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Research Report

A 20-year Clinical and Genetic Neuromuscular Cohort Analysis in Lebanon: An International Effort

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Abstract.
Background: Clinical and molecular data on the occurrence and frequency of inherited neuromuscular disorders (NMD) in the Lebanese population is scarce.
Objective: This study aims to provide a retrospective overview of hereditary NMDs based on our clinical consultations in Lebanon.
Methods: Clinical and molecular data of patients referred to a multi-disciplinary consultation for neuromuscular disorders over a 20-year period (1999–2019) was reviewed.

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Results: A total of 506 patients were diagnosed with 62 different disorders encompassing 10 classes of NMDs. 103 variants in 49 genes were identified. In this cohort, 81.4% of patients were diagnosed with motor neuron diseases and muscular dystrophies, with almost half of these described with spinal muscular atrophy (SMA) (40.3% of patients). We estimate a high SMA incidence of 1 in 7,500 births in Lebanon. Duchenne and Becker muscular dystrophy were the second most frequently diagnosed NMDs (17% of patients). These disorders were associated with the highest number of variants (39) identified in this study. A highly heterogeneous presentation of Limb Girdle Muscular Dystrophy and Charcot-Marie-Tooth disease was notably identified. The least common disorders (5.5% of patients) involved congenital, metabolic, and mitochondrial myopathies, congenital myasthenic syndromes, and myotonic dystrophies. A review of the literature for selected NMDs in Lebanon is provided.

Conclusions: Our study indicates a high prevalence and underreporting of heterogeneous forms of NMDs in Lebanon- a major challenge with many novel NMD treatments in the pipeline. This report calls for a regional NMD patient registry.

Keywords: Genetics, Lebanon, neuromuscular, SMA, DMD, LGMD, CMT, FSHD

INTRODUCTION

Neuromuscular disorders (NMDs) constitute a group of heterogeneous conditions that affect different components of the neuromuscular system, involved in controlling body movements. NMDs are characterized by dysfunction or damage of muscle or peripheral nerves, which results in partial or total loss of voluntary movement, and more seldom, leads to fatal complications such as cardiac or respiratory insufficiency. Clinical characteristics observed in NMD patients develop due to the dysfunction of motor units - composed of the lower motor neurons, the neuromuscular junction, and the skeletal muscle itself. The underlying cause for this dysfunction can be inherited or acquired [1]. To date, 1079 NMDs, classified into 16 categories, have been associated with 608 nuclear and mitochondrial genes (http://musclegenetable.fr/) [2].

The overlapping and often non-specific symptoms observed in NMD patients have made the process of differential diagnosis rather complex [1]. Moreover, the large number of genes and wide range of genetic alterations linked to these diseases have further complicated the diagnosis. Advances in the molecular biology field and the emergence of high-throughput approaches such as microarray analysis and next generation sequencing (NGS) have significantly enhanced the identification of novel NMD genes and improved the diagnostic process [3]. A systematic approach towards the diagnosis and management of hereditary NMDs is needed, especially in the Middle East, where the prevalence of these diseases is thought to be high and underestimated [4, 5]. The lack of NMDs-related data in Lebanon makes it difficult to understand and quantify their socio-economic impact, concurrent with retrospective studies calling for a much needed infrastructure for data collection and sharing in the Middle East [5, 6]. Establishing a patient registry would address this challenge and enable clinicians, researchers, policy makers, and industry experts to unify their efforts in introducing new treatment methods and providing better patient care [7]. In an effort towards starting a regional patient registry, we report herein a 20-year retrospective overview of a cohort of NMD patients from Lebanon.

METHODS

This study was conducted over a period of twenty years, from 1999 to 2019, by a multidisciplinary team from Lebanon and France that included clinicians, mainly neurologists, orthopedists, pediatricians, genetic counsellors, biologists, psychologists, and psychomotor therapists. It was further supported by two patient advocacy groups, AFM-Telethon (France) and Sesobel (Lebanon). Patients were mainly referred by neuropediatricians, adult neurologists, pathologists, and orthopedic surgeons from various Lebanese hospitals and clinics. Clinical and genetic reports of patients have been compiled from available medical records. Female and male patients of any age presenting with neuromuscular phenotypes, confirmed only clinically or coupled with molecular diagnosis, were included in this study. Ataxias, spastic paraplegias, primary cardiomyopathies, or mitochondrial diseases without neuromuscular expression were excluded. Genetic testing was carried out in different Lebanese private laboratories specialized in molecular genetic

diagnosis, as well as in public or private laboratories abroad, whenever the required test was not available in Lebanon. This study was approved by the institutional ethics review board. Blood samples for DNA extraction were obtained upon informed consent. All procedures performed in studies involving the human participants were in accordance with the ethical standards of the 1975 Helsinki declaration and molecular testing was performed in distinct laboratories by different biologists specialized in molecular and human genetics. All patients underwent thorough clinical and medical investigations, before being recommended suitable molecular testing. WES analysis was performed for cases with unclear clinical diagnosis and for patients with a suspected genetically heterogeneous disease. Up until 2014, tools used for molecular diagnostics included Multiplex PCR, Southern blot, Multiplex Ligation-Dependent Probe Amplification (MLPA), as well as Restriction Fragment Length Polymorphism PCR mainly for the diagnosis of Spinal Muscular Atrophy (SMA) and Duchenne/Becker Muscular Dystrophy (DMD; BMD). Sanger sequencing was applied for all other monogenic diseases. The use of Next Generation Sequencing (NGS) techniques has gradually become routine practice since then. A panel including 378 genes associated with NMDs was sequenced by NGS in patients suspected to present a clinically or genetically heterogeneous disease. Genomic DNA obtained from the tested sample was enriched for targeted regions using a hybridization-based protocol, and sequenced. All targeted regions were sequenced with $\geq$ 50X depth. When the genetic panel failed to identify the molecular diagnosis, Whole Exome Sequencing (WES) was performed [8]. Approximately 37 Mb (214,405 exons) of the Consensus Coding Sequences (CCS) were enriched from fragmented genomic DNA by more than 340,000 probes designed against the human genome (Nextera Rapid Capture Exome, Illumina) and the generated library sequenced on an Illumina NextSeq or HiSeq 4,000 platform to an average coverage depth 70–100X. An end to end bioinformatics pipeline including base calling, primary filtering of low quality reads and probable artefacts, and annotation of variants was applied. To select the deleterious variants, variants with a frequency greater than 1% in public databases (including gnomAD and 1000 Genomes) were filtered out in order to only select rare variants that might be relevant. Furthermore, due to the challenging interpretation of variants found in non-coding areas such as UTRs and intronic regions, these were discarded from our analysis unless their implication in the disease was previously reported. Relevant genetic variants were only selected if their depth is $\geq$ 50X. All identified variants were evaluated with respect to their pathogenicity and causality and categorized based on the guidelines of the American College of Medical Genetics and Genomics (ACMG).

mtDNA sequencing was performed by Centogene AG, Germany. For select patients who underwent mitochondrial analysis, the entire mitochondrial genome was amplified using two overlapping long range PCRs. Amplicons were examined for commonly reported deletions within the mitochondrial genome using Bioanalyzer. Libraries generated using Illumina compatible adapters were sequenced on Illumina platforms to an average sequencing depth of $>$ 1000X.

All variants identified by means of NGS analysis were subsequently confirmed by Sanger sequencing. Segregation studies were performed for all families wishing to complete the genetic study for their genealogy as a part of the offered genetic counselling.

RESULTS

The NMD cohort we present here consisted of 506 patients, including 291 males (57.5%) and 215 females (42.5%) from referrals across clinics in Lebanon. Patients were diagnosed between the ages of 0 and 60, and mainly exhibited a pediatric disease presentation (median = 2yrs; mean = 8yrs). 62 genetic disorders and clinical presentations classified into 10 groups were identified in this study (Fig. 1). Motor Neuron Diseases (MND), Muscular Dystrophies (MD), and Charcot-Marie-Tooth disease (CMT), represented the bulk of disorders identified with 227 (44.9%), 170 (36.6%), and 66 (13%) patients respectively. The remaining 28 (5.5%) patients exhibited congenital, metabolic, and mitochondrial myopathies, congenital myasthenic syndromes, and myotonic dystrophy. Disorders identified in this study encompassed monogenic inherited disorders, mostly involving autosomal recessive (n = 33) and dominant (n = 10) NMDs; X-linked (n = 4) and mitochondrial (n = 1) disorders constituted the remaining disorders identified (Table 1). Expectedly, SMA, DMD, and BMD were diagnosed in over half of the patients (290 patients; 57.3%). 95 patients of our cohort were diagnosed clinically without molecular testing. The remaining 411 patients underwent molecular

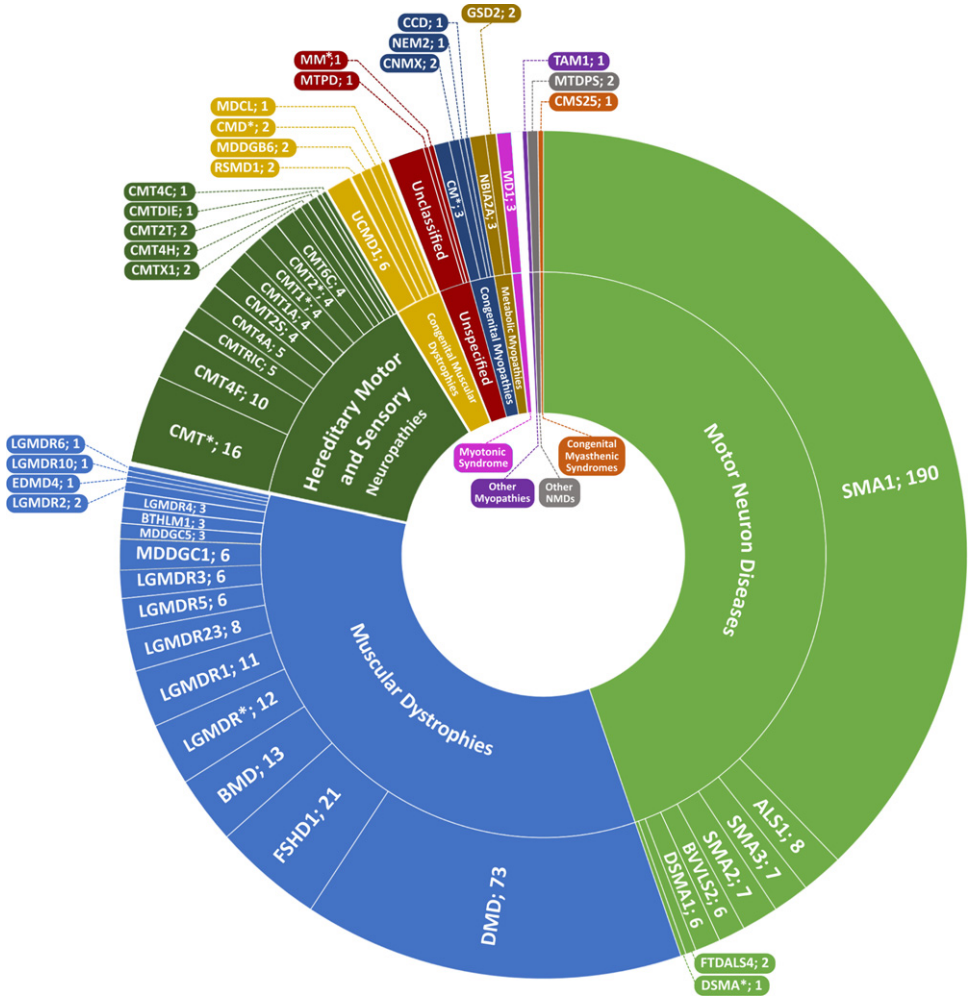

Fig. 1. Sunburst representing the spectrum of hereditary NMDs in the patient cohort. Arranged by number of patients clockwise, the inner ring represents NMD groups as classified by *The 2021 version of the gene table of neuromuscular disorders* [2]; the outer ring represents short names of NMD subtypes separated by number of patients. Full names and OMIM numbers for each disorder are included in Table 2. *Marked disorders indicate either an undetermined NMD subtype with all patients having been only clinically diagnosed, or disease-gene correlations not currently recognized as a subtype on OMIM.

diagnosis; variant data was available for 324 of those patients, thus generating a success rate of 79% for the diagnostic strategy followed. 103 distinct variants in 49 genes were identified. Based on zygosity, patients exhibited 35 homozygous, 14 heterozygous, 7 compounds heterozygous, 41 hemizygous variants, and 1 mitochondrial variant (Table 2). 39 distinct variants in *DMD* were notably identified (Fig. 2). A heterogeneous presentation of Limb-Girdle MD (LGMD) and congenital MD (CMD), involving 15 subtypes (Fig. 3), as well as CMT, involving 12 subtypes, was prominent. All families with no exceptions underwent genetic counselling and segregation studies.

DISCUSSION

5q-Spinal Muscular Atrophy

The most prevalent NMD in this study cohort was proximal SMA linked to chromosome 5q (40.3% of patients). SMA is an autosomal recessive lower alpha motor neuron disease globally estimated to affect ~1 in 4,000 to 20,000 births [9]. Patients in this study presented with characteristic features of acute infantile and intermediate forms of (SMA1-3). Assessment of *SMN1* variants in 185 patients (90.7% of SMA patients) from this cohort revealed homozygous deletions of exons 7 and 8 to be the most common

Table 1
List of hereditary NMDs identified in this study, distributed by modes of inheritance

Autosomal Recessive	
BVVL2	LGMDR5
CMS25	LGMDR6
CMT2S	LGMDR10
CMT2T	LGMDR23
CMT4A	MDCL
CMT4B3	MDDGB6
CMT4C	MDDGC1
CMT4F	MDDGC5
CMT4H	MTPD
CMT6C	NEM2
CMTRIC	RSMD1
DSMA1	SMA1
GSD2	SMA2
LGMDR1	SMA3
LGMDR2	UCMD1
LGMDR3	NBIA2A
LGMDR4	
Autosomal Dominant	
FSHD1	CMTDIE
BTHLM1	CCD
FTDALS4*	TAM1
ALS1*	MD1
CMT1A	EDMD4*
X-Linked Recessive	
BMD	
CNMX	
DMD	
X-Linked Dominant	
CMTX1	
Mitochondrial	
MM (*MTRNR2*)	

Full names of the disorders and respective OMIM numbers are included in Table 2. *Sporadic manifestations of genetic NMDs were identified in patients with EDMD4, ALS1, and FTDALS4, associated with *de novo* mutations in *SYNE1*, *SOD1*, and *TBK1* respectively.

molecular diagnosis. Assessment of *SMN2* gene copy number, which typically correlates inversely with disease severity [10], was not performed for most patients as it was not standard protocol at the time of the patients' diagnoses; access to second-tier investigations remains challenging for many Lebanese families. One of the patients in our cohort, presenting with SMA1, exhibited compound heterozygous mutations (c.549del; p.Lys184Serfs* and exon 7 and 8 deletion). Genotypes with deleterious point variants in addition to exon deletions are uncommon and homozygosity for such point variants are extremely rare. Interestingly, the same variant from our study (c.549del) was observed in an Iranian cohort where only one patient presenting with SMA1 exhibited it in homozygous state, whilst the remaining patients exhibited exon 7 and 8 deletions [11].

Published genetic studies on *SMN1*-associated SMA in Lebanon are limited to a few reports on patients with chronic forms of the disorder [12, 13]. These include a report on a Lebanese family with SMA3, described amongst 24 families in a linkage analysis study associating chronic proximal SMA with chromosome 5q preceding the discovery of the gene in 1994 [12]. Another study highlighted a homozygous variant (c.859G > C) in a Lebanese individual, which was also reported to be a protective marker against severe SMA [14, 15].

While the reported carrier frequency of SMA in the US, Australia, Europe, and the UK is 1:40–80, it has been reported to be much higher (∼1:20) in many parts of the Middle East and North Africa regions [16–21]. Based on a calculated average number of births per year for the 20-year period covered by this study using population (World Bank) and birth rate (UNICEF) data, we estimate a high annual incidence of ∼1 in 7,500 individuals with SMA in Lebanon. This is comparable to rates in Saudi Arabia (∼1 in 7000) [22], Egypt [17], and Libya (∼1 in 12500 for SMA1) [23]. Early treatment and intervention can improve clinical outcomes in SMA patients with the advent of new therapies [24]. Although Lebanon is far away from adopting SMA into neonatal screening programs, increased awareness about these disorders was noticed throughout the duration of this study. Additionally, the importance of pre-implantation and prenatal genetic screening in families with a history of SMA has become more highlighted in the general community.

Non-5q Spinal Muscular Atrophy

Other MNDs in this study included Amyotrophic Lateral Sclerosis (ALS), which has a reported prevalence and annual incidence of 3–5:100,000 and 1.5–2.7:100,000 individuals respectively [25]. In our study, six families were described with ALS1 and two families with FTDALS4 (Frontotemporal dementia and/or amyotrophic lateral sclerosis 4). Notably, a novel homozygous *TBK1* mutation (c.2079_2082del; p.Glu695Argfs*16) was identified in a 35 year old patient who presented with an abnormal gait and walking difficulty. Another 39 year old patient, heterozygous for the same variant presented with an abnormal gait, juvenile arthritis, dysphagia, dysarthria, and respiratory symptoms.

Table 2
List of hereditary NMDs with associated genes and variants identified in the patient cohort

NMD group	Disorder (Short name; MIM#)	Patients (#); Families (#)	Ages at Diagnosis (years or range in years with avgerage)†	Associated Gene or Genomic Region (MIM#)	Mutations identified (homozygous; heterozygous; *compound heterozygous*; hemizygous)†	References†
Motor Neuron Diseases	Amyotrophic Lateral Sclerosis 1 (ALS1; 105400)	8; 6	50-51	*SOD1* (147450)	c.352C > G p.Leu118Val	–
	Brown-Vialetto-Van Laere syndrome 2 (BVVL2; 614707)	6; 2	12–19 (avg. 16)	*SLC52A2* (607882)	**c.916G4A p.Gly306Arg**	[28, 29]
	Distal Spinal Muscular Atrophy 1 (DSMA1; 604320)	6; 3	< 1	*IGHMBP2* (600502)	**c.1540G > A p.Glu514Lys**	[14]
	Distal Spinal Muscular Atrophy (DSMA; Unknown subtype)	1; 1	–	–	–	
	Frontotemporal Dementia and/or Amyotrophic Lateral Sclerosis 4 (FTDALS4; 616439)	2; 2	35; 39	*TBK1* (604834)	**c.2079_2082del p.Glu695Argfs*16** c.2079_2082del p.Glu695Argfs*16	[8]
	Spinal Muscular Atrophy 1 (SMA1; 253300)	190; –	< 1	*SMN1*[a] (600354)	**exon 7 & 8 deletion**	–
	Spinal Muscular Atrophy 2 (SMA2; 253550)	7; 7	2–15 (avg. 6)		**exon 8 deletion**	
	Spinal Muscular Atrophy 3 (SMA3; 253550)	7; 4	7–33 (avg. 20)		*c.549del; p.Lys184Serfs* and exon 7 & 8 deletion*	
Hereditary Motor and Sensory Neuropathies	Charcot-Marie-Tooth Disease, Axonal, Type 2S (CMT2S; 616155)	4[b]; 2	32–53	*IGHMBP2* (600502)	**c.62G > T p.Arg21Ile**[b]	[8]
	Charcot-Marie-Tooth Disease, Axonal, Type 2T (CMT2T; 617017)	2; 2	–	*MME* (120520)	Under publication (Delague V. et al.)	
	Charcot-Marie-Tooth Disease, Demyelinating, Type 1A (CMT1A; 118220)	4; 4	6	*PMP22* (601097)	1.5-Mb duplication at 17p11.2	–
	Charcot-Marie-Tooth Disease, Type 4A (CMT4A; 214400)	5; 4	7–26 (avg. 13)	*GDAP1* (606598)	**c.668T > A p.Leu223***	[78]
	Charcot-Marie-Tooth Disease, Type 4B3 (CMT4B3; 615284)	2; 1	7; 12	*SBF1* (615284)	**c.1004T > C p.Lys335Pro**	[65]
	Charcot-Marie-Tooth Disease, Type 4C (CMT4C; 601596)	1; 1	–	*SH3TC2* (608206)	Unpublished data (Delague V. et al.)	
	Charcot-Marie-Tooth Disease, Demyelinating, Type 4F (CMT4F; 614895)	10; 3	0–19 (avg. 6)	*PRX* (605725)	**c.586C > T p.Arg196***	[67]
	Charcot-Marie-Tooth Disease, Demyelinating, Type 4H (CMT4H; 609311)	2; 2	17	*FGD4 (611104)*	**c.1698G > A p.Met566Ile**	[79]
	Charcot-Marie-Tooth Disease, Type 6C (CMT6C; 618511)	4; 2	–	*PDXK* (179020)	**c.628G > A p.Ala228Thr**	Under publication (Delague V. et al.)
	Charcot-Marie-Tooth Disease, Dominant Intermediate E (CMTDIE; 614455)	1; 1	–	*INF2* (610982)	c.312C > G p.Cys104Trp	–

	Charcot-Marie-Tooth Disease, Recessive Intermediate C (CMTRIC; 615376)	5; 4	7–15 (avg. 12)	*PLEKHG5* (611101)	**c.909C > A p.Tyr303*** **c.1452_1453del p.His485Profs*169**	[14] Under publication (Delague V. et al.)
	Charcot-Marie-Tooth Disease Type I (CMT1; Unknown subtype)	3; 2	13; 28; 37	–	–	
	Charcot-Marie-Tooth Disease Type I (CMT1; Associated with *MTMR4* mutation)	1; 1	0	*MTMR4* (603559)	Unpublished data (Delague V. et al.)	
	Charcot-Marie-Tooth Disease Type 2 (CMT2; Unknown subtype)	3; 3	23; 44	–	–	
	Charcot-Marie-Tooth Disease Type 2 (CMT2; Associated with *BAG3* mutation)	1; 1	25	*BAG3* (603883)	Under publication (Delague V. et al.)	
	Charcot-Marie-Tooth Disease, X-Linked Dominant, 1 (CMTX1; 302800)	2; 2	7; 34	*GJB1* (304040)	c.164_184dup p.Thr55_Asn61dup c.G139A p.Glu47Lys	[8]
	Charcot-Marie-Tooth Disease (CMT; Associated with *DNAJB2* mutation)	1; 1	–	*DNAJB2* (604139)	Under publication (Delague V. et al.)	
	Charcot-Marie-Tooth Disease (CMT; Associated with *VRK1* mutation)	1; 1	–	*VRK1* (602168)		
	Charcot-Marie-Tooth Disease (CMT; Unknown subtype)	14; 14	14–50	–	–	
Muscular Dystrophies	Becker Muscular Dystrophy (BMD; 300376)	13[c]; 5	6–30 (avg. 15)	*DMD* (300377)	See Fig. 2 for exonal deletions	–
	Bethlem Myopathy 1 (BTHLM1; 158810)	3; 2	12; 15; 43	*COL6A1* (120220)	c.928_930delAAG p.Lys310del c.868G > A p.Gly290Arg	–
	Duchenne Muscular Dystrophy (DMD; 310200)	73[c]; 62	0–41 (avg. 10)	*DMD* (300377)	See Fig. 2 for exonal deletions and exonal positions of truncating variants exon 53 duplication t(X;1)(p21;q23) c.94-1G > T c.4071 + 1G > A	[8]
	Emery-Dreifuss Muscular Dystrophy 4 (EDMD4; 612998)	1; 1	7	*SYNE1* (608441)	c.18445C > A p.Arg6149Ser	[14]
	Facioscapulohumeral Muscular Dystrophy 1 (FSHD1; 158900)	21[d]; 13	12–52 (avg. 27)	Chr.4q35 [*DUX4*] (606009)	4qA haplotype with 3–6 D4Z4 microsatellite repeats	–
	Limb Girdle Muscular Dystrophy Recessive 1 (LGMDR1; 253600)	11; 5	7–22 (avg. 19)	*CAPN3* (114240)	**c.257C > T p.Ser86Phe** *c.[257C > T];[956C > T]* *p.[Ser86Phe];[Pro319Leu]* *c.[956C > T;* *310-4_310-1delACAG]* *p.[Pro319Leu];[?]*	–

(Continued)

Table 2
List of hereditary NMDs with associated genes and variants identified in the patient cohort

NMD group	Disorder (Short name; MIM#)	Patients (#); Families (#)	Ages at Diagnosis (years or range in years with avgerage)†	Associated Gene or Genomic Region (MIM#)	Mutations identified (homozygous; heterozygous; *compound heterozygous*; hemizygous)†	References†
	Limb Girdle Muscular Dystrophy Recessive 2 (LGMDR2; 253601)	2; 2	21; 42	*DYSF* (603009)	**c.5438T > C p.Leu1813Pro**	–
	Limb Girdle Muscular Dystrophy Recessive 3 (LGMDR3; 608099)	6; 4	3–15 (avg. 11)	*SGCA* (600119)	*c.[157G > A];[c.574C > T] p.[Ala53Thr];[Arg192*]*	[8]
	Limb Girdle Muscular Dystrophy Recessive 4 (LGMDR4; 604286)	3; 3	10	*SGCB* (600900)	**c.-10_22dup p.Ala8fs**	–
	Limb Girdle Muscular Dystrophy Recessive 5 (LGMDR5; 253700)	6; 4	2–24 (avg. 13)	*SGCG* (608896)	**exon 7 deletion**	[8]
	Limb Girdle Muscular Dystrophy Recessive 6 (LGMDR6; 601287)	1; 1	8	*SGCD* (601411)	**c.784G > A p.Glu262Lys**	–
	Limb Girdle Muscular Dystrophy Recessive 10 (LGMDR10; 608807)	1; 1	6	*TTN* (188840)	**c.36040A > T; p.Lys12014***	[8]
	Limb Girdle Muscular Dystrophy Recessive 23 (LGMDR23; 618138)	8; 7	4–30 (avg. 11)	*LAMA2* (156225)	**c.8244 + 3_8244 + 6del** **c.8244 + 2dupT** *c.[3829C > T];[1300C > T] p.[Arg1277*];[Arg434*]*	[8]
	Limb Girdle Muscular Dystrophy Recessive (LGMDR; Unknown Subtype)	12; 7	10–45 (avg. 22)	–	–	
	Muscular Dystrophy-Dystroglycanopathy, Limb-Girdle, Type C, 1 (MDDGC1; 609308)	6; 3	4–22 (avg. 12)	*POMT1* (607423)	*c.[1858C > T];[221C > T] p.[Arg620*];[Ala74Val]* *c.[200C > A];[2005G > A] p.[Pro67Gln];[Ala669Thr]*	[8, 14]
	Muscular Dystrophy-Dystroglycanopathy, Limb-Girdle, Type C, 5 (MDDGC5; 607155)	3; 3	1; 6; 15	*FKRP* (606596)	**c.823C > T p.Arg275Cys**	[8]
Congenital Muscular Dystrophies	Muscular Dystrophy-Dystroglycanopathy, Congenital with Impaired Intellectual Development, Type B, 6 (MDDGB6; 608840)	2; 1	4; 8	*LARGE1* (603590)	**Intron 10 42.9-KB insertion/4.1-KB deletion**	[80]
	Muscular Dystrophy, Congenital, LMNA-Related (MDCL; 613205)	1; 1	6	*LMNA* (150330)	**c.1867A > G p.Thr623Ala**	[14]

	Muscular Dystrophy, Congenital (CMD; Unknown subtype)	4; 4	8	–	–	
	Rigid Spine Muscular Dystrophy 1 (RSMD1; 602771)	2; 2	14	*SELENON* (606210)	**c.1405C > T p.Arg469Trp**	–
	Ullrich Congenital Muscular Dystrophy 1 (UCMD1; 254090)	6[e]; 3	0–3	*COL6A1* (120220)	*COL6A1*: **c.2923G > C p.Gly975Arg**	[14]
				COL6A2 (120240)	*COL6A2*: **c.2611G > A p.Asp871Asn**[e]	
				COL6A3 (120250)	*COL6A3:* **c.8136del p.Arg2713Glyfs*3**	
Congenital Myopathies	Central Core Disease of Muscle (CCD; 117000)	1; 1	25	*RYR1* (180901)	c.8758C > T p.Arg2920*	[8]
	Myopathy, Centronuclear, X-Linked (CNMX; 310400)	2; 2	0	*MTM1* (300415)	c.969dupA p.Val324Serfs*8 c.1190A > G; p.Tyr397Cys	–
	Nemaline Myopathy 2 (NEM2; 256030)	1; 1	5	*NEB* (161650)	**c.5452-1G > A**	–
	Congenital Myopathy (CM; Unknown subtype)	3; 3	1; 7; 9	–	–	
Metabolic Myopathies	Glycogen Storage Disease II (GSD2; 232300)	2; 2	7	*GAA* (606800)	**c.266G > A p.Arg89His** **c.1927G > A p.Gly643Arg**	[8]
	Neurodegeneration with Brain Iron Accumulation 2A (NBIA2A; 256600)	3; 3	3; 4; 5	*PLA2G6* (603604)	**c.2257G > T p.Val753Phe** **c.2370T > G p.Tyr790***	[8]
Other Myopathies	Tubular Aggregate Myopathy 1 (TAM1; 160565)	1; 1	12	*STIM1* (605921)	c.57G > C p.Gln19His	–
Myotonic Syndromes	Myotonic Dystrophy 1 (MD1; 160900)	3; 2	30; 33; 60	*DMPK* (605377)	3' CTG triplet repeat expansion (> 400 repeats)	–
Other Neuromuscular Disorders	Mitochondrial DNA depletion syndrome (MTDPS; Unknown subtype)	2; 1	15	–	–	
Congenital Myasthenic Syndromes	Myasthenic Syndrome, Congenital, 25, Presynaptic (CMS25; 618323)	1; 1	14	*VAMP1* (185880)	**c.97C > T p.Arg33***	[8]
Unspecified	Mitochondrial Myopathy (MM; Associated with *MTRNR2* mutation)	1; 1	11	*MTRNR2* (561010)	m.2119T > C[f]	–
	Mitochondrial Trifunctional Protein Deficiency (MTPD; 609015)	1; 1	28	*HADHA* (600890)	**c.703C > T p.Arg235Trp**[g]	–
	Unclassified NMDs	7; 6	3–52 (avg. 27)	–	–	

[†]Patient data on age, variants, and relevant published studies are available only for independent subsets of patients. **a**: Spinal Muscular Atrophy patients presented with varying copy numbers of *SMN2* **b**: One patient presented with uncharacteristic syndromic features including delayed motor language development, intellectual disability, microcephaly, and dysmorphic facial features; **c**: All female patients were manifesting carriers of *DMD* mutations; **d**: One patient presented with typical FSHD1 symptoms and a negative molecular test; **e**: One patient presented with uncharacteristic cognitive features in the form of psychomotor delay; **f**: Heteroplasmic *MTRNR2* variant in a patient exhibiting features of mitochondrial myopathy; **g**: Variant position is based on the precursor from of the mitochondrial protein.

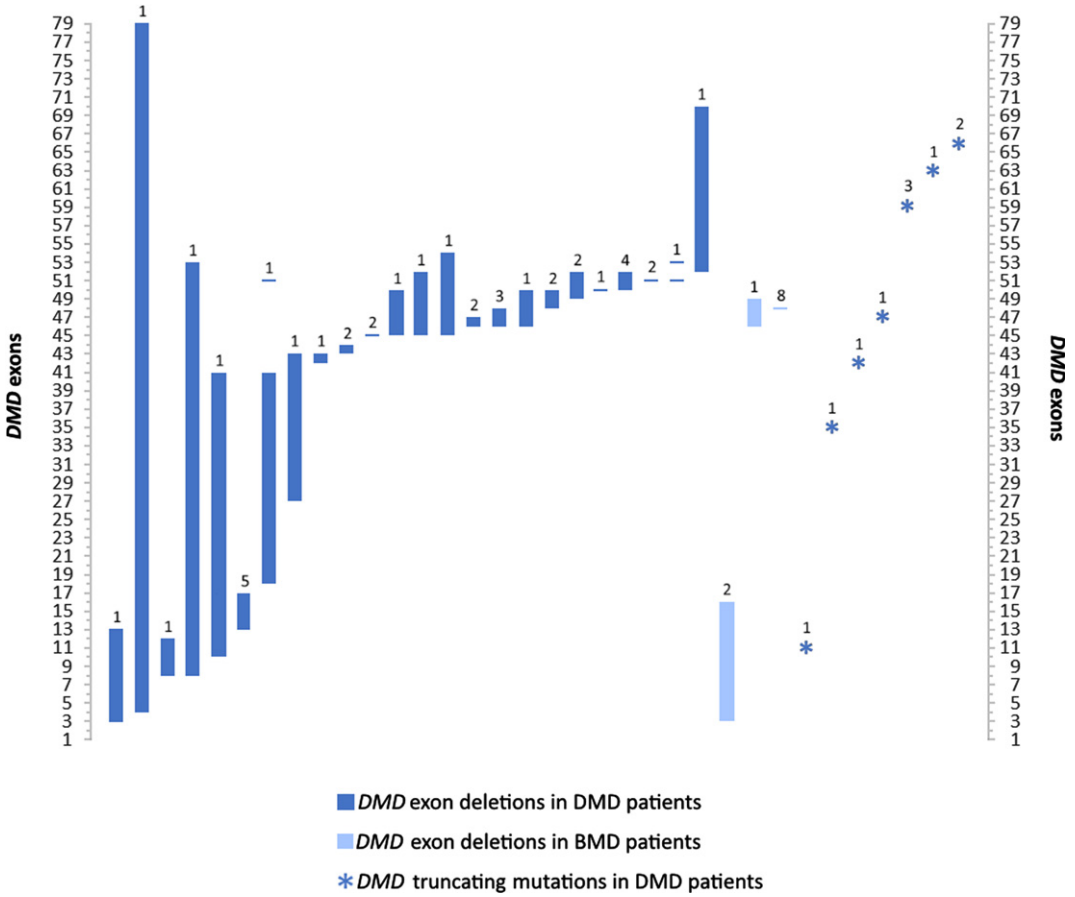

Fig. 2. Exonal dystrophin (*DMD*) deletion and truncating mutation patterns in patients with DMD and BMD in this study. Values over each bar represent the number of patients for each of the 36 variants. Remaining *DMD* mutations including duplication, translocation, and splice site variants are described in Table 2.

Literature from Lebanon is lacking, however the country has joined other regions in clinical trials for ALS modifying treatments [26]. A recent clinical study of ALS, which described 140 patients from Lebanon, revealed 113 patients with ALS and a lower mean age of onset relative to global reports, with the remaining patients presenting with six subtypes of atypical ALS [27].

Other non-5q MND disorders identified in this cohort included Brown-Vialetto-Van Laere syndrome 2 (BVVLS2), diagnosed in six patients from two families in our cohort [28]. All patients exhibited the same homozygous mutation (c.916G > A) in the riboflavin transporter gene *SLC52A2*. Interestingly, six other Lebanese patients from three families were described with BVVLS2 attributed to the same mutation [29]; haplotype and functional analysis revealed a shared ancestral allele highlighting the c.916G > A as a founder mutation in the Lebanese population. A subsequent study identified the same mutation in another large consanguineous Lebanese family [30].

Distal Spinal Muscular Atrophy 1 (DSMA1), another non-5q SMA associated with mutations in the *IGHMBP2* gene, was diagnosed in six patients from three families in this cohort. In the literature, a Lebanese family was among the first three families presenting with SMA features and normal *SMN1*, with haplotype analysis revealing linkage at 11q13-q21 [31]. Subsequent studies have identified homozygous mutations in *IGHMBP2* in the same [32]. Interestingly, a homozygous mutation in *IGHMBP2* (c.449 + 1G > T) was more recently associated with CMT2S in two Lebanese siblings [33].

Duchenne and Becker Muscular Dystrophies

MDs constituted the second most prevalent group of disorders among patients in this study, with

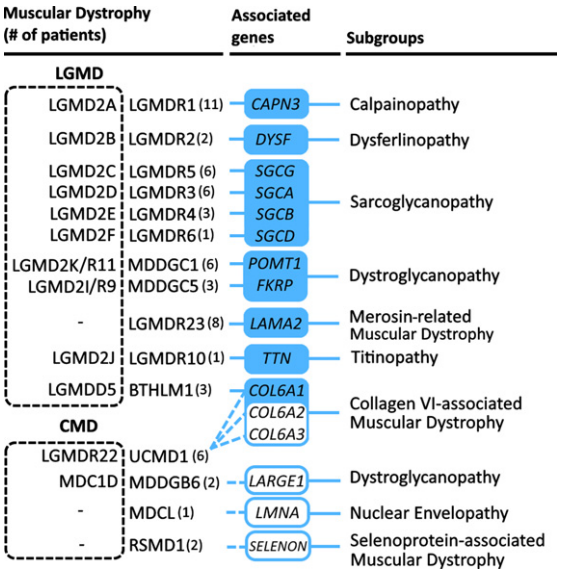

Fig. 3. Distribution of Limb Girdle Muscular Dystrophies and Congenital Muscular Dystrophies in this cohort. MD disorders listed are based on the current revised classification of LGMD [81]; known aliases are included in a dotted box to the left of the official OMIM disease name. Disease subgroups are based on associated genes, derived from Liewluck and Milone [82].

a predominantly male cohort of DMD and BMD patients (78 males and 8 females; 17% of patients). DMD is an X-linked progressive muscle wasting disorder with a global prevalence of 1 in 4000–6000 live male births [34]. The average age of confirmatory diagnosis for DMD in our cohort was 10 years, with half of the patients diagnosed at the age of eight or below. This is above the general global average and median diagnosis age in Western countries [35] and in the Middle East [5]. The average age of diagnosis for BMD was also higher than the general mean age of 12 [35]. *DMD* molecular data in our study was available for 61 patients, encompassing single and multiple exon deletions (71.1%), truncating mutations (18.4%), one duplication (exon 53), two splice site mutations, as well as a balanced translocation (Table 2; Fig. 2). Multi-exon deletions in our study encompassed most of the dystrophin gene, with the most common regions (exons 45–52 and 3–17) falling within previously characterized hotspot regions identified in large databases [36]. Interestingly, despite the rarity of DMD diagnoses in females, we report a manifesting female carrier with an 8–53 exon deletion. BMD-associated molecular data involved multi-exon deletions (exons 3–16 and 46–49) identified in three patients from two unrelated families (Fig. 2). Eight patients, including four males and four female carriers, harboring a deletion of exon 48 exhibited a very mild BMD phenotype, consistent with previous reports [37].

Comparable to our molecular findings, a recent study described a male cohort of 52 unrelated Lebanese DMD patients with a relatively lower average age of diagnosis (7 years) [38]. They described similar hotspot regions of multi-exon and single-exon deletions (in addition to exons 19, 52, and 53).

With Lebanese patients representing the typical DMD cohort, there is potential benefit from recent disease-modifying treatments, especially those targeting exon skipping [39]. Truncating point variants were more prominent than expected in our cohort relative to the previous Lebanese study [38]. These mutations are also targets of approved drug therapies [40]. An early diagnosis of DMD however is better and sometimes necessary to ensure drug efficacy. The adoption of promising therapies is contingent on the systematic overhaul of the DMD diagnostic process in Lebanon. To date, only a handful of male DMD patients have had access to these costly therapies.

Facioscapulohumeral Muscular Dystrophy

Facioscapulohumeral Muscular Dystrophy Type 1 (FSHD1) was the third most diagnosed MD and NMD in this study (4.2% of patients). Globally, FSHD has an estimated prevalence range of 1 in 8000 to 20000 individuals [41]. We report on 21 patients, 17 of whom had a confirmed genetic diagnosis with distal subtelomeric contractions of polymorphic repeats in chromosome 4. All patients exhibited the FSHD1 4qA allele with D4Z4 microsatellite deletions resulting in a range of 3 to 6 repeats. One of the patients in our cohort exhibited typical FSHD features, however no structural anomalies were detected on chromosome 4, suggesting that he may have FSHD2 or a mimicking muscle disorder.

Published data on FSHD in Lebanon is scarce. In a recent in-depth genetic analysis study on a large cohort of FSHD patients, a Lebanese family with five affected individuals was discussed, four of whom were diagnosed with distal 4qA repeat deletions [42]. Literature on FSHD in the Middle East is limited to a few studies from Iran, Egypt and Libya reporting relatively low numbers of patients in NMD cohorts [43–46]. The molecular diagnosis of FSHD1 remains technically challenging even with NGS, thus explaining in part the low rate of detection of this condition in Middle Eastern populations.

Limb Girdle Muscular Dystrophy

LGMD affects approximately 1 in 61,000 (35,000–106,000) individuals globally [47], with 34 subtypes currently associated with 34 genes (OMIM 2020). 11 subtypes of LGMD were identified in this study. LGMDR1 was the most frequently diagnosed subtype, associated with homozygous mutations in *CAPN3*. Another notable subtype from our study was LGMDR5, identified in four families, exhibited a recurring exon 7 deletion in *SGCG*. Published literature on hereditary LGMD in Lebanon is limited, mainly involving familial case reports of LGMDR1 with variants described in this study (c.257C > T and c.956C > T). Additionally, c.717ΔT and c.2242C > G variants were reported in Lebanese families presenting with mild and severe features respectively [48, 49]. Novel polymorphisms in *LAMA2* and *SGCG*, potentially influencing gene expression profiles, were identified in 35 healthy Lebanese individuals among other ethnicities [50].

The cohort described in our study is reflective of global reports, with LGMDR1 being the most frequently observed subtype [51, 52]. In the Middle East, a study on 60 Iranian families with LGMD showed a broadly similar pattern of LGMD relative to our cohort, involving – in order of frequency – LGMDR1, LGMDR4, followed by other sarcoglycanopathies and dysferlinopathy [53]. Clinics throughout the MENA region including Algeria, Tunisia, and Egypt have notably reported other trends [54]. More recently, a review of muscle biopsies from NMD patients in Saudi Arabia revealed mainly dysferlinopathies, sarcoglycanopathies, and congenital MDs including merosin-deficient CMD and Walker Warburg Syndrome [6].

Congenital Muscular Dystrophy

CMDs involve heterogeneous autosomally inherited disorders with severe features (relative to LGMD) typically manifesting at birth or infancy. Limited epidemiological data suggest a low prevalence of 0.68–2.5:100,000 [55]. We report on four forms of CMD including Rigid Spine MD (associated with *SEPN1* or *SELENON*), *LMNA*-associated CMD, Ullrich Congenital Muscular Dystrophy 1 (UCMD1; associated with *COL6*), and *LARGE1*-related CMD. In addition, other dystroglycanopathies considered to be CMDs, such as *FKRP* and *POMT1* related MDs, were included among the LGMD group in this cohort due to prominent features of slowly progressive or late-onset limb-girdle muscle weakness (Fig. 3). Notably, a novel homozygous variant in *COL6A3* (c.8136del) was identified in association with UCMD1; another variant in *COL6A1*, which we report for the first time at the homozygous state (c.2923G > C), was also associated with UCMD1. Literature on CMD in Lebanon is scarce involving multiple families with distinct genotype-phenotype correlations mainly involving dystroglycanopathies. Variants in *POMT1* were reported in Lebanese patients with MDDGB1 (MIM# 613155) and Walker-Warburg Syndrome [56, 57]. A Lebanese family was reported with MDDGA12 (MIM# 615249), associated with homozygous mutations in the *POMK* gene [58]. Additionally, heterozygous *LMNA* variants were associated with cardiomyopathy and with Emery-Dreifuss Muscular Dystrophy 2 [59, 60].

Charcot-Marie-Tooth disease

The remaining major group of NMDs identified in our study involved CMT disease. CMT is the most common hereditary neuropathy estimated to affect ∼1 in 1,200 to 10,000 individuals [61]; it involves a large group of genetically and clinically heterogeneous HMSN disorders. Before the emergence of NGS, molecular diagnostic rates for CMT were relatively poor. The adoption of whole exome sequencing has rapidly increased gene discovery, with multigene-panels set to cover future diagnoses [3, 62]. There are currently 75 CMT subtypes associated with 58 genes (OMIM 2020). We report 12 subtypes including recessive demyelinating forms of CMT (CMT4A, CMT4B3, CMT4F, CMT4H, CMT4C, and CMT6C); followed by axonal (CMT2S and CMT2T); recessive and dominant intermediate forms (CMTRIC and CMTDIE); dominant demyelinating CMT1A; as well as X-linked axonal and demyelinating form - CMTX1. A recently characterized subtype, CMT6C, was identified in two families from our cohort, associated with homozygous mutations in *PDXK* (under publication; Dr. Delague). Additionally, CMT patients in this study were observed with mutations in *BAG3*, *DNAJB2*, *MTMR4,* and *VRK1* (under publication; Dr. Delague); CMT genotype-phenotype correlations with variants in *BAG3* and *DNAJB2* genes were recently described [63, 64]. Our cohort also included CMT4B3 patients presenting with unique syndromic features. These patients – from a single family – lacked sensory symptoms and exhibited a fork and bracket MRI sign related to brainstem abnormalities [65]. A recent

study in the Lebanese population involving 116 sporadic and familial cases with CMT revealed CMT1A (associated with a commonly described duplication variant in the *PMP22* gene) to be the most prevalent type, followed by CMT4H and CMT4F [66]. Loci associated with the latter two subtypes (*FGD4* and *PRX* respectively) were first identified in Lebanese patients [67, 68]. Novel CMT genes continue to be identified in the Lebanese population, including the second reported study on *MCM3AP*-associated CMT in a Lebanese family [69].

Comparison with other NMD reports

Global reports have shown markedly different spectrums of NMDs. A study on hereditary NMDs in North England identified 31 disorders in 1,100 patients, most commonly involving myotonic dystrophy followed by dystrophinopathies and FSHD. In contrast to our cohort, the study reported fewer cases of SMA [51]. A review of NMD registry data in the Netherlands revealed ALS and myotonic dystrophy to be most common, whereas SMA was diagnosed as frequently as CMT in their study [70].

Clinically focused community surveys and hospital studies have offered brief insight into the spectrum of NMDs in the Middle East, including studies in Egypt, Saudi Arabia, and Libya, with sparse reporting on a multitude of NMD subtypes [22, 44–46, 71]. While large, in-depth, and locus-specific mutational studies are gaining prominence in the region [72, 73], extensive studies are needed to address the broad spectrum of genetic NMDs that are rapidly being identified and incorporated into globally recognized patient registries. A few reports have revealed the prevalence and profiles of NMDs in the region: in a 43-year study with 823 NMD patients from Cairo, DMD and autosomal MDs were reported as most frequent followed by lower rates of SMA and congenital myopathies. CMT disorders were less frequent, along with myotonic dystrophy identified in relatively few patients [74]. In Kuwait, muscular dystrophies are the most frequently diagnosed NMDs; this is followed by SMA and less frequently by myotonic syndromes, congenital and mitochondrial myopathies, CMT, and congenital myasthenic syndrome (unpublished data; personal communication Dr. Laila Bastaki). Another study analyzing 337 adult Iranian patients with NMDs revealed a similar constellation to our cohort of muscular dystrophies and mild proximal SMA [43]. Most recently, a 30-year overview of muscle biopsy analysis from 461 NMD patients in Saudi Arabia delineated multiple forms of MDs, as well as congenital and metabolic myopathies, with the study calling for a national NMD registry [6].

CONCLUSION

The efforts throughout the 20 years have successfully identified and helped NMD patients in Lebanon primarily because of the multidisciplinary approach that our team and collaborators have followed. In addition, our partner patient support organizations have been a tremendous help in supporting patients and their families, advocating for patients' social rights and lobbying for more support and treatment coverage by the government. With significant efforts made towards streamlining and standardizing the collection of patient data over the last two decades [75], the retrospective study presented here could form the backbone of a future Lebanese neuromuscular registry. This is a contribution to a better understanding of the epidemiology of neuromuscular diseases in the region, which is necessary with many novel NMD therapies in the pipeline.

Limitations

The cohort described here consists predominantly of genetic NMD cases, mostly due to recruitment bias, as our team rarely receives referrals of patients with non-genetic disorders. With the exception of SMA, for which we have been the main diagnostic centre in Lebanon to the best of our knowledge, we were unable to determine an accurate prevalence of NMDs in general or that of particular disorders within the Lebanese population. This study also overlooks patients diagnosed by other genetic centres. Although we do not report on parental consanguinity, inherited NMDs in this study constituted a majority of autosomal recessive disorders; this may be attributed to high rates of consanguinity, estimated to be an average between 28.4% and 35.5% in Lebanon [76, 77]. The limited access to genetic testing, mainly due to the associated costs given the lack of coverage by insurance companies and the government, is the reason why some of the patients described here received just a clinical diagnosis without a molecular confirmation. Additionally, seven patients exhibited presentations compatible with primary NMDs, however a specific diagnosis could not be reached. With the advent of NGS and its gradual affordability, the number of such patients has slowly decreased during

the period of this study. It is important to note that even with genetic sequencing, some NMDs such as mitochondrial disorders remain challenging to diagnose due to difficulties in variants' interpretation or due to phenotypic complexity. The literature review above does not cover uncommonly described NMDs in Lebanon; more details can be found on the Catalogue of Transmission Genetics in Arabs database: https://www.cags.org.ae/en/ctga-overview.

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AUTHOR CONTRIBUTIONS

All authors have made substantial contributions to conception and design and for important intellectual content. SB, AD, EC, SEH, JAU, GL, AM have made substantial contributions in acquisition of data, analysis and interpretation of data. SB, AD, CM, EC, SEH, VD, AU, AM, have been involved in drafting the manuscript and revising it critically. All authors have given final approval of the version to be published.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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Annex 1: Neuromuscular Diseases panel genes

AAAS, AARS1, ABCC9, ABHD12, ABHD5, ACAD9, ACADL, ACADM, ACADS, ACADVL, ACTA1, ACTN2, ADCY6, ADGRG6, ADSS1, AGL, AGRN, AIFM1, ALDOA, ALG14, ALG2, ALG3, AMPD1, ANO5, ARHGEF10, ASAH1, ASCC1, ATL1, ATL3, ATP1A1, ATP1A2, ATP2A1, ATP7A, B3GALNT2, B4GAT1, BAG3, BICD2, BIN1, BSCL2, BVES, C12orf65, C1orf194, CACNA1E, CACNA1S, CAPN3, CASQ1, CAV3, CAVIN1, CCDC78, CCT5, CFL2, CHAT, CHCHD10, CHKB, CHRNA1, CHRNB1, CHRND, CHRNE, CHRNG, CHST14, CLCN1, CNTN1, CNTNAP1, COA7, COL12A1, COL13A1, COL6A1, COL6A2, COL6A3, COLQ, COX6A1, COX6A2, CPT2, CRYAB, CTDP1, DAG1, DCAF8, DCTN1, DCTN2, DES, DGAT2, DHTKD1, DMD, DNA2, DNAJB2, DNAJB5, DNAJB6, DNM2, DNMT1, DOK7, DPAGT1, DPM1, DPM2, DPM3, DRP2, DST, DYNC1H1, DYSF, ECEL1, EGR2, ELP1, EMD, EMILIN1, ENO3, ERBB3, ERGIC1, ETFA, ETFB, ETFDH, EXOSC3, EXOSC8, FBLN5, FBN2, FBXO38, FGD4, FHL1, FIG4, FKBP10, FKBP14, FKRP, FKTN, FLAD1, FLNB, FLNC, FXR1, G6PC, GAA, GAN, GARS1, GBE1, GDAP1, GFPT1, GJB1, GJB3, GLA, GLDN, GLE1, GMPPB, GNB4, GNE, GOLGA2, GSN, GYG1, GYS1, HACD1, HADH, HADHA, HADHB, HARS1, HEXA, HINT1, HK1, HNRNPDL, HOXD10, HSPB1, HSPB3, HSPB8, IGHMBP2, INF2, INPP5K, ISCU, ISPD, ITGA7, KARS1, KBTBD13, KCNE3, KCNJ2, KCNJ5, KIF1A, KIF1B, KIF5A, KLHL40, KLHL41, KLHL9, KY, LAMA2, LAMA5, LAMP2, LARGE1, LAS1L, LDB3, LDHA, LGI4, LIMS2, LITAF, LMNA, LMOD3, LPIN1, LRP12, LRP4, LRSAM1, MAP3K20, MARS1, MATR3, MB, MCM3AP, MED25, MEGF10, MET, MFN2, MICU1, MME, MORC2, MPV17, MPZ, MSTO1, MTM1, MTMR14, MTMR2, MUSK, MYBPC1, MYH14, MYH2, MYH3, MYH7, MYH8, MYL1, MYO9A, MYOD1, MYOF, MYOT, MYPN, NAGLU, NALCN, NDRG1, NEB, NEFH, NEFL, NEK9, NGF, NPL, NTRK1, NUP88, OPA1, ORAI1, PABPN1, PAX7, PDHA1, PDK3, PDXK, PFKM, PGAM2, PGK1, PGM1, PHKA1, PHKB, PHKG2, PIEZO2, PIP5K1C, PLEC, PLEKHG5, PLOD2, PMP2, PMP22, PNPLA2, POGLUT1, POLG, POLG2, POMGNT1, POMGNT2, POMK, POMT1, POMT2, POPDC3, PPP3CA, PRDM12, PREPL, PRKAG2, PRPS1, PRX, PUS1, PYGM, PYROXD1, RAB7A, RAPSN, RBCK1, RBM7, REEP1, RETREG1, RRM2B, RXYLT1, RYR1, RYR3, SBF1, SBF2, SCARF2, SCN10A, SCN11A, SCN4A, SCN9A, SCO2, SCYL2, SELENON, SEPTIN9, SETX, SGCA, SGCB, SGCD, SGCG, SGPL1, SH3TC2, SIGMAR1, SIL1, SLC12A6, SLC16A1, SLC18A3, SLC22A5, SLC25A1, SLC25A20, SLC25A21, SLC25A42, SLC25A46, SLC5A7, SLC9A3R1, SMCHD1, SMPD4, SNAP25, SOD1, SORD, SOX10, SPEG, SPG11, SPTAN1, SPTBN4, SPTLC1, SPTLC2, STAC3, STIM1, SUCLA2, SURF1, SYNE1, SYNE2, SYT2, TAZ, TCAP, TECPR2, TFG, TIA1, TIMM22, TK2, TMEM43, TMEM65, TNNI2, TNNT1, TNNT3, TNPO3, TOR1A, TOR1AIP1, TPM2, TPM3, TRAPPC11, TRIM2, TRIM32, TRIM54, TRIM63, TRIP4, TRPA1, TRPV4, TTN, TTR, TWNK, TYMP, UBA1, UBA5, UNC50, VAMP1, VAPB, VCP, VIPAS39, VMA21, VPS33B, VRK1, WARS1, WNK1, YARS1, YARS2, ZC4H2, ZFHX2.