzing index cases (data not shown).

3.7 | Genotype-phenotype correlations

LGMD2A/LGMD-R1-calpain3-related patients with one (N = 22), two (N = 39) or none (N = 49) truncating variants presented different ages at onset ($F = 10.122$, $p < 0.001$). LGMD2A/LGMD-R1-calpain3-related patients with one or two truncating variants had similar ages at onset (difference of 2.5 years; 95% CI, −2.17-7.13; p = 0.415), whereas patients with one (difference of 4.48 years; 95% CI, 0.01-8.96; p = 0.049) or two (difference of 6.96 years; 95% CI, 3.23-10.71; $p < 0.001$, **Figure 4A**) truncating variants started earlier than patients without truncating variants. Due to similar progression behavior of LGMD2A/LGMD- R1-calpain3-related patients with one or two truncating variants, we grouped them together (N = 61). LGMD2A/LGMD-R1-calpain3-related patients with any truncating variant started 6.07 years (95% CI, 3.27-8.87, $p < 0.001$, **Figure 4B**) before patients without truncating variants. Patients with LGMD2B/LGMD-R2-dysferlin-related (N = 45) and sarcoglycanopathies (N = 46) had similar ages at onset across genotypes ($p > 0.05$ for all comparisons, **Figure S3**). We also performed subgroup analysis comparing disease progression according to genotype for LGMD2A/LGMD-R1-calpain3-related (N = 106), LGMD2B/LGMD-R2-dysferlin-related (N = 45) and sarcoglycanopathies (N = 46). Median age at walking aid dependency was different across genotypes of LGMD2A/LGMD-R1-calpain3- related (p = 0.003, **Figure S4A**), occurring at 25 years (95% CI, 19.6-30.3) for patients with one (N = 20), at 28 years (95% CI, 12.2-43.7) for patients with two (N = 38) and at 43 years (95% CI, 33.9-52) for patients without truncating variants (N = 48). Median age walking aid dependency of LGMD2A/LGMD-R1-calpain3-related patients with any truncating variant (N = 58) occurred 16 years before (27 years; 95% CI, 18.2-37.7) patients without truncating variants (43 years; 95% CI, 33.9-52; p = 0.001; **Figure S4B**). Median disease duration at walking aid dependency was different across genotypes of LGMD2A/LGMD-R1-calpain3-related (p = 0.032, **Figure S4C**), occurring at 13 years (95% CI, 11.2-14.8) for patients with one,

at 20 years (95% CI, 14.4-25.5) for patients with two and at 25 years (95% CI, 19.9-30) for patients without truncating variants. Median disease duration at walking aid dependency for patients with any truncating variant occurred 8 years before (17 years; 95% CI, 11.4-22.5) patients without truncating variants (25 years; 95% CI, 19.9-30; p = 0.055; **Figure S4D**). Median age at wheelchair dependency was similar for patients with LGMD2A/LGMD-R1-calpain3-related with one (N = 20; 45 years; 95% CI, not-available) or two (N = 38; 38 years; 95% CI, 29.1-46.8) truncating variants, which occurred earlier than for patients without truncating variants (N = 48; 52 years; 95% CI, 48.4-55.6; p < 0.001 for overall comparison; **Figure 4C**). Median age at wheelchair dependency of LGMD2A/LGMD-R1-calpain3-related patients with any truncating variant occurred 14 years before (38 years; 95% CI, 31.1-44.8) patients without truncating variants (52 years; 95% CI, 48.4-55.6; p < 0.001, **Figure 4D**). The same pattern occurred for disease duration at wheelchair dependency, where median age at wheelchair dependency was similar for patients with LGMD2A/LGMD- R1-calpain3-related with one (21 years; 95% CI, 10.1-31.9) or two (23 years; 95% CI, 13.7-32.3) truncating variants, which occurred earlier than for patients without truncating variants (36 years; 95% CI, 19.5-52.4; p = 0.026 for overall comparison; **Figure 4E**). Median disease duration at wheelchair dependency of LGMD2A/LGMD- R1-calpain3-related patients with any truncating variant occurred 13 years before (23 years; 95% CI, 15.2-30.7) patients without truncating variants (36 years; 95% CI, 19.5-52.4; p = 0.01, **Figure 4F**). There was no difference on age or disease duration at wheelchair or walking aid dependency for patients with LGMD2B/LGMD-R2-dysferlin-related and sarcoglycanopathies with one, two or none truncating variants (p > 0.1 for all comparisons, **Figure S5A-H**).

3.8 | Cardiac and respiratory involvement

Frequencies of likely disease-related and significant cardiac and respiratory involvement according to LGMD2/LGMD-R subtype is shown in **Table 1**.

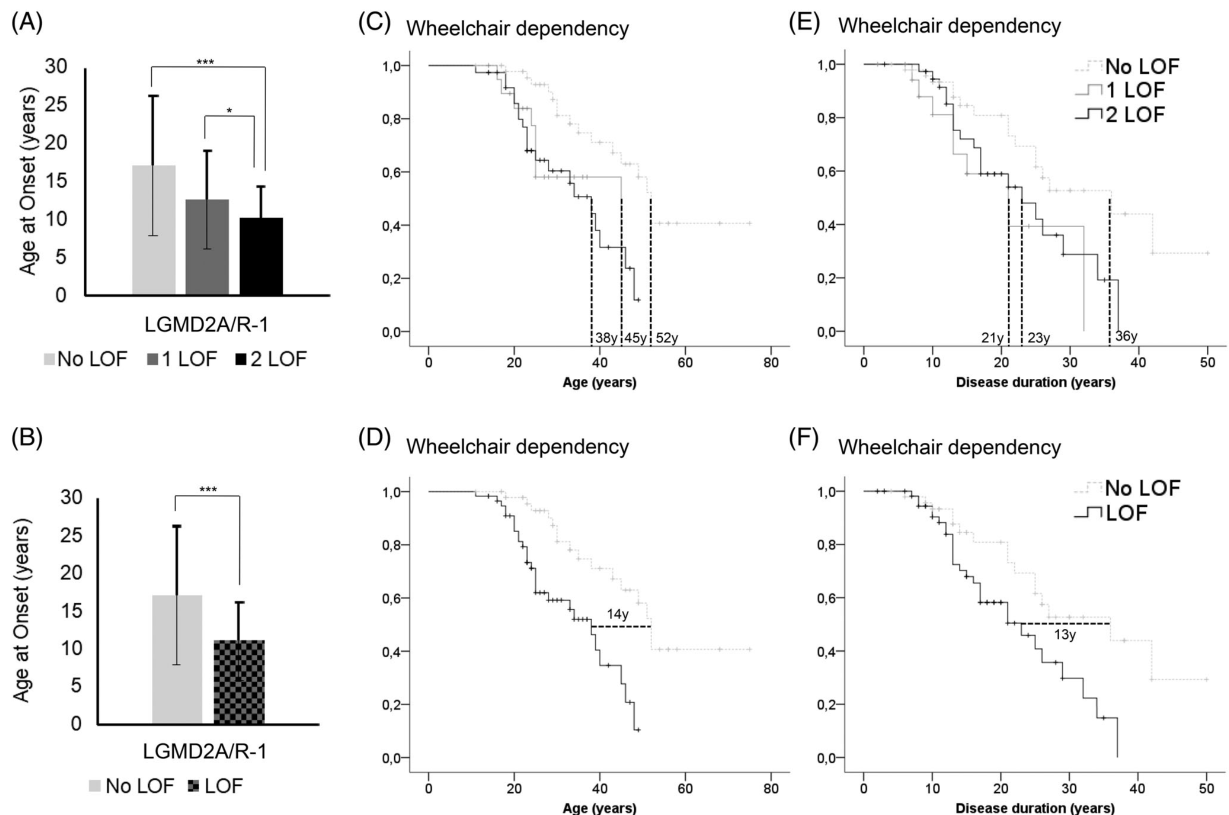

FIGURE 4 Genotype-phenotype correlations in LGMD2A/LGMD-R1-calpain3-related. The figure shows differences in the age at onset of LGMD2A/LGMD-R1-calpain3-related patients with 1, 2 or none truncating variants (A), and in the age at onset of patients with at least one truncating and none truncating variants (B). Bars represent means and lines SDs. Kaplan-Meier analysis of wheelchair dependency according to age in LGMD2A/LGMD-R1-calpain3-related patients with 1, 2 or none truncating variants (C) and patients with at least one truncating and none truncating variants (D), and wheelchair dependency according to disease duration in patients with 1, 2, or none truncating (E) and patients with at least one truncating and none truncating variants (F). Median values of progressions to wheelchair dependency are informed and highlighted by dashed lines (C,E). Differences in median values of progressions to wheelchair dependency between groups are informed and highlighted by dashed lines (D,F). LOF, loss-of-function or truncating variant; *$P < 0.05$; ***$P < 0.001$

4 | DISCUSSION

In the present study, we have comprehensively described clinical and molecular data of a large Brazilian cohort of patients with LGMD2/LGMD-R. Our study indicates that LGMD2A/LGMD-R1-calpain 3-related and LGMD2B/LGMD-R2-dysferlin-related are the most frequent subtypes in Brazil, and that sarcoglycanopathies are the most frequent childhood-onset subtypes. We have described 37 novel variants in LGMD2/LGMD-R genes, the progression to major handicap, genotype-phenotype correlations and sex influences on age at onset and disease progressions of most frequent LGMD2/LGMD-R subtypes.

4.1 | Relative frequency of LGMD2/LGMD-R in Brazil

With our large sample, we were able to define that LGMD2A/LGMD-R1-calpain3-related and LGMD2B/LGMD-R2-dysferlin-related are the most frequent LGMD2/LGMD-R subtypes in Brazil. When we stratified subtypes according to age at onset, sarcoglycanopathies were the most frequent subtypes before 10 years old; LGMD2A/ LGMD-R1-calpain3-related when disease starts between 11 and 20 years old; LGMD2B/LGMD-R2-dysferlin-related when disease starts between 21 and 40 years old, and both LGMD2B/LGMD- R2-dysferlin-related and LGMD2L/LGMD-R12-anoctamin5-related were the most

frequent subtypes starting after 40 years old. Regional relative frequencies of LGMD2/LGMD-R subtypes were similar to frequencies in the overall country, except for the North region where sarcoglycanopathies and LGMD2A/LGMD-R1-calpain3-related were the most frequent subtypes. North region had the smallest sample size and most cases were recruited in a child neurology center (3/7 index cases started symptoms before the age of 10), suggesting a selection bias toward early-onset forms. Our epidemiological profile is similar to recent large samples studies from Italy and the United States;[8,9] however, it partially differed from previous Brazilian reports more focused on the pediatric population. A previous single center study in São Paulo reported that sarcoglycanopathies were diagnosed in 40.5% of a series of 37 children with LGMD2/LGMD-R.14 A single center study from southern Brazil evaluated 56 patients with LGMD only with immunohistochemistry and reported sarcoglycanopathies (32%) as the most common diagnosis, followed by LGMD2B/LGMD-R2-dysferlin-related (14.3%) and LGMD2A/LGMD-R1-calpain3-related (8.9%).[13] Another study from São Paulo evaluated 40 families with LGMD2/LGMD-R and reported sarcoglycanopathies as the most frequent subtype (55%), followed by LGMD2B/LGMD-R2-dysferlin-related (25%) and LGMD2A/LGMD-R1-calpain3-related (17.5%).[12] The same group also evaluated 115 unrelated families from different centers in Brazil and reported that sarcoglycanopathies were responsible for 20% of the diagnosis, which is similar to what we have found (21%).30 Interestingly, 5% of our families had LGMD-R7-telethonin-related, an ultrarare subtype worldwide. LGMD2G/LGMD-R7-telethonin-related was first described in Brazilian families, and all families presented here were homozygous for the p.Gln53* variant in *TCAP*, described in the gene discovery report.[31]

4.2 | Sex effect

We found higher proportion of female patients with sarcoglycanopathies (2.2-fold), and a higher proportion of males in LGMD2L/LGMD-R12-anoctamin5-related (4.5-fold) and LGMD2I/LGMD-R9-FKRP-related (2.8-fold). Some previous studies found higher proportion of females with sarcoglycanopathies,[12,32] while other studies did not;[8,30,33,34] and male predominance in LGMD2I/LGMD-R9-FKRP-related was not previously reported. [35,36] Further larger cohorts on these subtypes should clarify if there are sex differences in these autosomal recessive diseases or whether these findings reflect selection bias. The finding of male predominance in LGMD2L/LGMD-R12-anoctamin5-related is well established, although little is understood.[8,37,38]

4.3 | Molecular analysis

We have found 119 different disease-related variants in the studied genes, 37 of them not previously described. Except from *SGCD* and *TCAP*, in which all cases carried the same pathogenic variant, we have found different causal variants in different exons of these genes (**Table S1**).

4.4 | CK levels

Information regarding CK levels (**Table 1**, **Figure S1**) and age at onset (**Figure 2**) might give valuable clues for the specific subtype of LGMD2/LGMD-R diagnosis. The pattern of CK elevation in LGMD2/ LGMD-R subtypes in our study was like previous studies reporting measures at disease onset.[8]

4.5 | Disease progression and its modifiers

We built survival curves to major handicap for LGMD2A/LGMD-R1-calpain3-related, LGMD2B/LGMD-R2-dysferlin-related and sarcoglycanopathies. Walking-aid and wheelchair dependency occurred earlier for patients with sarcoglycanopathies, followed by patients with LGMD2A/LGMD-R1-calpain3-related and later by patients with LGMD2B/LGMD-R2-dysferlin-related. Patients with sarcoglycanopathies also had faster disease progressions according to disease duration than the other forms, while patients with LGMD2A/LGMD-R1-calpain3-related and LGMD2B/LGMD-R2- dysferlin-related progressed in a similar way (**Figure 3**). These data suggest that the major difference of LGMD2A/LGMD-R1-calpain3-related and LGMD2B/LGMD-R2-dysferlin-related severity is related to the early age at onset of the former subtype and not to a more aggressive disease progression.

Sex effect on disease progression of these forms was assessed and we have found earlier ages and disease durations at walking aid dependency for men than for women with LGMD2B/LGMD-R2-dysferlin-related. We were not able to find studies that compared LGMD2B/LGMD-R2-dysferlin-related progression across sexes in the literature. Recent pathological studies described higher fiber atrophy factor in males than females with LGMD2B/LGMD-R2-dysferlin-related. Male patients had lower muscle fiber diameter and cross-sectional area and higher atrophic factor than male controls, while there were no differences in these morphological parameters between affected and control females.[39]

We also performed a genotype-phenotype correlation with disease progression and age at onset. LGMD2A/LGMD-R1-calpain3-related patients with one or two truncating variants started earlier than patients without truncating variants. The same happened for walking aid and wheelchair dependency, which occurred many years earlier and at shorter disease durations for patients with one or two truncating variants when compared to patients without truncating variant. There was no genotype-phenotype correlation with disease progression for sarcoglycanopathies and LGMD2B/LGMD-R2-dysferlin-related, however, we only had available genotypes of 45 patients with LGMD2B/LGMD-R2-dysferlin-related and this association should be searched in future larger sample collaborative studies. In fact, a large Italian cohort found a genotype-phenotype correlation with age at onset for LGMD2A/LGMD-R1-calpain3-related and also for LGMD2B/LGMD-R2-dysferlin-related, with earlier onsets in patients with truncating when compared to patients with non-truncating variants.[8] Differences in age at onset according to genotype for LGMD2A/LGMD-R1-calpain3-related were similar to what we have found, and the criteria for truncating and non-truncating variants of that study was similar to ours. Another large sample study in LGMD2A/LGMD-R1-calpain3-related (113 patients for genotype-phenotype correlations) found that patients with at least one missense variant started the disease later than patients with two truncating mutations.[40] This study also evaluated the effect of truncating variants on progression to disease related

handicap and they did not find statistically significant differences in the proportion of wheelchair-bound patients with two missense (5/14, 35.7%), one missense and one truncating variant (9/29, 31%) and two truncating variants (31/60, 51.7%). However, they did not consider disease duration for these comparisons. Remarkably, when these authors evaluated only patients with >25 years of disease duration, they have found that more patients with two truncating variants were wheelchair bound than patients with at least one missense variant.[40] Survival analysis allow to compare patients with different disease durations, without losing too much information and study power, as with stratification approaches like that. Another smaller sample study reported that all four LGMD2A/LGMD-R1-calpain3-related patients homozygous or compound heterozygous for truncating variants had absent calpain-3 protein, early-onset and rapid progression, whereas patients with two missense variants or compound heterozygous for a missense and a truncating variant had extremely variable levels of protein and rates of disease progression.[41] A Japanese and an Italian study did not find differences in the rate of disease progression according to genotype in LGMD2B/LGMD-R2-dysferlin-related.[42,43]

4.6 | Cardiac and respiratory involvement

Frequencies of cardiac and respiratory abnormalities in our study were similar to other large cohorts.[8] Heart involvement was frequent in LGMD2I/LGMD-R9-FKRP-related and sarcoglycanopathies. Respiratory involvement was frequent in all LGMD2/LGMD-R subtypes, being less severe in LGMD2G/LGMD-R7-telethonin-related and LGMD2L/LGMD-R12-anoctamin5-related, reinforcing the importance of periodical monitoring of respiratory function for LGMD2/LGMD-R.

4.6.1 | Study limitations

Our study represents one of the largest reported cohorts with comprehensive clinical and molecular data on LGMD2/LGMD-R; however, there was some heterogeneity of diagnostic evaluation across centers. For instance, the frequency of immunohistochemical diagnosis greatly varied. Nevertheless, the criteria for genetic diagnosis were standardized and all variants were reviewed by the same medical geneticists (RBR, JAMS). Clinical descriptions and survival curves were only performed for patients with defined or likely diagnosis. We also had significant missing data for some variables, for instance, we only had genotypes of 49/109 patients with LGMD2B/LGMD-R2-dysferlin-related, which might have limited genotype-phenotype correlation analysis for this subtype. Because all cases were recruited at specialized hospital centers, relative frequencies of LGMD2/LGMD-R in Brazil based on population-based studies might be different, especially concerning milder forms.

5 | CONCLUSION

In conclusion, we were able to define LGMD2A/LGMD-R1-calpain3-related and LGMD2B/LGMD-R2-dysferlin-related as the most frequent subtypes of LGMD2/LGMD-R in Brazil. We found that females with LGMD2B/LGMD-R2-dysferlin-related had a less severe progression to handicap than males and that

LGMD2A/LGMD-R1-calpain3-related patients with truncating variants had earlier disease onset and presented a more severe progression to handicap than patients without truncating variants. These results are of great importance to understand the epidemiology of LGMD2/LGMD-R in Brazil, as well as to understand better these diseases progression and its modifiers, and to better design future natural history studies and clinical trials for LGMD2/LGMD-R.

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CONFLICT OF INTEREST

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DATA ACCESSIBILITY

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

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Table S1 – Most frequent variants in LGMD2/LGMD-R genes

Gene	Most common variants	Exon	Families
ANO5	c.1359C>G (p.Tyr453*)	14	4/13 (30.7%)
	c.191dupA (p.Asn64Lysfs*15)	5	5/13 (38.4%)
CAPN3	c.328C>T (p.Arg110*)	2	18/97 (18.5%)
	c.2306G>A (p.Arg769Gln)	22	14/97 (14.4%)
	c.2362_2363delinsTCATCT (p.Arg788Serfs*14)	22	12/97 (12.3%)
	c.1468C>T (p.Arg490Trp)	11	10/97 (10.3%)
	c.258dupT (p.Leu87Serfs*4)	1	5/97 (5.1%)
	c.412dupC (p.Leu138Profs*15)	3	5/97 (5.1%)
	c.550A>G (p.Thr184Ala)	4	5/97 (5.1%)
	c.759_761delGAA (p.Lys254del)	5	5/97 (5.1%)
	c.2288A>G (p.Tyr763Cys)	22	5/97 (5.1%)
	c.60delA (p.Pro22Glnfs*35)	1	4/97 (4.1%)
	c.550delA (p.Thr184Argfs*36)	4	4/97 (4.1%)
	c.1714C>T (p.Arg572Trp)	13	4/97 (4.1%)
DYSF	c.5979_5980insA (p.E1994Rfs*3)	53	10/45 (22.2%)
	c.2875C>T (p.Arg959Trp)	27	5/45 (11.1%)
	c.5429G>A (p.Arg1810Lys)	48	5/45 (11.1%)
	c.2901dupC (p.M968Hfs*3)	27	4/45 (8.8%)
	c.701G>A (p.Gly234Glu)	7	3/45 (6.6%)
	c.2076delC (p.H693Tfs*4)	22	3/45 (6.6%)
	c.2996G>A (p.Trp999*)	28	3/45 (6.6%)
	c.3805G>T (p.E1269*)	34	3/45 (6.6%)
	c.6124C>T (p.Arg2042Cys)	54	3/45 (6.6%)
FKRP	c.826C>A (p.Leu276Ile)	4	17/21 (74%)
SGCA	c.229C>T(p.Arg77Cys)	3	7/18 (38.8%)
	c.850C>T (p.Arg284Cys)	7	7/18 (38.8%)
	c.724G>T (p.Val242Phe)	6	4/18 (22.2%)
SGCB	c.299T>A (p.Met100Lys)	3	9/13 (69.2%)
SGCG	c.525delT (p.Phe175Leufs*20)	6	9/10 (90%)
SGCD	c.657delC (p.Thr220Profs*6)	8	4/4 (100%)
TCAP	c.157C>T (p.Gln53*)	2	15/15 (100%)

Table S2 – Variants in LGMD2/LGMD-R genes

SUBJECT	GENE	NUCLEOTIDE CHANGE (AA CHANGE)	VARIANT CLASSIFICATION*	DIAGNOSIS
LGMD1	*CAPN3*	c.692T>C (p.Phe231Ser)/c.1999dupG (p.Glu667Glyfs*6)	Likely pathogenic (PM1,PM2,PM3,PP1)/Pathogenic	LGMD2A likely
LGMD2	*CAPN3*	c.692T>C (p.Phe231Ser)/c.1999dupG (p.Glu667Glyfs*6)	Likely pathogenic (PM1,PM2,PM3,PP1)/Pathogenic	LGMD2A likely
LGMD3	*DYSF*	c.2875C>T (p.Arg959Trp)/c.5979dupA (p.Glu1994Argfs*3)	Pathogenic/Pathogenic	LGMD2B
LGMD4	*DYSF*	c.2875C>T (p.Arg959Trp)/c.3137G>A (p.Arg1046His)	Pathogenic/Pathogenic	LGMD2B
LGMD5	*DYSF*	c.701G>A (p.Gly234Glu)x2	Pathogenic	LGMD2B
LGMD6	*DYSF*	NA	NA	LGMD2B[1]
LGMD7	*DYSF*	c.2875C>T (p.Arg959Trp)/c.3805G>T (p.Glu1269*)	Pathogenic/Pathogenic	LGMD2B
LGMD8	*DYSF*	NA	NA	LGMD2B[1]
LGMD9	*CAPN3*	c.412dupC (p.Leu138Profs*15)x2	Pathogenic (PVS1,PS4, PM2)	LGMD2A
LGMD10	*CAPN3*	NA	NA	LGMD2A[1]
LGMD11	*CAPN3*	c.1448C>A (p.Ala396Asp)x2	Pathogenic	LGMD2A
LGMD12	*DYSF*	c.2643+1G>A/c.2875C>T (p.Arg959Trp)	Pathogenic/Pathogenic	LGMD2B
LGMD13	*CAPN3*	c.328C>T (p.Arg110*)/c.412dupC (p.Leu138Profs*15)	Pathogenic/Pathogenic (PVS1,PS4, PM2)	LGMD2A
LGMD14	*TCAP*	c.157C>T (p.Gln53*)x2	Pathogenic	LGMD2G
LGMD15	*SGCA*	c.739G>A (p.Val247Met)/c.850C>T (p.Arg284Cys)	Pathogenic/Pathogenic	LGMD2D
LGMD16	*DYSF*	c.5979dupA (p.Glu1994Argfs*3)x2	Pathogenic	LGMD2B
LGMD17	*DYSF*	c.5979dupA;p (Glu1994Argfs*3),	Pathogenic	LGMD2B
LGMD18	*DYSF*	c.2810+1G>A/c.3805G>T (p.Glu1269*)	Pathogenic/Pathogenic	LGMD2B
LGMD19	*FKRP*	c.826C>A (p.Leu276Ile)x2	Pathogenic	LGMD2I
LGMD20	*DYSF*	c.5979dupA (p.Glu1994Argfs*3)x2	Pathogenic	LGMD2B
LGMD21	*CAPN3*	c.640G>A (p.Gly214Ser)x1	Likely pathogenic	LGMD2A possible
LGMD22	*SGCG*	c.629A>G (p.His210Arg)x2	Likely pathogenic (PS3, PM2, PP3)	LGMD2C likely
LGMD23	*CAPN3*	c.509dupA (p.Tyr170*)/c.1782+1G>T	Pathogenic/Pathogenic (PVS1, PM2, PP1)	LGMD2A
LGMD24	*CAPN3*	c.509dupA (p.Tyr170*)/c.1782+1G>T	Pathogenic/Pathogenic (PVS1, PM2, PP1)	LGMD2A
LGMD25	*DYSF*	c.5429G>A (p.Arg1810Lys)x2	Pathogenic/Pathogenic	LGMD2B
LGMD26	*CAPN3*	c.1355-1G>C x2	Pathogenic	LGMD2A
LGMD27	*CAPN3*	c.1250C>T (p.Thr417Met)x1	Pathogenic	LGMD2A possible
LGMD28	*DYSF*	c.701G>A (p.Gly234Glu)x2	Pathogenic	LGMD2B
LGMD29	*SGCA*	c.850C>T (p.Arg284Cys)x1	Pathogenic	LGMD2D possible

LGMD30	*DYSF*	c.757C>T (p.Arg253Trp)/c.3112C>T p.Arg1038*	Pathogenic/Pathogenic	LGMD2B
LGMD31	*DYSF*	NA	NA	LGMD2B[2]
LGMD32	*CAPN3*	c.390G>A (p.Try130*)	Likely Pathogenic (PVS1, PM2)	LGMD2A possible
LGMD33	*CAPN3*	c.1193+6T>A (p.Met399*)	Pathogenic	LGMD2A possible
LGMD34	*CAPN3*	c.1193+6T>A (p.Met399*)	Pathogenic	LGMD2A possible
LGMD35	*ANO5*	c.692G>T (p.Gly231Val)/c.1359C>G (p.Tyr453*)	Pathogenic/Pathogenic (PVS1, PS4, PM2)	LGMD2L
LGMD36	*ANO5*	c.692G>T (p.Gly231Val)x2	Pathogenic	LGMD2L
LGMD37	*CAPN3*	c.759_761delGAA (p.Lys253del)x1	Pathogenic	LGMD2A possible
LGMD38	*FKRP*	c 826C>A (p.Leu276Ile)x2	Pathogenic	LGMD2I
LGMD39	*FKRP*	c 826C>A (p.Leu276Ile)/c.1180T>A (p.Trp394Arg)	Pathogenic/VUS (PM2, PP3)	LGMD2I possible
LGMD40	*DYSF*	c.3235_3236insAGGCGG (p.Phe1079*)x2	Likely Pathogenic (PVS1, PM2)	LGMD2B likely
LGMD41	*DYSF*	NA	NA	LGMD2B[2]
LGMD42	*DYSF*	c.1163_1165dupCCG (p.Ala388dup)/c.1639-6T>A	Likely Pathogenic (PM1, PM2, PM4)/Pathogenic	LGMD2B likely
LGMD43	*CAPN3*	c.60delA (p.Pro22Glnfs*35)/c.759_761delGAA (p.Lys254del)	Pathogenic/Pathogenic	LGMD2A
LGMD44	*CAPN3*	c.2362_2363delinsTCATCT (p.Arg788Serfs*14)x2	Pathogenic	LGMD2A
LGMD45	*CAPN3*	c.550delA (p.Thr184Argfs*36)/c.1343G>A (p.Arg448His)	Pathogenic/Pathogenic	LGMD2A
LGMD46	*CAPN3*	c.1194-9A>G/c.2362_2363delinsTCATCT (p.Arg788Serfs*14)	Pathogenic/Pathogenic	LGMD2A
LGMD47	*CAPN3*	c.328C>T (p.Arg110*)x2	Pathogenic	LGMD2A
LGMD48	*CAPN3*	c.328C>T (p.Arg110*)/c.1468C>T (p.Arg490Trp)	Pathogenic/Pathogenic	LGMD2A
LGMD49	*CAPN3*	c.2362_2363delinsTCATCT (p.Arg788Serfs*14)x2	Pathogenic	LGMD2A
LGMD50	*CAPN3*	c.328C>T (p.Arg110*)x2	Pathogenic	LGMD2A
LGMD51	*CAPN3*	c.2288A>G (p.Tyr763Cys)/c.2362_2363delinsTCATCT (p.Arg788Serfs*14)	Pathogenic/Pathogenic	LGMD2A
LGMD52	*CAPN3*	c.328C>T (p.Arg110*)x2	Pathogenic	LGMD2A
LGMD53	*DYSF*	c.3149_3150delTC (p.Leu1050Glnfs*63)x2	Pathogenic	LGMD2B
LGMD54	*DYSF*	c.5979dupA (p.Glu1994Argfs*3)x2	Pathogenic	LGMD2B
LGMD55	*DYSF*	c.792+1G>A x2	Pathogenic	LGMD2B
LGMD56	*DYSF*	c.792+1G>A x2	Pathogenic	LGMD2B
LGMD57	*DYSF*	c.6124C>T (p.Arg2042Cys)x2	Pathogenic	LGMD2B
LGMD58	*DYSF*	c.6124C>T (p.Arg2042Cys)x2	Pathogenic	LGMD2B
LGMD59	*DYSF*	c.2901dupC (p.Met968Hisfs*3)/c.2996G>A (p.Trp999*)	Pathogenic (PVS1, PS3, PM2)/Pathogenic	LGMD2B
LGMD60	*SGCG*	c.525delT (p.Phe175Leufs*20)x2	Pathogenic	LGMD2C
LGMD61	*DYSF*	c.5429G>A (p.Arg1810Lys)x2	Pathogenic	LGMD2B

LGMD62	*SGCA*	c.724G>T (p.Val242Phe)/c.850C>T (p.Arg284Cys)	Pathogenic/Pathogenic	LGMD2D
LGMD63	*SGCB*	c.299T>A (p.Met100Lys)x2	Pathogenic	LGMD2E
LGMD64	*SGCB*	c.299T>A (p.Met100Lys)x2	Pathogenic	LGMD2E
LGMD65	*SGCB*	c.662-2A>G x2	Pathogenic	LGMD2E
LGMD66	*SGCB*	c.299T>A (p.Met100Lys)x2	Pathogenic	LGMD2E
LGMD67	*TCAP*	c.157C>T (p.Gln53*)x2	Pathogenic	LGMD2G
LGMD68	*SGCA*	c.229C>T (p.Arg77Cys)x2	Pathogenic	LGMD2D
LGMD69	*FKRP*	c.826C>A;(p.Leu276Ile)x2	Pathogenic	LGMD2I
LGMD70	*ANO5*	c.191dupA (p.Asn64Lysfs*15)/c.2012A>G (p.Tyr671Cys)	Pathogenic/VUS (PM2, PP3)	LGMD2L possible
LGMD71	*ANO5*	c.191dupA (p.Asn64Lysfs*15)x2	Pathogenic	LGMD2L
LGMD72	*TCAP*	c.157C>T (p.Gln53*)x2	Pathogenic	LGMD2G
LGMD73	*FKRP*	c.826C>A;(p.Leu276Ile)x2	Pathogenic	LGMD2I
LGMD74	*FKRP*	c.826C>A;(p.Leu276Ile)x2	Pathogenic	LGMD2I
LGMD75	*CAPN3*	c.328C>T (p.Arg110*)/c.1468C>T (p.Arg490Trp)	Pathogenic/Pathogenic	LGMD2A
LGMD76	*CAPN3*	c.328C>T (p.Arg110*)/c.1468C>T (p.Arg490Trp)	Pathogenic/Pathogenic	LGMD2A
LGMD77	*TCAP*	c.157C>T (p.Gln53*)x2	Pathogenic	LGMD2G
LGMD78	*DYSF*	c.5979dupA (p.Glu1994Argfs*3)x2	Pathogenic	LGMD2B
LGMD79	*CAPN3*	c.550A>G (p.Thr184Ala)/c.631A>G; (p.Lys211Glu)	Pathogenic (PS4,PM1,PM2,PM5,PP1,PP3)/Pathogenic	LGMD2A
LGMD80	*CAPN3*	c.506G>A (p.Arg169His)/c.631A>G (p.Lys211Glu)	Pathogenic/Pathogenic	LGMD2A
LGMD81	*CAPN3*	c.506G>A (p.Arg169His)/c.631A>G (p.Lys211Glu)	Pathogenic/Pathogenic	LGMD2A
LGMD82	*ANO5*	c.692G>T (p.Gly231Val)/c.1359C>G (p.Tyr453*)	Pathogenic/Pathogenic (PVS1, PS4, PM2)	LGMD2L
LGMD83	*CAPN3*	c.2050+1G>A/c.2288A>G (p.Tyr763Cys)	Pathogenic/Pathogenic	LGMD2A
LGMD84	*SGCD*	c.657delC (p.Thr220Profs*6)x2	Pathogenic	LGMD2F
LGMD85				LGM2B[2]
LGMD86	*FKRP*	c.826C>A (p.Leu276Ile)x2	Pathogenic	LGMD2I
LGMD87	*CAPN3*	c.2362delA (p.Arg788Glyfs*95)/x2	Likely Pathogenic (PVS1, PM2)	LGMD2A likely
LGMD88	*CAPN3*	c.245C>T (p.Pro82Leu)x2	Pathogenic	LGMD2A
LGMD89	*CAPN3*	c.258dupT (p.Leu87Serfs*4)/c.2362_2363delinsTCATCT (p.Arg788Serfs*14)	Pathogenic/Pathogenic	LGMD2A
LGMD90	*FKRP*	c.826C>A (p.Leu276Ile)x2	Pathogenic	LGMD2I
LGMD91	*ANO5*	c.172C>T (p.Arg58Trp)x2	Pathogenic	LGMD2L
LGMD92	*DYSF*	c.5979dupA (p.Glu1994Argfs*3)x2	Pathogenic	LGMD2B

LGMD93	*SGCA*	c.229C>T (p.Arg77Cys)/c.850C>T (p.Arg284Cys)	Pathogenic/Pathogenic	LGMD2D
LGMD94	*CAPN3*	c.1622G>A (p.Arg541Gln)x2	Pathogenic	LGMD2A
LGMD95	*FKRP*	c.899T>C (p.Val300Ala)/c.1403T>C (p.Phe468Ser)	Pathogenic/VUS (PM2, PP3)	LGMD2I possible
LGMD96	*ANO5*	c.191dupA (p.Asn64Lysfs*15)x2	Pathogenic	LGMD2L
LGMD97	*CAPN3*	c.328C>T (p.Arg110*)/c.2362_2363delinsTCATCT (p.Arg788Serfs*14)	Pathogenic/Pathogenic	LGMD2A
LGMD98	*FKRP*	c.826C>A (p.Leu276Ile)x2	Pathogenic	LGMD2I
LGMD99	*CAPN3*	c.328C>T (p.Arg110*)/c.550delA (p.Thr184Argfs*36)	Pathogenic/Pathogenic	LGMD2A
LGMD100	*DYSF*	c.2901dupC (p.Met968Hisfs*3)x2	Pathogenic (PVS1, PS3, PM2)	LGMD2B
LGMD101	*SGCA*	c.850C>T (p.Arg284Cys)x2	Pathogenic	LGMD2D
LGMD102	*SGCB*	c.299T>A (p.Met100Lys)x2	Pathogenic	LGMD2E
LGMD103	*ANO5*	c.191dupA (p.Asn64Lysfs*15)x2	Pathogenic	LGMD2L
LGMD104	*CAPN3*	c.328C>T (Arg110*)x1	Pathogenic	LGMD2A possible
LGMD105	*DYSF*	c.2875C>T (p.Arg959Trp)/c.5429G>A (p.Arg1810Lys)	Pathogenic/Pathogenic	LGMD2B
LGMD106	*DYSF*	NA	NA	LGMD2B[1]
LGMD107	*DYSF*	c.2077delC (p.His693Thrfs*4)/c.4872_4976delinsCCCC (p.Glu1624Aspfs*10)	Pathogenic/Pathogenic	LGMD2B
LGMD108	*DYSF*	c.2077delC (p.His693Thrfs*4)/c.4872_4976delinsCCCC (p.Glu1624Aspfs*10)	Pathogenic/Pathogenic	LGMD2B
LGMD109	*DYSF*	c.1165G>T (p.Glu389*)/c.5979dupA (p.Glu1994Argfs*3)	Likely Pathogenic (PVS1, PM2)/Pathogenic	LGMD2B likely
LGMD110	*DYSF*	NA	NA	LGMD2B likely[1]
LGMD111	*DYSF*	c.4003G>A (p.Glu1335Lys)/c.5594delG (p.Gly1865Alafs*101)	Pathogenic/Pathogenic	LGMD2B
LGMD112	*DYSF*	c.5429G>A (p.Arg1810Lys)x2	Pathogenic	LGMD2B
LGMD113	*DYSF*	c.5429G>A (p.Arg1810Lys)x2	Pathogenic	LGMD2B
LGMD114	*DYSF*	NA	NA	LGMD2B[1]
LGMD115	*DYSF*	c.5713C>T (p.Arg1905*)x2	Pathogenic	LGMD2B
LGMD116	*CAPN3*	c.550delA (p.Thr184Argfs*36)/c.2263G>C (p.Gly755Arg)	Pathogenic/VUS (PM2, PP3)	LGMD2A possible
LGMD117	*CAPN3*	c.2180_2183dupGGCA (p.Lys729Alafs*8)x2	Likely Pathogenic (PVS1, PM2)	LGMD2A likely
LGMD118	*CAPN3*	c.60delA (p.Pro22Glnfs*35)/c.412dupC (p.Leu138Profs*15)	Pathogenic/Pathogenic (PVS1,PS4, PM2)	LGMD2A
LGMD119	*CAPN3*	c.664G>A (p.Gly222Arg)/c.1468C>T (p.Arg490Trp)	Pathogenic/Pathogenic	LGMD2A
LGMD120	*CAPN3*	c.328C>T (p.Arg110*)x2	Pathogenic	LGMD2A
LGMD121	*CAPN3*	c.412dupC (p.Leu138Profs*15)/c.1711delC (p.Leu571Serfs*24)	Pathogenic (PVS1,PS4, PM2)/Pathogenic	LGMD2A
LGMD122	*CAPN3*	c.2242C>T (p.Arg748*)/c.2362_2363delinsTCATCT (p.Arg788Serfs*14)	Pathogenic/Pathogenic	LGMD2A
LGMD123	*CAPN3*	c.743T>G (p.Met248Arg)/c.1468C>T (p.Arg490Trp)	Pathogenic/Pathogenic	LGMD2A

LGMD124	*ANO5*	c.172C>T (p.Arg58Trp)x2	Pathogenic	LGMD2L
LGMD125	*TCAP*	c.157C>T (p.Gln53*)x2	Pathogenic	LGMD2G
LGMD126	*TCAP*	NA	NA	LGMD2G[1]
LGMD127	*FKRP*	c.826C>A (p.Leu276Ile)x2	Pathogenic	LGMD2I
LGMD128	*CAPN3*	c.2362_2363delinsTCATCT (p.Arg788Serfs*14)x2	Pathogenic	LGMD2A
LGMD129	*ANO5*	c.191dupA (p.Asn64Lysfs*15)/c.1359C>G (p.Tyr453*)	Pathogenic/Pathogenic (PVS1, PS4, PM2)	LGMD2L
LGMD130	*CAPN3*	c.412dupC (p.Leu138Profs*15)x2	Pathogenic (PVS1,PS4, PM2)	LGMD2A
LGMD131	*DYSF*	c.701G>A (p.Gly234Glu)/c.3805G>T (p.Glu1269*)	Pathogenic/Pathogenic	LGMD2B
LGMD132	*TCAP*	c.157C>T (p.Gln53*)x2	Pathogenic	LGMD2G
LGMD133	*CAPN3*	c.1333G>A (p.Gly445Arg)/c.1354+21G>A	Pathogenic/VUS (PM2)	LGMD2A possible
LGMD134	*CAPN3*	c.1621C>T (p.Arg541Trp)/c.2360_2361insTTCA (p.Arg788Serfs*2)	Pathogenic/Likely Pathogenic (PVS1, PM2)	LGMD2A likely
LGMD135	*TCAP*	c.157C>T (p.Gln53*)x2	Pathogenic	LGMD2G
LGMD136	*ANO5*	c.191dupA (p.Asn64Lysfs*15)/c. 2190G>T (p.Trp730Cys)	Pathogenic/VUS (PM2,PP3)	LGMD2L possible
LGMD137	*DYSF*	c.2996G>A (p.Trp999*)x2	Pathogenic	LGMD2B
LGMD138	*FKRP*	c.826C>A;(p.Leu276Ile)x2	Pathogenic	LGMD2I
LGMD139	*CAPN3*	c.743T>G (p.Met248Arg)/c.1240T>C (p.Cys414Arg)	Pathogenic/Likely Pathogenic (PM1,PM2,PP1,PP3)	LGMD2A likely
LGMD140	*CAPN3*	c.550A>G (p.Thr184Ala)/c.759_761delGAA (p.Lys254del)/	Pathogenic (PS4,PM1, PM2, PM5, PP1, PP3)/Pathogenic	LGMD2A
LGMD141	*SGCB*	c.272G>T (p.Arg91Leu)x2	Pathogenic	LGMD2E
LGMD142	*SGCB*	c.272G>T (p.Arg91Leu)x2	Pathogenic	LGMD2E
LGMD143	*DYSF*	c.5979dupA (p.Glu1994Argfs*3)x2	Pathogenic	LGMD2B
LGMD144	*CAPN3*	c.2306G>A (p.Arg769Gln)x2	Pathogenic	LGMD2A
LGMD145	*DYSF*	c.5429G>A (p.Arg1810Lys)x2	Pathogenic	LGMD2B
LGMD146	*DYSF*	c.2076delC (p.His693Thrfs*4)/c.3280T>C (p.Trp1094Arg)	Likely Pathogenic (PVS1, PM2)/VUS (PM2, PP3)	LGMD2B possible
LGMD147	*DYSF*	c.2077delC (p.His693Thrfs*4)x2	Pathogenic	LGMD2B
LGMD148	*SGCA*	c.229C>T (p.Arg77Cys)/c.850C>T (p.Arg284Cys)	Pathogenic/Pathogenic	LGMD2D
LGMD149	*SGCA*	c.724G>T (p.Val242Phe)/c.739G>A (p.Val247Met)	Pathogenic/Likely Pathogenic	LGMD2D likely
LGMD150	*CAPN3*	c.550A>G (p.Thr184Ala)x2	Pathogenic (PS4,PM1, PM2,PM5, PP1,PP3)	LGMD2A
LGMD151	*CAPN3*	c.2306G>A (p.Arg769Gln)x2	Pathogenic	LGMD2A
LGMD152	*CAPN3*	c.2306G>A (p.Arg769Gln)x2	Pathogenic	LGMD2A
LGMD153	*CAPN3*	c.258dupT (p.Leu87Serfs*4)/c.2306G>A (p.Arg769Gln)	Pathogenic/Pathogenic	LGMD2A
LGMD154	*CAPN3*	NA	NA	LGMD2A[1]
LGMD155	*CAPN3*	c.328C>T (p.Arg110*)x2	Pathogenic	LGMD2A

LGMD156	*CAPN3*	c.328C>T (p.Arg110*)/c.2306G>A (p.Arg769Gln)	Pathogenic/Pathogenic	LGMD2A
LGMD157	*CAPN3*	c.328C>T (p.Arg110*)x2	Pathogenic	LGMD2A
LGMD158	*CAPN3*	c.2243G>A (p.Arg748Gln)x2	Pathogenic	LGMD2A
LGMD159	*CAPN3*	c.2306G>A (p.Arg769Gln)x2	Pathogenic	LGMD2A
LGMD160	*CAPN3*	c.2288A>G (p.Tyr736Cys)x2	Pathogenic	LGMD2A
LGMD161	*CAPN3*	c.145C>T (p.Arg46Cys)/c.620 A>C (p.Lys207Thr)	Pathogenic/Pathogenic	LGMD2A
LGMD162	*CAPN3*	c.550A>G (p.Thr184Ala)x2	Pathogenic (PS4,PM1, PM2,PM5, PP1,PP3)	LGMD2A
LGMD163	*CAPN3*	c.1468C>T (p.Arg490Trp)x2	Pathogenic	LGMD2A
LGMD164	*CAPN3*	c.2306G>A (p.Arg769Gln)x2	Pathogenic	LGMD2A
LGMD165	*CAPN3*	c.2306G>A (p.Arg769Gln)x2	Pathogenic	LGMD2A
LGMD166	*CAPN3*	c.2288A>G (p.Tyr736Cys)x2	Pathogenic	LGMD2A
LGMD167	*CAPN3*	c.2306G>A (p.Arg769Gln)x2	Pathogenic	LGMD2A
LGMD168	*CAPN3*	c.865C>T (p.Arg286Trp)/c.1314G>T (p.Trp438Cys)	Pathogenic/ Likely Pathogenic (PM1, PM2, PP1, PP3)	LGMD2A likely
LGMD169	*CAPN3*	c.865C>T (p.Arg286Trp)/c.1314G>T (p.Trp438Cys)	Pathogenic/ Likely Pathogenic (PM1, PM2, PP1, PP3)	LGMD2A likely
LGMD170	*CAPN3*	c.2362_2363delinsTCATCT (p.Arg788Serfs*14)x2	Pathogenic	LGMD2A
LGMD171	*CAPN3*	c.1314G>A (p.Trp438*)	Likely Pathogenic (PVS1, PM2)	LGMD2A possible
LGMD172	*CAPN3*	c.759_761del (p.Lys254del)x2	Pathogenic	LGMD2A
LGMD173	*CAPN3*	c.759_761del (p.Lys254del)x2	Pathogenic	LGMD2A
LGMD174	*CAPN3*	c.759_761del (p.Lys254del)x2	Pathogenic	LGMD2A
LGMD175	*CAPN3*	c.2306G>A (p.Arg769Gln)x2	Pathogenic	LGMD2A
LGMD176	*CAPN3*	c.2306G>A (p.Arg769Gln)x2	Pathogenic	LGMD2A
LGMD177	*CAPN3*	c.2306G>A (p.Arg769Gln)x2	Pathogenic	LGMD2A
LGMD178	*CAPN3*	c.2306G>A (p.Arg769Gln)x2	Pathogenic	LGMD2A
LGMD179	*CAPN3*	c.2306G>A (p.Arg769Gln)x2	Pathogenic	LGMD2A
LGMD180	*CAPN3*	c.2306G>A (p.Arg769Gln)x2	Pathogenic	LGMD2A
LGMD181	*CAPN3*	c.2306G>A (p.Arg769Gln)x2	Pathogenic	LGMD2A
LGMD182	*CAPN3*	c.60delA (p.Pro22Gln*35)/c.2243G>A (p.Arg748Gln)	Pathogenic/Pathogenic	LGMD2A
LGMD183	*CAPN3*	NA	NA	LGMD2A[1]
LGMD184	*CAPN3*	c.328C>T (p.Arg110*)x2	Pathogenic	LGMD2A
LGMD185	*CAPN3*	c.2243G>A (p.Arg748Gln)x2	Pathogenic	LGMD2A
LGMD186	*CAPN3*	NA	NA	LGMD2A[1]
LGMD187	*CAPN3*	c.1468C>T (p.Arg490Trp)x2	Pathogenic	LGMD2A

LGMD188	*CAPN3*	c.2306G>A (p.Arg769Gln)x2	Pathogenic	LGMD2A
LGMD189	*CAPN3*	c.258dupT (p.Leu87Serfs)/c.2306G>A (p.Arg769Gln)	Pathogenic/Pathogenic	LGMD2A
LGMD190	*DYSF*	NA	NA	LGMD2B[2]
LGMD191	*DYSF*	NA	NA	LGMD2B[2]
LGMD192	*DYSF*	NA	NA	LGMD2B[2]
LGMD193	*DYSF*	NA	NA	LGMD2B[2]
LGMD194	*DYSF*	NA	NA	LGMD2B[2]
LGMD195	*DYSF*	NA	NA	LGMD2B[2]
LGMD196	*DYSF*	NA	NA	LGMD2B[2]
LGMD197	*DYSF*	NA	NA	LGMD2B[2]
LGMD198	*DYSF*	NA	NA	LGMD2B[2]
LGMD199	*DYSF*	NA	NA	LGMD2B[2]
LGMD200	*DYSF*	NA	NA	LGMD2B[2]
LGMD201	*DYSF*	NA	NA	LGMD2B[2]
LGMD202	*DYSF*	NA	NA	LGMD2B[2]
LGMD203	*DYSF*	NA	NA	LGMD2B[2]
LGMD204	*DYSF*	NA	NA	LGMD2B[2]
LGMD205	*DYSF*	NA	NA	LGMD2B[2]
LGMD206	*DYSF*	NA	NA	LGMD2B[2]
LGMD207	*DYSF*	NA	NA	LGMD2B[2]
LGMD208	*DYSF*	NA	NA	LGMD2B[2]
LGMD209	*DYSF*	NA	NA	LGMD2B[2]
LGMD210	*DYSF*	NA	NA	LGMD2B[2]
LGMD211	*DYSF*	NA	NA	LGMD2B[2]
LGMD212	*DYSF*	NA	NA	LGMD2B[2]
LGMD213	*DYSF*	NA	NA	LGMD2B[2]
LGMD214	*DYSF*	NA	NA	LGMD2B[2]
LGMD215	*DYSF*	NA	NA	LGMD2B[2]
LGMD216	*DYSF*	NA	NA	LGMD2B[2]
LGMD217	*DYSF*	NA	NA	LGMD2B[2]
LGMD218	*DYSF*	NA	NA	LGMD2B[2]
LGMD219	*DYSF*	NA	NA	LGMD2B[2]

LGMD220	*DYSF*	NA	NA	LGMD2B[2]
LGMD221	*DYSF*	NA	NA	LGMD2B[2]
LGMD222	*DYSF*	NA	NA	LGMD2B[2]
LGMD223	*DYSF*	NA	NA	LGMD2B[2]
LGMD224	*DYSF*	NA	NA	LGMD2B[2]
LGMD225	*DYSF*	NA	NA	LGMD2B[2]
LGMD226	*DYSF*	NA	NA	LGMD2B[2]
LGMD227	*DYSF*	NA	NA	LGMD2B[2]
LGMD228	*DYSF*	NA	NA	LGMD2B[2]
LGMD229	*SGCB*	c.299T>A (p.Met100Lys)x2	Pathogenic	LGMD2E
LGMD230	*SGCB*	c.299T>A (p.Met100Lys)x2	Pathogenic	LGMD2E
LGMD231	*SGCG*	c.525delT (p.Phe175Leufs*20)x2	Pathogenic	LGMD2C
LGMD232	*SGCB*	c.299T>A (p.Met100Lys)/c.323T>G, p.(Leu108Arg)	Pathogenic/Pathogenic	LGMD2E
LGMD233	*SGCG*	c.525delT (p.Phe175Leufs*20)x2	Pathogenic	LGMD2C
LGMD234	*SGCG*	c.525delT (p.Phe175Leufs*20)x2	Pathogenic	LGMD2C
LGMD235	*SGCG*	c.525delT (p.Phe175Leufs*20)x2	Pathogenic	LGMD2C
LGMD236	*SGCG*	c.525delT (p.Phe175Leufs*20)x2	Pathogenic	LGMD2C
LGMD237	*NA*	NA	NA	LGMD2C-F[2]
LGMD238	*NA*	NA	NA	LGMD2C-F[2]
LGMD239	*NA*	NA	NA	LGMD2C-F[2]
LGMD240	*NA*	NA	NA	LGMD2C-F[2]
LGMD241	*NA*	NA	NA	LGMD2C-F[2]
LGMD242	*NA*	NA	NA	LGMD2C-F[2]
LGMD243	*NA*	NA	NA	LGMD2C-F[2]
LGMD244	*NA*	NA	NA	LGMD2C-F[2]
LGMD245	*NA*	NA	NA	LGMD2C-F[2]
LGMD246	*NA*	NA	NA	LGMD2C-F[2]
LGMD247	*NA*	NA	NA	LGMD2C-F[2]
LGMD248	*NA*	NA	NA	LGMD2C-F[2]
LGMD249	*NA*	NA	NA	LGMD2C-F[2]
LGMD250	*NA*	NA	NA	LGMD2C-F[2]
LGMD251	*NA*	NA	NA	LGMD2C-F[2]

LGMD252	*SGCA*	c.724G>T (p.Val242Phe)/c.850C>T (p.Arg284Cys)	Pathogenic/Pathogenic	LGMD2D
LGMD253	*SGCG*	c.525delT (p.Phe175Leufs*20)x2	Pathogenic	LGMD2C
LGMD254	*SGCG*	c.525delT (p.Phe175Leufs*20)x2	Pathogenic	LGMD2C
LGMD255	*SGCB*	c.299T>A (p.Met100Lys)x2	Pathogenic	LGMD2E
LGMD256	*SGCB*	NA	NA	LGMD2E[1]
LGMD257	*NA*	NA	NA	LGMD2C-F[2]
LGMD258	*NA*	NA	NA	LGMD2C-F[2]
LGMD259	*TCAP*	c.157C>T (p.Gln53*)x2	Pathogenic	LGMD2G
LGMD260	*TCAP*	c.157C>T (p.Gln53*)x2	Pathogenic	LGMD2G
LGMD261	*TCAP*	c.157C>T (p.Gln53*)x2	Pathogenic	LGMD2G
LGMD262	*TCAP*	c.157C>T (p.Gln53*)x2	Pathogenic	LGMD2G
LGMD263	*TCAP*	c.157C>T (p.Gln53*)x2	Pathogenic	LGMD2G
LGMD264	*FKRP*	c.826C>A (p.Leu276Ile)x2	Pathogenic	LGMD2I
LGMD265	*FKRP*	c.826C>A (p.Leu276Ile)x2	Pathogenic	LGMD2I
LGMD266	*FKRP*	c.400C>T (p.Arg134Trp)x2	Likely Pathogenic	LGMD2I likely
LGMD267	*FKRP*	c.400C>T (p.Arg134Trp)x2	Likely Pathogenic	LGMD2I likely
LGMD268	*FKRP*	c.400C>T (p.Arg134Trp)x2	Likely Pathogenic	LGMD2I likely
LGMD269	*FKRP*	c.400C>T (p.Arg134Trp)x2	Likely Pathogenic	LGMD2I likely
LGMD270	*FKRP*	c.826C>A (p.Leu276Ile)x2	Pathogenic	LGMD2I
LGMD271	*FKRP*	c.826C>A (p.Leu276Ile)x2	Pathogenic	LGMD2I
LGMD272	*FKRP*	NA	NA	LGMD2I[1]
LGMD273	*CAPN3*	c.550delA (p.Thr184Argfs*36)	Pathogenic	LGMD2A possible
LGMD274	*CAPN3*	c.1456C>T (p.Gln486*)/c.1979A>G (p.Gln660Arg)	Likely Pathogenic (PVS1, PM2)/Pathogenic	LGMD2A likely
LGMD275	*CAPN3*	c.1456C>T (p.Gln486*)/c.1979A>G (p.Gln660Arg)	Likely Pathogenic (PVS1, PM2)/Pathogenic	LGMD2A likely
LGMD276	*DYSF*	NA	NA	LGMD2B[2]
LGMD277	*NA*	NA	NA	LGMD2C-F[2]
LGMD278	*NA*	NA	NA	LGMD2C-F[2]
LGMD279	*FKRP*	c.826C>A (p.Leu276Ile)/c.1384C>T (p.Pro462Ser)	Pathogenic/Pathogenic	LGMD2I
LGMD280	*SGCB*	c.299T>A (p.Met100Lys)/c.753+5G>A; p.[(?)]	Pathogenic/VUS (PM2, PP3)	LGMD2E possible
LGMD281	*CAPN3*	c.328C>T (p.Arg110*)/c.2031_2032insC (p.Asn678Glnfs*22)	Pathogenic/Pathogenic (PVS1, PS3, PM2, PP1)	LGMD2A
LGMD282	*CAPN3*	c.328C>T (p.Arg110*)/c.2031_2032insC (p.Asn678Glnfs*22)	Pathogenic/Pathogenic (PVS1, PS3, PM2, PP1)	LGMD2A
LGMD283	*CAPN3*	c.133G>A (p.Ala45Thr)/c.2306 G>A (p.Arg769Gln)	Pathogenic/Pathogenic	LGMD2A

LGMD284	*DYSF*	NA	NA	LGMD2B[2]
LGMD285	*CAPN3*	c.328C>T (p.Arg110*)/c.759_761delGAA (p.Lys254del)	Pathogenic/Pathogenic	LGMD2A
LGMD286	*CAPN3*	c.328C>T (p.Arg110*)/c.759_761delGAA (p.Lys254del)	Pathogenic/Pathogenic	LGMD2A
LGMD287	*CAPN3*	c.743T>G (p.Met248Arg)/c.1240T>C (p.Cys414Arg)	Pathogenic/Likely Pathogenic (PM1,PM2,PP1,PP3)	LGMD2A likely
LGMD288	*CAPN3*	c.60delA (p. Pro22Glnfs*35)/c.1714C>T (Arg572Trp)	Pathogenic/Pathogenic	LGMD2A
LGMD289	*CAPN3*	c.328C>T (p.Arg110*)x2	Pathogenic	LGMD2A
LGMD290	*CAPN3*	c.328C>T (p.Arg110*)x2	Pathogenic	LGMD2A
LGMD291	*DYSF*	NA	NA	LGMD2B[2]
LGMD292	*DYSF*	NA	NA	LGMD2B[2]
LGMD293	*DYSF*	NA	NA	LGMD2B[2]
LGMD294	*DYSF*	c.2996G>A (p.Trp999*)/c.855+1G>A	Pathogenic (PVS1, PS3, PM2)/Pathogenic (PVS1, PS3, PM2)	LGMD2B
LGMD295	*DYSF*	c.2996G>A (p.Trp999*)/c.855+1G>A	Pathogenic (PVS1, PS3, PM2)/Pathogenic (PVS1, PS3, PM2)	LGMD2B
LGMD296	*DYSF*	NA	NA	LGMD2B[2]
LGMD297	*FKRP*	NA	NA	LGMD2I[3]
LGMD298	*SGCG*	c.525delT (p.Phe175Leufs*20)x2	Pathogenic	LGMD2C
LGMD299	*ANO5*	c.1210 C>T (p.Arg404*)x2	Pathogenic	LGMD2L
LGMD300	*CAPN3*	c.550A>G (p.Thr184Ala)x2	Pathogenic (PS4,PM1,PM2,PM5,PP1,PP3)	LGMD2A
LGMD301	*NA*	NA	NA	LGMD2C-F[2]
LGMD302	*DYSF*	NA	NA	LGMD2B[2]
LGMD303	*DYSF*	NA	NA	LGMD2B[2]
LGMD304	*TCAP*	NA	NA	LGMD2G[2]
LGMD305	*CAPN3*	c.1536+1G>T/c.2362_2363delinsTCATCT (p.Arg788Serfs*14)	Pathogenic/Pathogenic	LGMD2A
LGMD306	*CAPN3*	c.258dupT (p.Leu87Serfs*4)x1	Pathogenic	LGMD2A[2]
LGMD307	*CAPN3*	c.258dupT (p.Leu87Serfs*4)x2	Pathogenic	LGMD2A
LGMD308	*DYSF*	NA	NA	LGMD2B[2]
LGMD309	*DYSF*	NA	NA	LGMD2B[2]
LGMD310	*SGCA*	c.229C>T (p.Arg77Cys)x2	Pathogenic	LGMD2D
LGMD311	*SGCA*	c.229C>T (p.Arg77Cys)x2	Pathogenic	LGMD2D
LGMD312	*TCAP*	c.157C>T (p.Gln53*)x2	Pathogenic	LGMD2G
LGMD313	*TCAP*	c.157C>T (p.Gln53*)x2	Pathogenic	LGMD2G
LGMD314	*TCAP*	c.157C>T (p.Gln53*)x2	Pathogenic	LGMD2G
LGMD315	*TCAP*	c.157C>T (p.Gln53*)x2	Pathogenic	LGMD2G

LGMD316	*FKRP*	c.545A>G (p.Tyr182Cys)x2	Likely Pathogenic	LGMD2I likely
LGMD317	*FKRP*	c.545A>G (p.Tyr182Cys)x2	Likely Pathogenic	LGMD2I likely
LGMD318	*FKRP*	NA	NA	LGMD2I likely[1]
LGMD319	*FKRP*	c.826C>A (p.Leu276Ile)x2	Pathogenic	LGMD2I
LGMD320	*CAPN3*	c.1468C>T (p.Arg490Trp)/c.2362_2363delinsTCATCT (p.Arg788Serfs*14)	Pathogenic/Pathogenic	LGMD2A
LGMD321	*SCGB*	c.551A>G (p.Tyr184Cys)x2	Likely Pathogenic	LGMD2E likely
LGMD322	*CAPN3*	c.328C>T (Arg110*)x2	Pathogenic	LGMD2A
LGMD323	*CAPN3*	c.328C>T (Arg110*)x2	Pathogenic	LGMD2A
LGMD324	*CAPN3*	c.1714C>T (p.Arg572Trp)/c.2275A>T (p.Asn759Tyr)	Pathogenic/VUS (PM1, PM2, PP3)	LGMD2A possible
LGMD325	*NA*	NA	NA	LGMD2C-F[2]
LGMD326	*ANO5*	c.1210 C>T (p.Arg404*)x2	Pathogenic	LGMD2L
LGMD327	*DYSF*	c.2996G>A (p.Trp999*)x2	Pathogenic	LGMD2B
LGMD328	*DYSF*	NA	NA	LGMD2B[1]
LGMD329	*FKRP*	c.826C>A (p.Leu276Ile)x2	Pathogenic	LGMD2I
LGMD330	*SGCA*	c.271G>A (p.Gly91Ser)x2	Pathogenic	LGMD2D
LGMD331	*SGCA*	c.271G>A (p.Gly91Ser)x2	Pathogenic	LGMD2D
LGMD332	*SGCA*	c.271G>A (p.Gly91Ser)x2	Pathogenic	LGMD2D
LGMD333	*SGCA*	c.271G>A (p.Gly91Ser)x2	Pathogenic	LGMD2D
LGMD334	*DYSF*	NA	NA	LGMD2B[2]
LGMD335	*DYSF*	NA	NA	LGMD2B[2]
LGMD336	*DYSF*	NA	NA	LGMD2B[2]
LGMD337	*CAPN3*	c.328C>T (p.Arg110*)x2	Pathogenic	LGMD2A
LGMD338	*DYSF*	NA	NA	LGMD2B[2]
LGMD339	*DYSF*	NA	NA	LGMD2B[2]
LGMD340	*DYSF*	c.6124C>T (p.Arg2042Cys)x2	Pathogenic	LGMD2B
LGMD341	*DYSF*	c.5979dupA (p.Glu1994Argfs*3)	Pathogenic	LGMD2B possible
LGMD342	*DYSF*	c.5979dupA (p.Glu1994Argfs*3)x2	Pathogenic	LGMD2B
LGMD343	*DYSF*	c.5979dupA (p.Glu1994Argfs*3)x2	Pathogenic	LGMD2B
LGMD344	*CAPN3*	c.60delA (p.Pro22Glnfs*35)/c.2306G>A (p.Arg769Gln)	Pathogenic/Pathogenic	LGMD2A
LGMD345	*CAPN3*	c.1714C>T (p.Arg572Trp)/c.2288A>G;(p.Tyr763Cys)	Pathogenic/Pathogenic	LGMD2A
LGMD346	*FKRP*	c.826C>A (p.Leu276Ile)x2	Pathogenic	LGMD2I
LGMD347	*SGCA*	c.229C>T (p.Arg77Cys)x2	Pathogenic	LGMD2D

LGMD348	*ANO5*	c.689A>G (p.Asp230Gly)/c.1359C>G (p.Tyr453*)	VUS (PM1, PM2)/ Pathogenic (PVS1,PS4, PM2)	LGMD2L possible
LGMD349	*SGCD*	c.657delC (p.Thr220Profs*6)x2	Pathogenic	LGMD2F
LGMD350	*SGCA*	c.229C>T (p.Arg77Cys)x2	Pathogenic	LGMD2D
LGMD351	*DYSF*	c.2779delG (p.Ala927Leufs*21)/c.3115C>T (p.Arg1039Trp)	Pathogenic/ Likely Pathogenic (PS3,PM2,PP3)	LGMD2B likely
LGMD352	*CAPN3*	c.245C>T (p.Pro82Leu)x2	Pathogenic	LGMD2A
LGMD353	*DYSF*	c.2218del (p.Leu740Cysfs*9)	Likely Pathogenic (PVS1, PM2)	LGMD2B possible
LGMD354	*CAPN3*	c.1714C>T (p.Arg572Trp)x2	Pathogenic	LGMD2A
LGMD355	*DYSF*	c.3071C>T (p.Pro1024Leu)/c.3486_3487delGG,(p.Asp1163Profs*11)	VUS (PM2,PP3)/ Likely Pathogenic (PVS1, PM2)	LGMD2B possible
LGMD356	*CAPN3*	c.1468C>T (p.Arg490Trp)x2	Pathogenic	LGMD2A
LGMD357	*SGCD*	c.657delC (p.Thr220Profs*6)x2	Pathogenic	LGMD2F
LGMD358	*SGCD*	c.657delC (p.Thr220Profs*6)x2	Pathogenic	LGMD2F
LGMD359	*SGCD*	c.657delC (p.Thr220Profs*6)x2	Pathogenic	LGMD2F
LGMD360	*SGCG*	c.525delT (p.Phe175Leufs*20)x2	Pathogenic	LGMD2C
LGMD361	*CAPN3*	c.550A>G (p.Thr184Ala)x2	Pathogenic (PS4,PM1,PM2,PM5,PP1,PP3)	LGMD2A
LGMD362	*CAPN3*	c.1468C>T (p.Arg490Trp)x2	Pathogenic	LGMD2A
LGMD363	*DYSF*	c.5836_5839del (p.Gln1946Trpfs*19)x2	Pathogenic	LGMD2B
LGMD364	*SGCA*	c.241C>T (p.Arg81Cys)	Likely Pathogenic	LGMD2D[2]
LGMD365	*CAPN3*	c.2440-6_2440-3del/	Likely Pathogenic	LGMD2A possible
LGMD366	*SGCA*	c.229C>T (p.Arg77Cys)x2	Pathogenic	LGMD2D
LGMD367	*SGCA*	c.241C>T (p.Arg81Cys)/c.502G>A (p.Gly168Arg)	Likely Pathogenic/ VUS (PM2)	LGMD2D possible
LGMD368	*SGCA*	c.724G>T (p.Val242Phe)x2	Pathogenic	LGMD2D
LGMD369	*CAPN3*	c.1468C>T (p.Arg490Trp)x2	Pathogenic	LGMD2A
LGMD370	*CAPN3*	c.1468C>T (p.Arg490Trp)x2	Pathogenic	LGMD2A

*Variant classification according to the American College of Medical Genetics and Genomics criteria; [1]clinical diagnosis with confirmed molecular diagnosis in the family; [2]pathological diagnosis only; [3] confirmed molecular diagnosis, but genotype not informed; AA aminoacid; NA not available; VUS variant of unknown significance; x2 homozygous for the variant. Novel variants are depicted in bold.

Figura S1

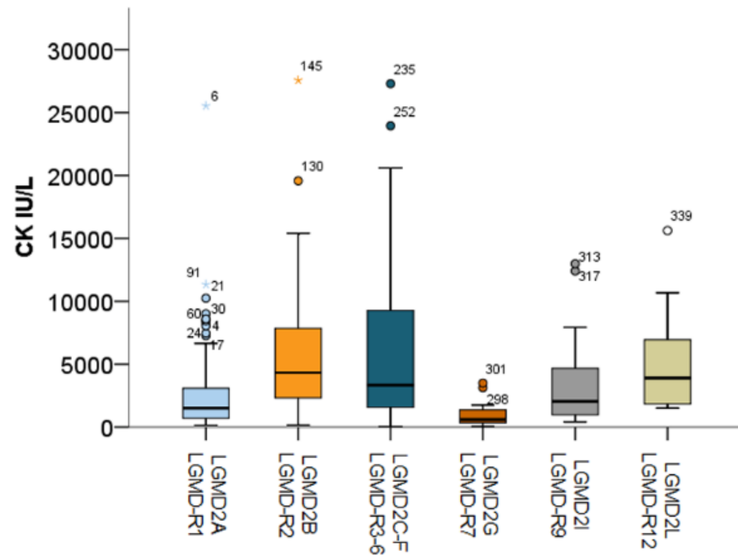

Figure S1: Creatine kinase levels in LGMD2/LGMD-R.
Box-plots show median, interquartile and range of maximum CK levels in different LGMD2/LGMD-R subtypes. The dots and the individual´s numbers

Figura S2

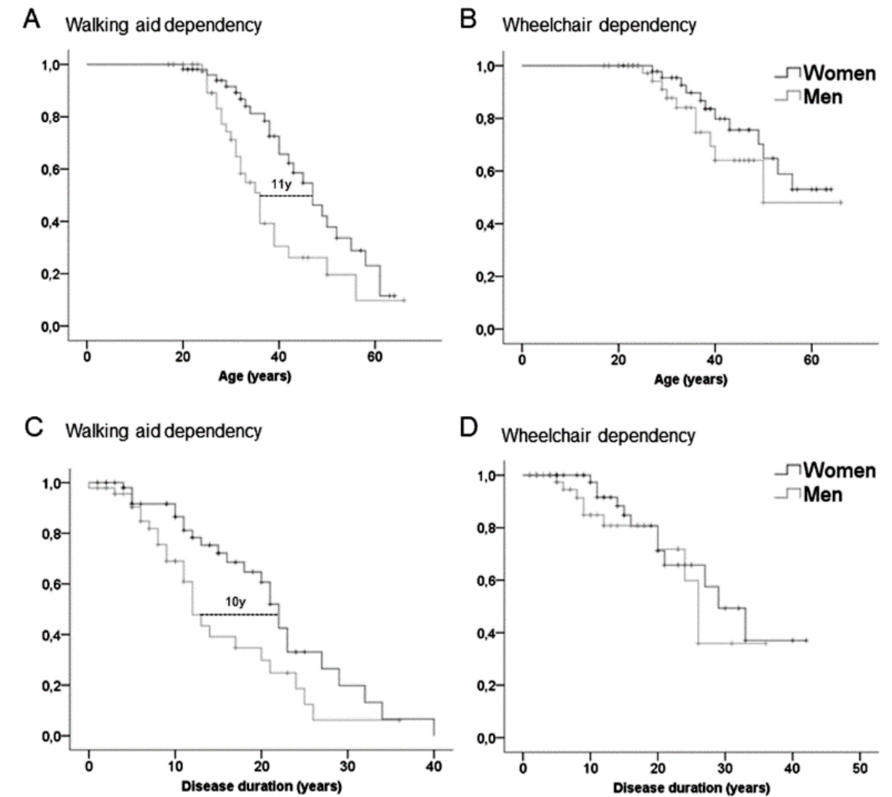

Figure S2: Sex effect on LGMD2B/LGMD-R2-dysferlin-related progression to major handicap
The figure shows Kaplan-Meier analysis of loss of independent walking **(A)** and wheelchair dependency **(B)** according to age in male and female individuals with LGMD2B/LGMD-R2-dysferlin-related, and loss of independent walking **(C)** and wheelchair dependency **(D)** according to disease duration. Differences in median values of progressions to loss of independent walking between groups are informed and highlighted by dashed lines **(A,C)**.

Figura S3

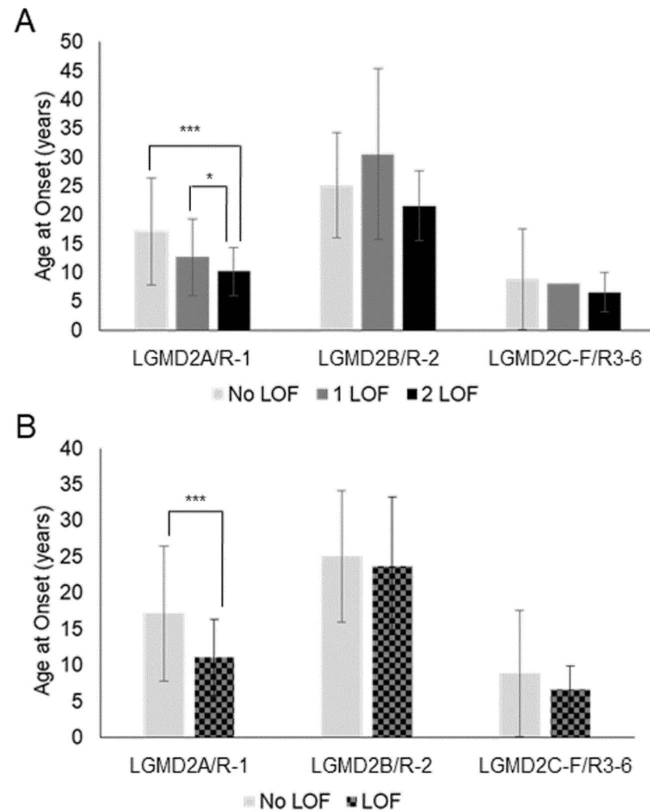

Figure S3: Genotype-phenotype correlations in most frequent LGMD2/LGMD-R subtypes

The figure shows differences in the age at onset of LGMD2A/LGMD-R1-calpain3-related and LGMD2B/LGMD-R2-dysferlin-related patients and patients with sarcoglycanopathies (LGMD2C-F) with 1, 2 or none truncating variants **(A)**, and in the age at onset of patients with at least one truncating and none truncating variants **(B)** with these subtypes. Bars represent means and lines standard deviations. LOF, loss-of-function or truncating variant; *p<0.05; ***p<0.001.

Figura S4

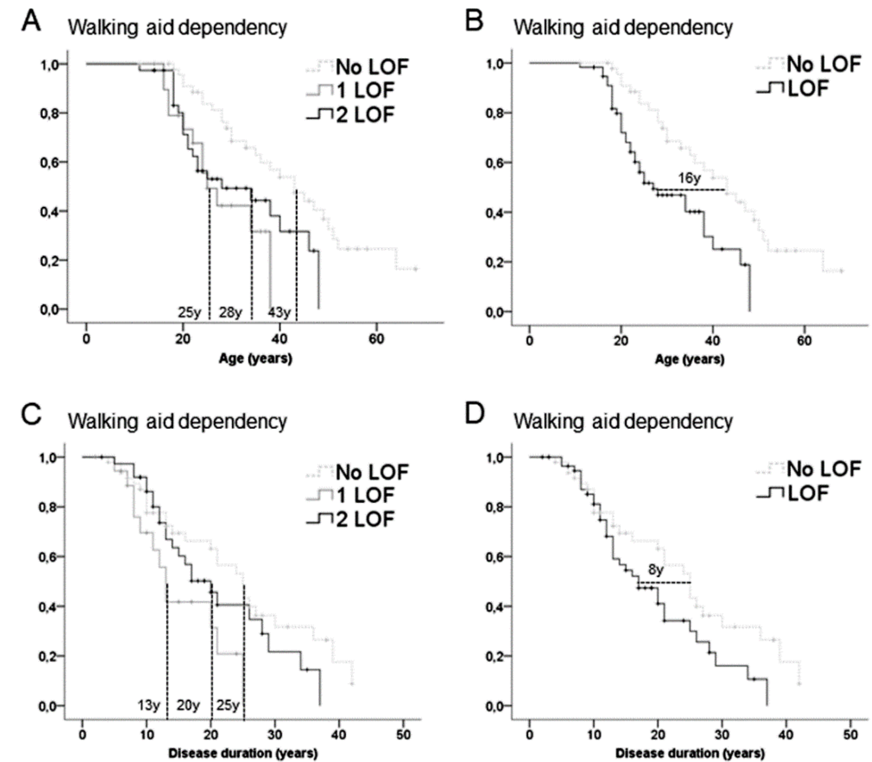

Figure S4: Genotype-phenotype correlation with progression to loss of independent walk in LGMD2A/LGMD-R1-calpain3-related

Kaplan–Meier analysis of loss of independent walk according to age in LGMD2A/LGMD-R1-calpain3-related patients with 1, 2 or none truncating (**A**) and patients with at least one truncating and none truncating variants (**B**), and of loss of independent walk according to disease duration in patients with 1, 2 or none truncating (**C**) and patients with at least one truncating and none truncating variants (**D**). Median values of progressions to wheelchair dependency are informed and highlighted by dashed lines (**A**,**C**). Differences in median values of progressions to wheelchair dependency between groups are informed and highlighted by dashed lines (**B**,**D**). LOF, loss-of-function or truncating variant.

Figura S5

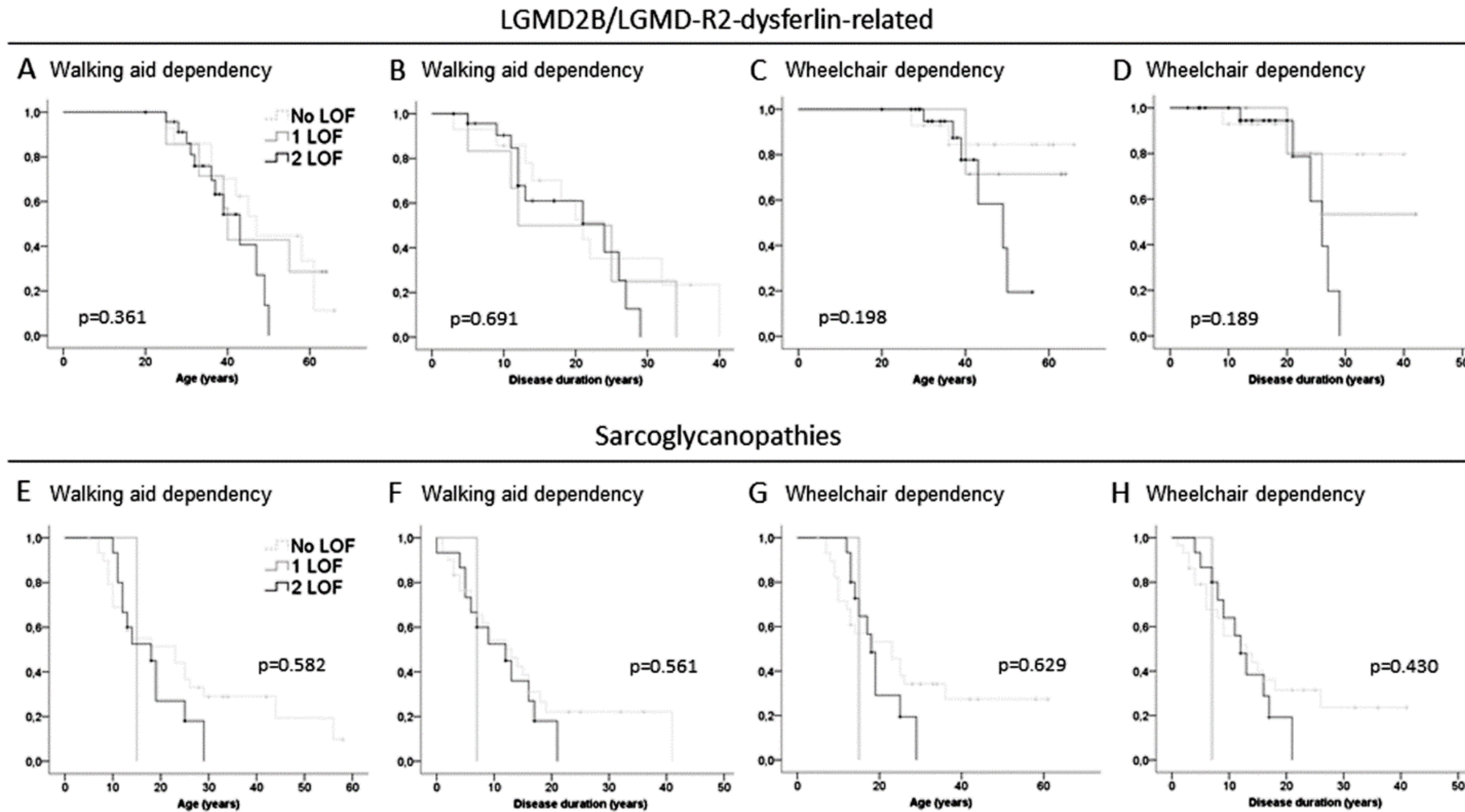

Figure S5: Genotype-phenotype correlation with progression to major handicap in LGMD2B/LGMD-R2-dysferlin-related and sarcoglycanopathies
The figure shows Kaplan–Meier analysis of loss of independent walking according to age (**A**) and disease duration (**B**), and of wheelchair dependency according to age (**C**) and disease duration (**D**) in LGMD2B/LGMD-R2-dysferlin-related patients with 1, 2 or none truncating variants; and of loss of independent walking according to age (**E**) and disease duration (**F**), and of wheelchair dependency according to age (**G**) and disease duration (**H**) in patients with sarcoglycanopathies with 1, 2 or none truncating variants. p values of Log Rank comparisons were given.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	42, 44
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	44
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	45
Objectives	3	State specific objectives, including any prespecified hypotheses	45
Methods			
Study design	4	Present key elements of study design early in the paper	45-46
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	45-46
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	45
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	45-46
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	45-46
Bias	9	Describe any efforts to address potential sources of bias	45-46,59
Study size	10	Explain how the study size was arrived at (CONVENIENCE SAMPLING)	45-46
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	46-47
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	46-47
		(*b*) Describe any methods used to examine subgroups and interactions	46-47
		(*c*) Explain how missing data were addressed	59
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	47-48
		(b) Give reasons for non-participation at each stage	59
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table1
		(b) Indicate number of participants with missing data for each variable of interest	47,56-59
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	Partially Table 1
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	47-59
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	NA
		Cross-sectional study—Report numbers of outcome events or summary measures	NA
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	47-59
		(*b*) Report category boundaries when continuous variables were categorized	47-59
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	49-56
Discussion			
Key results	18	Summarise key results with reference to study objectives	56-59
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	56-59
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	56-59
Generalisability	21	Discuss the generalisability (external validity) of the study results	56-60
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	43

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at ttp://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

8. ARTIGO II

Diagnostic yield of multi-gene panel for muscular dystrophies and hereditary myopathies

Running Title: NGS panel for muscular dystrophies and hereditary myopathies

Pablo Brea Winckler [1,2]
Bruna Cristine Chwal [3]
Marco Antonnio Rocha Dos Santos [1,2]
Daniela Burguêz [3]
Marcia Polese-Bonato [3]
Edmar Zanoteli [4]
Marina Siebert [5,6,7]
Filippo Pinto e Vairo [8]
Márcia Lorena Fagundes Chaves [1,2,9]
Jonas Alex Morales Saute [1,2,3,9*]

1. Graduate Program in Medicine: Medical Sciences, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil
2. Neurology Division, Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre, Brazil
3. Medical Genetics Division, HCPA, Porto Alegre, Brazil
4. Universidade de São Paulo (USP), Faculdade de Medicina, Departamento de Neurologia, São Paulo, SP, Brazil
5. Graduate Program in Science in Gastroenterology and Hepatology, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil
6. Unit of Laboratorial Research, Experimental Research Center, Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre, Brazil
7. BRAIN (Basic Research and Advanced Investigations in Neurosciences) Laboratory, Experimental Research Center, Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre, Brazil
8. Center for Individualized Medicine and Department of Clinical Genomics, Mayo Clinic, Rochester, MN, USA
9. Department of Internal Medicine, UFRGS, Porto Alegre, Brazil

*Corresponding author: Jonas Alex Morales Saute Medical Genetics Divison, HCPA Ramiro Barcelos, 2350, Zip-code 90035-903, Porto Alegre, Brazil Phone:+55-051-33598011

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Conflict of interest statement
Winckler PB, BC Chwal, dos Santos MAR, Burguêz D, Polese-Bonato M, Zanoteli Siebert M, Vairo FP and Chaves MLF have no disclosures related to this study. Saute JA received research grants from PTC Therapeutics. Study design; collection, analysis and interpretation of data; writing of the report and the decision to submit the report for publication had no participation of the study sponsors.

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

ABSTRACT
BACKGROUND: Genetic testing is being considered the first step in the etiological investigation of muscular dystrophies (MD) and hereditary myopathies (HM). However, only a few studies established the performance of the different testing approaches. The aims of the present study were to evaluate the diagnostic yield of a customized next generation sequencing (NGS) panel comprising 39 genes as the first-tier test for MD/HD diagnosis, and to characterize clinical and molecular findings of MD/HM families from southern Brazil. METHODS: Fifty-one consecutive index cases with clinical suspicion of MD/HM were recruited from October 2014 to March 2018 at a specialized single center in a cross-sectional study. RESULTS: The overall diagnostic yield of the NGS-MD/HM panel was 52.9%, increasing to 60.8% when including cases of MD/HM with candidate variants. Multi-gene panel solved the diagnosis of 12/25 (48%) probands with limb girdle muscular dystrophies (LGMD), of 7/14 (50%) with congenital muscular diseases (CMD), and of 7/10 (70%) with muscular dystrophy with prominent joint contractures (MDJC). The most frequent diagnosis for LGMD were LGMD2A/LGMD-R1-calpain3-related and LGMD2B/LGMD-R2-dysferlin-related; for CMD, RYR1-related disorders; and for MDJC, Emery Dreifuss muscular dystrophy type 1 and COL6A1-related disorders. Sixteen novel variants were reported. CONCLUSION: In summary, customized NGS panel when applied in the initial investigation of MD/HM results in a high diagnostic yield, likely reducing patient´s diagnostic odyssey and providing important information for genetic counselling and for participation in disease-specific clinical trials.

Keywords: diagnosis, next generation sequencing, muscular dystrophy, hereditary myopathy.

1 | INTRODUCTION

Muscular dystrophies (MD) and hereditary myopathies (HM) are heterogeneous group of diseases caused by pathogenic variants in multiple genes, inherited in all Mendelian patterns, with symptoms starting from birth to late adulthood.[1] Epidemiological data on MD/HM are scarce and variable worldwide, with prevalence estimations ranging from <1 to 7 in 100,000 individuals, when not accounting for dystrophinopathies, myotonic dystrophy and facioscapulohumeral muscular dystrophy.[2-8] There is a lack of epidemiological data of MD/HD in Latin America, with recent studies only defining the relative frequencies of different subtypes of autosomal recessive limb girdle muscular dystrophy (LGMD) in Brazil.[9]

Proximal weakness is the main clinical presentation of MD/HM, which may be accompanied by atrophy or pseudohypertrophy of calves. Cardiac and respiratory symptoms or signs may be present and should be screened during the initial clinical evaluations. The disease severity may range from mild to severe generalized weakness with early loss of walking capacity. [10-12] After clinical suspicion of MD/HD, complementary tests such as creatine phosphokinase (CK) and electromyography (EMG) are usually performed to confirm the anatomical diagnosis. Muscle magnetic resonance and ultrasound may also be helpful to narrow the diagnostic hypothesis. [13,14] Historically, when there was a clinical suspicion of MD/HM, a muscle biopsy was performed to confirm the diagnosis, searching for specific structural and immunohistochemistry features of a specific MD/HM. However, due to the reduction in genetic testing costs and the increase in its availability in the last years, coupled with its lack of invasiveness, most contemporary diagnostic approaches of MD/HM suggest starting the investigation with genetic testing, either with single-gene, multiple genes panels or exome sequencing (ES), considering muscle biopsy as a second-tier approach for investigation of unsolved cases.[15,16] Nonetheless, there are only a few studies that reported the diagnostic yield of the different testing approaches.[17,18] and most of the studies that applied next generation sequencing (NGS)-based tests did not used the test as a first-tier approach. Therefore, the aims of the present study were to evaluate the diagnostic yield of a NGS panel which includes 39 genes associated with the most prevalent types of MD/HD worldwide as the first-tier approach for MD/HD diagnosis, and to characterize clinical, molecular and histologic findings of MD/HM families from southern Brazil.

2 | MATERIALS AND METHODS

2.1 | Design and subjects

A convenience sample of 51 consecutive index cases with clinical suspicion of MD/HM according to expert clinical and neurophysiological evaluation and pedigree analysis were recruited from October 2014 to March 2018 at the Neuromuscular Genetics outpatients' clinic of Hospital de Clínicas de Porto Alegre (HCPA), in a cross-sectional study. Patients with diagnosis or suspicion of immune-mediated myopathies, myopathies secondary to endocrine diseases, toxic myopathies, dystrophinopathies, myotonic dystrophy,

oculopharyngeal muscular dystrophy and facioscapulohumeral muscular dystrophy were excluded. The study was approved by the Ethics in Research Committee of our institution (GPPG-HCPA/17-0340). Informed written consent was obtained from all individuals'/guardians' prior participation.

2.2 | Clinical and histological data collection

Data regarding sex, age, parental consanguinity, family recurrence, age at onset (first sign of weakness or respiratory/cardiac related symptoms), age at walking aid (AWA) and wheelchair (AWC) dependency, and highest CK levels were collected from patients and relatives or retrieved from electronic medical records. When AWC was provided, but AWA was not, the same value for both variables were considered and when information was not provided data was censored. Muscle Immunohistochemistry / Western Blot (IHC/WB) data were used either as a diagnostic standard or as functional evidence of variant pathogenicity. Cardiac and respiratory function was accessed by Doppler echocardiography and in some cases by cardiac magnetic resonance imaging and respiratory involvement by pulmonary function tests. Conduction abnormalities were reported only when categorized as clinically significant, and structural cardiac abnormalities were classified as related and non-related to MD/HM by an internal medicine experienced physician. Respiratory function was investigated by spirometry with forced vital capacity (FVC) and forced expiratory volume (FEV1). Necessity of non-invasive or invasive ventilatory support was also assessed. All reported data were from the most recent tests.

2.5 | Diagnostic strategy

2.5.1 | Genetic analysis

Genomic DNA was extracted from peripheral blood using salting out method and kept at -20°C (Miller SA et al, 1988). A NGS panel was designed using the Ion AmpliSeq™ Designer software (http://www.ampliseq.com/) to target all coding DNA sequences and flanking regions of the 39 MD/HM genes: *ACTA1, ALDOA, AMPD1, ANO5, BIN1, CAPN3, CAV3, COL6A1, COL6A2, COL6A3, DES, DNAJB6, DNM2, DYSF, EMD, ENO3, FHL1, FKRP, FKTN, GYS2, LAMA2, LAMP2, LMNA, MTM1, MYOT, NEB, PFKM, POMGNT1, POMT1, POMT2, PYGM, RYR1, SELENON, SGCA, SGCB, SGCD, SGCG, TCAP*, and *TPM3*. The customized panel consisted of two primer pools with 1065 different amplicons (295.18 Kb). NGS was performed using the Ion Torrent Personal Genome Machine (Ion Torrent™). The raw data generated was processed by the Torrent Suite Software v5.0 (Thermo Fisher Scientific). Reads were mapped to the hg19 reference genome using the Torrent Mapping Alignment Program (TMAP). Coverage assessment was performed using the Coverage Analysis plugin available in the Torrent Browser. Mean coverage on target was 99.17%, with 100% coverage for all included genes, except *COL6A1* (95.61%), *ALDOA* (92.5%) and *SELENON* (89.86%). Variants were annotated using Ion Reporter (http://www.ionreporter.thermofisher.com/, Thermo Fisher Scientific), Enlis Genome Research software (Enlis-LLC, Berkeley, CA.) and VarSome.[20] Integrative Genomics Viewer (IGV) [21] was used for

variant visualization. All genes were considered for analysis, regardless inheritance pattern classification. Copy number variation in *CAPN3* was searched by multiplex ligation-dependent probe amplification (SALSA MLPA kit P176 *CAPN3*, MRC Holland).

2.5.2 | Variant analysis

Sequences were obtained from the National Center for Biotechnology Information (NCBI) protein database, and variants were described in relation to the canonical transcripts. Sequence variations were compared to data available in the Human Gene Mutation Database (HGMD®, http://www.hgmd.cf.ac.uk/), Leiden Open Variation Database (LOVD,http://www.dmd.nl) and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar). Variants were classified according to the 2015 American College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology (AMP) criteria[22] and the updated recommendations for PVS[23], PP5[24] and PS3 criteria[25]. PolyPhen-2[26], SIFT[27], CADD[28], M-CAP[29], Mutation-Taster[30], REVEL[31] and Human-Splicing- Finder-v3.0[32] were used for in silico analysis. Phylogenetic conservation was estimated with Genomic Evolutionary Rate Profiling (GERP++)[33], and allele frequencies were searched on gnomAD[34]. Copy number variants (CNV) were called using DECoN[35] and the software's default parameters. For the first 25 index cases (family 1 to 25), pathogenic or likelypathogenic variants found on the NGS panel results were confirmed by Sanger sequencing using the ABI 3130xl Genetic Analyzer (Thermo Fisher Scientific). Since the NGS results were consistent with Sanger sequencing when there were ≥30 reads at the variant position, Sanger sequencing was performed only when there were ≤30 reads at the position of interest from family 26 to 51. Sanger sequencing or other orthogonal methods for regions not covered in the NGS panel were not performed.

2.5.3 | Muscle biopsy

Muscle biopsy with IHC/WB was performed for some cases to aid in variant classification as well as for some unsolved cases after uninformative multi-gene MD/HM panel.

2.6 | Statistical analysis

Data are reported as mean and standard deviation (SD) for continuous variables and as frequencies and percentages for categorical variables.

3 | RESULTS

Twenty-five probands with LGMD, 14 with congenital muscular diseases (CMD), 10 with muscular dystrophy with prominent joint contractures (MDJC) and one each with metabolic myopathy (MM) and distal myopathy (DM) were included. Demographical characteristics of the 51 probands and the overall sample of 68 individuals (including 17 additional affected relatives) with MD/HM are shown in **Table 1**. **Supplemental Table 1** present clinical, the genetic variants that were deemed causal for patients' phenotypes, neurophysiological, CK levels, and histologic data of all the 68 evaluated individuals.

TABLE 1 Clinical characteristics of subjects with hereditary myopathies/muscular dystrophies

	Overall	LGMD	CMD	MDJC
Number of patients (families)	68 (51)	38 (25)	16 (14)	11*(10)
Female (%)	25 (36.8%)	13 (34.2%)	8 (50%)	3(27.2%)
Age in years	35.7 (19.9)	42 (16.2)	23.3(16.5)	25.1(17.6)
Age at onset	14.9 (15.5)	20.5 (14.8)	4.3 (8.3)	6.83 (9.5)
Disease duration	20.9 (12.6)	22.3 (12.7)	19 (10.8)	15 (7.8)
Walking aid (%)	-	17/35 (48.5%)	11/16 (68.7%)	4/11 (36.3%)
Wheelchair bound (%)	-	16/37 (43.2%)	11/16 (68.7%)	3/11 (27.27%)
GMWS	-	5.2 (2.46)	5.8 (2.7)	3.9 (2.58)
Max CK levels IU/L	-	2205 (2484)	1091 (2150)	872 (932)
Respiratory involvement (%)	-	14/28 (50%)	10/12 (83.3%)	3/9 (23.3%)
Ventilatory support (%)	-	1/35 (2.8%)	3/15 (20%)	0/10 (0%)
Structural heart disease (%)	-	5/33 (15.1%)	0/14 (0%)	0/10 (0%)
Heart conduction disorder(%)	-	4/30 (13.3%)	0/13 (0%)	4/10 (40%)

Data are shown as mean (standard deviation). *One asymptomatic mutation carrier was evaluated for molecular diagnosis, but she was not included in this analysis. CMD; congenital muscular diseases; LGMD; limb-girdle muscular dystrophy; Max, maximum; MDJC, muscular dystrophy with prominent joint contractures; GMWS, modified Gardner-Medwin-Walton Scale.

3.1 | Diagnostic yield

The overall diagnostic yield of the NGS-MD/HM panel was 52.9% (27/51, **Figure 1**), increasing to 60.8% (31/51) when including cases with candidate variants for MD/HM. The highest diagnostic yield was for MDJC at 70% (7/10 individuals), followed by CMD at 50% (7/14 individuals) and LGMD (48% - 12/25 individuals). The diagnosis of the single patient with metabolic myopathy (MM) was solved, McArdle disease, and the single patient with distal myopathy (DM) remained with unknown etiological diagnosis (Figure 2). Thirteen out of 26 autosomal recessive cases (50%), 2/5 (40%) of the autosomal dominant cases, 2/3 (66%) of the X-linked, and 9/17 (52.9%) of the isolated cases were solved with the multi-gene panel.

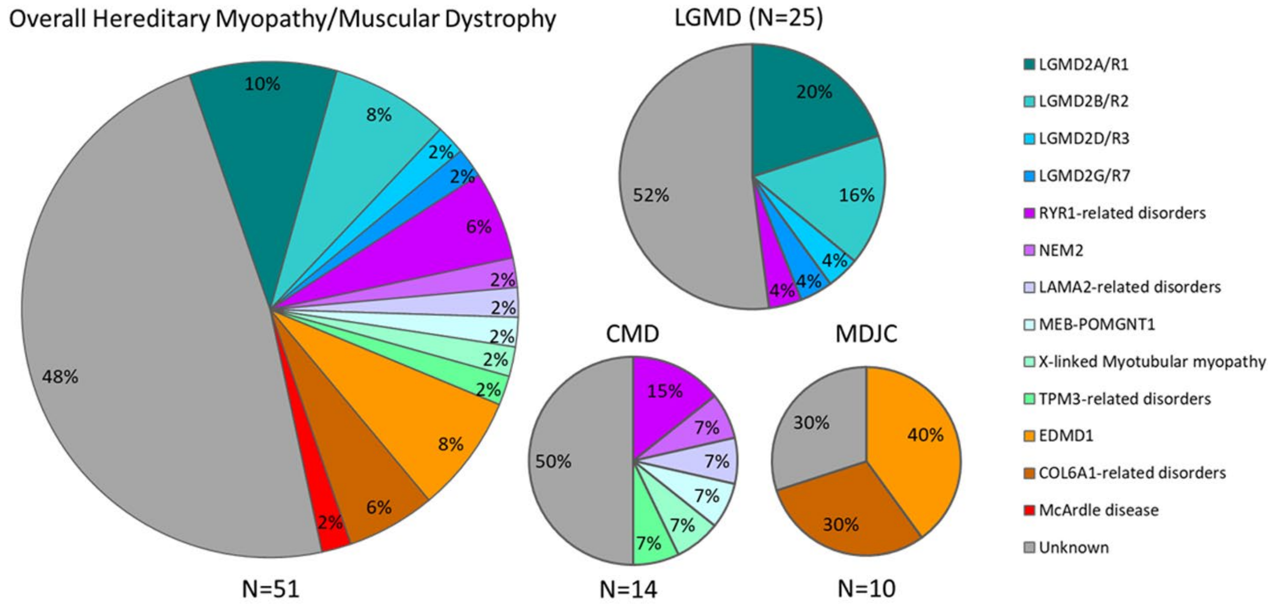

FIGURE 1 Diagnostic yield of the NGS panel for Hereditary Myopathy/Muscular Dystrophy. N indicates the number of indexes cases. CMD; congenital muscular diseases; EDMD, Emery-Dreifuss muscular dystrophy; LGMD, limb-girdle muscular dystrophy; MDJC, muscular dystrophy with prominent joint contractures; MEB, muscle-eye-brain disease; NEM, nemaline myopathy

3.2 | Novel variants

Sixteen novel variants were identified, being 1 classified as pathogenic, 10 as likely pathogenic, and 5 as VUS. All novel variants were present in index cases from single families, except for the NM_000070.2:c.692T>C - p.(Phe231Ser) in *CAPN3*, which was found in two individuals from the same family (this information was considered for attributing PP1 criteria for the variant). **Table 2** presents the detailed classification of the novel variants and **Supplemental Table 1** includes all genetic variants found in the cohort.

TABLE 2 Novel variants in muscular dystrophies/hereditary myopathies genes

Gene (Transcript)	Nucleotide change	AA change	Variant type	AF[1]	SIFT	Poly Phen2	MT	MCAP	CADD	REVEL	GERP++[2]	Functional Evidence	LOVD	CLINVAR	ACMG classification[3]	ACMG criteria[3]
CAPN3 (NM_00070)	c.692T>C	p.(Phe231Ser)	Missense	0	0	0.997	0.999	0.673	28.8	0.981	5.41	NA	No	No	Likely pathogenic	PM1, PM2, PP1, PP3
COL6A1 (NM_001848.3)	c.956A>G	p.(Lys319Arg)	Missense	0	0.01	0.877	0.999	0.191	33	0.624	4.7	Collagen VI deficiency	No	Yes(1,VUS)	Likely Pathogenic	PS3 sup, PM1, PM2, PM5, PP3
COL6A1 (NM_001848.3)	c.1397delC	p.(Pro466Argfs*39)	Frameshift	0	0.01	0.877	0.999	0.191	NA	NA	4.7	NA	No	No	Likely Pathogenic	PVS1, PM2
COL6A2 (NM_001849.4)	c.1060G>A	p.(Asn354Asn)	Missense	0.0000611	0.12	0.997	0.999	0.555	22.9	0.326	4.46	No	No	Yes (2, VUS)	VUS	PM1, PM2, BP4
COL6A2 (NM_001849.4)	c.2927T>C	p.(Leu976Ser)	Missense	0.000213	0	0.643	0.999[4]	0.302	23.4	0.632	4	NA	No	Yes (2, VUS; 1 Likely Benign)	VUS	PM2, BP4
COL6A3 (NM_004369.4)	c.2801G>C	p.(Ser934Thr)	Missense	0	NA	0.999	0.999	0.194	24.9	0.614	5.5	NA	No	No	VUS	PM2, PP3
EMD (NM_000117.3)	c.146dupC	p.(Pro50Ala*11)	Frameshift	0	NA	NA	1	NA	NA	NA	3.03	NA	No	No	Likely Pathogenic	PVS1, PM2
EMD (NM_000117.3)	c.546T>A	p.(Tyr182*)	Nonsense	0	NA	NA	0.999	NA	25.4	NA	-6.08	NA	Yes	No	Likely Pathogenic	PVS1 strong, PM2
EMD (NM_000117.3)	c.581_582delCA	p.(Ser194Phefs*15)	Frameshift	0	NA	NA	1	NA	NA	NA	3.36	Absence of emerin	No	Yes (1,Pathogenic)	Likely Pathogenic	PVS1 strong, PM2, PP4
FKTN (NM_006731.2)	c.1270G>A	p.(Gly424Ser)	Missense	0.00000398	0.01	0.94	0.9999	0.211	33	0.886	6.04	NA	No	No	VUS	PM2, PP3
LAMA2 (NM_000426.4)	c.1255delA	p.(Ile419Leufs*4)	Frameshift	0	NA	NA	1	NA	NA	NA	2.28	NA	No	Yes (2, Pathogenic)	Pathogenic	PVS1, PM2, PP4
NEB (NM_001271208.2)	c.21203_21204insA	p.(Ser7068Argfs*4)	Frameshift	0	NA	NA	1	NA	NA	NA	6.07	NA	No	No	Likely pathogenic	PVS1, PM2
POMGNT1 (NM_001243766.1)	c.1694_1695delCT	p.(Ser565Phefs*20)	Frameshift	0	NA	NA	1	NA	NA	NA	2.08 (5.6)	NA	No	Yes (1, Likely pathogenic)	Likely Pathogenic	PVS1, PM2
POMT1 (NM_007171.4)	c.814A>G	p.(Ile272Val)	Missense	0	1	0.09	0.999[4]	0.030	0.001	0.294	-10.8	NA	No	No	VUS	PM2, BP1, BP4
RYR1 (NM_000540.3)	c.2533C>T	p.(Leu845Phe)	Missense	0.00000398	0.08	0.961	0.99	0.48	23.5	0.616	3.7	NA	No	Yes (1,VUS)	Likely pathogenic	PM1, PM2, PP2, PP3
SELENON (NM_206926.2)	c.598_599insC	p.(Tyr200Serfs*67)	Frameshift	0	NA	NA	1	NA	NA	NA	5.84	NA	No	No	Likely pathogenic	PVS1, PM2

Allele frequencies on [1]gnomAD; [2] GERP++ data is shown as mean (standard deviation) or raw value; [3]American College of Medical Genetics and Genomics criteria, Richards et al, 2015; [4]polymorphism prediction. AA, amino acid; AF, allele frequency; CADD, Combined Annotation Dependent Depletion; GERP, Genomic Evolutionary Rate Profiling; gnomAD, Genome Aggregation Database; LOVD, Leiden Open Variation Database; MCAP, Mendelian Clinically Applicable Pathogenicity; MT, Mutation Taster; NA, not available; SIFT, Sorting Intolerant From Tolerant; VUS, variant of unknown significance.

3.3 | Diagnosis of cases with uninformative NGS-MD/HM panel

Five index cases (20%) were diagnosed in the follow-up period, after uninformative NGS-MD/HM panel, being four with LGMD and one with CM (**Figure 2**). Regarding the individuals with LGMD,one was found to have tongue fasciculations and was ultimately diagnosed with genetically confirmed spinal muscular atrophy type 3 (case Myo5, **Supp. Table 1**); one individual had muscle biopsy depicting myosin storage and MYH7-related disease diagnosed after sequencing of the gene. (case Myo18, **Supp. Table 1**); one patient was diagnosed with LGMD2T/LGMD-R19-GMPPB-related by clinical ES (case Myo29, **Supp. Table 1**); one female individual was diagnosed with a dystrophinopathy based on immunohistochemistry analysis of muscle biopsy; however there were no pathogenic variants in DMD detected by multiplex ligation-dependent probe amplification (MLPA) or NGS (case Myo40, **Supp. Table 1**). One patient with CM was ultimately diagnosed with fiber-type disproportion congenital myopathy SELENON-related by clinical ES. After reviewing the panel raw data, the region where the missed pathogenic variant in *SELENON* lies, did not pass the quality control before variant calling due to suboptimal coverage. Thus, this was the only false negative result of the NGS-MD/HM panel (case Myo24, **Supp. Table 1**) in this cohort.

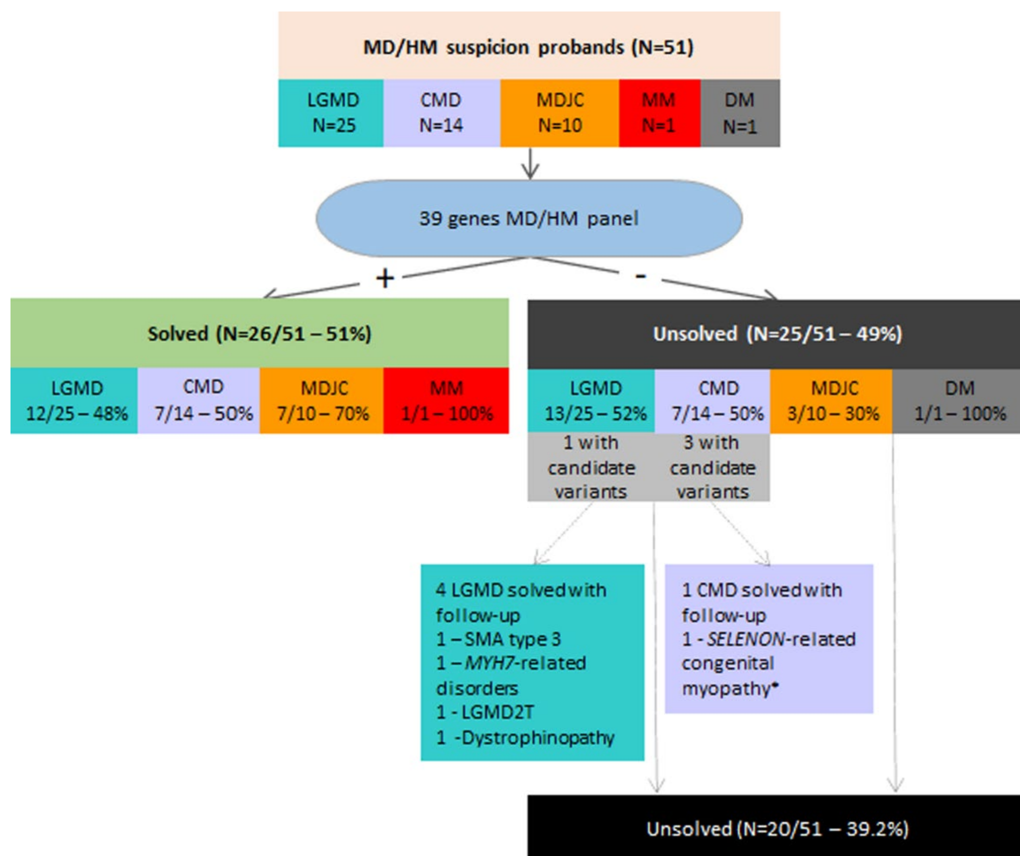

FIGURE 2 Flowchart of the genetic investigation. N indicates the number of indexes cases. CMD; congenital muscular diseases; DM, distal myopathy; LGMD, limb-girdle muscular dystrophy; MDJC, muscular dystrophy with prominent joint contractures; MM metabolic myopathy; SMA, spinal muscular atrophy. *false negative result with the NGS panel due to suboptimal read-depth.

4 | DISCUSSION

The present study described the diagnostic yield of a customized multi-gene NGS panel as the first-tier approach in the investigation of muscular dystrophies and hereditary myopathies. With this strategy, over half of the families received a firm genetic diagnosis and another 10% were found to have strong candidate variants for their phenotype. The highest diagnostic yield was found for congenital muscular dystrophies and congenital myopathies, and for muscular dystrophies with prominent joint contractures. Moreover, sixteen novel variants were detected and detailed phenotypic information was provided to aid in genotype-phenotype correlations for individuals from an admixture population from Southern Brazil.

Our diagnostic yield was similar to reports applying NGS technologies for similar purposes, but in heterogeneous populations with MD/HM. In Europe, a study reported that a customized panel including 93 genes solved 43% of diagnosis in a cohort of 504 individuals with MD/HM, most with LGMD and CM[18-36,37]. This study included individuals with previous negative results after genetic analysis of 1 to >5 genes, so it cannot be considered a true first-tier genetic approach, and the diagnostic performance of the customized NGS panel might have been overestimated, because authors considered solved diagnosis when variants predicted to affect function in genes corresponding to clinical suspicions were found. Another recent study found that a comprehensive NGS panel had a diagnostic yield of 49.3% in a cohort of 207 patients from Spain with congenital myopathies, distal myopathies, congenital and adult-onset muscular dystrophies, and congenital myasthenic syndromes [37]. Of note, almost half of individuals in this study had had a muscle biopsy before the genetic testing. A very large sample study evaluated a customized panel of 266 genes for the diagnosis of 25,356 unrelated individuals with suspected neuromuscular disorders, reporting a diagnostic performance of 33% among subjects with muscular dystrophy [38]. In an Indian study, ES solved 49% of the 207 unrelated cases with MD/HM from diverse regions of the country [18]. Most patients in this study had undergone ES as a first-tier or initial genetic approach (muscle biopsy was performed in 22% of cases before ES), and the criteria for solved diagnosis was similar to ours. Additionally, a high diagnostic yield, around 50%, was recently depicted for the genetic investigation of oligosymptomatic subjects with hyperCKemia with a customized NGS panel with most cases with muscle biopsy prior to sequencing [17], and an even higher diagnostic performance, around 87.5%, was found in a recent small case series of consanguineous families with muscle dystrophy investigated by ES [39]. Therefore, customized multi-gene panels and ES have similar diagnostic performances, with high yield, for patients with MD/HM suspicion.

LGMD2A/LGMD-R1-calpain3-related and LGMD2B/LGMD-R2-dysferlin-related were the most diagnosed subtypes of LGMD in the present study, an epidemiological profile similar to the findings from other Brazilian regions reported in a recent multicenter study [9] and to recent large samples studies from Italy and the United States [40,41] however, it differed from previous Brazilian reports focused on the pediatric population [42]. Of note, case Myo57, in which a single heterozygous missense pathogenic variant in *CAPN3* was found, presented a CNV depicted by DECoN that was confirmed by MLPA analysis, a deletion from exons 2 to 8 in *CAPN3* (c.(309+1_310-11) _1115+1_1116-1)del. The 48% solve rate for LGMD was

higher than in larger cohorts, which reported 27% of genetic diagnosis for a similar size NGS panel [41]; however, the later study recruited individuals that were referred by a program launched by MD associations/foundations for genetic testing in a reference laboratory, so a great heterogeneity on clinical and genetic evaluations was very likely, which might have underestimated the yield of such approach. The diagnostic performance of the multi-gene panel was similar to the 45% diagnostic yield of ES in Australian patients with LGMD. Of note in this study more than a half of the families with solved diagnosis had variants in disease-related genes not typically associated with LGMD [43]. The diagnostic yield for CMD/CM was consistent with previous studies on larger samples utilizing NGS technologies[44,45]. *RYR1* related disorders and *NEM2*, which were the most frequent diagnosis for CMD/CM, are also one of the most prevalent forms of CM in other populations [45,46].

EDMD1 and *COL6A1*-related disorders were the most frequently diagnosed subtypes of MDJC in our cohort and *EDMD1* is the most frequent subtype of *EDMD* in other populations as well [47]. Two recent studies with larger sample sizes reported the most frequently implicated genes in *COL6*-related disorders. A Brazilian study reported that *COL6A1* and *COL6A2* were the most frequently implicated genes [48] and a Chinese study reported that *COL6A2* was the most frequent [49].

Noteworthy, the diagnostic yield was similar for individuals with AR inheritance and for isolated cases, which were higher than for the families with AD inheritance. These data suggest that genetic testing should be performed when there is a suspicion of MD/HM regardless of the family history.

Interestingly, 6/20 (30%) index cases with uninformative NGS-MD/HM panel were diagnosed during the follow-up, one of them by phenotype review and targeted gene analysis, three of them by other NGS approaches, and two based on muscle biopsy results. For the remaining unsolved cases, muscle biopsy, when performed, only confirmed the broad diagnosis of muscular dystrophy or congenital myopathy. Our study had one false negative result in *SELENON*, which was the gene with lowest coverage (89.86%) by the NGS panel what reinforces those regions with suboptimal read-depth by NGS should be assessed by orthogonal methods to achieve reliable coverage.

4.1 | Study limitations

The study evaluated patients from a single center and the total sample size is limited. However due to this limitation, the evaluations and investigation protocols were more homogeneous. Since muscle biopsies with IHC/WB analysis were not performed for all probands, incorrect diagnosis of MD/HM might have been included in the sample; however, this would act as a conservative bias decreasing the diagnostic yield of the NGS panel. Due to the retrospective design for clinical data collection, some clinical variables data were missing. To allow the reader´s interpretation of the impact of missing data; the overall number of evaluated subjects for all variables was depicted throughout the paper. All cases were recruited at a reference center for rare diseases, specialized in neuromuscular disorders, thus the diagnostic yield for MD/HD using similar approaches might not be the same in different clinical contexts, especially at lower

complexity institutions. Additional limitations were that we were unable to define the phase of some variants by testing unaffected relatives, that additional functional studies were not available to validate the VUS found, that although we have used a CNV algorithm with great accuracy for panels we might have missed CNVs in the evaluated genes and the inherent limitation of the designed panel, which is unable to detect variants in deep intronic and non-coding regions.

5 | CONCLUSION

In our cohort, a genetic diagnosis was obtained in 53% of families using a multi-gene NGS panel as a first-tier approach in the investigation of MD/HM. A firm diagnosis obtained early in the investigation reduces the burden of patients facing diagnostic odysseys allowing them to receive proper genetic counselling and ultimately participating in gene specific clinical trials.

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ANEXOS

Supplementary TABLE 1
Available as an attachment in excel format due to its large size and to facilitate reader's consultation

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	81-82
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	82
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	83
Objectives	3	State specific objectives, including any prespecified hypotheses	83
Methods			
Study design	4	Present key elements of study design early in the paper	83-85
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	83-85
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	83-85
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	83-85
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	NA
Bias	9	Describe any efforts to address potential sources of bias	83-85,91
Study size	10	Explain how the study size was arrived at	83,84,91
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	85
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	85
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	91
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	91
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	85, Supp Table 1
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	Figure 2
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	Supp Table 1
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	NA
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	NA
		Cross-sectional study—Report numbers of outcome events or summary measures	85-89
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	NA
		(*b*) Report category boundaries when continuous variables were categorized	85-89, Table 1
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	85-89
Discussion			
Key results	18	Summarise key results with reference to study objectives	81

Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	15-16
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-16
Generalisability	21	Discuss the generalisability (external validity) of the study results	12-16
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	3

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at ttp://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

9. CONSIDERAÇÕES FINAIS E PERSPECTIVAS FUTURAS

Avanços científicos recentes têm permitido o acesso a testes genéticos que auxiliam o diagnóstico preciso de pacientes com DM/MH. Um diagnóstico genético foi obtido em 53% das famílias usando um painel NGS de 39 genes como uma abordagem de primeira linha na investigação de DM/MH. O presente estudo foi um dos primeiros a avaliar o uso do painel NGS como exame inicial para diagnóstico de pacientes com suspeita clínica de DM/MH, antes da biópsia muscular, mostrando um rendimento diagnóstico adequado para um teste único reduzindo a odisseia diagnóstica dos pacientes. A confirmação diagnóstica no início da investigação reduz desgastes na realização de múltiplos exames complementares desnecessários, possibilita o aconselhamento genético adequado e proporciona uma melhor assistência médica.

Pela primeira vez foram avaliadas todas as regiões do País com relação a LGMD2. Essa foi a maior série de casos já descrita na literatura mundial com dados clínicos e moleculares de LGMD2. Através dela foi possivel mudar o panorama epidemiológico das LGMD2 no Brasil, identificando-se que LGMD2A e LGMD2B são os subtipos mais frequentes de LGMD2 na popupação geral, similar ao que foi relatado em outros locais do mundo como Estados Unidos e Europa. A frequência relativa observada é diferente da previamente relatada em centros com foco em populações pediátricas, onde as sarcoglicanopatias são mais prevalentes, o que também foi reforçado neste trabalho. Mulheres com LGMD2B têm uma progressão menos grave para deficiência do que os homens. Pacientes com LGMD2A com variantes truncadas têm início da doença mais precoce além de apresentarem uma progressão mais grave para a deficiência quando comparado aos sem variantes truncadas. Esses resultados são de grande importância para o entendimento da epidemiologia das DM/MH no Brasil, bem como para entender melhor a progressão dessas doenças e seus modificadores, dados fundamentais que permitirão inserir o Brasil no mapa de populações em que se conhece a prevalência desse grupo de doenças, permitindo a colaboração em futuros ensaios clínicos.

Foi possível entregar a população local um diagnóstico molecular preciso, permitindo avanços na definição de práticas e protocolos assistenciais. Houve um marcado aprimoramento técnico da equipe assistencial uma vez que se passou a conhecer o diagnóstico etiológico final dessas patologias. Futuramente, espera-se incorporar essa abordagem diagnóstica no SUS e estabelecer parcerias entre centros acadêmicos assistenciais, além de aumentar a sinergia indústria-academia para buscar otimização de recursos, promover o avanço científico e principalmente melhorar assistência médica a população.

10 | OUTRAS PUBLICAÇÕES INDEXADAS E CAPÍTULOS DE LIVROS DURANTE O DOUTORADO

10.1 | Genetic profile of Brazilian patients with dystrophinopathies
Paper and publication date: Clin Genet. 2017 Aug
doi: 10.1111/cge.12975. Epub 2017 Feb 22. – QUALIS A3

10.2 | Clinical and molecular findings in a cohort of ANO5-related myopathy
Paper and publication date: Ann Clin Transl Neurol. 2019 Jul
doi: 10.1002/acn3.50801. Epub 2019 Jun 11. QUALIS A1

10.3 | Diagnostic yield of targeted sequential and massive panel approaches for inherited neuropathies
Paper and publication date: Clin Genet. 2020 Aug
doi: 10.1111/cge.13793. Epub 2020 Jun 29. QUALIS A3

10.4 | Dropped head syndrome as a manifestation of Charcot-Marie-Tooth disease type 4C
Paper and publication date: Neuromuscul Disord. 2019 Feb
doi: 10.1016/j.nmd.2018.11.010. Epub 2018 Nov 29. QUALIS A3

10.5 | Speech characteristics in individuals with myasthenia gravis: a case control study
Paper and publication date: Logoped Phoniatr Vocol. 2020 Oct
doi: 10.1080/14015439.2020.1834614. QUALIS A4

10.6 | Cognitive performance in patients with Myasthenia Gravis: an association with glucocorticosteroid use and depression
Paper and publication date: Dement Neuropsychol. 2020 Jul-Sep
doi: 10.1590/1980-57642020dn14-030013. QUALIS B3

10.7 | Autosomal recessive spastic ataxia of Charlevoix-Saguenay: a family report from South Brazil
Paper and publication date: Arq Neuropsiquiatr. 2017 Jun
doi: 10.1590/0004-282X20170044 QUALIS B2

10.8 | PRO NEURO Programa de Atualização em Neurologia da Academia Brasileira de Neurologia, 2021
Livro doi: 10.5935/978-65-5848-219-2.B0001