Inherited Myopathies and Congenital Myasthenic Syndromes in a Cohort from Different Regions of Turkey

by

Esmer Zeynep Duru Badakal

A Dissertation Submitted to the
Graduate School of Sciences and Engineering
in Partial Fulfillment of the Requirements for
the Degree of

Master of Science

in

Molecular Biology and Genetics

KOÇ
ÜNİVERSİTESİ

April 16, 2024

Inherited Myopathies and Congenital Myasthenic Syndromes in a Cohort from Different Regions of Turkey

Koç University

Graduate School of Sciences and Engineering

This is to certify that I have examined this copy of a master's thesis by

Esmer Zeynep Duru BADAKAL

and have found that it is complete and satisfactory in all respects,
and that any and all revisions required by the final
examining committee have been made.

Committee Members:

_____

Prof. Dr. A. Nazlı Başak (Advisor)

_____

Prof. Dr. Sibel Ertan

_____

Prof. Dr. Hilmi Uysal

Date: _____

to NDAL family…

ABSTRACT

Inherited Myopathies and Congenital Myasthenic Syndromes in a Cohort from Different Regions of Turkey

Esmer Zeynep Duru BADAKAL

Master of Science in Molecular Biology and Genetics

April 16, 2024

Myopathies include a clinically, genetically, and mechanistically heterogeneous group of diseases characterized by muscle weakness and fatigue. Congenital myasthenic syndromes (CMS) are characterized by symptoms such as easy fatigue, muscle weakness, and ptosis. Definitive diagnosis of the disease is often difficult, but next-generation sequencing technologies, especially whole exome sequencing (WES), have contributed to their diagnostic process. This thesis involves the use of WES, bioinformatic analysis, and wet-lab to understand the genetic basis of Turkish Myopathy and CMS patients. In total, 23 cases were solved, including eight novel variants, in 30 index patients. The number of unsolved cases was seven, including three sporadic cases.

With this thesis, in the cohort under study, the genetic causes of myopathies and congenital myasthenia syndrome have been revealed and differential diagnoses guided. WES is the current gold standard for genetic investigation of inherited disorders, but it has also limitations and challenges. The diagnosis rate is positively correlated with gene-disease associations, and it is suggested that the more associations, the higher the diagnostic yield. The discovery of new causal genes through large cohort studies is also important for this reason. This thesis is expected to contribute to a better understanding of the clinical and genetic picture of myopathy and CMS patients in Turkey, and thus to a better comprehension of the complicated phenotype-genotype correlations of muscle disorders. Such studies will help to shape a hopeful future in the genetic era.

ÖZETÇE

Türkiye'nin Farklı Bölgelerinden Bir Kohort Çalışması: Kalıtsal Miyopatiler ve Konjenital Miyastenik Sendrom'un Moleküler Analizi

Esmer Zeynep Duru BADAKAL

Moleküler Biyoloji ve Genetik, Yüksek Lisans

16 Nisan 2024

Miyopatiler, kas güçsüzlüğü ve yorgunlukla karakterize olan, klinik, genetik ve mekanik özelliklerine göre gruplandırılabilen heterojen bir hastalık grubudur. Konjenital miyastenik sendromlar (CMS) ise kolay yorulma, kas güçsüzlüğü ve pitoz gibi belirtilerle karakterizedir. Bu hastalıkların kesin tanısı genellikle zordur, ancak yeni nesil dizileme teknolojileri, özellikle tüm ekzom dizileme (WES), bu tanı sürecine katkıda bulunmuştur. Bu tez Türk Miyopati ve CMS hastalarında genetik bulguları anlamak amacıyla WES, biyoinformatik analiz ve wet-lab kullanımını içermektedir. Toplamda 31 ailede, sekiz yeni varyant tanımlanarak, 23 olgu çözülmüştür. Çözülemeyen vaka sayısı ise, üç sporadik hasta da dahil olmak üzere yedidir. Bu tez ile Miyopatilerin ve Konjenital Miyasteni Sendromu'nun genetik nedenleri ortaya çıkarılarak ayırıcı tanılara yol gösterilmiştir.

WES, günümüzde kalıtsal hastalıkların genetik araştırmasında kullanılan altın standarttır, ancak bazı sınırlamalar ve zorluklar içerir. Tanı oranı gen-hastalık ilişkisiyle pozitif koreledir' gen-hastalık ilişki sayısı arttıkça, tanıdaki verim de artacaktır. Büyük kohort çalışmaları ile yeni nedensel genlerin keşfedilmesi bu sebepten dolayı da önemlidir. Bu tezin Türkiye'de miyopati ve CMS hastalarının klinik ve genetik tablosunu daha iyi anlamaya katkı sağlaması ve karmaşık fenotip-genotip ilişkilerine ışık tutması beklenmektedir. Umutlu bir gelecek için, bu tür genetik-tabanlı çalışmaların önemi büyüktür.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my advisor Prof. A. Nazlı Başak. This transformative journey commenced with an internship in 2019, and I continued to learn during my master's study. Prof. Başak's valuable support, guidance, encouragement, and constructive criticism shaped my path.

Special thanks to Prof. Dr. Sibel Ertan and Prof. Dr. Hilmi Uysal for dedicating their time to evaluate my thesis. I am also appreciative of Prof. Dr. Hilmi Uysal for contributing to the majority of patients in my thesis cohort.

I extend my gratitude to Suna and İnan Kıraç Foundation and Koç University Research Center for Translational Medicine, KUTTAM, for their unwavering support. Heartfelt thanks to all NDAL members for fostering a productive working environment and genuine friendships. My appreciation goes to Tuğçe Gül and Ayça Şahin for their enduring friendship and outstanding collaboration as research partners. Special thanks to my friend Şeyma Tekgül for her assistance during my master's program. Being part of the NDAL team has been a life-changing experience.

I want to express my deepest thanks to my chosen family member Hazal Kıranbağlı for always being like a sister to me, and to another chosen family member, Ezgi Zümre Taşkın, for offering emotional and even financial support in all circumstances. I would also like to thank Necip for his endless support and unconditional love.

I express my gratitude to my family—Kaan Badakal, Berna Badakal, and Mercan Badakal—for teaching me and providing the space to pursue what I believe is right. They taught me the importance of being a comma in life, not a period, recognizing that the truth can evolve over time. I also want to thank myself for my stance in life. Finally, I pray for God's mercy upon my dear ALS patient, photographer, and pharmacist uncle H. Kürşat Badakal, who served as the emotional motivation for embarking on this journey. I send him a lot of love.

As I write this letter in the morning on my way to the lab, the road ahead feels open, and I eagerly anticipate walking it alongside countless wonderful souls.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

AAA	ATPase family associated with various cellular activities domain
ACMG	American College of Medical Genetics and Genomics
AD	Autosomal Dominant
ADCAs	Autosomal Dominant Cerebellar Ataxias
ADCK3	AARF Domain-Containing Kinase 3
ADDH	Ataxia, Delayed Dentition, and Hypomyelination
AFG3L2	ATPase Family Gene 3-like 2
AFP	Alpha-Fetoprotein
ALS	Amyotrophic Lateral Sclerosis
ANO10	Anoctamin 10
AO	Age of Onset
AOA	Ataxias with Oculomotor Apraxia
APTX	Aprataxin
AR	Autosomal Recessive
ARCAs	Autosomal Recessive Cerebellar Ataxias
ARSACS	Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay
AT	Ataxia Telangiectasia
ATCAY	Caytaxin
ATLD	AT like Disorder
ATM	Ataxia-Telangiectasia Mutated Gene
ATN1	Atrophin 1
ATP13A2	ATPase 13A2
ATX	Ataxia
ATXN	Ataxin
AVED	Ataxia with Isolated Vitamin E Deficiency
B	Benign
BEAN1	Brain-Expressed, Associated with Nedd4, 1
BSCL2	Seipin
C19orf12	Chromosome 19 Open Reading Frame 12
CACNA1A	Calcium Channel, Voltage-Dependent, P/Q Type, Alpha-1a Subunit
CADD	Combined Annotation-Dependent Depletion
CANVAS	Cerebellar Ataxia, Neuropathy, and Vestibular Areflexia Syndrome

CAPN1	Calpain 1
CCDC88C	Coiled-Coil Domain-Containing Protein 88C
CH	Cerebellar Hypoplasia
CHIP-seq	Chromatin Immunoprecipitation Sequencing
Chr	Chromosome
CMT	Charcot-Marie-Tooth
CNS	Central Nervous System
CNVs	Copy Number Variations
Conc	Concentration
COX20	Cytochrome c Oxidase Assembly Factor COX20
CTX	Cerebrotendinous Xanthomatosis
CYP27A1	Cytochrome P450, Subfamily XXVIIA
D	Damaging
DNA	Deoxyribonucleic Acid
DYS	Dystonia
EA	Episodic Ataxia
ExAC	Exome Aggregation Consortium
F	Female
Fam	Family
FGF14	Fibroblast Growth Factor 14
FRDA	Friedreich's Ataxia
FXN	Frataxin
GAN	Gigaxonin
GAN1	Giant Axonal Neuropathy-1
GERP	Genomic Evolutionary Rate Profiling
gnomAD	The Genome Aggregation Database
HEPN	Higher eukaryotes and prokaryotes nucleotide-binding domain
het	Heterozygous
HGP	Human Genome Project
hom	Homozygous
HSP	Hereditary Spastic Paraplegias
IGV	Integrative Genomics Viewer

IMNEPD1	Neurologic, Endocrine, and Pancreatic Disease, Multisystem, Infantile-Onset 1
ITPR1	Inositol 1,4,5-Triphosphate Receptor, Type 1
KCNC3	Potassium Channel, Voltage-Gated, Shaw-Related Subfamily, Member 3
KCND3	Potassium Voltage-Gated Channel, Shal-Related Subfamily, Member 3
LP	Likely Pathogenic
M	Male
MC4DN11	Mitochondrial Complex IV Deficiency, Nuclear Type 11
MGCA3	3-Methylglutaconic Aciduria, Type III
MRE11	MRE11 Homolog, Double-Strand Break Repair Nuclease
MRI	Magnetic Resonance Imaging
n	Novel
n.a	Not Available
NADGP	Neurodegeneration with Ataxia, Dystonia, and Gaze Palsy, Childhood-Onset
NBIA4	Neurodegeneration with Brain Iron Accumulation 4
NDs	Neurodegenerative Diseases
NGS	Next Generation Sequencing
NIPA1	Nipa Magnesium Transporter 1
NOP56	NOP56 Ribonuclear Protein
OMA	Oculomotor Apraxia
OPA3	Outer Mitochondrial Membrane Lipid Metabolism Regulator OPA3
P	Patient
Pth	Pathogenic
PCH2D	Pontocerebellar Hypoplasia Type 2D
PCR	Polymerase Chain Reaction
PD	Parkinson’s Disease
PNP	Polyneuropathy
PNS	Peripheral Nervous System
POLR3A	Polymerase III, RNA, Subunit A
Polymorp	Polymorphism
PPP2R2B	Protein Phosphatase 2, Regulatory Subunit B, Beta
PRKCG	Protein Kinase C, Gamma

PTRH2	Peptidyl-tRNA Hydrolase 2
RD	Rare Diseases
REVEL	Rare Exome Variant Ensemble Learner
RFC1	Replication Factor C, Subunit 1
RNA	Ribonucleic Acid
SACS	Sacsin
SCAN1	Spinocerebellar Ataxia, Autosomal Recessive, with Axonal Neuropathy 1
SCAR	Spinocerebellar Ataxia, Autosomal Recessive
SCAs	Spinocerebellar Ataxias
Seg	Segregation
SEPSECS	O-Phosphoserine tRNA-Selenocysteine tRNA Synthase
SETX	Senataxin
SIFT	Sorting Intolerant from Tolerant
SMA	Spinal Muscular Atrophy
SPAX	Spastic Ataxia
SPG	Spastic Paraplegia
SPG11	Spatacsin
SPG7	Paraplegin
SPTBN2	Spectrin, Beta, Nonerythrocytic, 2
SQSTM1	Sequestosome 1
STUB1	Stip1 Homologous and U Box-Containing Protein 1
SV	Structural Variations
SYNE1	Spectrin Repeat-Containing Nuclear Envelope Protein 1
T	Tolerated
TBE	Tris/Borate/EDTA
TBP	TATA Box-Binding Protein-Like Protein 1
TDP1	Tyrosyl-DNA Phosphodiesterase 1
TGM6	Transglutaminase 6
Tm	Melting Temperature
TNR	Trinucleotide Repeat
TTBK2	Tau Tubulin Kinase 2
TTPA	Tocopherol Transfer Protein, Alpha
UBL	Ubiquitin like domain

UV	Ultraviolet
VUS	Variants of Uncertain Significance
WES	Whole Exome Sequencing
WGS	Whole Genome Sequencing

Chapter 1: INTRODUCTION

The nervous and muscular systems fundamental to the human body, detect and respond to signals from internal and external stimuli. This action-response mechanism works by gathering information about itself and its environment through sensory nerves and initiating action through motor nerves and muscles. As a result of a problem in this communication, the muscle loses its ability to perform movements and may eventually undergo atrophy.

Muscle contraction is associated with the corresponding nerve cell. Each anterior horn cell of the spinal cord and brainstem, known as the motor neuron, stimulates numerous muscle fibers. Every part of the process, from the spinal cord to the activation of a muscle cell, represents a motor unit. In this system, damaged areas have the ability to compensate for each other, but complete recovery cannot be observed. For instance, if a motor neuron or its axon sustains damage, the associated muscle may eventually undergo atrophy. However, muscle cells on the periphery might adapt to receive signals from another intact motor neuron. Conversely, if the damage occurs within the muscle cell itself and there are intact cells nearby, they attempt to reorganize the affected area.

The connection between nerve and muscle is called the neuromuscular junction and has a special one-way conduction of nerve impulses. This conduction continues when an electrical signal from the nerve to the muscle changes the Ca^{2+} balance in the presynaptic region of the neuromuscular junction (Ca^{2+} is taken up), and acetylcholine, a neurotransmitter, is released and binds to acetylcholine-sensitive receptors located in postsynaptic muscle fibers. These chemically stimulated receptors then release the stored calcium and activate the muscle fibers, producing the contractile action of the muscle.

The smallest unit responsible for the contractile action of a muscle is called a myofibril. Myofibrils consist of thick filaments (composed of myosin) and thin filaments (composed of actin and tropomyosin), which combine to form muscle fibers. With an average diameter of 1μ, myofibrils exhibit alternating dark (highly diffractive, A band) and light (isotropic, I band) bands, creating the characteristic striations of striated muscles. The thicker myofilaments are found within the A band, while the thinner ones extend from the Z lines to the center of the A band.

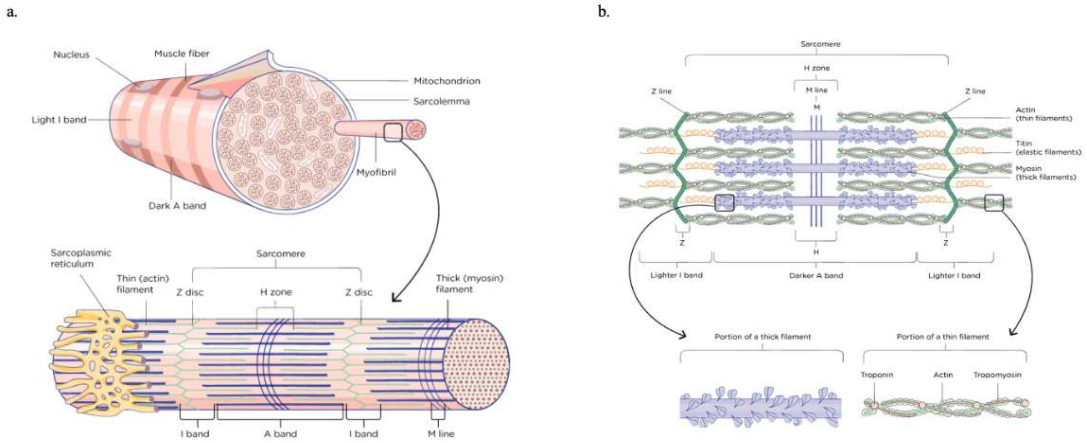


Figure 1.1 a. Muscle structure and **b.** elements of contraction.

Muscle system - MCAT content. (2023, March 21). Jack Westin. https://jackwestin.com/resources/mcat-content/muscle-system/muscle-structure-and-control-of-contraction

Consequently, the isotropic bands are also comprised of thin myofilaments. The central region of the A band, which thin myofilaments do not reach, is referred to as the H band (Figure 1). The area between the Z lines is known as the sarcomere, which shortens during muscle contraction.

This thesis will examine diseases that affect movement due to a hereditary problem in the muscle, including myopathies, which are caused by issues in the mechanism that comprises the muscle, and Myasthenia gravis, a condition caused by problems in the connection mechanism between the nerve and the muscle cell, known as the neuromuscular junction.

1.1. Myopathies

Myopathy is composed of the Greek words "myo" - muscle, and "patheia" – pathy for suffering, which signifies “muscle disease” (Nagy & Veerapaneni, 2023). Myopathies refer to a group of diseases caused by progressive or reversible dysfunction of the various muscle fibers. This disease group has been widely researched since millions of people are affected; cases vary depending on age, race, and gender. Symptoms of myopathies are muscle weakness, muscle stiffness, cramps, spasms, and decreased muscle tone. The most common problems include, but are not limited to, gait abnormalities and difficulty in running, climbing stairs, chewing, swallowing, and even breathing (Querin & Colella, 2023). Myopathies are classified into two main groups according to their clinical and pathological findings:

i) Hereditary Myopathies: Muscular dystrophies are the most common type of inherited myopathies (https://my.clevelandclinic.org/health/diseases/17256-myopathy). The second most common hereditary myopathy group is mitochondrial myopathies, affecting one in every 4300 people. Other forms are extremely rare (Mathis et al., 2018).

ii) Acquired myopathies: They may be caused by different factors, such as inflammation, infection, drug toxicity, and impairment of the endocrine system (Nagy & Veerapaneni, 2023). Studies have shown that the incidence of inflammatory myopathies ranges from 1.16 - 19 in one million people per year, and the prevalence of this disease in the population ranges from 2.4 to 33.8 in 100,000 people (Meyer et al., 2015).

The diagnosis of myopathies consists of several steps. The first step is the physical examination in which EMG, serum creatine kinase enzyme level detection, and muscle biopsy are performed. In addition to these, routine microscopic histologic examination, Western blot, and electron microscopy are conducted (Bayındır, Y. 2019). However, diagnosis in myopathies is difficult. Since the symptoms of diseases can be common, differential diagnosis can be challenging. Consequently, genetic testing has become very important in differential diagnosis. Moreover, invasive procedures, such as muscle biopsy, are no longer mandatory for people with a firm genetic diagnosis (Saat & Sahin, 2021).

1.1.1. Classification of Myopathies

Hereditary myopathies are divided into five classes: Congenital myopathies, Muscular dystrophies, Channelopathies, Metabolic myopathies, and Mitochondrial myopathies. These, usually acquired at birth, can be transmitted from generation to generation in an autosomal dominant, autosomal recessive, or X-linked inheritance (Ogasawara & Nishino, 2023).

Table 1.1 Classification of Myopathies, formed from (González-Jamett et al., 2018; Nagy & Veerapaneni, 2023; Romero & Clarke, 2013).

Hereditary Myopathies

Congenital Myopathies

- Congenital Myopathies with Protein Aggregates
- Congenital Myopathies with Core
- Congenital Myopathies with Central Nuclei
- Congenital Myopathies with Abnormal Fiber Ratios or Sizes
- Congenital Fiber-Type disproportion Myopathy
- Congenital Myopathies with Myosin Storage

Muscular Dystrophies

- Myotonic Dystrophy
- Duchenne Muscular Dystrophy (DMD)/ Becher Muscular Dystrophy (BMD)
- Facioscapulohumeral muscular dystrophy (FSHD)
- Emery-Dreifuss muscular dystrophy (EDMD)
- Limb-Girdle Muscular Dystrophy (LGMD)

Mitochondrial Myopathies

- Kearns-Sayre syndrome (KSS)
- Chronic progressive external ophthalmoplegia (CPEO)
- Leigh syndrome
- Mitochondrial DNA depletion syndromes (MDDS)
- Mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS)
- Myoclonic epilepsy with ragged-red fibers (MERRF)

Metabolic Myopathies

- Glycogen Storage Diseases
- Lipid Storage Diseases

Channelopathies

- Myotonia congenita (MC)
- Paramyotonia congenita (PMC)
- Hypokalemic Periodic Paralysis (HypoPP)
- Hyperkalemic Periodic Paralysis (HyperPP)

Acquired Myopathies

- Infectious Myopathies
- Toxic myopathies
- Endocrine Myopathies
- Immune-mediated or Idiopathic Inflammatory Myopathies
- Electrolyte-mediated Myopathies
- Myopathies associated with systemic disease

1.1.2. Hereditary Myopathies

With the muscular dystrophic disease described by Duchenne in the mid-1800s as muscle weakness, it has started to be thought that myopathies may be familial. Over time, it has become known that myopathies can in fact be inherited. Inherited myopathies, or hereditary myopathies, are genetic conditions that primarily affect the skeletal muscle tissue due to mutations in specific genes responsible for encoding proteins for muscle structure and function with X-linked, autosomal dominant, and autosomal recessive states. Hereditary myopathies are typically either present at birth or may become manifest later in childhood or adulthood. The severity of these conditions varies, with early-onset forms often being more severe in clinical presentation (Cassandrini et al., 2017). Dystrophinopathies are the most common among other inherited myopathies. Mitochondrial myopathies are the second most common inherited myopathies after Dystrophinopathies. Other inherited myopathies, by comparison, are of rare prevalence (Mathis et al., 2018).

Initial counseling, laboratory tests, imaging tests, muscle biopsy, and genetic examination are very important for diagnosing hereditary myopathies. However, the methods used in treating hereditary myopathies (physical therapy, exercise, orthotics, and medications) are aimed at the symptoms rather than eliminating the disease. Early diagnosis is crucial for managing symptoms and preventing complications (Mammen et al., 2018; Ogasawara & Nishino, 2023a).

Some specific types of myopathies will be briefly described in this part to support the Result section.

1.1.2.1. Congenital Myopathies

Congenital myopathies constitute a heterogeneous group of rare genetic neuromuscular disorders that manifest clinically with muscle weakness, hypotonia, and motor developmental delays, typically emerging in the neonatal or early infancy period. The onset of the disease generally occurs in the neonatal period, with an estimated incidence of 1:25,000 live births (Cassandrini et al., 2017). Historically, the term "congenital myopathy" was introduced by Shy and Magee in 1956, marking the onset of systematic delineation of these conditions. Early classification relied heavily on histopathological findings from muscle biopsies, which exhibited varying patterns such as Congenital Myopathies With Protein Aggregates, Congenital Myopathies With Cores, Congenital Myopathies With Central Nuclei, Congenital Myopathies With Abnormal Fiber Ratios Or Sizes, and Myosin Storage Myopathy (Romero & Clarke, 2013) leading to subclassifications like nemaline myopathy, core myopathy, and centronuclear myopathy, among others (Schorling et al., 2017).

Advancements in genetic sequencing techniques have revolutionized our understanding of congenital myopathies, revealing a complex genetic landscape with diverse mutations impacting proteins integral to muscle structure, contraction, and function. (North et al., 2014). This genomic complexity often translates into phenotypic variability, with distinct mutations within the same gene yielding differing clinical presentations (Gonorazky et al.,2018). Furthermore, mutations across different genes may produce overlapping clinical and histological features. Some individuals may present with overlapping elements between different subtypes. The nomenclature of congenital myopathies is continually evolving as advancements in genetic sequencing and molecular diagnostics unravel new subtypes and elucidate the intricate relationship between genotype and phenotype.

Accurate diagnosis often necessitates a comprehensive evaluation, combining clinical assessment, muscle biopsy analysis, and genetic testing to identify specific genetic mutations. This multidisciplinary approach aids in tailoring individualized treatment strategies and genetic counseling, providing valuable insights for affected individuals and their families.

1.1.2.1.1. Congenital Myopathies with Protein Aggregates

Nemaline myopathy (NM) represents a diverse group of congenital myopathies characterized by muscle weakness. For the first time in 1963, Shy et al. described Nemaline Myopathy, which has a different pathophysiologic course from the previously described core disease (Greenfield et al., 1958; Shy et al., 1963). It ranges from severe forms with early lethality to milder forms manifesting in childhood or adulthood. NM is caused by various genetic mutations, leading to structural or regulatory protein abnormalities in muscle fibers. These mutations often affect genes responsible for thin filament components, such as nebulin (*NEB*) and skeletal muscle alpha-actin (*ACTA1*), among others (Romero & Clarke, 2013).

The first mutations associated with nemaline myopathy (NM) were reported in 1995 with the *TPM3* gene (Laing et al., 1995). Since then, researchers have identified at least eleven additional genes associated with autosomal dominant or recessive forms of NM. Twelve known genes associated with NM encode proteins within the muscle sarcomere, impacting muscle weakness through mechanisms, like altered thin filament length or calcium sensitivity. *NEB* mutations are the most common cause of NM. Less frequent gene mutations, such as in *TPM3* and *TPM2*, affect tropomyosin structure, resulting in NM (Laitila & Wallgren-Pettersson, 2021). While mutations in *NEB* and ACTA1 genes have been commonly reported in the literature as significant contributors to NM, a study conducted in Turkey in 2008 has suggested the presence of a major mutation related to *TPM3* (Lehtokari et al., 2008).

Despite ongoing research, there is currently no specific curative treatment for NM. Management focuses on symptomatic care, maintaining muscle strength, monitoring respiratory function, and aiding in daily activities (Laitila & Wallgren-Pettersson, 2021).

1.1.2.1.2. Congenital Myopathies with Core

Core myopathies constitute a diverse group of congenital muscle disorders characterized by structural and functional irregularities within muscle fibers (Ogasawara & Nishino, 2021). These conditions often present a common pathological feature, displaying areas lacking mitochondrial enzyme activity, observed in varied forms like central core and minicore (Dubowitz & Everson Pearse, 1960). There are presently 15 recognized genes associated with core formation in muscle fibers. However, *RYR1* and *SELENON* mutations typically account for Central Core Disease (CCD) and Multiminicore Disease (MmD), respectively (Ogasawara & Nishino, 2023b).

Central Core Disease (CCD) is a cornerstone in myology's history, the first disease identified within the congenital myopathy group. Initially described in 1956 by Shy and Magee as a "new congenital nonprogressive myopathy," its inheritance pattern can be autosomal dominant or recessive with variable penetrance (SHY & MAGEE, 1956).

A prevalence study in the pediatric United States population reported *RYR1* mutations as the most common cause of congenital myopathies (Topaloglu, 2020). The skeletal muscle isoform of the ryanodine receptor, encoded by the *RYR1* gene, plays a crucial role in skeletal muscle excitation-contraction coupling. Both autosomal dominant and autosomal recessive inheritance patterns have been observed in CCD, with dominant inheritance being more prevalent. While most dominant mutations occur in the C-terminal region, recessive mutations are dispersed throughout the *RYR1* gene (Amburgey et al., 2011). Patients with CCD due to *RYR1* mutations are at risk of developing Malignant Hyperthermia (MH), a life-threatening pharmacogenetic complication triggered by certain anesthetics and muscle relaxants (Ogasawara & Nishino, 2021).

Multiminicore Disease (MmD) was initially documented in 1971 by Engel et al. It represents an autosomal recessive congenital myopathy, marked by shortened and irregular sarcomere regions, containing fewer mitochondria in skeletal muscle fibers (Topaloglu, 2020). Its manifestations may include neonatal hypotonia, generalized muscle weakness, amyotrophy, or motor developmental delay. Most MmD cases stem from mutations in *SELENON/SEPN1* or *RYR1* genes. *SELENON*, encoding selenoprotein N, plays a role in endoplasmic reticulum oxidoreduction and calcium homeostasis (Ogasawara & Nishino, 2023b).

1.1.2.1.3. Congenital Myopathies with Central Nuclei

In 1966, Spiro and colleagues introduced the term "myotubular myopathy" to delineate distinctive histopathological changes observed in muscle fibers, resembling the structural characteristics of fetal myotubes. Individuals with myotubular myopathy commonly display severe generalized hypotonia and weakness from birth, often accompanied by profound respiratory insufficiency necessitating ventilator support. Additionally, swallowing difficulties often require gastric tube feeding to prevent aspiration (Romero & Clarke, 2013).

These myopathies exhibit three primary forms, based on inheritance mode and clinical manifestations:

1. X-linked recessive myotubular myopathy (XL-MTM or XL-CNM), typically appearing as severe prenatal or neonatal myopathy.

2. Autosomal dominant and sporadic forms (AD-CNM), associated with mild, moderate, and severe phenotypes.
3. Autosomal recessive form (AR-CNM), presenting with severe and moderate phenotypes.

Causative genes for centronuclear myopathy include *DNM2, BIN1, TTN, RYR1*, and *SPEG.* Autosomal dominant centronuclear myopathy arises from mutations in the *DNM2* gene, encoding dynamin-2 (Bitoun et al., 2005), while autosomal recessive centronuclear myopathy results from mutations in the gene encoding amphiphysin 2 (*BIN1*) (Bitoun et al., 2007). *DNM2*-related myopathy typically showcases clinically mild forms or late-childhood and adult-onset manifestations, with rare occurrences of severe early-onset forms due to de novo mutations (Bitoun et al., 2007). Patients with recessive *BIN1*-related myopathy usually present an intermediate form between severe X-linked neonatal and autosomal dominant forms (Nicot et al., 2007).

1.1.2.1.4. Congenital Myopathies with Abnormal Fiber Ratios or Sizes

Skeletal muscles are made up of different types of fibers. These fiber types differ in the way they contract and in their properties. For example, fast-twitch fibers contract faster than slow-twitch fibers. The fiber diameter of skeletal muscles and the proportion of different fiber types present secondary changes in neuromuscular disorders and systemic conditions. These changes can impair the function of muscle fibers, causing weakness, muscle wasting, or other symptoms. In congenital myopathies, a frequent secondary feature is the predominance of type 1 muscle fibers coupled with the characteristic smaller size of type 1 fibers compared to type 2 fibers. **Congenital Fiber- Type Disproportion Myopathy (CFTD)** is the most prevalent type of this myopathy subgroup, and different causative genes have been identified: *ACTA1, RYR1, TPM2*, and *TPM3* (Romero & Clarke, 2013).

1.1.2.1.5. Congenital Myopathies with Myosin Storage

Myosin storage myopathy (MSM) is a well-described but rare distinct subgroup of congenital myopathies characterized by the presence of hyaline bodies within type 1 muscle fibers, initially known as myopathy with hyaline bodies (Rafay et al., 2005; Shingde et al., 2006). The *MYH7* gene plays a crucial role in MSM formation as it encodes myosin. Clinical manifestations of myosin storage myopathy vary in their severity and onset. Severe neonatal forms are characterized by contractures and require early ventilatory support, while adult-onset forms exhibit slowly progressing generalized weakness. Common clinical features observed in

individuals with MSM include weakness in the shoulder-hip girdle muscles, polt-foot, calf hypertrophy, scoliosis, and respiratory failure (Rafay et al., 2005; Romero & Clarke, 2013).

1.1.2.2. Muscular Dystrophies

Muscular Dystrophy (MD) encompasses a group of inherited neuromuscular disorders characterized by progressive muscle weakness, atrophy, and degeneration of skeletal muscles, affecting limbs, axial, and facial muscles. Other organs can also be affected by some forms of the disease. This weakness results from a complex interplay of muscle degeneration, regeneration, and fibrosis within the muscle tissue.

Broadly, muscular dystrophies can be categorized clinically into two main groups: Limb Girdle Muscular Dystrophies and Muscle Dystrophies Showing a Special Muscle Weakness Distribution or Clinical Picture. While numerous forms exist, the most common include:

- **Myotonic Dystrophy Type 1 (DM1)** has an autosomal dominant inheritance. It is a complex disorder affecting multiple systems, characterized by symptoms including muscular dystrophy, myotonia (prolonged muscle contractions), cataracts, testicular atrophy, frontal balding, and cardiac conduction defects. DM1 is caused by the expansion of a CTG repeat in the 3' untranslated region (UTR) of the *DMPK* gene. When this CTG repeat exceeds 50 triplets, it becomes pathogenic, leading to the manifestation of the disease. Intermediate alleles, which have 35–49 CTG triplets, are not typically disease-causing but may exhibit instability when transmitted between generations. The effects of these expansions depend on various factors, such as the type, position, and length of the repeat sequence.

Despite being less common than DM1, another type of myotonic dystrophy is myotonic dystrophy type 2 (DM2). DM2, as a genetic abnormality, involves a noncoding nucleotide expansion within an unrelated gene called zinc finger 9 (*ZNF9*). This expansion specifically consists of CCUG repeats within RNA and leads to the formation of RNA foci within the cell nucleus, similar to DM1 (Todd & Paulson, 2010).

- **Duchenne Muscular Dystrophy (DMD)**, the most prevalent form of muscular dystrophy, is a rare genetic neuromuscular disorder primarily affecting males due to its recessive X-linked nature (Xp2.1). However, in exceptional instances of X-chromosome inactivation, females can also experience the impacts of DMD. Typically commencing around the age of four, this condition manifests with muscle weakness, particularly in the thighs and pelvic region, significantly hindering standing (LaPelusa & Kentris, 2023). DMD is attributed to mutations in the dystrophin gene, often resulting in substantial deletions that tend to cause disruptions in the protein-building process, leading to progressive muscle deterioration and

weakness. Studies indicate a genetic continuum between Duchenne (DMD) and the milder Becker (BMD) muscular dystrophy, with BMD displaying comparably milder symptoms and often a longer lifespan.

- **Facioscapulohumeral muscular dystrophy (FSHD)** impacts an estimated 870,000 individuals globally, ranking among the more prevalent types of muscular dystrophy following Duchenne muscular dystrophy (Schätzl et al., 2021). The condition manifests as facial muscle weakness, scapular winging, and foot drop. Progressive involvement of other musculature, notably the truncal muscles, may precipitate functional impairment (Johnson et al., 2020). FSHD comprises two delineated subtypes, FSHD1 and FSHD2, each resulting from abnormal Double Homeobox 4 (*DUX4*) gene expression despite disparate genetic etiologies (Schätzl et al., 2021).
- **Emery-Dreifuss muscular dystrophy (EDMD)** is a rare, genetic, relatively mild muscle disorder mainly affecting skeletal muscles and the heart (Yunisova et al., 2022). The condition is characterized by the weakening and decline of muscles over time. Symptoms of EDMD usually begin in childhood or adolescence and manifest as muscle weakness and wasting, particularly in the upper arms and lower legs. In addition, contractures in more than one place are common (Brisset et al., 2019).

EMDN displays two primary inheritance patterns: X-Linked and Autosomal Dominant (Helbling-Leclerc et al., 2002). EDMD is caused by mutations in certain genes, such as *STA* (EMD) and *LMNA*, which produce proteins necessary for maintaining the structure and function of muscle cells. There is currently no cure for EDMD, but symptom-relieving treatments are available, especially heart involvement, which usually arises between the ages of 20 and 40. It affects all individuals within this age bracket and represents a major cause of sudden death. The majority of patients typically require pacemaker implantation in their 30s, a crucial intervention that serves to save lives (Oflazer et al., 2020.).

- **Limb-Girdle Muscular Dystrophy (LGMD)** is characterized by the involvement of the pelvic and shoulder girdles, generally manifests in the second or third decade, and often presents with a milder course in comparison to Duchenne Muscular Dystrophy (DMD) (Laval & Bushby, 2004). However, there is considerable variability in terms of onset age, severity, and detailed clinical profile. The most significant symptoms are muscle stiffness and contractures, limiting joint mobility, exercise intolerance, and fatigue. In some cases, elevated muscle enzymes and rhabdomyolysis (muscle breakdown) can be observed. A recent study conducted on a Northern England population estimated the overall prevalence of LGMD at 2.27 cases per 100,000 individuals (Mohassel & Bönnemann, 2015). Additionally, autosomal recessive

LGMDs were found to be more prevalent compared to autosomal dominant types (Mercuri & Muntoni, 2013). Several other conditions, including inflammatory, metabolic, mitochondrial, congenital/structural, toxic, paraneoplastic, and endocrine myopathies, along with neurogenic diseases like spinal muscular atrophy (SMA), share some similar symptoms with LGMDs and consequently are prone to misdiagnosis. This finding underscores the complexity of definitively diagnosing LGMD (Mohassel & Bönnemann, 2015). Approximately 30 forms of LGMD have been reported to date (Table 1.2.), with a unique genetic fingerprint and its own pattern of weakness and progression. Genetic causes, inheritance patterns, and diverse manifestations of various LGMD types have been listed in Table 1.3.

Table 1.2 Classification of Limb-girdle muscular dystrophies (LGMD)

LGMD Type	Inheritance	OMIM Number	Gene	Protein	Localization
Type 1A	AD	159000	*MYOT*	Myotilin	Sarcomere-associated protein (Z disc) (Cross-links actin filaments + sarcomere assembly)
Type 1B	AD	159001	*LMNA*	Lamin A/C	Nuclear lamina-associated protein (Nuclear envelope in fibroblasts)
Type 1C	AD	607780	*CAV3*	Caveolin-3	Sarcolemma-associated protein (Skeletal muscle mitochondria form and function)
Type 1D (LGMDD1)	AD	603511	*DNAJB6*	DNAJ homologue, family B, member 6 (Co-chaperone DNAJB6)	Sarcomere-associated protein (Z disc) (Maintenance and aggregation of sarcomeric protein)
Type 1E	AD	602067	*DES*	Desmin	Intermediate filament protein (Maintain muscular function and structure)

Table 1.2 cont. Classification of Limb-girdle muscular dystrophies (LGMD)

LGMD Type	Inheritance	OMIM Number	Gene	Protein	Localization
Type 1F (LGMDD2)	AD	608423	*TNP3*	Transportin 3	Sarcomeric assembly
Type 1G (LGMDD3)	AD	609115	*HNRNPDL*	Heterogenous nuclear ribonucleoprotein D-like	Regulate transcription and alternative splicing
Type 1H	AD	613530	*NA*	NA	NA
Type 1I (LGMDD4)	AD	618129	*CAPN3*	Calpain 3	Sarcomere regulation
Bethlem myopathy dominant (LGMDD5)	AD	158810	*COL6A1* *COL6A2* *COL6A3*	Collagen 6	Extracellular matrix structure and muscle regeneration
Type 2A (LGMDR1)	AR	253600	*CAPN3*	Calpain-3	Myofibril-associated proteins (Sarcomere regulation)
Type 2B (LGMDR2)	AR	253601	*DYSF*	Dysferlin	Sarcolemma-associated protein (Sarcomere stability and muscular repair)
Type 2C (LGMDR5)	AR	253700	*SGCG*	y-sarcoglycan	Sarcolemma-associated protein (Cytoskeleton extracellular matrix link)
Type 2D (LGMDR3)	AR	608099	*SGCA*	a-sarcoglycan	Sarcolemma-associated protein (Cytoskeleton extracellular matrix link)
Type 2E (LGMDR4)	AR	604286	*SGCB*	ß-sarcoglycan	Sarcolemma-associated protein (Cytoskeleton extracellular matrix link)

Table 1.1 cont. Classification of Limb-girdle muscular dystrophies (LGMD)

LGMD Type	Inheritance	OMIM Number	Gene	Protein	Localization
Type 2F (LGMDR6)	AR	601287	*SGCD*	5-sarcoglycan	Sarcolemma-associated protein (Cytoskeleton extracellular matrix link)
Type 2G (LGMDR7)	AR	601954	*TCAP*	Titin cap (telethonin)	Sarcomere-associated protein (Z disc) (Myofibrillogenesis)
Type 2H (LGMDR8)	AR	254110	*TRIM32*	Tripartite motif-containing 32 (ubiquitin ligase)	Sarcomeric-associated protein (Z disc) (Ca2+ movement of in skeletal muscles)
Type 2I (LGMDR9)	AR	607155	*FKRP*	Fukutin-related protein	Putative glycosyltransferase enzymes (Glycosylation of ox-dystroglycan)
Type 2J (LGMDR10)	AR	608807	*TTN*	Titin	Sarcomeric protein (Muscle tension)
Type 2K (LGMDR11)	AR	609308	*POMTI*	Protein-I-O-mannosyl-transferase 1	Glycosyltransferase enzymes (O-mannosylation in)
Type 2L (LGMDR12)	AR	611307	*AN05*	Anoctamin 5	Transmembrane protein, possible sarcoplasmic reticulum (Calcium-activated chloride channels)
Type 2M (LGMDR13)	AR	611588	*FKTN*	Fukutin	Putative glycosyltransferase enzymes (Glycosylation of)
Type 2N (LGMDR14)	AR	613158	*POMT2*	Protein-O-mannosyl-transferase 2	Glycosyltransferase enzymes (O-mannosylation in ox-dystroglycan)

Table 1.2 cont. Classification of Limb-girdle muscular dystrophies (LGMD)

LGMD Type	Inheritance	OMIM Number	Gene	Protein	Localization
Type 2O (LGMDR15)	AR	613157	*POMGNT1*	Protein-O-linked mannose ß 1,2-N-aminyltransferase 1	Glycosyltransferase enzymes (Catalyzes reactions in)
Type 2P (LGMDR16)	AR	613818	*DAG1*	Dystrophin-associated glycoprotein 1	Sarcomeric-associated protein (Part of the dystroglycan complex)
Type 2Q (LGMDR17)	AR	613723	*PLEC1*	Plectin 1	Sarcolemma-associated protein (Z disc) (Cell survival, cell growth, actin organization and T-cell activation)
Type 2R	AR	125660	*DES*	Desmin	Connect sarcomeres to form myofibrils
Type 2S (LGMDR18)	AR	615356	*TRAPPC11*	Transport protein particle complex 11	Transport between endoplasmic reticulum and Golgi apparatus
Type 2T (LGMDR19)	AR	615352	*GMPPB*	GDP-mannose pyrophosphorylase B	Production of GDP-mannose for the O-mannosylation of
Type 2U (LGMDR20)	AR	614631	*ISPD*	Isoprenoid synthase	Glycosylation of
Type 2V	AR	606800	*GAA*	Alpha-1,4-glucosidase	Lysozyme function
Bethlem myopathy recessive (LGMDR22)	AR	158810	*COL6A1* *COL6A2* *COL6A3*	Collagen 6	Extracellular matrix structure and muscle regeneration
Laminin muscular dystrophy (LGMDR23)	AR	618138	*LAMA2*	Laminin	Myotubes stability and apoptosis
POMGNT2-re1ated muscular dystrophy (LGMDR24)	AR	618135	*POMGNT2*	Protein O-linked Mannose ß-1,4-NAcetylglucosaminyl-transferase 2	Catalyzes reactions in

Table 1.3 Genetic causes, inheritance patterns, and diverse manifestations of various LGMD types.

LGMD Types	Genetic Cause	Manifestations
Autosomal Dominant (LGMD1-14)		
LGMD1A (Myotubular Myopathy)	Mutations in the *MYOTUBULIN* gene. Myotubulin is a protein crucial for muscle cell development and function.	- Early onset, often in infancy or childhood. - Muscle weakness primarily affecting shoulders, hips, and neck. - Delayed motor development and difficulty with activities like walking and climbing stairs. - Facial weakness and drooping eyelids. - Respiratory problems and feeding difficulties in severe cases. - Intellectual disability in some individuals.
LGMD1B (Calpain 3 Deficiency)	Mutations in the *CAPN3* gene. Calpain 3, an enzyme essential for muscle protein breakdown and repair.	- Variable onset, from childhood to adulthood. - Progressive muscle weakness, initially affecting shoulders and hips, later spreading to other muscles. - Contractures (muscle shortening and tightening) leading to joint stiffness. -Exercise intolerance and fatigue. - Heart rhythm abnormalities in some individuals.
LGMD1C (Dysferlinopathy)	Mutations in the *DYSF* gene. Dysferlin is a protein involved in muscle membrane repair and stabilization.	- Onset typically in adolescence or young adulthood. - Gradual muscle weakness in the shoulders, hips, and pelvic girdle. - Muscle stiffness and contractures, limiting joint mobility. -Exercise intolerance and fatigue. -Elevated muscle enzymes and rhabdomyolysis (muscle breakdown) in some cases.
LGMD1D (Emery- Dreifuss Muscular Dystrophy)	Mutations in genes like *LMNA, EMD* and others. Proteins involved in nuclear structure and function.	- Variable onset, ranging from childhood to adulthood. - Muscle weakness in shoulders, hips, and neck. - Contractures and joint stiffness. - Frequent heart rhythm abnormalities, including conduction block and cardiomyopathy. -Facial weakness and drooping eyelids.

Table 1.3 cont. Genetic causes, inheritance patterns, and diverse manifestations of various LGMD types

Autosomal Recessive (LGMD20-33)		
LGMD2A (Calpain 3 deficiency)	Similar to LGMD1B but requires biallelic *CAPN3* genes.	- Typically, earlier onset than LGMD1B, often in childhood. - More severe muscle weakness and contractures, affecting a wider range of muscles. - Respiratory and cardiac complications are more common.
LGMD2A (Calpain 3 deficiency)	Similar to LGMD1B but requires biallelic *CAPN3* genes.	- Typically, earlier onset than LGMD1B, often in childhood. - More severe muscle weakness and contractures, affecting a wider range of muscles. - Respiratory and cardiac complications are more common.
LGMD2B (Dysferlinopathy)	Similar to LGMD1C but requires two mutated *DYSF* genes.	- Onset typically childhood or adolescence. - Can involve more severe muscle weakness, contractures, and rhabdomyolysis compared to LGMD1C.
LGMD2C-E (*SGCG, BCL2L1, TTN* Myopathies)	Mutations in different genes *(SGCG, BCL2L1, TTN).* Proteins crucial for muscle structure and function.	- Variable onset and severity, with muscle weakness, contractures, and potential heart involvement depending on the specific gene affected.
Other LGMD2 types *(Dystrophin, GGPS1, ANO5, etc.)*	Mutations in various genes encoding diverse muscle proteins.	- Diverse presentations with muscle weakness, contractures, and potential involvement of other organs, depending on specific type.
X-Linked LGMDs		
LGMD2D (Dystrophin Myopathy)	Mutations in the *DYSTROPHIN* gene on the X chromosome.	- Variable onset and severity, often starting in childhood or adolescence. - Muscle weakness, contractures, and potential heart involvement.
LGMD2E (Miyoshi Myopathy)	Mutations in the *BCL2L1* gene on the X chromosome.	- Variable onset and severity, often starting in childhood or adolescence. - Muscle weakness, contractures, and potential heart involvement.

1.1.2.3. Mitochondrial Myopathies

Mitochondrial myopathy arises from genetic mutations that hinder mitochondrial function, particularly ATP production, impacting either nuclear DNA (nDNA) or mitochondrial DNA (mtDNA) (Parsons, 2014). These mutations disrupt oxidative phosphorylation and ATP synthesis, leading to energy depletion, notable in energy-intensive tissues like skeletal muscles. The condition's genetic variability results in a spectrum of clinical manifestations in terms of onset age, severity, and affected tissues. Sporadic instances stem from de novo mutation in mtDNA or nDNA, while hereditary cases involve maternal transmission of altered mtDNA or the inheritance of nDNA mutations affecting mitochondrial function(Milone & Wong, 2013).

- **Kearns-Sayre syndrome (KSS):** A rare condition marked by progressive external ophthalmoplegia (PEO), pigmentary retinopathy, usually initiated before age 20, potentially accompanied by cardiac conduction issues and atypical myopathy symptoms.
- **Chronic progressive external ophthalmoplegia (CPEO):** Presents as progressive extraocular muscle weakness, leading to drooping eyelids and impaired eye movements, frequently linked to not just mitochondrial DNA deletions but also to nuclear DNA mutations.
- **Leigh syndrome:** A severe neurological disorder associated with more than 85 gene mutations primarily impacting brain mitochondrial function, causing progressive loss of intellectual and motor abilities, often commencing in infancy or early childhood.
- **Mitochondrial DNA depletion syndromes (MDDS):** A group of disorders involving substantial reductions in mitochondrial DNA content that causes maternally inherited disease, resulting in severe multisystemic symptoms affecting various organs and tissues. Disease manifesting in childhood.
- **Mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS):** Characterized by stroke-like episodes, seizures, headaches, and lactic acidosis due to mitochondrial DNA mutations affecting brain energy production.
- **Myoclonic epilepsy with ragged-red fibers (MERRF):** Typically featuring myoclonic epilepsy alongside ataxia, muscle weakness, and observable ragged-red fibers in muscle biopsies, often associated with mitochondrial DNA single nucleotide (m.8344A > G) mutations.

1.1.2.4. Metabolic Myopathies

Metabolic myopathies encompass irregularities in carbohydrate and lipid metabolism due to deficiencies in enzymes, causing aberrant substrate accumulation or insufficient synthesis of enzymatic pathway products. These anomalies affect the functionality of skeletal muscles and are classified into glycogen and lipid storage diseases (Table 1.4) (Srinivasan & Amato, 2003).

Table 1.4 Metabolic Myopathies subgroups.

Glycogen Storage Diseases			
Disease	**Age of Onset**	**Causes**	**Clinical Phenotype**
Pompe Disease	Childhood to Adult	Deficiency of acid α-glucosidase (GAA) enzyme	Childhood: myocardiopathy, hypotonia, muscle weakness, respiratory failure. Adult: progressive muscle weakness, altered movements.
Cori Disease	Childhood	Glycogen debranching enzyme deficiency (*AGL* gene)	Hypotonia, distal weakness, possible cardiac and hepatic issues.
McArdle Disease	Adolescence	Myophosphorylase deficiency (*PYGM* gene mutations)	Fatigue, exercise-induced myalgia, myoglobinuria, renal failure.
Lipid Storage Diseases			
Disease	**Age of Onset**	**Causes**	**Clinical Phenotype**
Neutral Lipid Storage Disease	Childhood to Adult	Mutations in *PNPLA2* gene (adipose triglyceride lipase)	Proximal muscle weakness, particularly affecting upper limbs, elevated CK levels, potential cardiomyopathy.
Primary Carnitine Deficiency	Childhood to Adult	Deficiency in carnitine transport	Muscle weakness, hypoglycemia, cardiomyopathy
Multiple Acyl-CoA Dehydrogenase Deficiency	Infancy to Childhood	Deficiency in enzymes involved in fatty acid metabolism	Hypotonia, metabolic crises, hypoglycemia, cardiomyopathy, liver problems

Varied glycogen storage diseases, such as Pompe, Cori, and McArdle, manifest due to deficiencies in enzymes or proteins vital for metabolism. McArdle Disease is indeed considered as one of the more prevalent forms of muscle glycogen storage disorders. Typically affecting between 1 in 67,000 to 1 in 100,000 individuals in the general population and resulting from

mutations in the *PYGM* gene which causes myophosphorylase deficiency (Urtizberea et al., 2023), McArdle Disease manifests in patients with fatigue, exercise-induced myalgia, and, in severe cases, myoglobinuria and acute renal failure due to rhabdomyolysis, accompanied by heightened susceptibility to malignant hyperthermia (González-Jamett et al., 2018).

Even with extensive molecular investigations, the genetic association can only account for four types of lipid storage myopathies (LSMs) (Liang & Nishino, 2011). These conditions exhibit specific clinical features, including Neutral Lipid Storage Disease, Primary Carnitine Deficiency, and Multiple Acyl-CoA Dehydrogenase Deficiency.

1.1.2.5. Channelopathies

Channelopathies encompass a wide range of disorders resulting from dysfunctions of ion channels in cell membranes that affect ions such as sodium, chloride, calcium, and potassium, with symptoms typically appearing in the first or second decade of life (Statland & Barohn, 2013). These conditions, caused by genetic or acquired factors, are particularly evident in primary skeletal muscle disorders and show considerable clinical and genotypic variability even within the same family (Kim, 2014).

The hallmark of muscle ion channel diseases is their paroxysmal nature, primarily exhibiting myotonia (muscle stiffness) and episodic weakness. These disorders are represented along a spectrum, varying from myotonia-dominant conditions like potassium-enhanced myotonia to weakness-predominant ones, such as hypokalemic periodic paralysis, with some displaying a mix of both symptoms (Oflazer et al., 2020). These conditions are linked to mutations in genes responsible for sodium channels (*SCN4A*), chloride channels (*CLCN1*), calcium channels (*CACNA1S*), or potassium channels (*KCNJ2* and *KCNJ18*) (Phillips & Trivedi, 2018).

Within non-dystrophic myotonias (NDM), myotonia congenita (MC) emerges as the most prevalent skeletal muscle channelopathy caused by *CLCN-1* gene mutations (González-Jamett et al., 2018). It can be inherited as autosomal dominant (Thomsen's disease) or recessive (Becker's disease) traits (Kim, 2014). Typically, patients exhibit muscle stiffness, especially in the legs, triggered by rapid voluntary movements, with clinical heterogeneity even within families. Dominantly inherited MC (Thomsen's disease) usually lacks muscle weakness, while recessively inherited MC (Becker's disease) may present a transient weakness that improves with exercise (Phillips & Trivedi, 2018).

1.2. Acquired Myopathies

Acquired myopathies, which can be acute (sudden onset) or chronic (long-lasting), are non-hereditary muscle diseases that occur later in life and can have optimal results with early diagnosis and treatment. There are different types of acquired myopathies depending on their causes:

- **Infectious Myopathies:** Viruses such as influenza virus, bacteria such as HIV, parasites such as trichinosis, and fungi such as Aspergillus.
- **Toxic myopathies:** Alcohol, heavy metals, some chemicals, and medicine.
- **Endocrine Myopathies:** Hypothyroidism, Cushing's syndrome, and hypokalemia.
- **Immune-mediated or Idiopathic Inflammatory Myopathies:** Lupus, rheumatoid arthritis, and polymyositis.
- **Electrolyte-mediated Myopathies:** Hypokalemia, hypomagnesemia, and hypophosphatemia.
- **Myopathies associated with systemic disease:** Amyloidosis, sarcoidosis, vitamin D deficiency, critical care myopathy, idiopathic eosinophilic myopathy, paraneoplastic.

1.3. Myasthenia Gravis and Congenital Myasthenic Syndrome

Unlike typical myopathy, myasthenia gravis and congenital myasthenic syndrome, caused by the disruption of the neuromuscular junction, are rare chronic diseases that specifically affect the eye and facial muscles, causing fluctuating muscle weakness throughout the day (Wiendl et al., 2023)(Navarro-Martínez et al., 2023).

Myasthenia Gravis represents an autoimmune condition primarily targeting nicotinic acetylcholine receptors (AChRs),—mostly situated postsynaptically, while Congenital Myasthenic Syndrome is generally an inherited disorder. (Gilhus et al., 2019); (Wiendl et al., 2023) Different genetic mutations and inheritance patterns contribute to diversity in symptom presentation and disease severity. Myasthenia Gravis often has a double-peak pattern in the majority of the population, with a minor peak around age 30 and a significant peak between 70 and 80 years old (Gilhus et al., 2019). Meanwhile, Congenital Myasthenic Syndrome typically emerges during childhood or adolescence, although occurrences in adults have been documented (Engel, 2020). Both conditions share similar phenotypes, ranging from mild to moderate, with potentially severe complications, in particular high mortality due to respiratory crises. Effective treatment allows patients with Myasthenia Gravis to have nearly normal lives, whereas symptom-relieving medications are currently the basis for managing Congenital Myasthenic Syndrome (Oflazer et al., 2020). Ocular symptoms, particularly asymmetric ptosis, commonly mark the onset for both diseases, followed by involvement of bulbar muscles and challenges in extremity muscles such as stair or hill climbing. Unlike Myasthenia Gravis, daily fluctuations in muscle strength and fatigue are not consistently observed in Congenital Myasthenic Syndrome. Patients often report feeling better in the morning, with symptoms worsening during the day or upon fatigue, representing a defining characteristic of the condition (Gilhus et al., 2019).

Myasthenia Gravis (MG) is the most researched and best-understood autoantibody-mediated neurological disorder (Gilhus et al., 2019). The first documentation of myasthenia gravis dates back to 1972 when Thomas Willis described its symptoms. In the second phase (1930-1960), the identification of acetylcholine as a neuromuscular neurotransmitter marked a significant progress. The third period (1960-1990) was characterized by very important breakthroughs. Key immunological mechanisms, such as the isolation of acetylcholine receptors and the discovery of anti-acetylcholine receptor antibodies, emphasized the autoimmune nature of MG. The fourth phase, from 1990 to 2020, witnessed further steps forward in immunology, particularly with the identification of anti-Musk antibodies (Deymeer,

2020). MG-related autoantibodies can generally be divided into two main groups: those against transmembrane or extracellular autoantigens and those against intracellular autoantigens. Some antibodies are clearly pathogenic, while others are probably not directly involved in the disease process (Gilhus et al., 2019).

The primary autoantigen in Myasthenia Gravis (MG) is the muscle's Nicotinic Acetylcholine Receptor (AChR), mainly concentrated at the ends of folds in the postsynaptic membrane. This AChR is a 250 kDa pentameric glycoprotein that covers the cell membrane. It consists of two α1-subunits, a β1-subunit, a δ-subunit, and a γ-subunit (in embryonic AChR) or ε-subunit (in adult AChR). These subunits together form a cation channel. After binding acetylcholine (ACh) to two binding sites on the α1-subunits, this channel opens and allows cations (such as Na^+, Ca^{2+}, and K^+) to move across the membrane. Anti-AChR antibodies can be identified in 80% of individuals diagnosed with MG. MuSK, a protein with a single subunit lining the cell membrane, plays a crucial role in the recruitment of AChR to the neuromuscular junction and in maintaining the integrity of the postsynaptic membrane. Approximately 1-10% of individuals diagnosed with MG have detectable levels of Anti-MuSK antibodies (Gilhus et al., 2019).

Congenital Myasthenic Syndromes (CMS) originate from the influence of 35 distinct genes, which can be divided into 14 groups according to their pathomechanisms. The variability in pathomechanisms across these groups contributes to widely diverse clinical features and treatment responses observed among different categories of CMS. Here are some of the subtypes and the genes involved:

- Endplate AChR Deficiency (*CHRNA1, CHRNB1, CHRND, CHRNE,* and *RAPSN*)
- Escobar Variant of Multiple Pterygium Syndrome (EVMPS, Escobar Syndrome) (*CHRNG*)
- Lethal Form of Multiple Pterygium Syndrome (LMPS)/Fetal Akinesia Deformation Sequence (FADS) (*CHRNA1, CHRND, MUSK, RAPSN, DOK7*, and *SLC18A3*)
- Slow-Channel CMS (SCCMS) and Fast-Channel CMS (FCCMS) (*CHRNA1, CHRNB1, CHRND,* and *CHRNE*)
- Synaptic CMS (*COLQ, LAMB2*, and *COL13A1*)
- Sodium Channel CMS (*SCN4A*)
- Defective AChR Clustering (*AGRN, LRP4, MUSK*, and *DOK7*)

Most CMS patients have autosomal recessive inheritance and carry biallelic pathogenic loss-of-function variants, whereas autosomal dominant inheritance or hemiallelic pathogenic variants are also seen in CMS patients. (Ohno et al., 2023)

1.4. Differential Diagnosis of Neuromuscular Disorders in the Genomic Era

Clinical and genetic heterogeneities make the diagnosis of overlapping disorders of muscle weakness complicated and challenging. Muscle weakness is one of the first signs of many neuromuscular diseases, such as Myopathies, Muscular Dystrophies, or Neuropathies. After clinical examinations, genetic analysis plays a crucial role in the differential diagnosis. Next-generation sequencing (NGS) technology has improved the rate of accurate diagnosis of many neurogenetic disorders. This technology uniquely analyzes DNA and RNA molecules, completely changing genetic studies. By reforming the processing of genetic material, this advanced technology has transformed the analysis of DNA and RNA molecules due to its cost-effectiveness and high efficiency (Satam et al., 2023). The revolutionary change brought by NGS has resulted in a fundamental shift in genomic research. Next-generation sequencing (NGS) includes several types of sequencing methods that differ in their approaches and applications. Some of the primary types of NGS include Whole Genome Sequencing (WGS), Whole Exome Sequencing (WES), Targeted Sequencing, RNA Sequencing (RNA-Seq) and ChIP Sequencing (ChIP-Seq) (Table 1.6).

Table 1.5 NGS technologies adapted from (Pang et al., 2017).

Sequencing Type	Description	Advantages	Disadvantages
Whole Genome Sequencing (WGS)	Involves sequencing an organism's entire genome, providing comprehensive genetic information about an individual's DNA, including both coding and non-coding regions.	- Provides comprehensive genetic information. - Encompasses both coding and non-coding regions	- Can be costly and time-consuming. - Requires processing large amounts of data
Whole Exome Sequencing (WES)	Focuses specifically on sequencing the protein-coding regions of the genome known as exons. It is a more targeted approach compared to WGS, aiming to identify variations or mutations within the exome, which are often associated with genetic disorders.	- More cost-effective and targeted. - Effective in identifying significant variations associated with genetic disorders	- Does not cover non-coding regions. - Might overlook some genetic changes (e.g., variations in non-coding regions)

Table 1.5 cont. NGS technologies adapted from (Pang et al., 2017).

Sequencing Type	Description	Advantages	Disadvantages
Targeted Sequencing	Involves sequencing specific regions or genes of interest rather than the entire genome or exome. It allows for a more cost-effective and focused analysis of particular genomic regions relevant to certain diseases or research questions.	– More cost-effective and focused analysis. – Provides detailed information on specific diseases or genes	- May lack general information about the rest of the genome. - May miss important variations outside the selected targets. - Cannot provide an overall view of comparison with other species or the general structure of the genome

1.5. Whole Exome Sequencing (WES)

The DNA of a human consists of 3.2 billion nucleotides. The number of genes, which was previously known to be approximately 23,500, was reported to be 60,900 (Nurk et al., 2022), while the number of protein-coding genes was reported to be 19,890, according to The Complete Sequence of a Human Genome. There are two main groups that make up a gene: Protein-coding and non-coding. The protein-coding genomic region is called an exon, and the sum of all exons in the genome is called the exome, which makes up about 1-2% of the entire genome. However, almost 75% of the disease-causing variants that have so far been linked to a gene are found in the exonic region. This is one of the main reasons why WES is ergonomically preferable to WGS.

WES analysis;

1. Genomic DNA is fragmented into short fragments of approximately 300 base pairs.
2. The resulting fragments are adaptor-ligated to enable sequencing (this can be performed before or after enrichment).
3. A series of steps are required to enrich the exon sequences:
    a. Enriching the library by hybridizing to targets called "baits" that select only exon regions.
    b. Washing of unselected regions.
    c. Elution of selected fragments to create a library enriched in protein-coding sequences.
4. Large-scale second-generation sequencing generates billions of base pair data. This step allows DNA sequences to be read.
5. Processing the sequenced data:
    a. Alignment of sequences.
    b. Quality control.
    c. Variant calling.

Selection of candidate variant(s).

Platform selection for WES analysis is important in terms of targeting, read depth, and coverage, along with cost vs. efficacy (Yohe et al., 2017). Read depth demonstrates how many times a certain nucleotide has been read. In clinical sequencing, 100x read depth is usually suggested. Read depth and cost have a positive correlation: although increasing read depth increases cost as well, it could decrease false positive results (Marian, 2014).

As mentioned earlier, physiological, and even pathologic differentiation between neuromuscular diseases is very difficult. For example, clinical assessment alone is insufficient to identify the subtype to which a patient with limb-girdle muscular dystrophy belongs, and therefore, clinicians consult genetic testing (Nallamilli et al., 2018). Today, genetic diagnosis is recognized as the gold standard for diagnosing a disease, and WES has particular potential in diseases that do not exhibit a simple Mendelian mode of inheritance or in diseases caused by de-novo mutations. Today, the proportion of patients diagnosed with WES is, on average, around 30% (up to 60% depending on various factors such as the age of the patient, the presence of another patient in the family, or consanguinity) (Burdick et al., 2020; Krenn et al., 2023).

However, WES has its limitations. For example, expansions in non-coding protein regions in a particular gene or deep intronic variants that may affect splicing may be responsible for the occurrence of a particular disease. Such variants may not be detected by WES. Furthermore, Copy Number Variations (CNVs) are also analyzed with WES data, but their reliability may be limited (van Dijk et al., 2018).

The sample selection for WES analysis has great importance, since inclusion criteria regarding sample selection can profoundly affect the analysis results. For instance, a family consisting of multiple affected members can be a more promising candidate for WES analysis than a single patient. At this point, evaluating the pedigree could inform about the inheritance pattern, which is a key step in the detection of the candidate variant. An important point is to check large databases such as 1000 genome and gnomAD to see the frequency of the candidate variant in ostensibly healthy populations. Screening the own population, e.g. in-home databases is also of prime importance. If the variant is present in many unaffected individuals, this would decrease the possibility of the candidate variant being disease-causing. However, it is important to keep in mind the possibility of incomplete penetrance and asymptomatic individuals. A careful analysis of the pedigree, as well as clinical examination of the family members, plays a significant role in genotype/phenotype correlation. In addition to frequency, *in-silico* tools such as DANN, CADD, Mutation Taster, REVEL, SIFT, and GERP assist in the determination of pathogenicity.

Chapter 2: PURPOSE

Neuromuscular disorders (NMD) can seriously affect the quality of life of affected individuals. Myopathy and Congenital Myasthenic Syndromes are types of NMD. The classification of myopathies is challenging due to the overlapping nature of symptoms across different groups. They overlap not only with each other but also with other neuromuscular conditions, making their diagnosis challenging.

Turkey, characterized by its extensive geographical expanse and notable prevalence of consanguinity, exhibits heightened rates of both birth and ethnic diversity. Many neuromuscular disease sub-groups prevalent in the region are inherited in an autosomal recessive manner.

The purpose of this thesis is to understand the genes that cause Myopathy and Congenital Myasthenic Syndrome (CMS) and to identify novel variants in the Turkish cohort under study. The investigative approach integrates PCR-based Sanger and Whole-Exome Sequencing (WES), bioinformatics methodologies, and relevant computational tools.

Understanding the complicated genetic processes behind myopathy and CMS is expected to help us find new ways to treat these conditions. Findings from this research will hopefully provide new insights into how genes and traits are linked in neuromuscular disorders among a Turkish cohort of 30 families.

Chapter 3: MATERIALS

3.1. Subjects

In this thesis research, 30 index cases were investigated and categorized across various muscle diseases: 15 families with 18 patients initially diagnosed with myopathy, nine families with 10 patients having LGMD, and six families with nine patients clinically diagnosed with myasthenia gravis. Patients were classified based on their inheritance pattern: autosomal dominant, autosomal recessive, or sporadic., Seven out of 30 families have autosomal dominant inheritance, while 20 followed autosomal recessive patterns, and three were apparently sporadic cases. Consanguinity or same-small village marriage was found in 18 families. The average age of disease onset of the index patients was 15.5 years, with a standard deviation of 14.7 years, expanding from birth to 58 years. In addition to 30 index cases, there are seven affected family members in this thesis cohort (Table 3.1).

Expert clinicians from different centers conducted thorough clinical evaluations, and an informed consent was acquired from all participants. Blood samples were collected using EDTA-containing tubes for subsequent DNA analysis. Extensive clinical characteristics of the patients were gathered from the clinicians, and a thorough investigation into the family's medical history, related to the disease, was carried out.

Table 3.1 Demographic and Clinical Information of the Cohort under Study.

Family No	NDAL-ID	Index/ Family member	Gender	Date of Birth	Origin	Consanguinity	AO	Phenotype	Inheritance	Clinical information
1	MYO134	index	M	1984	Istanbul	same village	22	MYAS	Sporadic	- Preliminary diagnosis: Myasthenia Gravis.
2	MYO139	index	M	1967	Sivas	first cousins	40	MYO	AR	- Progressive gait disturbance persisting for 15 years. - Complaints specifically in neuromuscular and lower extremity functions.
	MYO146	affected sister	F	?	Sivas	first cousins	?	MYO	AR	- Similar symptoms with her brother.
3	MYO141	index	M	1974	Mersin	no	46	LGMD	AR	- Atrophy in the proximal muscles of the left leg after intense exercise - Elevated levels of creatine kinase (CK), alanine transaminase (ALT), and aspartate transaminase (AST). - Preliminary diagnosis: LGMD
4	MYO149	index	M	1996	Siirt (mother) Batman (father)	bilateral first cousins	childhood	MYO	AR	- Walking at the age of 18 months. - At the age of 5-6, he was behind his peers and got tired quickly. -Increased muscle enzymes. - Diagnosed with rhabdomyolysis in 2014.
5	MYO150	index	M	2010	Malatya (mother) Yozgat (father)	no	congenital	MYO	AD	- Difficulty in walking and climbing stairs. - Hearing impairment in one ear and color blindness. - Demyelinating Polyneuropathy and Subacute Combined Degeneration (B12 deficiency) and a balance disorder starting in March 2022 were reported in the father.

Table 3.1 cont. Demographic and Clinical Information of the Cohort under Study.

Family No	NDAL-ID	Index/ Family member	Gender	Date of Birth	Origin	Consanguinity	AO	Phenotype	Inheritance	Clinical information
6	MYO156	index	M	1988	Rize	first cousins	congenital	MYAS	AR	- Droopy eyelids - Fatigue during sucking. - Motor neuron involvement in the upper and lower extremities. - Mild involvement in horizontal gaze. - Preliminary diagnosis: Congenital Myasthenia
	MYO157	affected step-niece	F	1996	Rize	first cousins	congenital	MYAS	AR	- Droopy eyelids - Fatigue during sucking - Fatigue while walking. - Partial limitation in horizontal gaze. - Also diagnosed with focal epilepsy.
	MYO158	affected step-niece	F	1991	Rize	first cousins	congenital	MYAS	AR	- Muffled crying, droopy eyelid, fatigue during sucking. - Around 1.5 years of age, heel predominant walking - Difficulty and fatigue during running, playing games, and climbing stairs. - Partial limitations in horizontal gaze.
7	MYO162	index	F	2004	Mardin	first cousins	11	MYO	AR	- Progression of external ophthalmoplegia for seven years. - Involvement in CN3/4/6 or conjugated gaze/following. - Normal MRI and CT scans. - Very thin and short stature. - Preliminary diagnosis: Progressive External Ophthalmoplegia (PEO).

Table 3.1 cont. Demographic and Clinical Information of the Cohort under Study.

Family No	NDAL-ID	Index/ Family member	Gender	Date of Birth	Origin	Consanguinity	AO	Phenotype	Inheritance	Clinical information
8	MYO163	index	M	1990	Adıyaman	first cousins	21	MYO	AR	- Muscle pain and leg weakness. - EMG: Myogenic involvement in the upper extremities. - Muscle biopsy indicated myopathy. - Scapula alata observed - Atrophy in right biceps and triceps. - Absent DTRs in right biceps, triceps normal. - Preliminary diagnosis: Myopathy.
9	MYO168	index	F	1981	Greece	no	25	MYO	AD	- Difficulty in climbing stairs for the first time after childbirth and progressive worsening. - Non-progressive tetraparesis in the proximal muscles, no myotonia, and diffuse myalgia. - EMG: Occasional myotonic discharges. - Similar complaints in the son.
	MYO177	affected son	M	?	Greece	no	?	MYO	AD	- Difficulty in running
10	MYO169	index	M	1911	Batman	distant relatives	21	MYO	AD	- Bilateral hearing loss following a seizure at the age of 1-1.5 years. - Gait problems and weakness starting from birth - Toe walking after the age of 5-6 years. - Inability to rise from a seated position and worsening of walking.
11	MYO189	index	M	1998	Nevsehir	distant relatives	1	MYO	AD	- Challenges holding his head during the newborn period. - Walking at the age of 1, but frequent falls. - Difficulty in climbing stairs and getting up from a seated position without support. - Upper and lower extremity involvement. - Preliminary diagnosis: Dystrophies and/or congenital myopathy.

Table 3.1 cont. Demographic and Clinical Information of the Cohort under Study.

Family No	NDAL-ID	Index/ Family member	Gender	Date of Birth	Origin	Consanguinity	AO	Phenotype	Inheritance	Clinical information
12	MYO191	index	F	2009	Azerbaijan	no	6	MYO	Sporadic	- Limitations in eye movement and poor vision when looking away at the age of 5-6. - Preliminary diagnosis: Chronic Progressive External Ophthalmoplegia (CPEO) and myoclonic epilepsy with ragged-red fibers (MERF2).
13	MYO194	index	M	1989	Azerbaijan	same village	12	MYO	AR	- Strabismus in multiple family members. - A progressive increase in ptosis over the last 10 years. - Blurred vision in the right and sometimes left eye, occurring 4-5 times in a month and lasting for 40 minutes, followed by severe headaches. - Preliminary diagnosis: CPEO.
14	MYO198	index	M	1999	Antalya	first cousins	16 months	MYAS	AR	- Difficulty in walking at the age of 16 months. - Preliminary diagnosis: Congenital Myasthenia.
15	MYO200	index	M	1996	Elazıg	second degree	childhood	MYO	AR	- Issues with muscle relaxation and maintenance of contraction. - A similar disease in the sister. - Preliminary diagnosis: Thomsen or Becker Disease.
16	MYO207	index	M	1984	Antalya	first cousins	7	MYO	AR	- Muscle-related concerns at the age of 7. - Difficulty in muscle relaxation. - Preliminary diagnosis: Thomsen or Becker Disease. - Similar symptoms in the son.
	MYO208	affected son	M	2010	Antalya	first cousins	2	MYO	AR	- Difficulty in muscle relaxation.

Table 3.1 cont. Demographic and Clinical Information of the Cohort under Study.

Family No	NDAL-ID	Index/ Family member	Gender	Date of Birth	Origin	Consanguinity	AO	Phenotype	Inheritance	Clinical information
17	MYO210	index	M	2002	Kayseri	distant relatives	21	MYO	AR	- Persistent complaints of rapid and excessive fatigue, no pain and cramps. - Elevated CK levels detected during a hospitalization in internal medicine for urinary bleeding. - Decreasing of the elevated CK after IV hydration observed. - Occasional essential tremors in the left hand - Irregular sleep patterns. - Muscle strength appears to be normal in both distal and proximal extremities - Reduced DTRs solely in the right lower extremity. - Preliminary diagnosis: Myopathy with rhabdomyolysis.
18	MYO211	index	M	1995	Balıkesir	no	12	MYO	AR	- Easily fatigued and difficulty in walking, accompanied by a foot deformity. - Progressive weakness in arms over the past 3-4 years. - No sensory deficit and normal DTRs. - A poor gait. - Muscle biopsy: Hypotrophic and scattered atrophic fibers. - Pacemaker implantation 6 years ago. - No similar disease in the family but deaths of his father's three older brothers and one older sister at the age of 2 for unknown reasons reported. - Preliminary diagnosis: Emery-Dreifuss.
19	MYO212	index	M	1960	Konya	same village	13	MYO	AR	- Challenges with running and walking. - Gradually emerging weakness in the upper extremities. - No swallowing difficulty. - Sleep disturbances due to shortness of breath. - Similar condition in his sister and brother. - Preliminary diagnosis: Myopathy.

Table 3.1 cont. Demographic and Clinical Information of the Cohort under Study.

Family No	NDAL-ID	Index/ Family member	Gender	Date of Birth	Origin	Consanguinity	AO	Phenotype	Inheritance	Clinical information
20	LGMD30	index	F	1990	Ankara	first cousins	27	LGMD	AR	- Progressive muscle weakness. - Preliminary diagnosis: LGMD.
21	LGMD31	index	M	1992	Batman	first cousins	29	LGMD	AR	- Progressive muscle weakness and gait disturbance. - Preliminary diagnosis: LGMD.
22	LGMD35	index	F	1985	Antalya	first cousins	28	LGMD	AD	- Progressive muscle weakness. - Preliminary diagnosis: LGMD.
23	LGMD36	index	M	1984	Elazıg	first cousins	congenital	LGMD	AR	- Difficulty in sucking during infancy. - Delayed head control. - Delayed walking. - Preliminary diagnosis: LGMD.
24	LGMD52	index	F	1989	Antalya	distant relatives	7	LGMD	AR	- Progressive Lower Motor Neuron (MN) involvement. - Preliminary diagnosis: LGMD.
25	LGMD55	index	F	1978	Bilecik	same village	25	LGMD	AR	- Progressive muscle weakness. - EMG: Myopathy - Preliminary diagnosis: LGMD.
	LGMD56	affected sister	F	-	Bilecik	same village	-	LGMD	AR	- Progressive muscle weakness.
26	LGMD64	index	F	1986	Tokat (mother) Malatya (father)	no	16	LGMD	AD	- Progressive muscle weakness. - Similar conditions in father and brother. - Preliminary diagnosis: LGMD.
27	LGMD67	index	M	1988	Burdur	first cousins	childhood	LGMD	AR	- Muscle weakness and gait disturbance. - Similar complaints in the older brother who passed away in 2009. - Preliminary diagnosis: LGMD.

Table 3.1 cont. Demographic and Clinical Information of the Cohort under Study.

Family No	NDAL-ID	Index/ Family member	Gender	Date of Birth	Origin	Consanguinity	AO	Phenotype	Inheritance	Clinical information
28	MYAS16	index	F	1991	Kayseri	first cousins	4	MYAS	AR	- Droopy eyelid and speech disorder. - Muscle biopsy revealed no distinctive features. - Mitochondrial disease and Progressive External Ophthalmoplegia (PEO) were definitively ruled out. - Similar symptoms in his cousin. - Preliminary diagnosis: Congenital Myasthenia.
	MYAS20	affected nephew	M	?	Kayseri	no	-	MYAS	AR	- Droopy eyelid and speech disorder.
29	MYAS18	index	F	1979	Isparta	no	2.5	MYAS	AR	- Bilateral ptosis arising during walking and in the evening hours, accompanied by weakness in the hands and feet, which resolved after 10-15 minutes of rest. - No relief of symptoms from Mestionon. - EMG and Muscle biopsy: Normal - Negative results from Ach-receptor and anti-MOG testing. - Preliminary diagnosis: Congenital Myasthenia.
30	MYAS27	index	F	1961	Bulgaria	no	58	MYAS	Sporadic	- Fatigue and weakness for approximately 10 years. - Severe low back pain. - Extreme fatigue and weight loss to the extent that walking became difficult, following a vaccine administration in 2020. - Improvement after receiving Intravenous Immunoglobulin (IVIG). - Preliminary diagnosis: Myasthenia gravis. - Brother and sister have been reported to be carriers for the activated PI3K delta syndrome (APDS) gene.

3.2. DNA Isolation

The genetic material extraction involved the utilization of the MagNA Pure Compact Nucleic Acid Isolation Kit I, along with the MagNA Pure Compact Instrument from Roche, and QIAGEN EZ1&2™ DNA Blood 350µl Kit, QIAGEN EZ1 *Advanced* XL to isolate genomic DNA from peripheral blood samples. The concentration and quality of the DNA were determined using the Thermo Scientific NanoDrop 2000c Spectrophotometer.

3.3. Whole Exome Sequencing Platforms

For the Whole Exome Sequencing process, Macrogen Inc, located in Amsterdam, handled the sequencing through the Illumina NovaSeq 6000 platform. The enrichment kits employed were the Agilent SureSelect Human All Exon V6 and IDT xGen Exome Research Panel V2.

3.4. Validation Experiments and Family Segregation Analysis

Validation experiments were conducted through PCR-based Sanger sequencing utilizing the MyTaq™ DNA Polymerase (Bioline, 5u/µL) and 5X MyTaq Reaction Buffer to amplify 10 ng of genomic DNA. The specific primer sequences employed for validating the experiments are listed in Table 3.2. Details regarding the PCR reagents and amplification conditions utilized are delineated in Tables 3.3 and 3.4, respectively.

Table 3.2 Primer sequences.

Gene	Exon	Primer F	Primer R	Tm(ºC) F	Tm (ºC) R
FUS	6	GGT TCC TGT CTT GTT TCC TAG C	CAG GCT CCC AAG TTC TCA CAA	60.3	59.8
DYSF	29	TTC AGA GAT GGT CCC AGG AGA	TGA TCT GTG AGC ACC TCC AGT	59.8	59.8
ANO5	13	GTG AAC CAT CCT GCA ACC AA	GCT GTC CGT TTC ACA GTT TTC T	57.3	58.4
DNM2	18	GTC ACC CCA TTG TTT GGG CAG	GTG CAT GAT GGT CTT TGG CAT	61.8	57.9
SGCA	5	CTG TGA TGG ATG GGG CAT TG	GGA CAA GGA GAT GCG GGT ATA G	59.4	62.1
DYSF	19	TGG CTT GTC ATA TCC CCC GA	GGA AGC AGG GCA AGT GTT GAT	59.4	59.8
GNE	10	TCA GGT AAA GCA CCC TCA GTG	GGG TCA GCC AAG AAG ACT GT	59.6	59.6

Table 3.2. cont. Primer sequences.

Gene	Exon	Primer F	Primer R	Tm(ºC) F	Tm (ºC) R
CHRNE	4-5	ATG GTG TGA GGT CTT GTC CA	GGC CTC GGA GTA GCT CTT	57.3	58.2
CHRNE	7	TAG ACA ACG ACG GCA AGA CC	AGA CAG CCA GAG CTT TTC CC	59.4	59.4
COL6A3	36	CCC CGT CTT CCC AAC AGA AC	GAA GCC ACA AAG GAG CAT GG	61.4	59.4
SCN9A	9	AGT CAG TGT CCA GAG GGG TA	CAG GCT CTT AAC ATA CAC CAG G	59.4	60.3
STIM1	3	GGG TAG ATG GAA TGT GTT ATG GC	CGC CAA CTC TGG AGA ACC TT	60.6	59.4
CHRNE	Intron 10	TCG TAC TAA CCT CGT CCC CT	GGG TCG GCA CAG TCA GTA AA	59.4	59.4
ANO5	5	CTT TTG TGC TTT TGC CAT TTC AG	TCC ATG TGA AAT TAT AAA ATG TGC TTC	57.1	57.4
ANO5	20	TTT GTG TTT CAG GAC AAA GAC TTG	GCT TAA GGG ATA AAA CTA AAA CC	57.6	55.3
TPM3	5	ACC GGG CCT TAA AAG ATG AAG	AGC CAA AGG GGA AGG GAT AAA T	57.9	58.4
CLCN1	3	AGC AAT CAG CAG AGG GGT AA	GGT AGA AAC TCC AGA AAC GGC	58.7	58.9
CLCN1	4	ACT CAG GCT TCC CAT GTG TC	CAG GGT CAA GGT GAA GGT GAA	59.6	59.8
CLCN1	22	TCC TGCT GTA TTG ACC AGT CTC	CCT GCT GCT CAA ATG GGA CA	59.5	60.6
COL6A2	16	TTG ACA TCA GGT GAA GGG GC	CTA AGC ACC CCT ATC TTG GGT	59.95	58.8

Table 3.3 PCR reagents for amplification.

Reagent	Volume (µL)	Stock concentration	Final concentration
dH_2O	16.8	-	-
Buffer	5	5X	1X
Forward Primer	1	10 µM	0.4 µM
Reverse Primer	1	10 µM	0.4 µM
MyTaq Polymerase	0.2	5 U/µL	1 U
DNA	1	10 ng/µL	10 ng/µL
Total	25		

Table 3.4 Conditions for PCR amplification.

Step	Temperature (°C)	Duration	# Of cycles
Initial denaturation	95	1 min	1
Denaturation	95	15 sec	35
Annealing	variable*	15 sec	
Extension	72	10 sec	
Final extension	72	10 min	1
Hold	24	∞	-
*Annealing temperature is specific for each primer pair			

3.4.1. Agarose Gel Electrophoresis

The components, substances, and tools utilized in agarose gel electrophoresis can be found in Table 3.5.

Table 3.5 Chemicals used in gel electrophoresis.

Components/ Substances/ Tools		Catalog No.	Company
10X Electrophoresis Buffer	89 mM Tris-base	1000258	Thermo Scientific, USA
	89 mM Boric acid		
	2 mM EDTA		
Agarose LE, 500G MERCK		11685678001	Sigma-Aldrich, ABD
GelRedTM Dropper Bottle		103.302-05	Olerup SSP, Sweeden
GeneRu1er 100 bp DNA Ladder		00767368	Thermo Scientific, USA
6X TriTrack DNA Loading Dye		00747349	Thermo Scientific, USA
Electrophoresis Tank, OWL EasyCast B1		349900	Thermo Scientific, USA
Power Supply, EC250-90		25090 ECA-LVD	Thermo Scientific, USA
ChemiDocTM MP Imaging System		17001402	Bio-Rad, USA

3.4.2. Laboratory Equipment and Kits

All laboratory equipment and kits used in the framework of this thesis are listed in Tables 3.6 and 3.7. respectively.

Table 3.6 Laboratory equipment.

Equipment	**Model**	**Catalog No.**	**Company**
Autoclave	HV-110L	30515011496	HMC HIRAYAMA
Balance	TE612	-	Sartorius, Germany
Centrifuge	Microfuge 16	A46473	Beckman Coulter, USA
DNA Extraction System	MagNa Pure Compact Instrument	03731146001	Roche, Switzerland
Electrophoretic Equipment	OWL EasyCast B1 Tank	349900	Thermo Scientific, USA
Falcon Tubes	50-ml Screw Cap Tubes	100619-02903	Axygen, USA
Gel Documentation System	ChemiDoc™ MP Imaging System	17001402	Bio-Rad, USA
Glassware	-	-	Isolab, Germany
Heat Block	ThermoMixer F1.5	5384000012	Eppendorf, Dubai
Microcentrifuge tubes	0.5 mL, 1.5 mL, 2.0 mL	-	Axygen, USA
Micropipettes	0.2 uL, 10 uL, 20uL, 200uL, 1000 uL	-	RaininPipet-Lite XLS, Mettler-Toledo International Inc., USA
Microwave oven	Intellowave MD554	7885270100	Arçelik, Turkey
Parafilm	-	PM-996	Bemis, USA
Power Supply	EC250-90	25090 ECA-LVD	Thermo Scientific, USA
Refrigerator	391640 EI	-	Arçelik, Turkey
Spectrophotometer	NanoDrop 2000c	E112352	Thermo Scientific, USA
Thermal Cycler	T100™	1861096	BioRad, USA
Tips/Filter tips	10 uL, 100 uL, 200 uL, 1000 uL	-	Axygen, USA
Vortex	REAX top	541-10000-00-0	Heidolph, Germany

Table 3.7 Commercially available kits, other than exome capture kits.

Kit	Catalog No.	Company
MagNA Pure Compact Nucleic Acid Isolation Kit I	3730972001	3730964001
QIAGEN EZ1&2^{TM} DNA Blood 350µl Kit	163033035	Qiagen, Germany
QIAquick PCR Purification Kit (250)	163018180	Qiagen, Germany
Genomic DNA Clean & Concentrator (25 preps)	ZRC175442	Zymo Research, USA

3.5. Online Databases and Bioinformatic Tools

The open-source NGS and bioinformatics databases, tools, and software used in WES analysis are listed in Table 3.8.

Table 3.8 Open-source databases, bioinformatics software and tools.

Database/Software/Tool	Definition
CADD (Rentzsch, Schubach, Shendure, & Kircher, 2021)	Assesses harmfulness of genetic variants in the human genome
CLC Main Workbench (Qiagen, Germany)	Platform for DNA, RNA, and protein sequence analyses
ClinVar (Landrum et al., 2020)	Archives human phenotypes and variations with supporting evidence
DANN (Quang, Chen, & Xie, 2015)	Predictive scoring tool for variant impact in the genome
dbSNP (Sherry et al., 2001)	Database housing SNPs, insertions/deletions, and microsatellites
FinchTV	Displays data from Sanger DNA sequencing
Franklin by Genoox	Variant interpretation software based on community insights
GeneCards	Integrated human gene database
GERP++ (Davydov et al., 2010)	Estimates conservation through substitution deficits
GnomAD	Database containing data from 76,156 unrelated genomes
Integrative Genomics Viewer (IGV)	Tool for visual exploration of genomic data
MetalR	Prediction tool for missense variants integrating scores

Table 3.7 cont. Open-source databases, bioinformatics software and tools

Database/Software/Tool	Definition
MutationTaster	Predicts variant pathogenicity based on various factors
NCBI Primer Blast (Ye et al., 2012)	Tool to search for PCR-specific primers
NetPrimer	Primer analysis tool
OligoCalc (Kibbe, 2007)	Primer analysis tool
Online Mendelian Inheritance in Man (OMIM) (McKusick-Nathans Institute of Genetic Medicine)	Online database containing human genes and diseases
REVEL (Ioannidis et al., 2016)	Prediction tool utilizing multiple scores for missense variants
RFFlow	Software for drawing pedigrees and diagrams
SEQ Platform by Genomize	Platform for genomic data storage, sharing, and usage
SIFT (P. C. Ng & Henikoff, 2003)	Predicts impact of amino acid substitutions on protein function
UCSC database (Kent et al., 2002)	Software from the University of California Santa Cruz
Varsome (Kopanos et al., 2019)	Search engine with data collection and impact analysis for human genomics

Chapter 4: METHODS

4.1. DNA isolation

From all participants, peripheral blood stored in EDTA tubes was collected for genomic DNA extraction. Using the MagNA Pure Compact Instrument, the DNA was isolated, and its concentration was subsequently determined at 260 nm optical density via a Nanodrop spectrophotometer. To assess potential chemical or protein contamination, the absorption values at 260/230 nm and 260/280 nm were recorded and documented.

4.2. Whole Exome Sequencing

Whole exome sequencing (WES) was conducted on DNA samples from all index patients. In selected cases, affected family members also underwent WES. WES comprises three primary stages, each punctuated by quality control checkpoints, followed by bioinformatic analysis.

1. Sample Preparation
   - DNA extraction and quantification
   - Quality control: Assessment of DNA integrity and purity
2. Exome Capture and Library Preparation
   - Enrichment of protein-coding regions (exons)
   - Library construction for sequencing
   - Quality control: Verification of library quality and yield
3. Sequencing
   - Determination of nucleotide sequences within captured exons
   - Quality control: Evaluation of sequencing data quality
4. Bioinformatics Analysis
   - Alignment of reads to reference genome.
   - Variant identification and interpretation

WES focuses on protein-coding regions, potentially identifying disease-causing variants. Thorough quality control measures ensure data reliability and accuracy. Bioinformatics analysis involves complex computational tools for variant interpretation.

4.3. Data Analysis

Raw reads undergo a meticulous transformation within the SEQ Platform, a comprehensive bioinformatics analysis tool developed by Genomize (Istanbul, Turkey). This intricate process encompasses:

- Data Cleaning: SEQ Platform filters and refines raw sequencing data, ensuring optimal quality for subsequent analysis.
- Alignment to Reference Genome: Reads are aligned to a reference genome, akin to piecing together a puzzle, revealing their precise position within the genetic blueprint.
- Variant Calling: Potential genetic variations are identified and flagged, paving the way for in-depth exploration of their significance.
- Annotation: Identified variants are enriched with detailed information, shedding light on their potential implications and functional relevance.

4.4. Variant Prioritization

The American College of Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) developed a thorough guideline to standardize variant interpretation in clinical genetic testing. These recommendations provide several methods for determining if gene variations linked to Mendelian illnesses are harmful. They provide five different scores:

- Pathogenic: There is a substantial probability of disease due to this variation.
- Likely pathogenic: More proof is required to demonstrate that the variation is likely causing disease.
- Variant of unknown significance (VUS): It is impossible to say for sure how harmful the variant is.
- Likely benign: additional investigation is required to determine that the variation is not likely to cause illness.
- Benign: There is very little chance of the variation causing illness.

Each score provides specific evidence regarding the variant's potential to cause disease, promoting accurate diagnosis and clinical management (Tavtigian et al., 2018). The ACMG-AMP guidelines are based on a variety of factors, including:

- The location of the variant in the gene. Variants that occur in essential genes or genes that are involved in disease pathways are more likely to be pathogenic.
- The type of variant. Certain types of variants, such as nonsense mutations and frameshift mutations, are more likely to be pathogenic than others.
- The frequency of the variant in the population. Variants that are rare in the population are more likely to be pathogenic than those that are common.
- The clinical presentation of the patient. If a patient has a clinical presentation that is consistent with a particular disease, this may suggest that a variant associated with that disease is pathogenic.

The ACMG-AMP guidelines are a valuable tool for clinical genetic testing laboratories. By following these guidelines, laboratories can improve the accuracy of variant interpretation and provide more reliable information to patients and their families. In addition to the ACMG-AMP scoring system, clinical labs employ a battery of computational tools to gain deeper insights into variant pathogenicity. These tools delve into the molecular and evolutionary aspects of the variation, providing valuable complementary evidence.

Probing Protein Effects: Mutation Taster, SIFT, CADD, DANN, and REVEL: These are tools that forecast how a variant might affect the structure and function of proteins. These algorithms' scores offer more proof in favor of or against variant pathogenicity, especially in the case of missense mutations that change the protein's amino acid sequence. A high CADD score, for instance, might imply a major disturbance in the structure of the protein, pointing to possible pathogenicity.

4.5. Determining Evolutionary Importance

GERP score: This score evaluates the region's rate of evolutionary conservation in relation to the variation. Protein function is more likely to depend on highly conserved areas, which have remained mostly intact across species. As a result, a variation in an area with high conservation is more likely to be pathogenic than one in a location with lower conservation.

By integrating data from ACMG-AMP scoring, computational tools like MutationTaster and GERP, and clinical data such as the patient's symptoms, labs can build a comprehensive picture of the variant's potential impact. This multifaceted

approach helps improve the accuracy of variant classification and informs informed clinical decisions for patient management.

4.6. PCR Experiments for Validation of Variants

4.6.1. PCR Conditions

Following Whole Exome Sequencing (WES) analysis, Sanger DNA sequencing via PCR serves as a validation step to confirm the existence and distinctness of identified variants. This ensures accuracy before downstream applications. Primer design for each variant employed two approaches:

- De novo design: NCBI's Primer Blast tool enabled the creation of specific primers for each variant target.
- Literature reference: Primers for some variants were sourced from published studies.

To optimize PCR performance and specificity, primer pairs were meticulously checked for potential issues using dedicated software:

- NetPrimer and OligoCalc: These tools identified and minimized the formation of hairpins, self-dimers, and cross-dimers within each primer pair.
- UCSC in-silico PCR: This online tool further validated the primers' target specificity within the human genome.

Sentromer (Istanbul, Turkey) synthesized the primers as lyophilized powders. We prepared working solutions by adding the recommended volume of dH_2O to achieve a 100µM concentration. Finally, PCR amplicons were visualized on a 2% agarose gel stained with GelStar fluorescent dye under ultraviolet light for confirmation.

4.7. Agarose Gel Electrophoresis

A 2% agarose gel was prepared with 0.5X TBE buffer and GelStar fluorescent DNA dye and then placed in an electrophoresis chamber filled with 0.5X TBE. Three volumes of PCR product were mixed with two volumes of loading dye and loaded onto the gel. The gel was run for an hour at 120A and visualized under UV light using a Bio-Rad ChemiDoc MP Imaging System. PCR products were outsourced to Macrogen (Amsterdam) for Sanger sequencing, and the sequencing results were analyzed using the FinchTV programs.

4.8. Purification of PCR Products for Sanger Sequencing

To minimize background noise in Sanger sequencing and remove potential contaminants like primer dimers and non-specific bands, PCR products underwent purification. Two approaches were employed:

Agarose gel extraction: QIAquick Gel Extraction Kit (Qiagen, Germany) was used to isolate target bands from the gel.

Direct PCR product purification: QIAquick PCR Purification Kit (Qiagen, Germany) directly cleaned the PCR product, bypassing the gel extraction step.

Chapter 5: RESULTS

In this study, WES analysis was conducted on 30 index patients plus seven affected family members, resulting in 37 patients presenting with either myopathy or congenital myasthenic syndrome phenotypes; WES results were validated by Sanger analysis in the probands and their affected relatives.

Twenty-one out of 30 families exhibited a recessive inheritance pattern, six displayed a dominant pattern, and three family trees looked apparently sporadic. Within these families, 27 different variants in 16 genes were identified. Eight variants were novel, and 19 variants were reported; their association with diseases in OMIM is detailed in Table 5.1.

Confirmation of variant presence and family segregation was accomplished through Sanger sequencing. Segregation results, prediction tool scores, and gnomAD frequencies of variants are summarized in Table 5.2.

Table 5.1 All patient results.

Family ID	NDAL ID	Result	Gene	Zygosity	Variant		Transcript ID	Reported/ Novel	OMIM
					Protein Sequence	Coding Sequence			
1	MYO134	Unsolved							
2	MYO139	Solved	*GNE*	Hom	p.Tyr621His	c.1861T>C	ENST00000396594.3	Novel	#605820
	MYO146	Solved	*GNE*	Hom	p.Tyr621His	c.1861T>C	ENST00000396594.3	Novel	#605820
3	MYO141	Solved	*ANO5*	Comp. Het	p.Asn64Lysfs*15 and p.Arg758His	c.191dupA and c.2273G>A	ENST00000324559.8	Reported & Novel	#608662
4	MYO149	Solved	*PYGM*	Hom	p.Phe710del	c.2128_2130del	ENST00000164139.3	Reported	#608455
5	MYO150	Solved	*RYR1*	Het	p.Arg4861His	c.14582G>A	ENST00000359596.3	Reported	#180901
6	MYO156	Solved	*CHRNE*	Hom		c.1219+2T>G	ENST00000293780.4	Reported	#100725
	MYO157	Solved	*CHRNE*	Hom		c.1219+2T>G	ENST00000293780.4	Reported	#100725
	MYO158	Solved	*CHRNE*	Hom		c.1219+2T>G	ENST00000293780.4	Reported	#100725
7	MYO162	Unsolved							
8	MYO163	Unsolved							
9	MYO168	Solved	*DNM2*	Het	p.Arg369Trp	c.1105C>T	ENST00000389253.4	Reported	#602378
	MYO177	Solved	*DNM2*	Het	p.Arg369Trp	c.1105C>T	ENST00000389253.4	Reported	#602378
10	MYO169	Solved	*STIM1*	Het	p.His109Pro	c.1105C>T	ENST00000300737.4	Novel	#605921
11	MYO189	Solved	*TPM3*	Het	p.Arg168Cys	c.502C>T	ENST00000368530.2	Reported	#191030
12	MYO191	Unsolved							

Table 5.1 cont. All patient results.

Family ID	NDAL ID	Result	Gene	Zygosity	Variant		Transcript ID	Reported/ Novel	OMIM
					Protein Sequence	Coding Sequence			
13	MYO194	Unsolved							
14	MYO198	Solved	*CHAT*	Hom	p.Ile620Leu	c.1858A>C	ENST00000337653.7	Reported	#118490
15	MYO200	Solved	*CLCN1*	Hom	p.Val536Ile	c.1606G>A	ENST00000343257.7	Reported	#118425
16	MYO207	Solved	*CLCN1*	Comp. Het.	p.Arg105Cys p.Phe167Leu p.Gly845Ser	c.313C>T c.501C>G c.2533G>A	ENST00000343257.7	Reported Reported Reported	#118425
	MYO208	Solved	*CLCN1*	Hom	p.Gly845Ser	c.2533G>A	ENST00000343257.7	Reported	#118425
17	MYO210	Solved	*PYGM*	Hom	p.Arg50Ter	c.148C>T	ENST00000164139.4	Reported	#232600
18	MYO211	Solved	*EMD*	Hem.	-	c.188-6A>G	ENST00000369842.9	Reported	#310300
19	MYO212	Solved	*CAPN3*	Hom	p.Phe200_Leu204del	c.598_612del	ENST00000397163.8	Reported	#253600
20	LGMD30	Solved	*DYSF*	Hom	p.Lys1050del	c.3149_3151del	ENST00000410020.3	Novel	#603009
21	LGMD31	Solved	*ANO5*	Hom	p.Glu419Ter	c.1255G>T	ENST00000324559.8	Novel	#608662
22	LGMD35	Solved	*DNM2*	Het	p.His681Arg	c.2042A>G	*ENST00000389253.4:*	Novel	#602378
23	LGMD36	Unsolved							
24	LGMD52	Solved	*SGCA*	Hom	p.Glu137Lys	c.409G>A	ENST00000262018.3	Reported	#600119
25	LGMD55	Solved	*DYSF*	Hom	p.Arg573Trp	c.1717C>T	ENST00000410020.3	Reported	#603009
	LGMD56	Solved	*DYSF*	Hom	p.Arg573Trp	c.1717C>T	ENST00000410020.3	Reported	#603009
26	LGMD64	Solved	*AARS1*	Het	p.Asn71Ser	c.212A>G	ENST00000261772.13	Reported	#01065

Table 5.1 cont. All patient results.

Family ID	NDAL ID	Result	Gene	Zygosity	Variant		Transcript ID	Reported/ Novel	OMIM
					Protein Sequence	Coding Sequence			
27	LGMD67	Solved	*COL6A2*	Hom	p.Gly445Glu	c.1334G>A	ENST00000300527.9	Novel	#620725
28	MYAS16	Solved	*CHRNE*	Hom	p. Cys148Ser	c.442T>A	ENST00000293780.4	Reported	#100725
	MYAS20	Unsolved	*CHRNE*	Het	p. Cys148Ser	c.442T>A	ENST00000293780.4	Reported	#100725
29	MYAS18	Solved	*CHRNE*	Comp. Het	p.Glu204Lys and p.Glu227ArgfsTer73	c.610G>A and c.675del	ENST00000293780.4	Reported & Novel	#100725
30	MYAS27	Unsolved							

Table 5.2 Online prediction tool scores and frequencies of the variants.

Gene	Associated Disease	Inheritance Pattern	Variant		ACMG (Varsome)	CADD	DANN	GERP	Mutation Taster	REVEL	SIFT	gnomAD
			Protein Sequence	Coding Sequence								
AARS1	CMT2N Axonal	AD	p.Asn71Ser	c.212A>G	PM5, PP3, PM2 **VUS++**	26.000	0.9989	5.9	Disease causing	Uncertain	Deleterious	-
ANO5	MMD 3	AR	p.Asn64Lysfs*15	c.191dupA	PVS1, PP5 **P**	NA	NA	NA	NA	NA	NA	0.001063
			p.Glu419Ter	c.1255G>T	PVS1, PM2 **LP**	41.0000	0.9981	5.1100	Disease causing	NA	NA	-
	LGMDR12	AR	p.Arg758His	c.2273G>A	PM5, PM2, PP3, PP5 **VUS++**	25.6000	0.9991	5.24	NA	Pathogenic	Deleterious	0.00003203

Table 5.2 cont. Online prediction tool scores and frequencies of the variants.

Gene	Associated Disease	Inheritance Pattern	Variant		ACMG (Varsome)	CADD	DANN	GERP	Mutation Taster	REVEL	SIFT	gnomAD
			Protein Sequence	Coding Sequence								
CAPN3	LGMDD4	AD	p.Phe200_Leu204del	c.598_612del	PP5, PM1, PM4, PM2 **P**	NA	NA	NA	NA	NA	NA	0.00002628
	LGMDR1	AR										
CHAT	CMS6, Presynaptic	AR	p.Ile620Leu	c.1858A>C	PP3, PM2 **VUS**	27.4000	0.9937	5.56	Uncertain	Pathogenic Moderate	Uncertain	NA
CHRNE	CMS4A Slow-channel	AD, AR	p. Cys148Ser	c.442T>A	PP3, PM2, PP5 **P**	26.4000	0.9948	4.7800	Disease causing	Pathogenic	Deleterious	NA
	CMS4B Fast-channel	AR	p.Glu204Lys	c.610G>A	PP3, PM2 **VUS+**	32.000	0.9991	4.6900	Disease Causing	Pathogenic Moderate	Deleterious	NA
			p.Glu227ArgfsTer73	c.675del	PVS1, PM2 **LP**	NA	NA	NA	NA	NA	NA	NA
	CMS4C Associated with acetylcholine receptor deficiency	AR	-	c.1219+2T>G	PVS1, PM2, PP5 **P**	NA	0.9782	4.90	Uncertain	NA	NA	NA

Table 5.2 cont. Online prediction tool scores and frequencies of the variants.

Gene	Associated Disease	Inheritance Pattern	Variant		ACMG (Varsome)	CADD	DANN	GERP	Mutation Taster	REVEL	SIFT	gnomAD
			Protein Sequence	Coding Sequence								
CLCN1	Thomsen Disease CMD	AD	p.Arg105Cys	c.313C>T	BP4, BP3, PM2, PP2, PP5 **LB**	23.600	0.9993	2.99	Disease causing	Pathogenic Supporting	Damaging	0.0001649
			p.Phe167Leu	c.501C>G	BS2, BP4, PM1, PP5 **LB**	NA	0.9652	5.03	Disease causing	Uncertain	Tolerated	0.0007950
	Becker Disease CMR	AR	p.Val536Ile	c.1606G>A	PP3, PM1, PM2, PP5 **LP**	28.0000	0.9992	5.91	Uncertain	Uncertain	Uncertain	0.00002829
			p.Gly845Ser	c.2533G>A	PP3, PM1, PM2 **VUS++**	33.000	0.9984	4.16	Disease causing	Pathogenic Moderate	Damaging	NA
COL6A2	Myosclerosis, Congenital	AR	p.Gly445Glu	c.1334G>A	PM1, PP3, PM2 **LP**	33.000	0.9953	4.37	Disease causing	Damaging	Damaging	NA
	BRHLM1B	AD, AR										
	UCMD1B	AD, AR										

Table 5.2 cont. Online prediction tool scores and frequencies of the variants.

Gene	Associated Disease	Inheritance Pattern	Variant		ACMG (Varsome)	CADD	DANN	GERP	Mutation Taster	REVEL	SIFT	gnomAD
			Protein Sequence	Coding Sequence								
DNM2	CNM1	AD	p.Arg369Trp	c.1105C>T*	PP5, PM5, PP3, PM1, PM2 **P**	29.2000	0.9993	4.93	Disease causing	Uncertain	Deleterious	0.00003187
	CMT2M, Axonal	AD										
	CMTDIB, dominant intermediate B	AD	p.His681Arg	c.2042A>G	PP3, PM2 **VUS**	26.3000	0.9975	5.27	Disease causing	7930	Damaging	-
	LCCS5	AR										
DYSF	MMD 1	AR	p.Arg573Trp	c.1717C>T	PP5, PP3, PM2, BP1 **P**	30.000	0.9992	5.4699	Uncertain	Pathogenic	Pathogenic	0.00003316
	LGMDR2	AR										
	DMAT	AR	p.Lys1050del	c.3149_3151del	PM1, PM4, PM2 **LP**	NA	NA	NA	NA	NA	NA	NA
EMD	EDMD1	XLR	-	c.188-6A>G	PP3, PS2, PM2, PP5 **LP**	NA	NA	NA	NA	NA	NA	NA
GNE	GNE myopathy	AR	p.Tyr621His	c.1861T>C	PM1, PP3, PM2 **VUS++**	24.7000	0.9986	5.75	Disease causing	Pathogenic Moderate	Tolerated	NA

Table 5.2 cont. Online prediction tool scores and frequencies of the variants.

Gene	Associated Disease	Inheritance Pattern	Variant		ACMG (Varsome)	CADD	DANN	GERP	Mutation Taster	REVEL	SIFT	gnomAD
			Protein Sequence	Coding Sequence								
PYGM	McArdle disease	AR	p.Phe710del	c.2128_2130del	PP5, PM4, PM2 **P**	NA	NA	4.7743	NA	NA	NA	0.00001193
RYR1	CMYP1A	AD	p.Arg4861His	c.14582G>A*	PS3, PM1, PM5, PP3, PP5, PM2 **P**	32.000	0.9856	4.71	Uncertain	Pathogenic	Pathogenic	-
	CMYP1B	AR										
SGCA	LGMDR3	AR	p.Glu137Lys	c.409G>A	PP5, PM5,PM2, PP2 **P**	22.2999	0.9979	4.3499	Uncertain	Pathogenic	Uncertain	0.00004366
STIM1	TAM1	AD	p.His109Pro	c.1105C>T	PM5, PP3, PM1 **LP**	25.7000	0.9860	5.3400	Disease causing	Pathogenic	Deleterious	-
TPM3	CMYP4A	AD	p.Arg168Cys	c.502C>T	PP5, PM5, PM1, PP3, PM2 **P**	32.000	0.9985	6.000	Disease causing	Pathogenic Moderate	Pathogenic Supporting	-
	CMYP4B	AR										

In the cohort under study, 23 out of 30 index cases were solved, the diagnostic yield was found to be 17 for autosomal recessive (AR) and six for autosomal dominant (AD) patterns. (Figure 5.1).

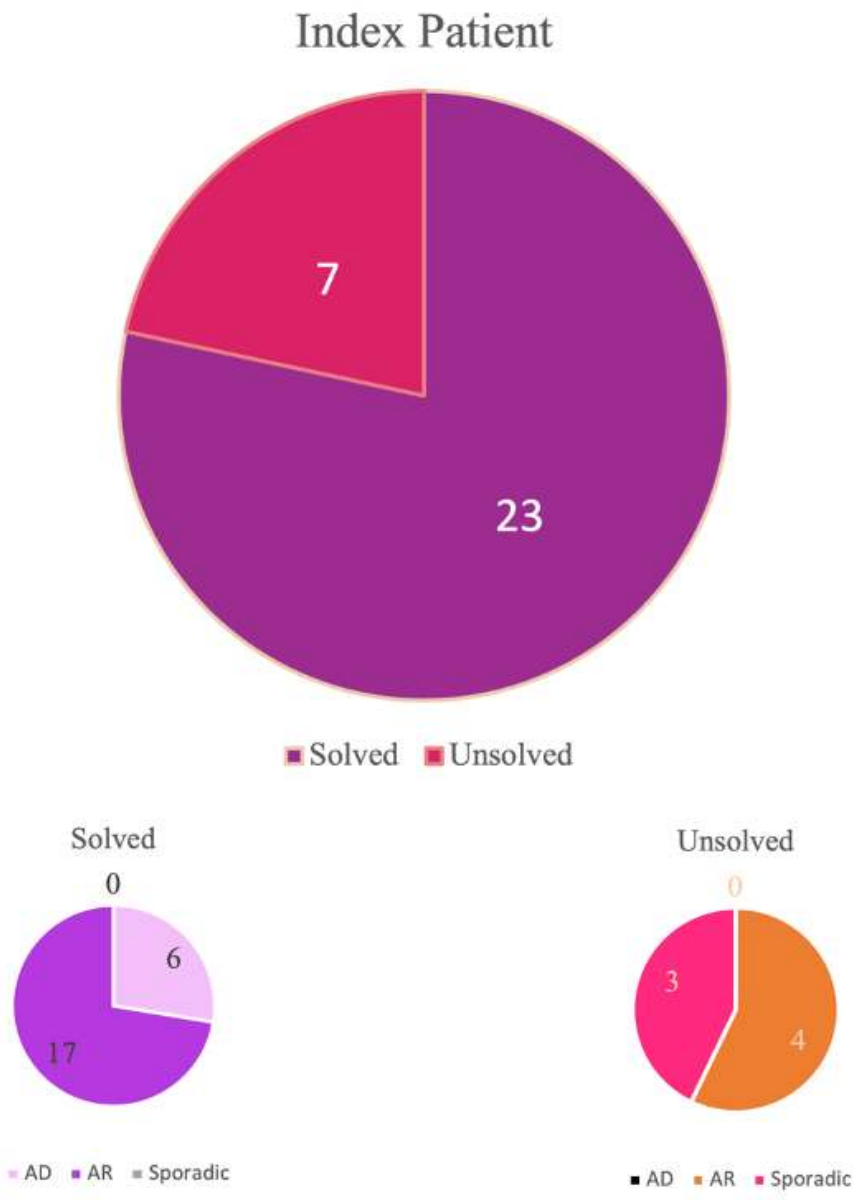


Figure 5.1 In this study, 23 of 30 families experienced solved, and seven remained unsolved. All families with autosomal dominance have solved.

Pedigrees

NDAL ID: MYO134
Date of Birth/Origin: 1984/ İstanbul
Age of Onset: 2006/ 22
Consanguinity: Same village
Clinician: Dr. Belgin Mutluay

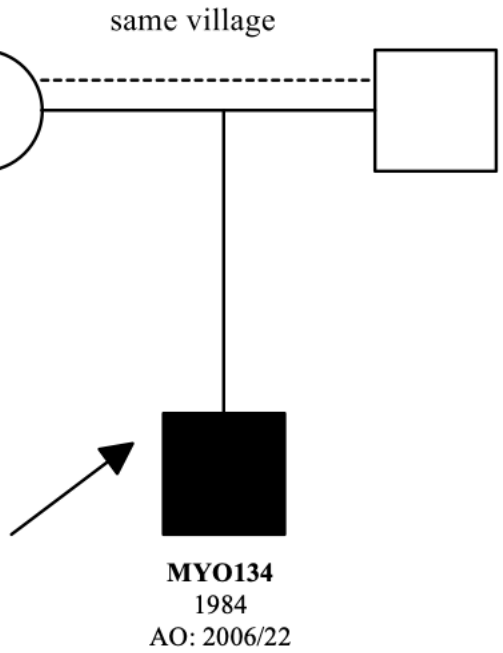


Figure 5.2 Family 1, unsolved case.

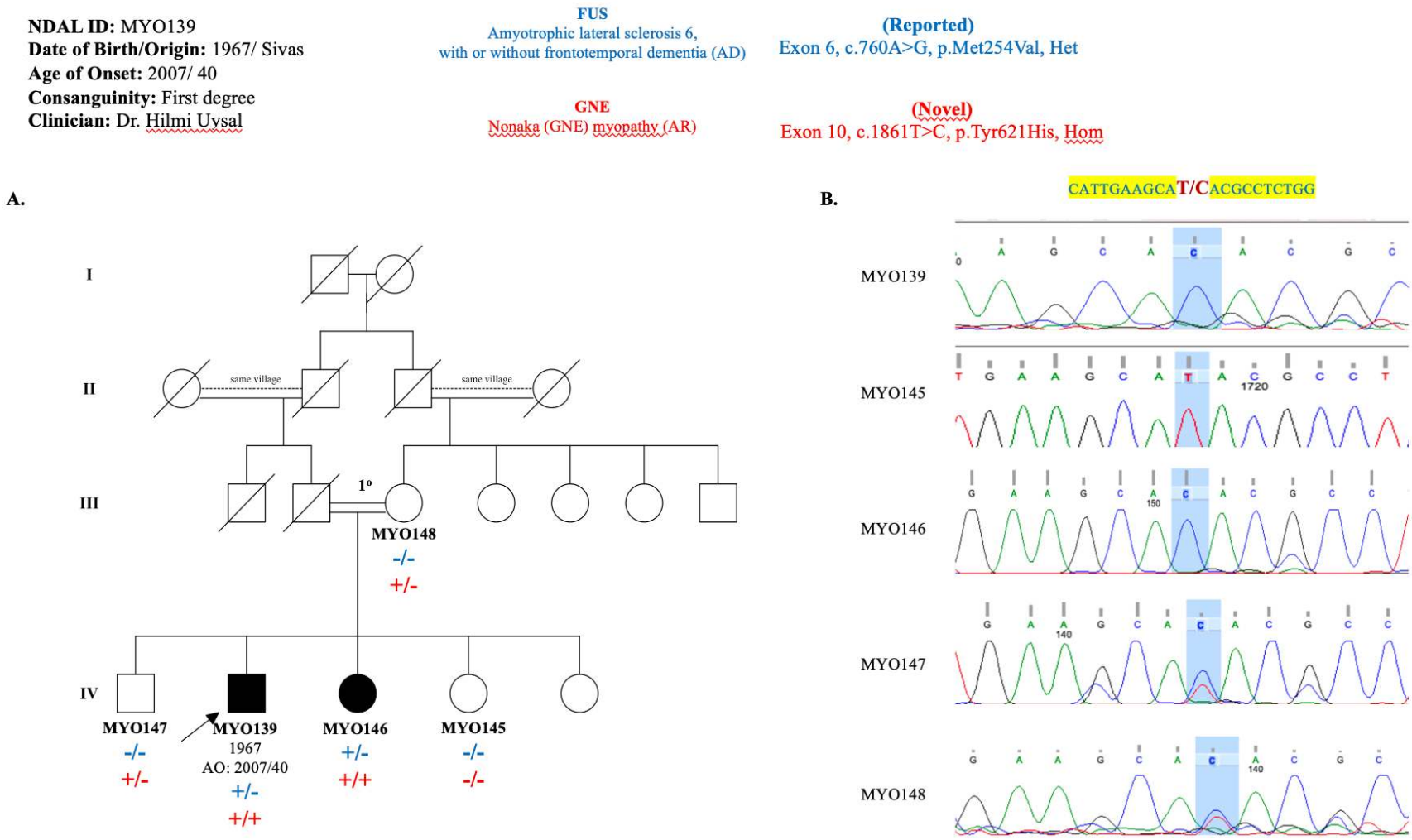


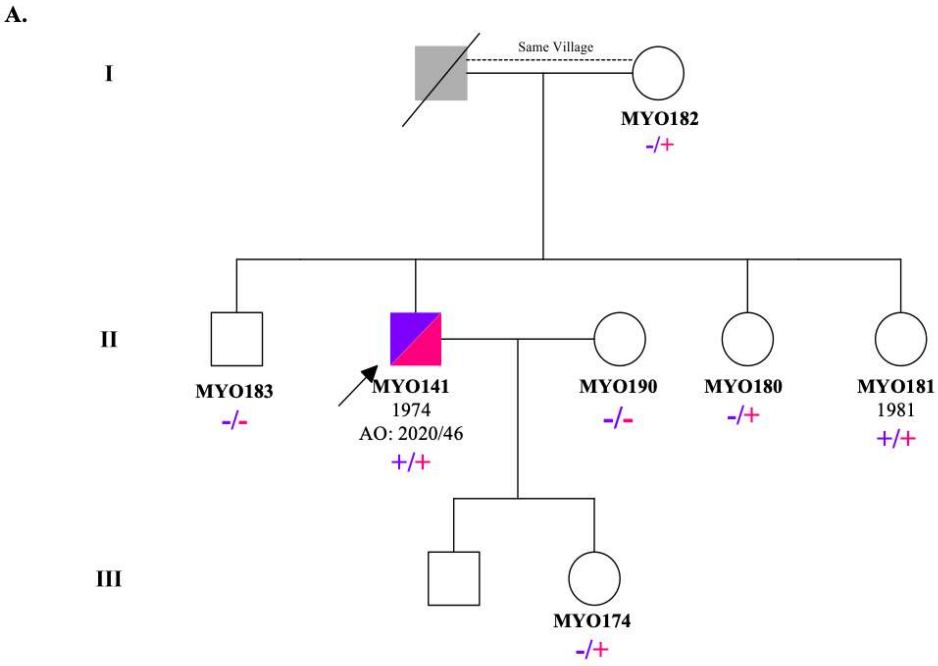
Figure 5.3 Family 2, solved with *GNE*. Additionally, a reported mutation in the *FUS* gene was observed in both siblings. However, since their clinical presentation did not align at the moment with ALS6, it can be an incidental finding. **A.** Family tree of MYO139. **B.** Sanger sequencing of *GNE* Exon 10 c.1861T>C.

NDAL ID: MYO141
Date of Birth/Origin: 1974/ Tarsus
Age of Onset: 2020/ 46
Consanguinity: Same Village
Clinician: Dr. Ayşe Cağlar Sarılar

ANO5
Muscular dystrophy, limb-girdle, autosomal recessive 12

(Reported)
Exon 5 , c.191dup, p.Asn64Lysfs*15, Het

(Novel)
Exon 20, c.2273G>A, p. Arg758His, Het



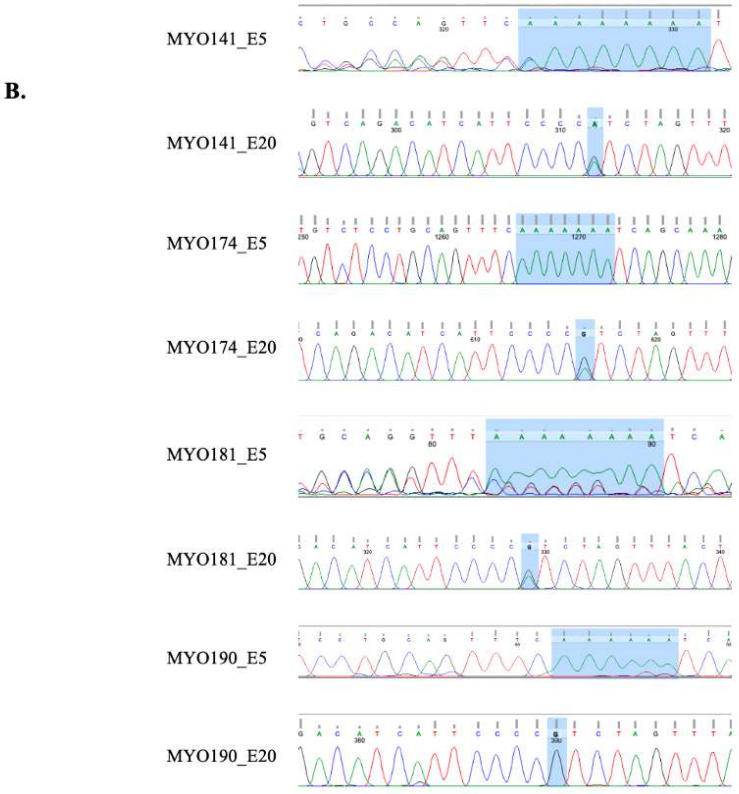


Figure 5.4 Family 3, solved with *ANO5*. MYO181 is presymptomatic cases.

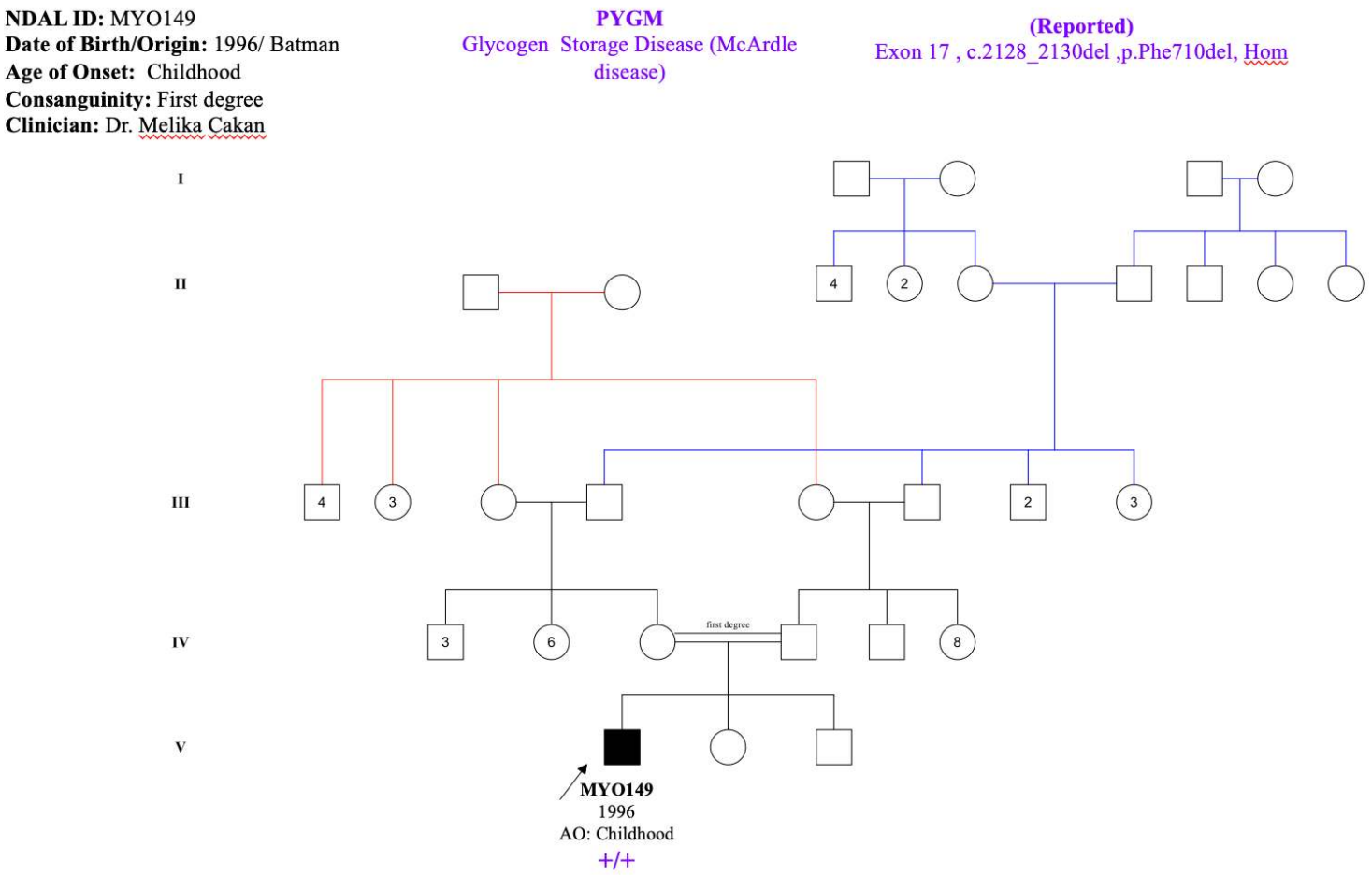


Figure 5.5 Family 4, solved with *PYGM*. Sanger validation was not conducted for this family.

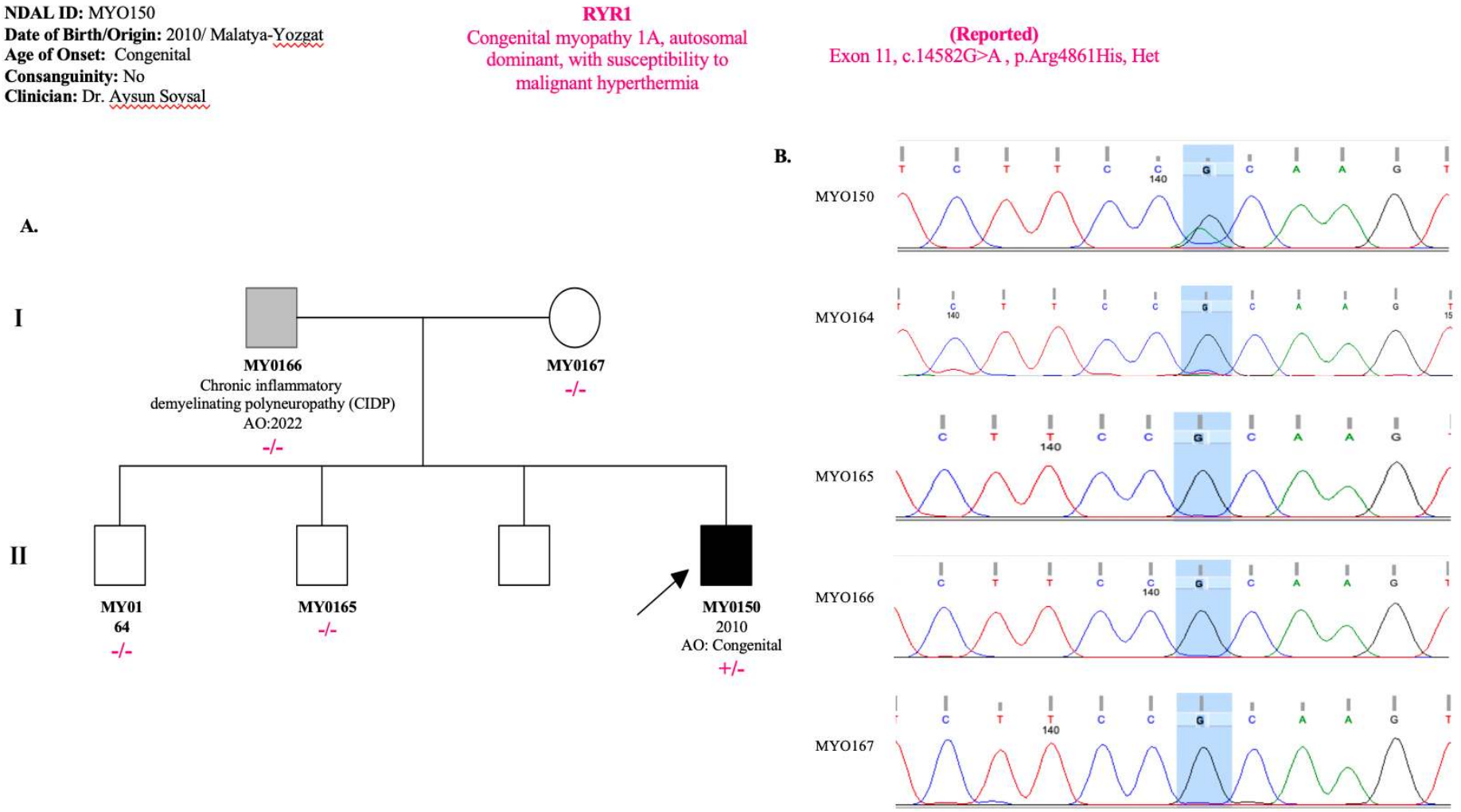


Figure 5.6 Family 5, solved with *RYR1*. During Sanger validation, no mutation was detected in any family member, suggesting that it is *de novo*.

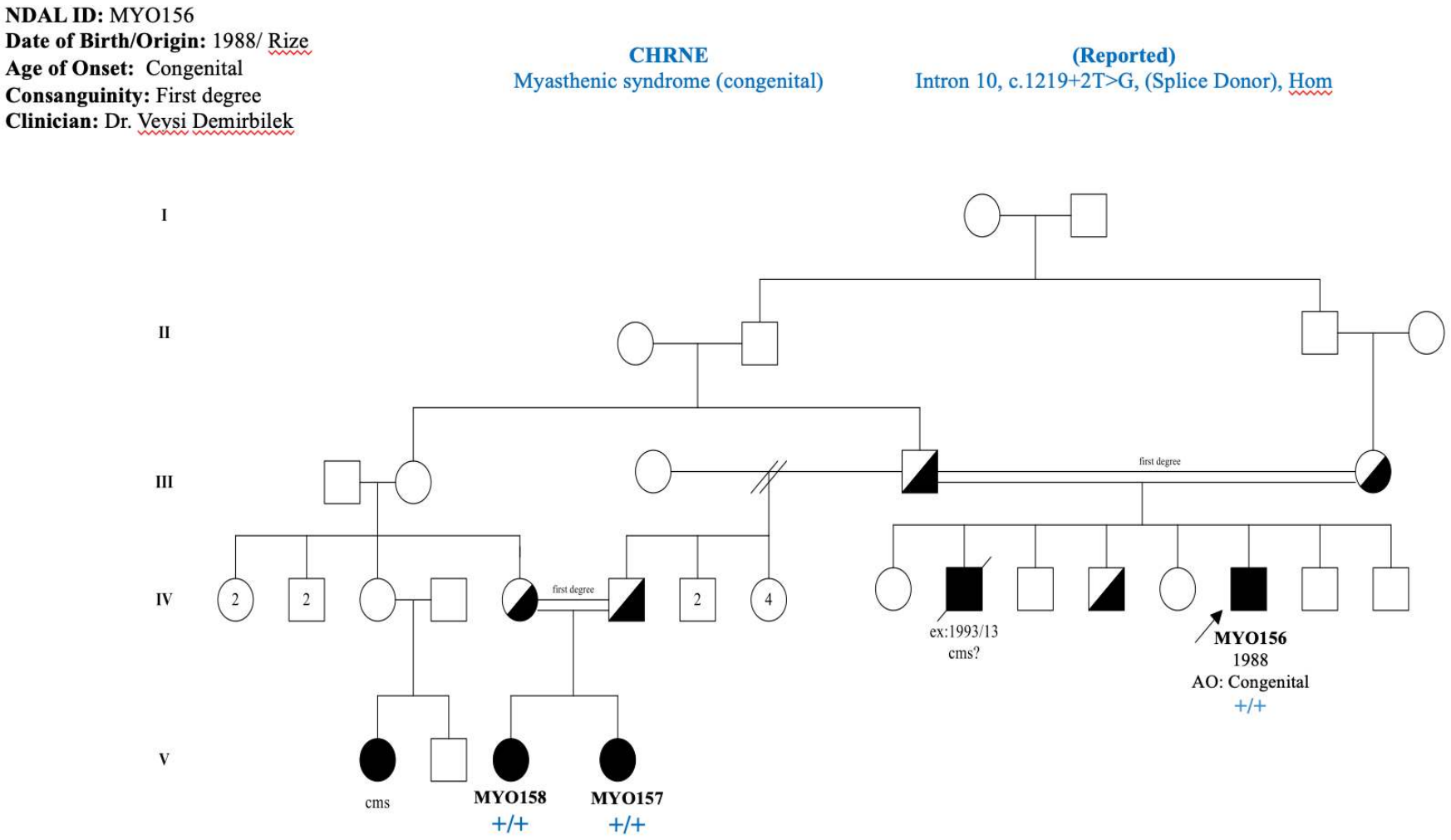


Figure 5.7 Family 6, solved with *CHRNE*. MYO156's affected step-nieces, MYO157 and MYO158 too.

NDAL ID: MYO162
Date of Birth/Origin: 2004/ Mardin
Age of Onset: 2015/ 11
Consanguinity: Second degree
Clinician: Dr. Hilmi Uysal

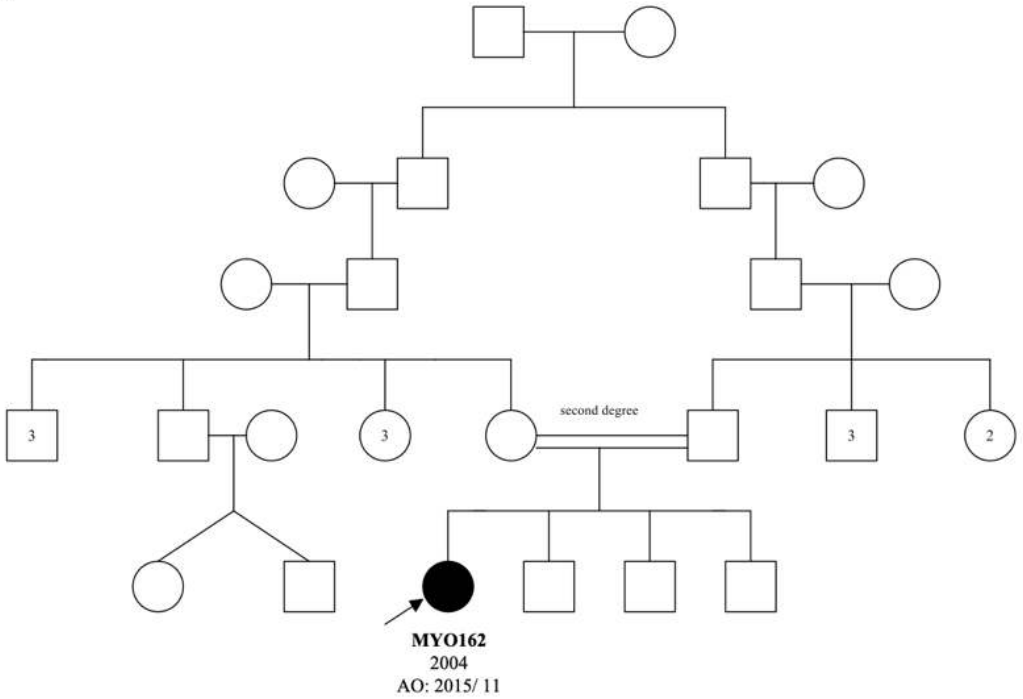


Figure 5.8 Family 7, unsolved case.

NDAL ID: MYO163
Date of Birth/Origin: 1990/ Adıyaman
Age of Onset: 2011/ 21
Consanguinity: First degree
Clinician: Dr. Aysun Soysal

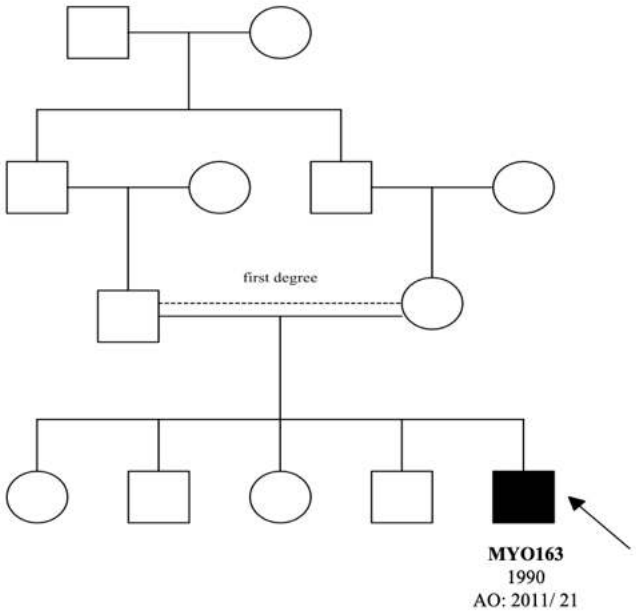


Figure 5.9 Family 8, unsolved case.

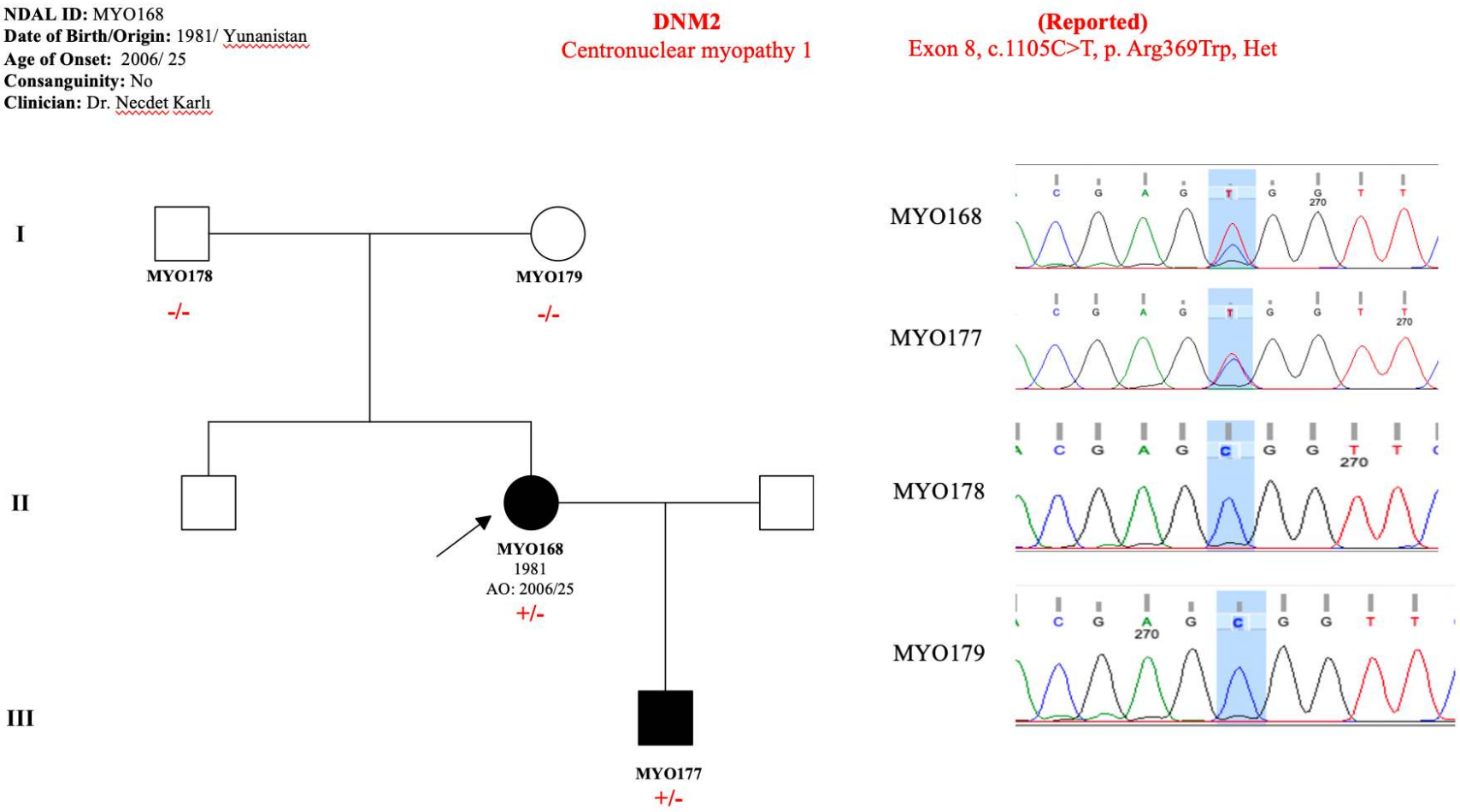


Figure 5.10 Family 9, solved with *DNM2*. During Sanger validation, no mutation was detected in index family member, suggesting that it is *de novo*.

NDAL ID: MYO169
Date of Birth/Origin: 1991/ Batman
Age of Onset: 2012/ 21
Consanguinity: Distant relative
Clinician: Dr. Aysun Soysal

STIM1
Myopathy, tubular aggregate, 1

(Reported)
Exon 3, c.326A>C, p. His109Pro, Het

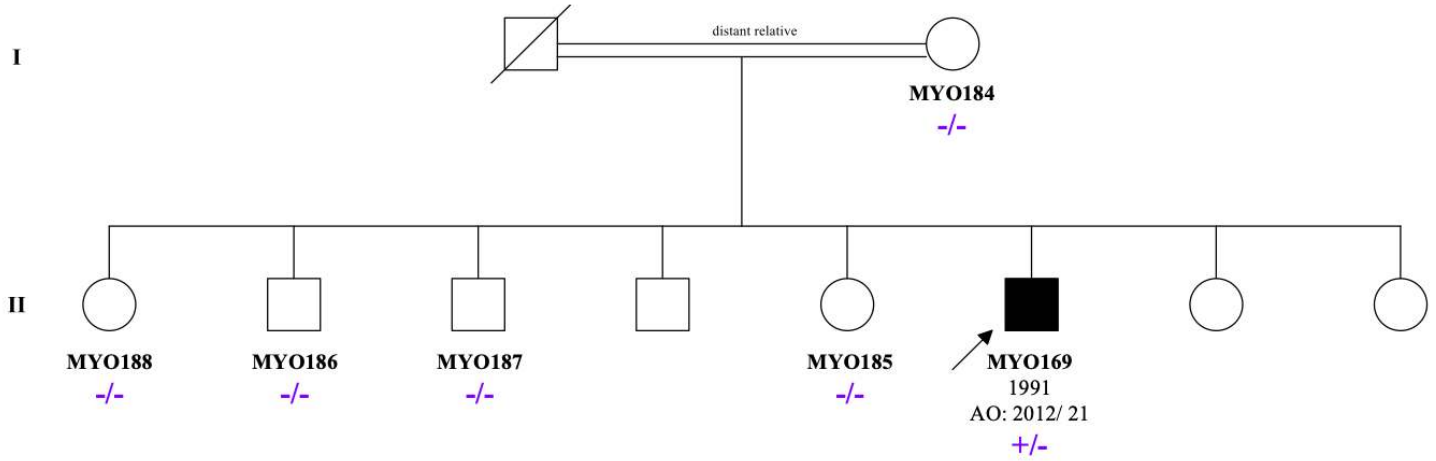


Figure 5.11 Family 10, solved with *STIM1*. During Sanger validation, the mutation was not detected in any family member, indicating that it is *de novo*.

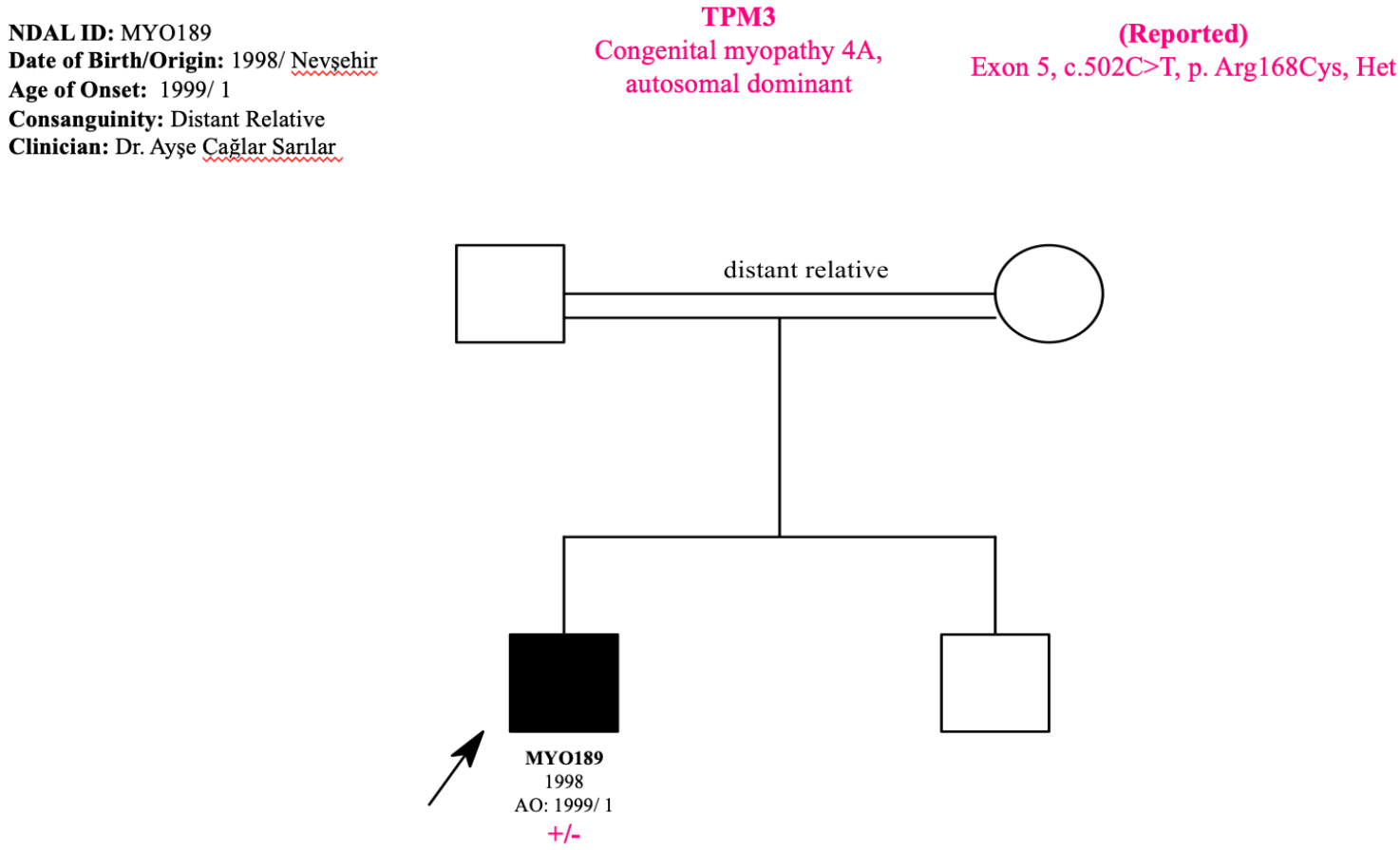


Figure 5.12 Family 11, solved with *TPM3*. Sanger validation was not conducted for this family; however, similar complaints were not present in the parents. Therefore, the mutation is presumed to be *de novo*.

NDAL ID: MYO191
Date of Birth/Origin: 2009/ Azerbaycan
Age of Onset: 2015/ 6
Consanguinity: No
Clinician: Dr. Vefa İsmayilova

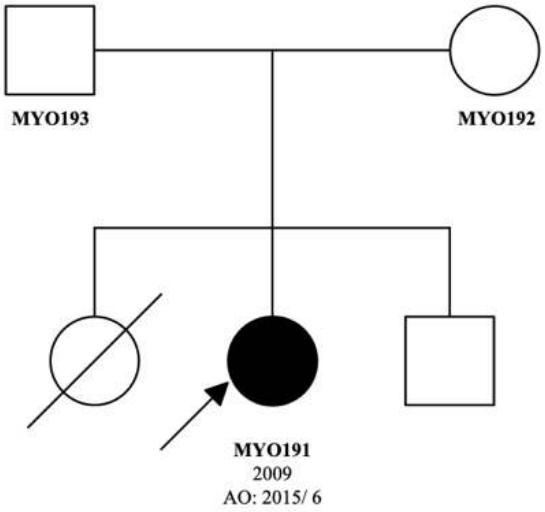


Figure 5.13 Family 12, sporadic unsolved case.

NDAL ID: MYO194
Date of Birth/Origin: 1989/ Azerbaycan
Age of Onset: 2001/ 12
Consanguinity: No
Clinician: Dr. Vefa İsmayilova

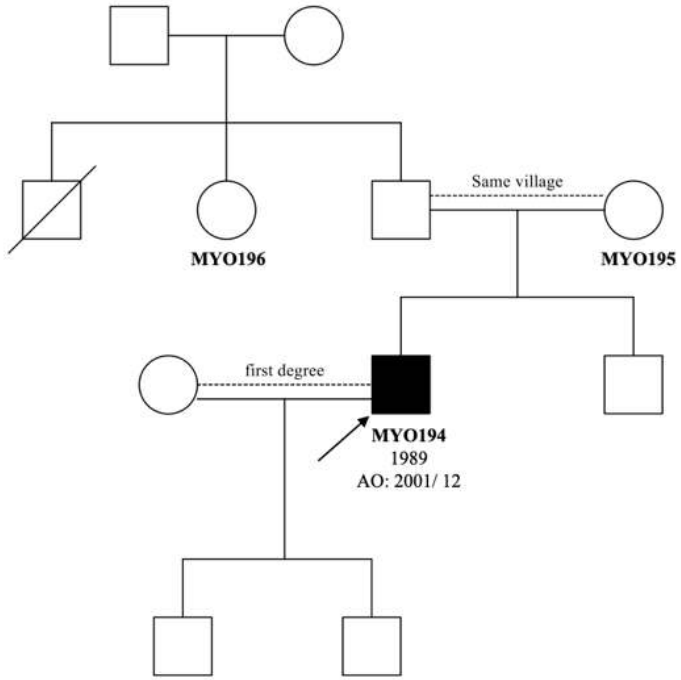


Figure 5.14 Family 13, unsolved case.

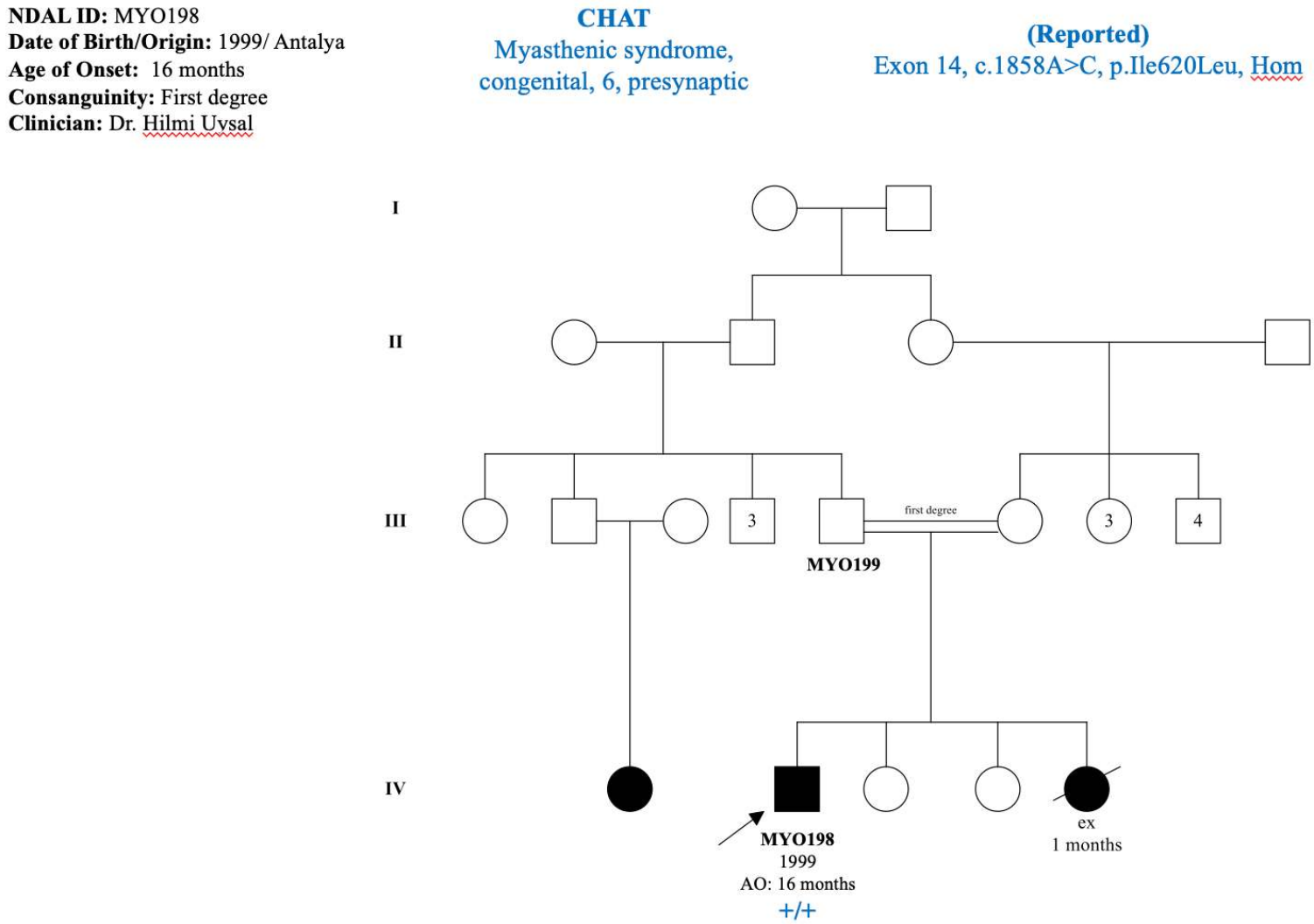


Figure 5.15 Family 14, solved with *CHRNE*. Sanger validation was not performed on this family.

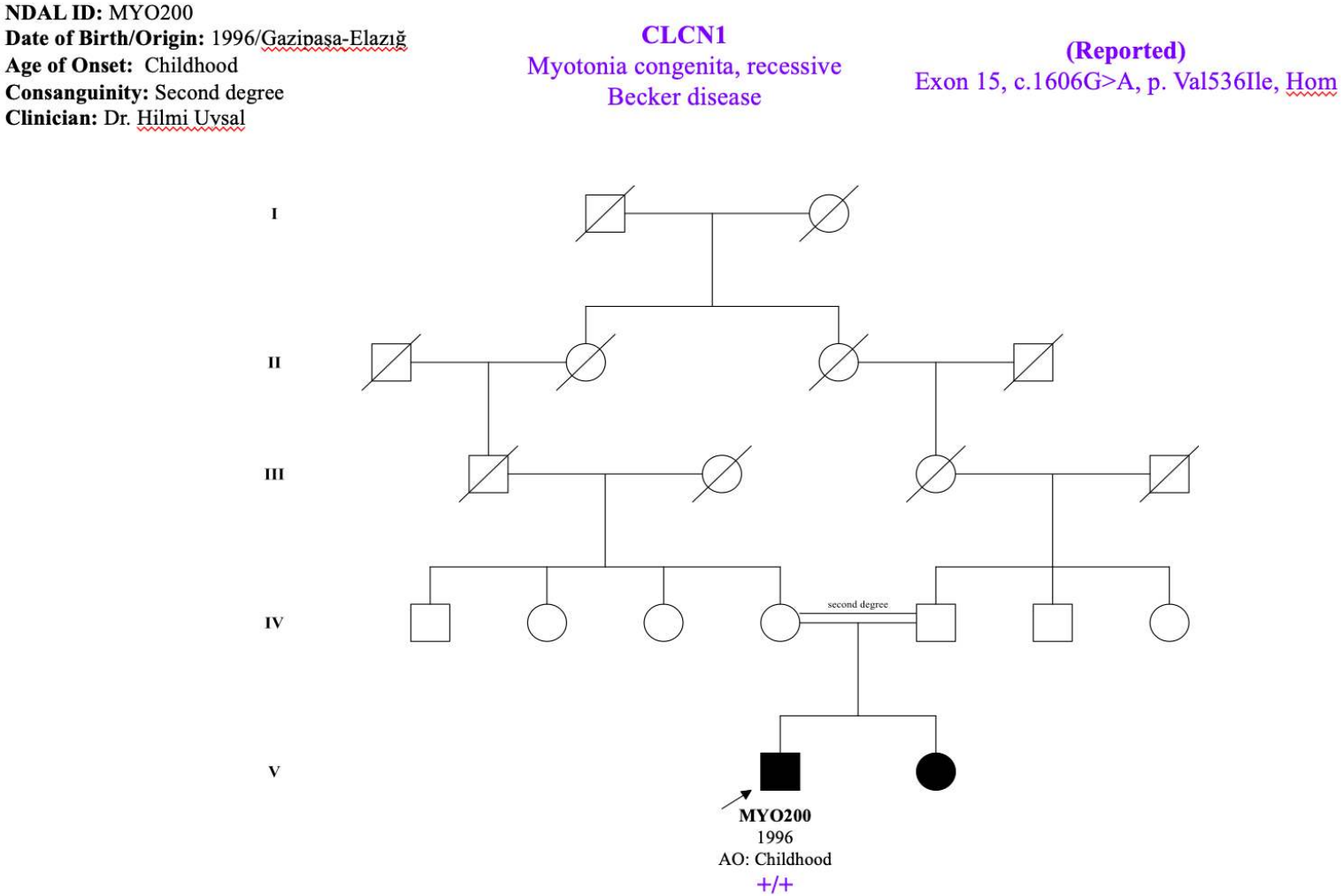


Figure 5.16 Family 15, solved with *CLCN1*. Sanger validation was not performed on this family.

NDAL ID: MYO207
Date of Birth/Origin: 1984/Antalya
Age of Onset: 1991/ 7
Consanguinity: First degree
Clinician: Dr. Hilmi Uysal

CLCN1
Myotonia congenita, recessive
Becker disease

(Reported)
Exon 3, c.313C>T, p. Arg105Cys, Het
(Reported)
Exon 4, c.501C>G , p.Phe167Leu, Het
(Reported)
Exon 22, c.2533G>A, p.Gly845Ser, Het

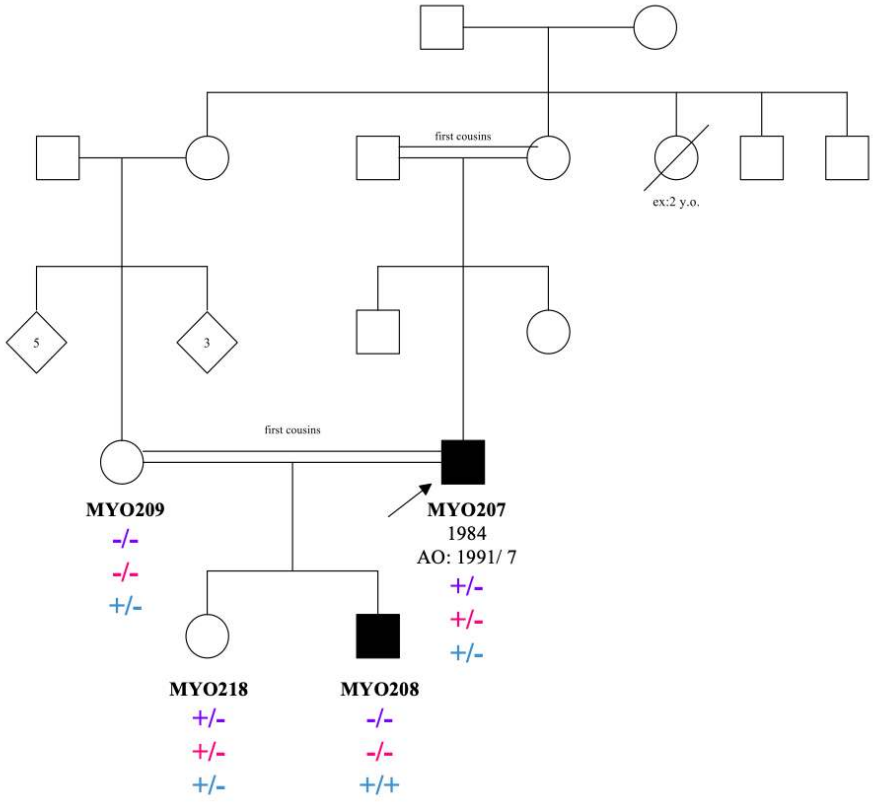


Figure 5.17 Family 16, solved with *CLCN1*.

NDAL ID: MYO210
Date of Birth/Origin: 2002/ Kayseri
Age of Onset: 2023/ 21
Consanguinity: Distant relative
Clinician: Dr. Ayşe Çağlar Sarılar

PYGM
Glycogen Storage Disease
(McArdle disease)

(Reported)
Exon 1, c.148C>T, p.Arg50Ter, Hom

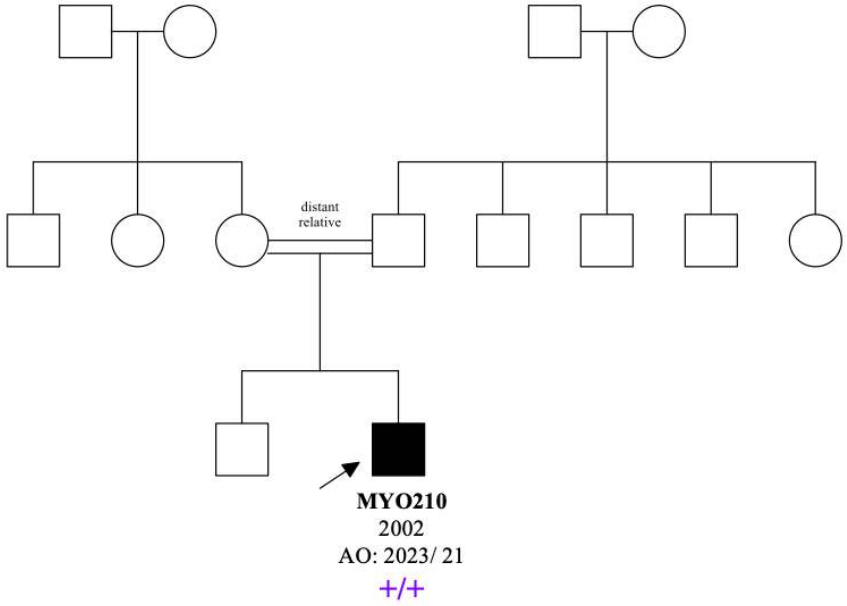


Figure 5.18 Family 17, solved with *PYGM*. Sanger sequencing was not performed on this family.

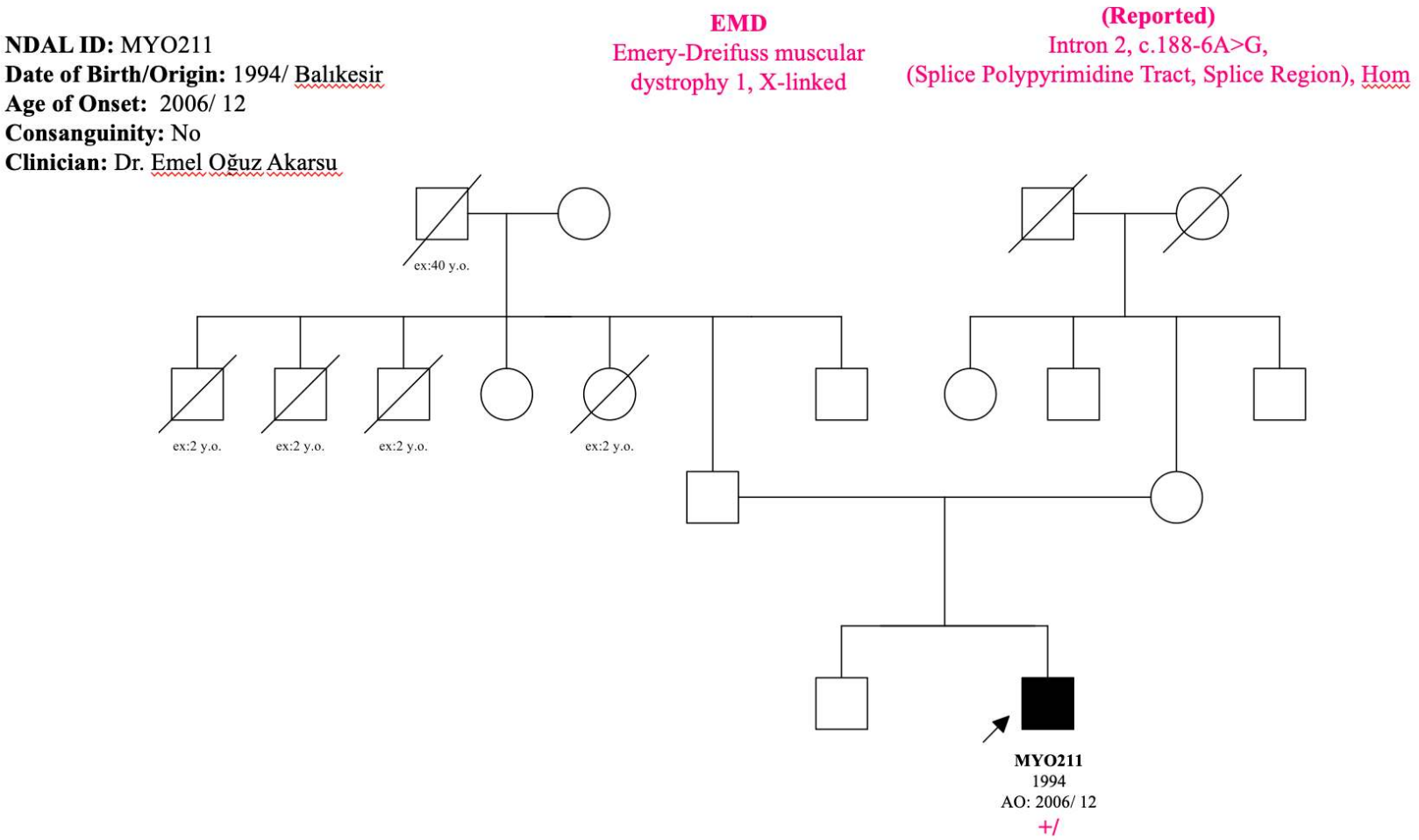


Figure 5.19 Family 18, solved with *EMD*. The *EMD* gene is located on the X chromosome. Because MYO211 is male, the mutation shows a hemizygous pattern.

NDAL ID: MYO212
Date of Birth/Origin: 1960/ Konya
Age of Onset: 1973/ 13
Consanguinity: Same village
Clinician: Dr. Hilmi Uysal

CAPN3
Muscular dystrophy, limb-girdle, autosomal recessive 1

(Reported)
Exon 4, c.598_612del, p.Phe200_Leu204del, Hom

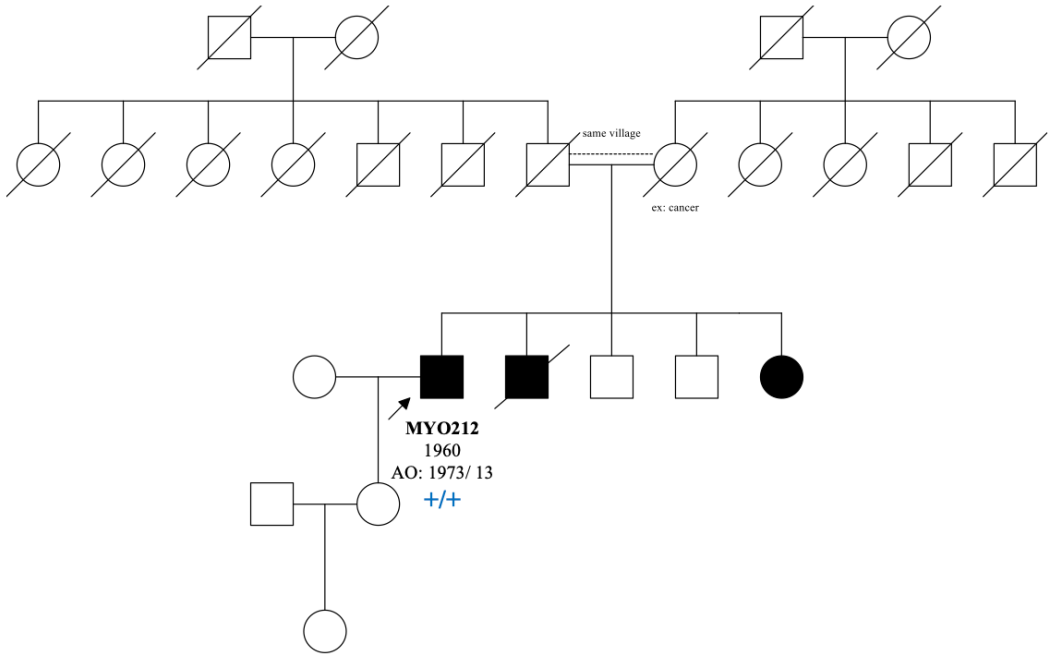


Figure 5.20 Family 19, solved with *CAPN3*. Sanger sequencing was not performed on this family.

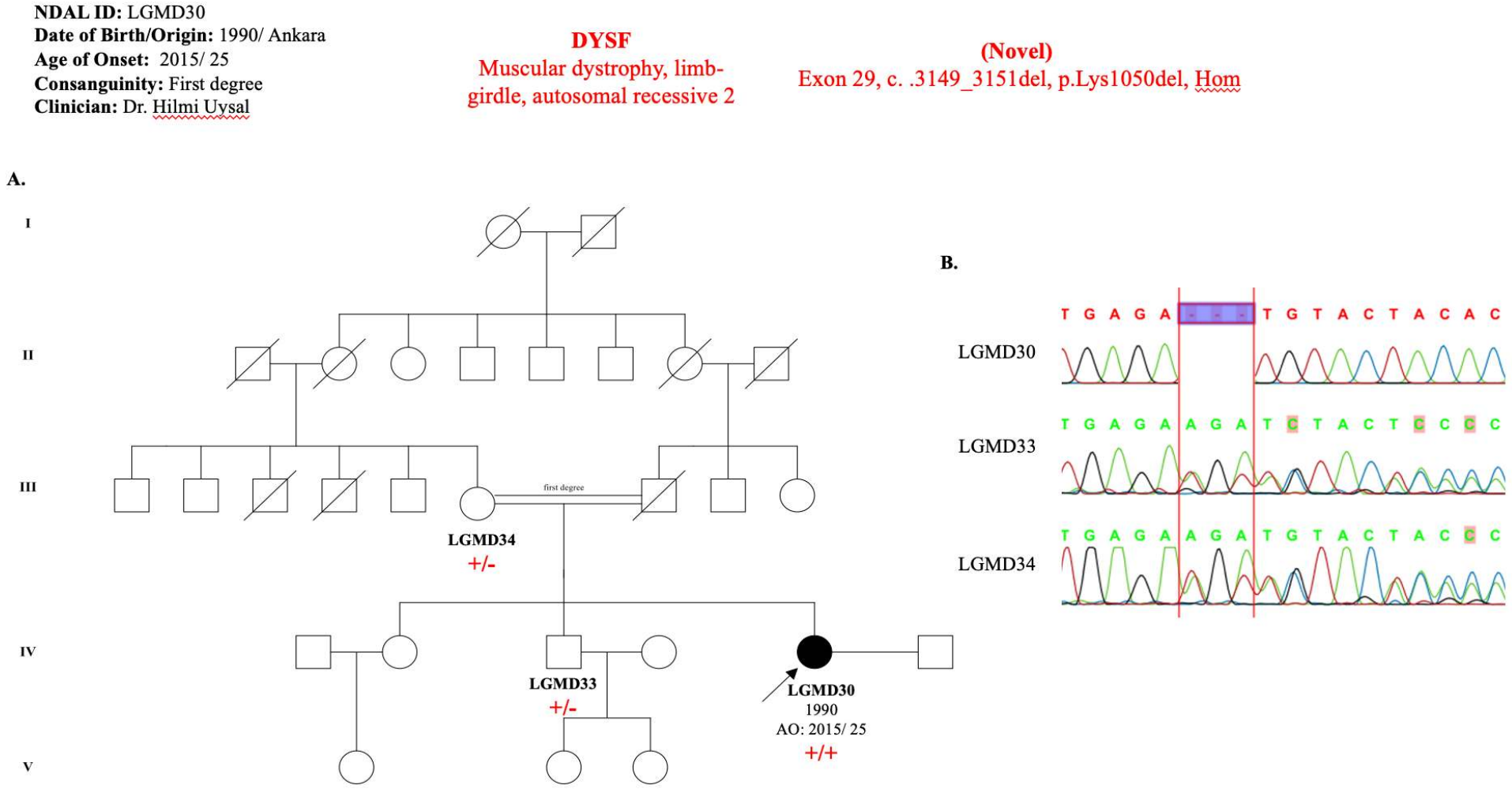


Figure 5.21 Family 20, solved with *DYSF*. **A.** Family tree of LGMD30. **B.** Sanger sequencing of mutation in *DYSF*.

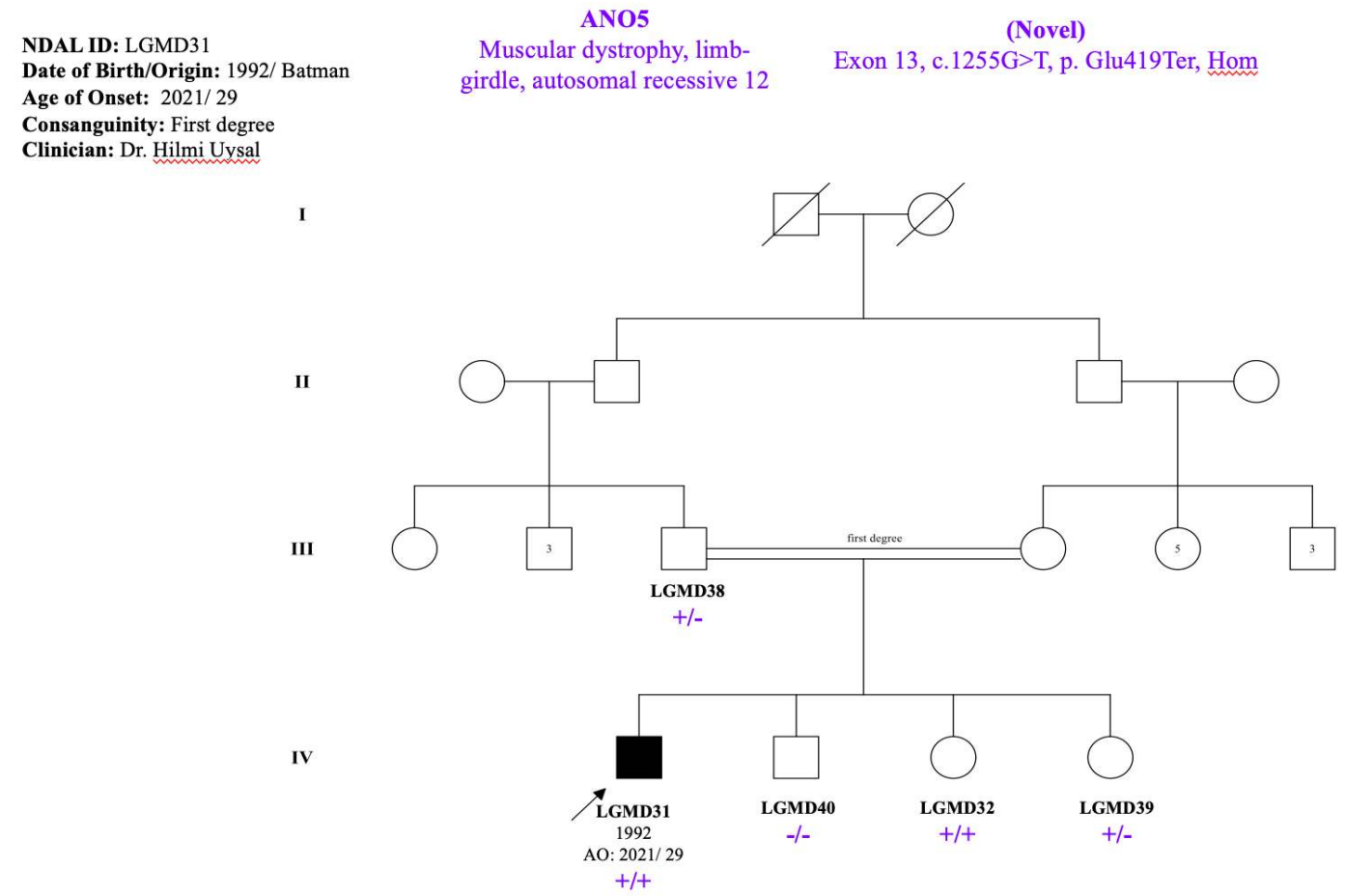


Figure 5.22 Family 21, solved with *ANO5* gene. According to Sanger validation LGMD32 is presymptomatic.

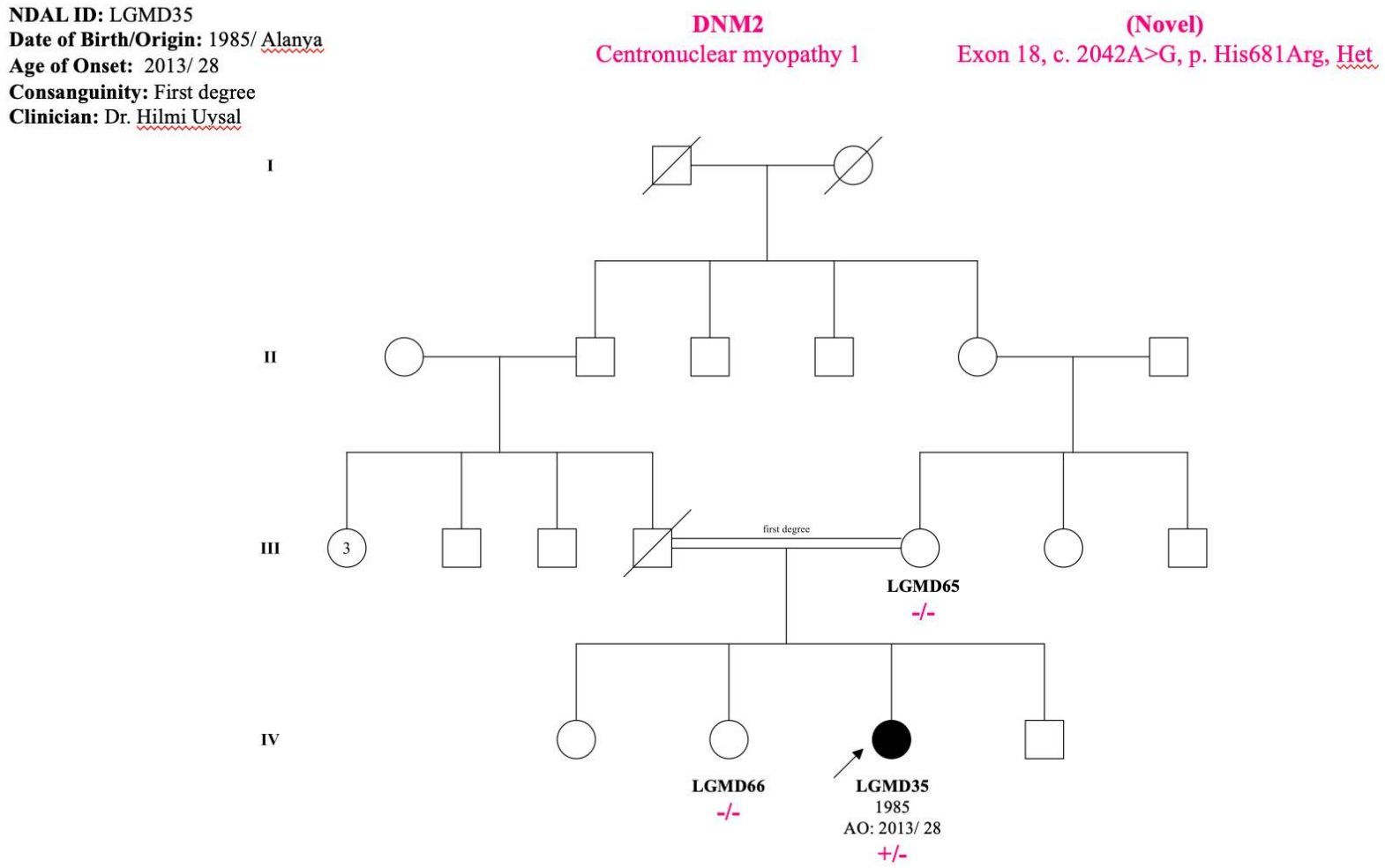


Figure 5.23 Family 22 solved with *DNM2*.

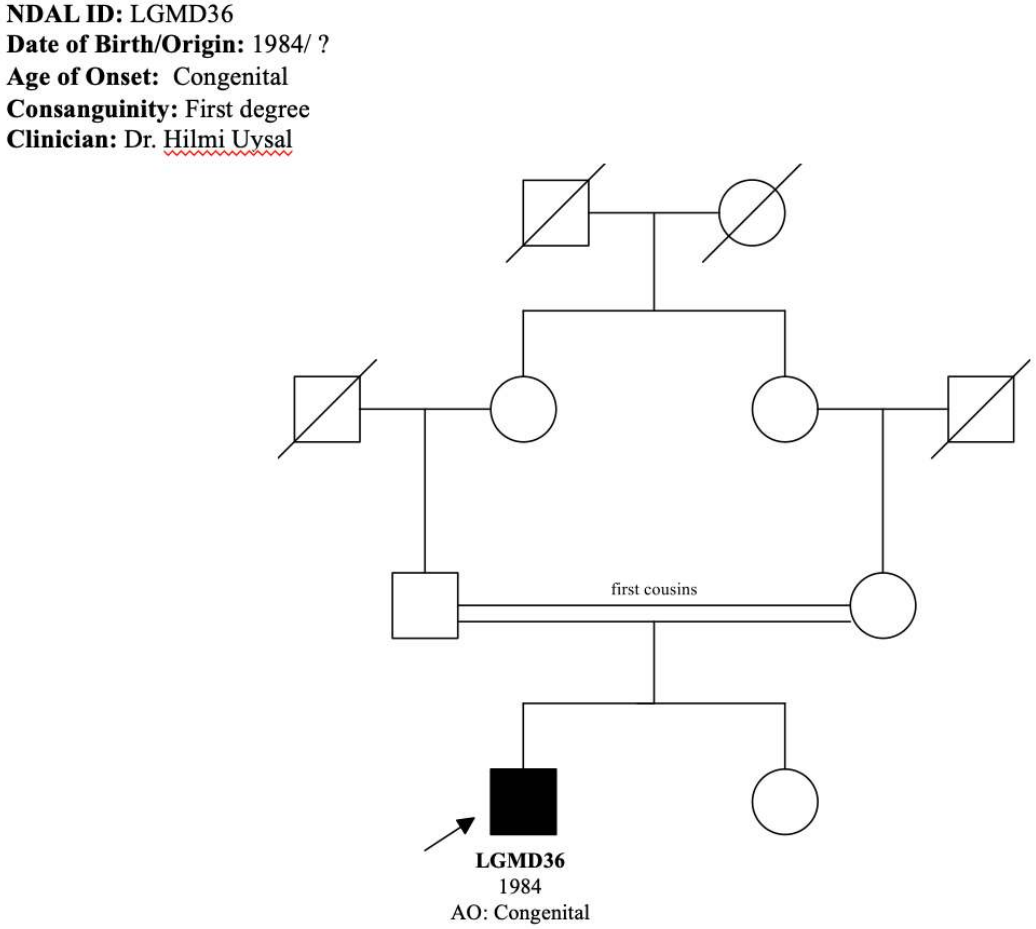


Figure 5.24 Family 23 unsolved case.

NDAL ID: LGMD52
Date of Birth/Origin: 1989/ Antalya
Age of Onset: 1996/ 7
Consanguinity: Distant relative
Clinician: Dr. Hilmi Uysal

SGCA
Muscular dystrophy, limb-girdle, autosomal recessive 3

(Reported)
Exon 5, c. 409G>A, p. Glu137Lys, Hom

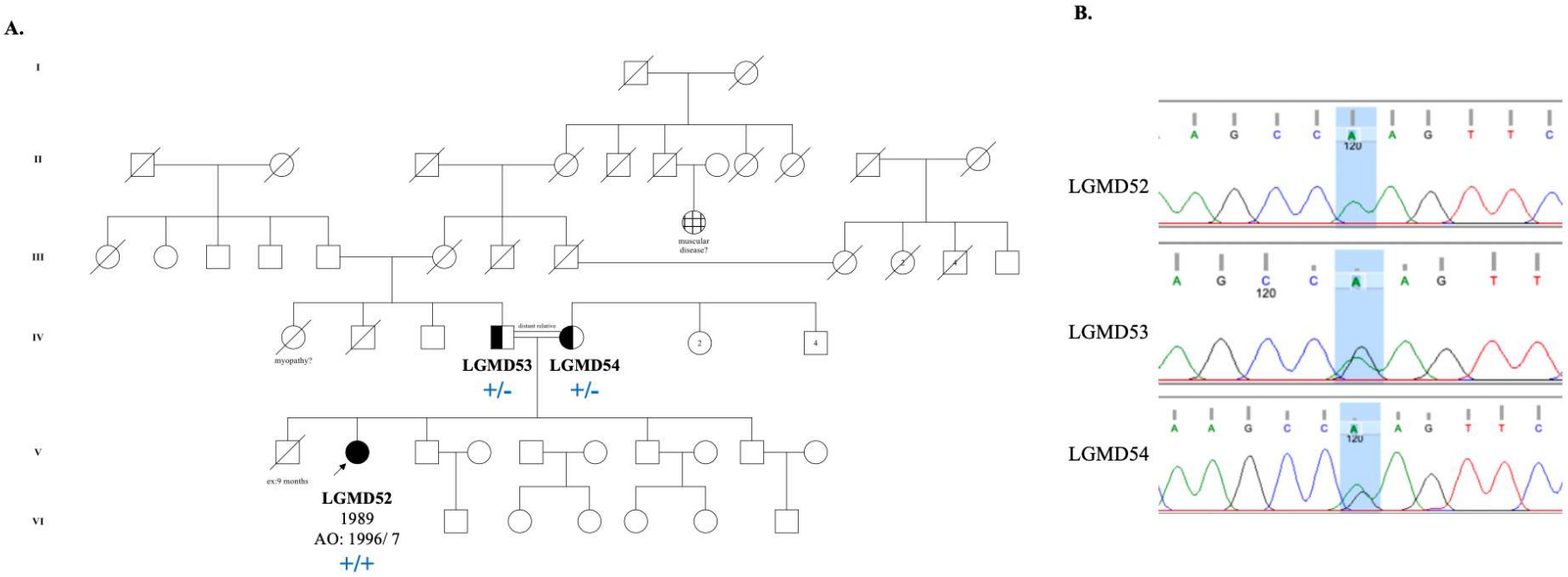


Figure 5.25 Family 24 solved with *SGCA*.

NDAL ID: LGMD55
Date of Birth/Origin: 1978/ ?
Age of Onset: 2003/ 25
Consanguinity: Same village
Clinician: Dr. Hilmi Uysal

DYSF
Muscular dystrophy, limb-girdle, autosomal recessive 2

(Reported)
Exon 19, c.1717C>T, p.Arg573Trp, Hom

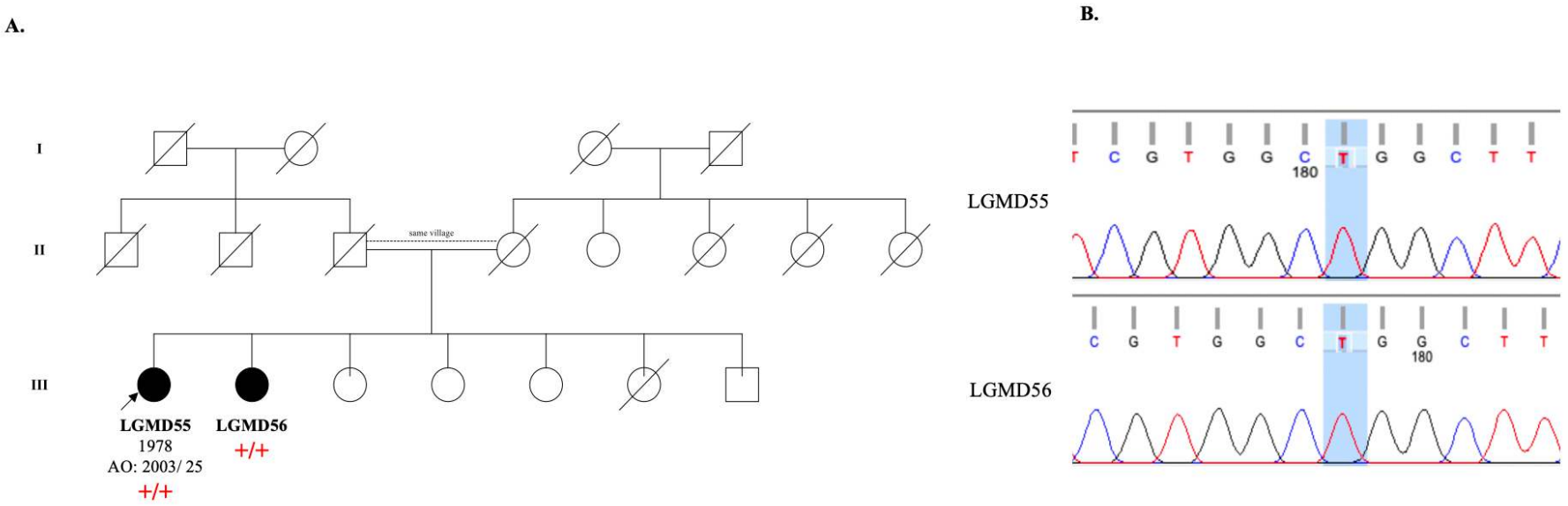


Figure 5.26 Family 25 solved with *DYSF*.

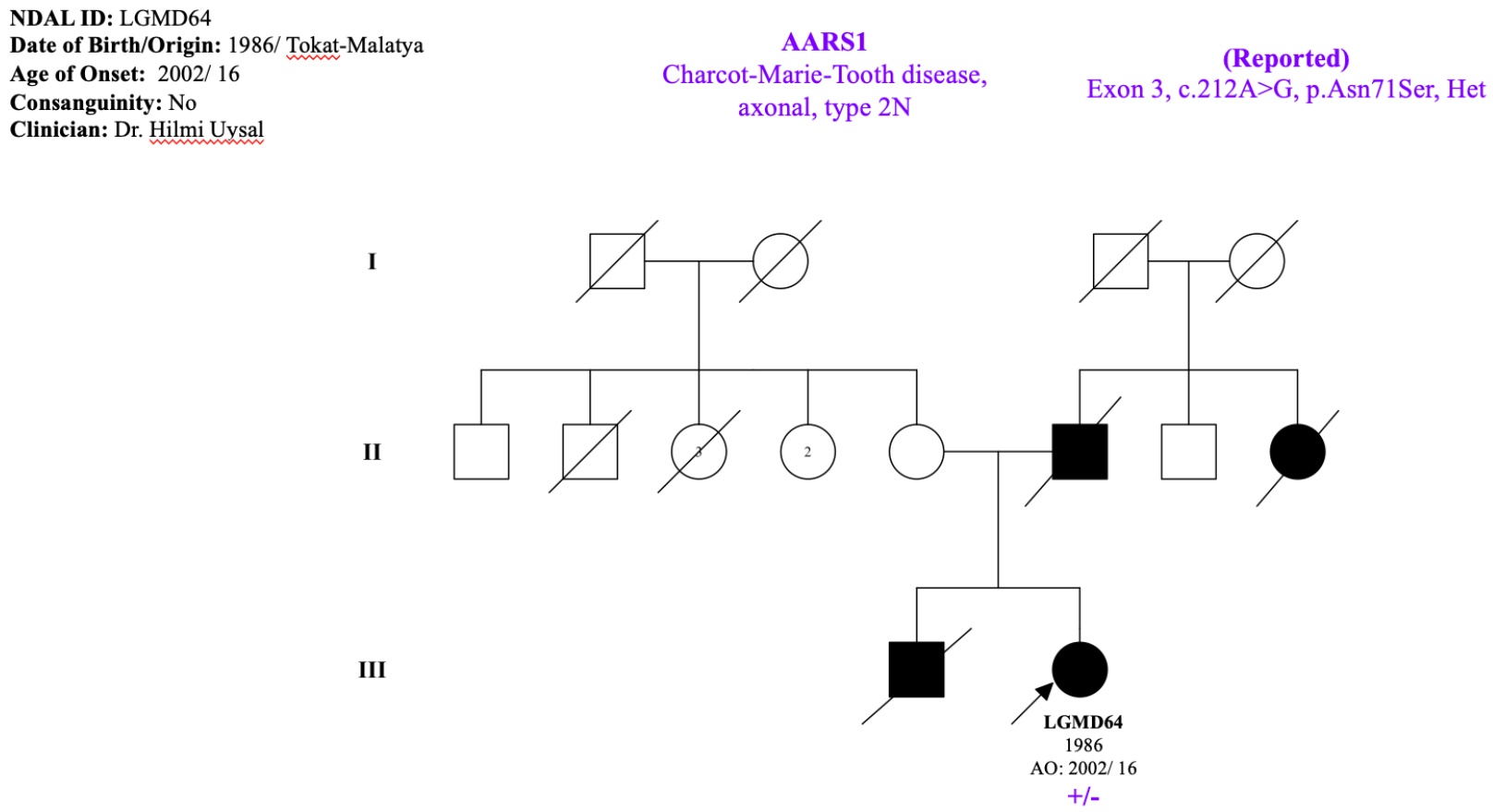


Figure 5.27 Family 26 solved with *AARS1*. Sanger validation was not performed on this family.

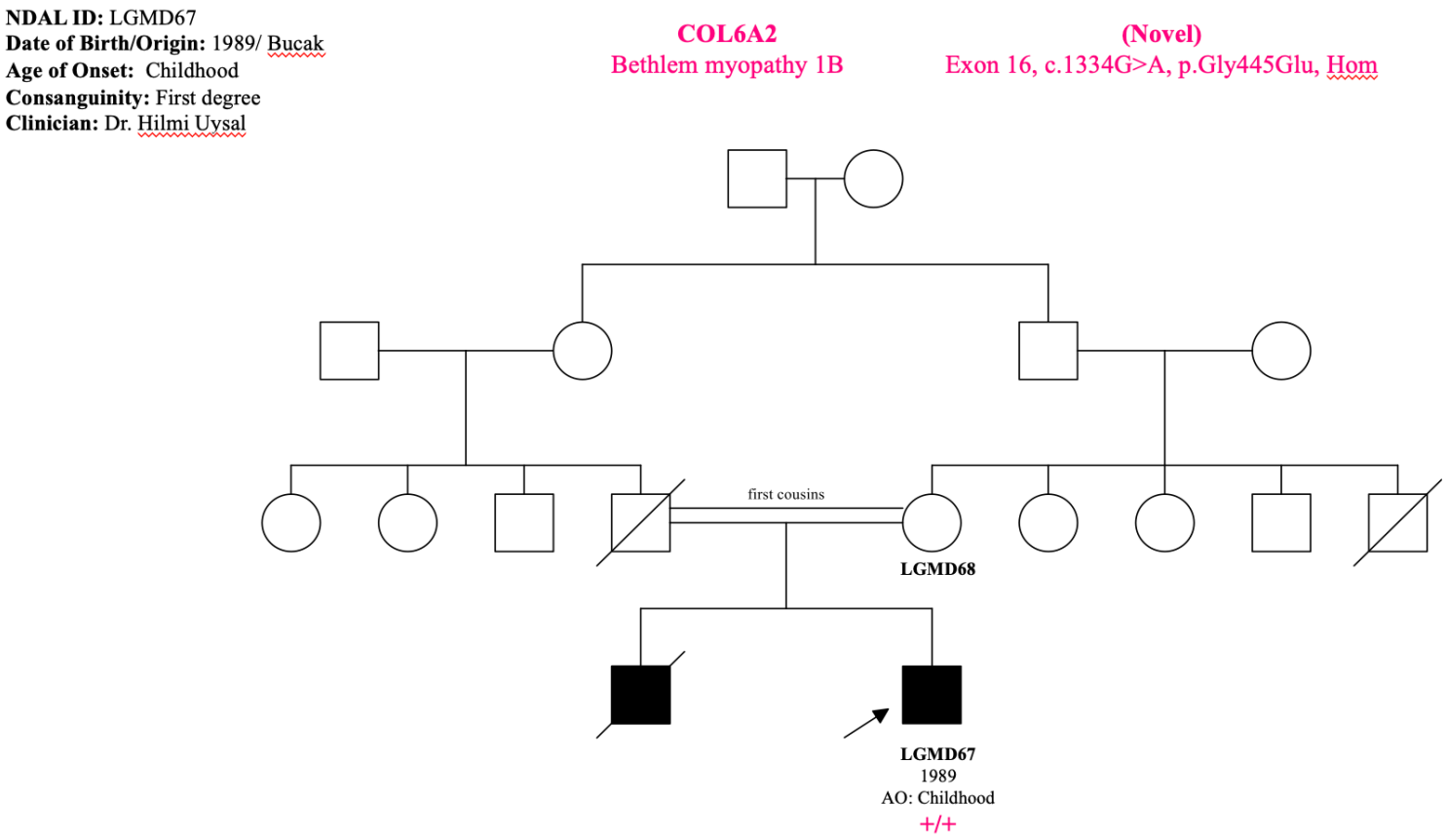


Figure 5.28 Family 27 solved with *COL6A2*. Sanger validation was not performed on this family.

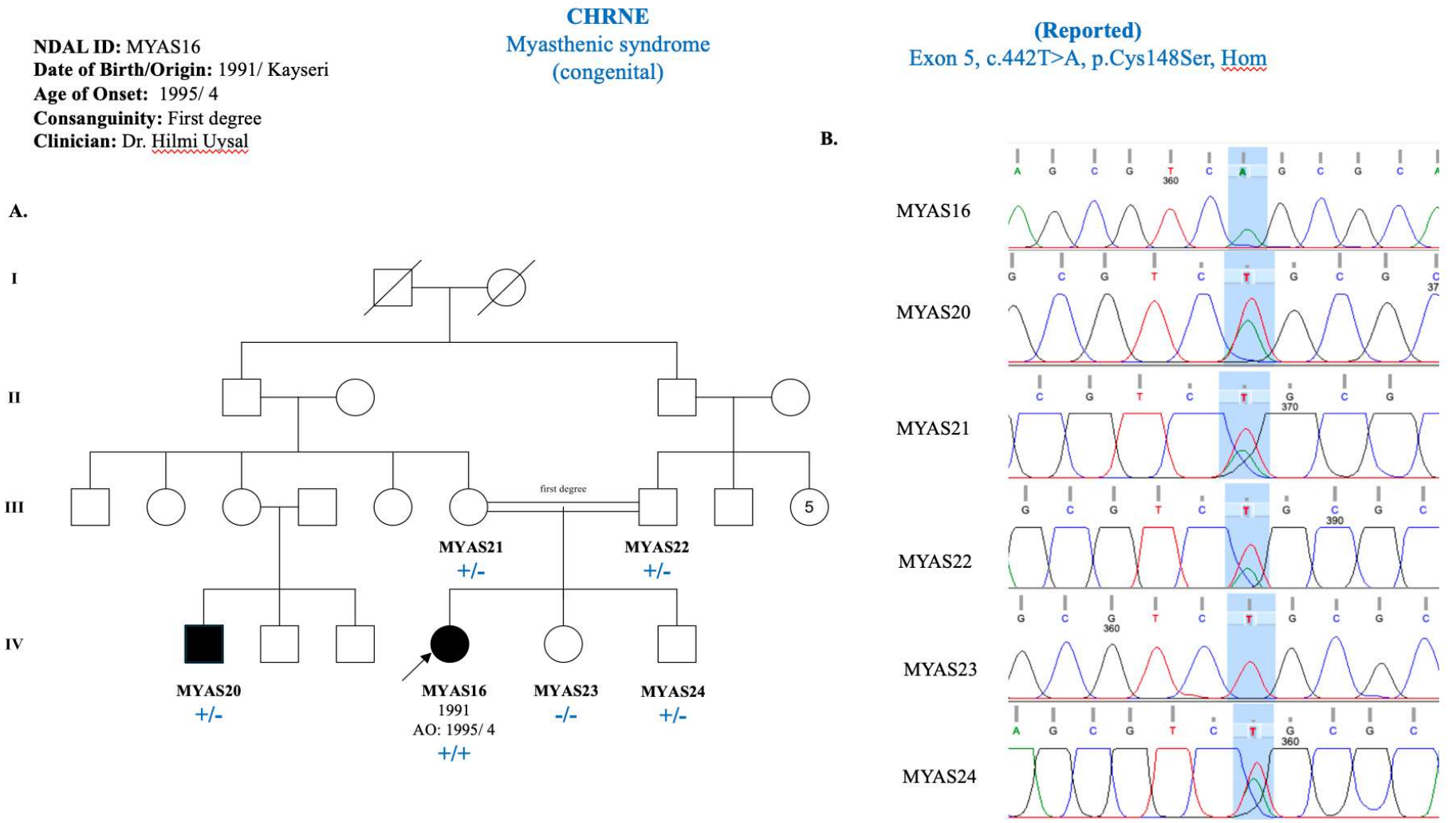


Figure 5.29 Family 28 solved with *CHRNE*. During Sanger validation, her affected cousin was found to carry the mutation in a heterozygous state. Unfortunately, for this family, a heterozygous mutation alone is not sufficient to explain the manifestation of symptoms.

NDAL ID: MYAS18
Date of Birth/Origin: 1979/ Isparta
Age of Onset: 1981/ 2.5
Consanguinity: No
Clinician: Dr. Hilmi Uysal

CHRNE
Myasthenic syndrome
(congenital)

(Reported)
Exon 7
c.610G>A
(Missense)
p.Glu204Lys
Het

(Novel)
Exon 7
c.675del(CT>C)
(Frameshift)
p.Glu227ArgfsTer73
Het

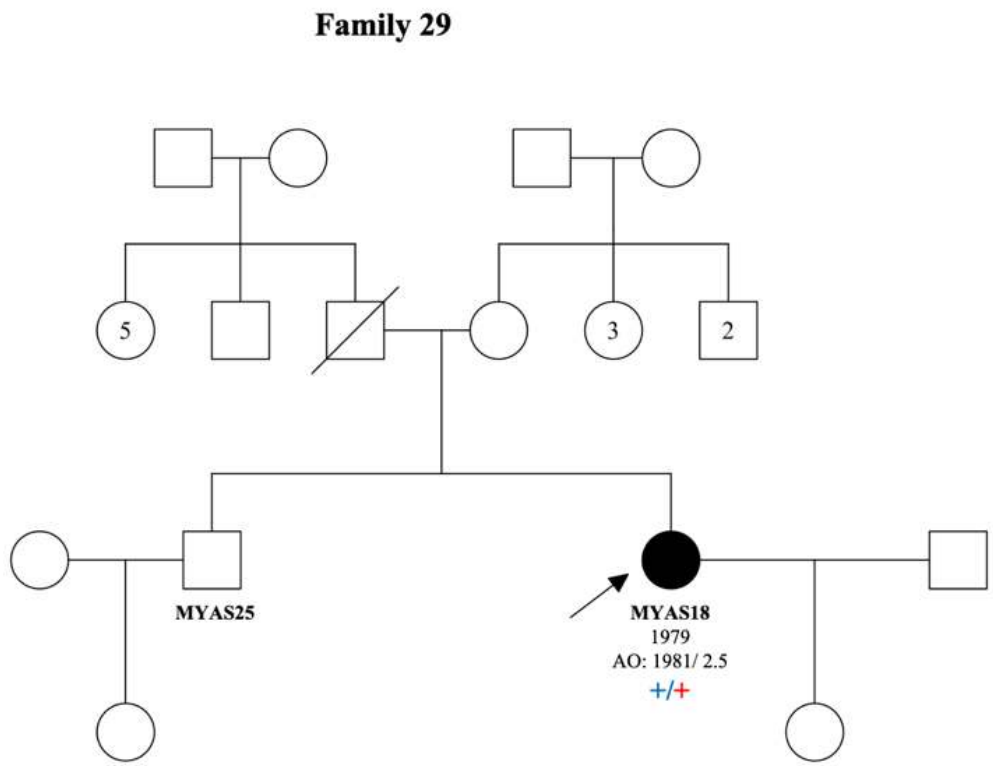


Figure 5.30 Family 29 solved with *CHRNE*.

NDAL ID: MYAS27
Date of Birth/Origin: 1961/ Bulgarisatan
Age of Onset: 2019/ 58
Consanguinity: No
Clinician: Dr. Mustafa Bakar

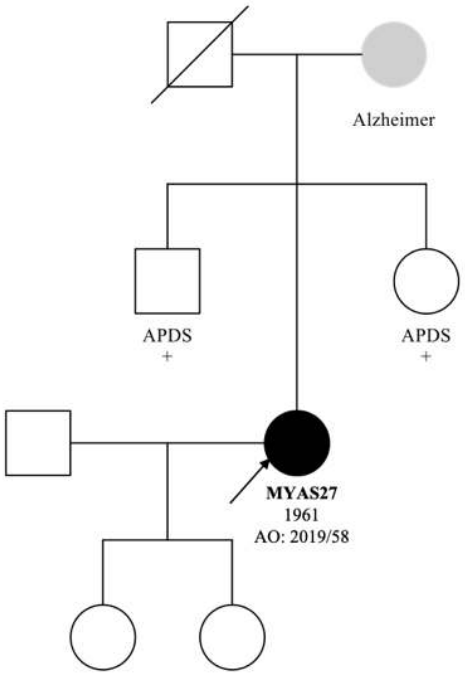


Figure 5.31 Family 30 sporadic unsolved case.

Chapter 6: DISCUSSION

In this thesis, a total of 30 index patients/ families underwent comprehensive whole exome sequencing. Seven affected family members were also included in the thesis. The analysis revealed that five families exhibited an autosomal dominant (AD), while 22 demonstrated an autosomal recessive (AR) inheritance. The remaining three families were categorized as apparently sporadic cases. Consanguinity was present in 60% of families within the studied cohort. Causal variants were successfully identified in 23 of the 30 cases analyzed. Specifically, 16 families presented with biallelic variants, and one presented as hemizygous, while five families exhibited heterozygous causative variants following an autosomal dominant pattern of inheritance. Sporadic cases remained unsolved in the study. Although the numbers here are small, the diagnostic rate aligns with the existing literature on the resolution of neuromuscular diseases through WES. (Ghaoui et al., 2015); (Krenn et al., 2023).

6.1. Decoding Genetic Complexity: Unveiling the Neuromuscular Landscape through Whole Exome Sequencing in a Turkish Cohort

In our study, encompassing a total of 30 families, we made substantial progress in unraveling the genetic landscape of observed pathogenic variants. A total of 23 different variants distributed across 16 distinct genes were identified in this cohort (Table 5.1). Our findings extended further with unique genetic markers identified in individual families, including *AARS1, ANO5, CAPN3, CHAT, CHRNE, CLCN1, COL6A2, DNM2, DYSF, EMD, GNE, PYGM, RYR1, SGCA, STIM1* and *TPM3.* These findings underscore the diverse genetic foundations and inheritance patterns present in the Turkish families studied, offering valuable insights for continued research and clinical applications.

6.1.1. Myopathy Genes

AARS1, ANO5, CAPN3, CLCN1, COL6A2, DNM2, DYSF, EMD, GNE, PYGM, RYR1, SGCA, STIM1, and *TPM3* are genes associated with myopathy and its subgroups, inherited in an autosomal dominant (AD) and/or autosomal recessive (AR) manner. Notably, despite the initial diagnosis of myopathy by the attending physician, WES results for patient LGMD64, with suspicion of myopathy, were found to be associated with a distinct neuromuscular disease (CMT2N). This family was resolved through the identification of a pathogenic variant in the AARS1 gene, as outlined in the article published by Heather M. McLaughlin et al. in 2012.

These instances underscore the overlapping symptoms of neuromuscular diseases. The genetic information provided to the physicians proved valuable for patients in the diagnostic and treatment processes.

6.1.1.1. Genes in Congenital Myopathies

DNM2

Autosomal Dominant Centronuclear Myopathy (CNM, OMIM 160150) is a rare congenital muscle disease caused by mutations in the DNM2 gene, which is responsible for encoding dynamin2 (DNM2). Zuchner et al. (2005) reported that the DNM2 gene comprises 22 exons, including 20 coding exons, and dynamins consist of five domains.

These diseases are characterized by progressive muscle weakness and wasting, in which symptoms usually manifest in late childhood or adolescence. Due to the diverse range of symptoms, prediction of disease course is challenging for each patient. DNM2 has the ability to bind to microtubules and is a protein involved in the formation of microtubule bundles by contributing to the function of hydrolyzing GTP. In addition, it is also involved in vesicular transport processes, especially endocytosis (Bitoun et al., 2005).

DNM2-associated CNM displays a wide spectrum of clinical features, ranging from severe neonatal to moderate adult-onset forms with diverse histopathologic phenotypes (Fujise et al., 2022). Alterations in the Pleckstrin homolog (PH) domain of DNM2 is associated with severe neonatal onset, whereas milder phenotypes with later onset are typically due to mutations in the middle domain (MD). Most CNM-associated DNM2 variants reported are associated with either early onset or a severe phenotype e.g., p.E368K, p.R369Q (Fujise et al., 2022).

In this thesis, two families with a preliminary diagnosis of myopathy and LGMD phenotypes were shown to have two heterozygous variants in the DNM2 gene. There is a missense mutation in LGMD35 and another missense mutation in MYO168 and her affected son MYO177. The mutation in LGMD35 is a novel p. His681Arg (c.2042A>C) mutation. This variant score is VUS on prediction tools. Another mutation identified is p.Arg369Trp (c.1105C>T), a pathogenic mutation that can occur de novo and is already known in the literature to be the most frequent and severe DNM2 variant. Family segregation analysis, performed on the patients and their families, was in line with their DNM2-related CNM and CMT; it was shown that both cases developed the variant de novo and that MYO168 had transmitted the variant to his son. According to the information provided, LGMD35's age of onset is 28. The patient has a rather slowly progressive prognosis. The ages of onset in diseases associated with DNM2 have been reported in the first or second decades. The variant, p.

His681Arg (c.2042A>C), in the PRD domain, may have delayed the disease's course. MYO168, although the age at which the patient saw a specialist clinician and was firmly diagnosed corresponds to young adulthood, the patient has reported complaints since childhood, consistent with CNM.

RYR1

Congenital myopathy-1A (CMYP1A), susceptible to malignant hyperthermia, is an autosomal dominant disorder characterized by muscle weakness. While symptoms may start at older ages in some cases, they generally begin in infancy or early childhood. This condition is caused by a mutation in the ryanodine receptor-1 gene (RYR1), which affects calcium regulation in skeletal muscle. The RYR1 gene encodes ryanodine, which is a calcium release channel found in the membrane of the skeletal muscle's sarcoplasmic reticulum. RYR1 mutation caused various phenotype severities. The disorder is static or slowly progressing. Affected individuals typically show delayed motor development and usually manage to walk independently, but many have difficulty running or climbing stairs.

MYO150, born in 2010, whose father had Demyelinating Polyneuropathy and Subacute Combined Degeneration due to B12 deficiency, began experiencing muscle issues shortly after birth. He has struggled with walking and climbing stairs since childhood. As of March 2022, he started experiencing balance problems. Additionally, he has hearing impairment in one ear and color blindness. WES analysis revealed that he carries the RYR1-the p. Arg4861His c.14582G>A variant in heterozygous state. This variant is located in a well-conserved region and was first reported by Monnier et al. in 2001. In their study, the same mutation was found in three unrelated patients. They noted that despite being unrelated, patients from families CCD07, CCD09, and CCD15 exhibited similar core patterns (Table 1). Since 2001, this mutation has been mentioned in numerous publications. Ultimately, the symptoms observed in our patient (MYO150) align with the disease description.

Table 6.1 The Most Common Genes in the Cohort and Their Associated Diseases and Clinical Manifestations.

	Age of Onset	**First described Symptoms**	**Latest examination**
CCD07	Infancy	Delay motor development (DMM) Started walking at 2 years 6 months	Age:31 Ambulatory, proximal weakness in lower and upper limbs, scoliosis, tendon contractures
CCD09	Birth	DMM Hypotonia	Age: 2 years 6 months Walking with help, muscle weakness, tendon contractures, scoliosis.
CCD15	Childhood	Difficulty running and climbing stairs	Age:31 Proximal weakness and myogenic EMG
MYO150	Birth	Hypotonia Difficulty walking and climbing stairs	Age:22 Balanced problems

STIM1

Tubular aggregate myopathy is a condition mainly affecting the skeletal muscles, which are responsible for body movement. It typically starts in childhood and worsens over time, causing muscle pain, cramps, or weakness. The legs are usually more affected, but the arms can be, too. People with this condition may find running, climbing stairs, or standing up difficult. It can also affect walking style and lead to joint deformities. Muscle biopsies show tubular aggregates, inner nuclei and rarely vacuoles. A specific genetic mutation in the STIM1 gene has been identified as the cause of tubular aggregate myopathy (TAM). STIM1 is crucial for calcium- sensing in the endoplasmic reticulum, and mutations in this gene were found in a highly conserved region involved in calcium binding.

MYO169, born in 1991 in Batman and of Mardin origin, experienced bilateral hearing loss after a seizure at around1.5 years old. He had walking difficulties and weakness since birth and started to walk on his fingers at the age of 5-6 years. Walking remained challenging until 21 years old, when he sought medical help due to worsening mobility and difficulty standing up. After WES analysis, a mutation, p. His109Pro, c.326A>C, was found in the STIM1 gene. This variation has been reported as pathogenic in the literature. Changes in the conserved region of p. His109 can lead to various clinical presentations.

TPM3

Congenital myopathy 4a, also known as CMYP4A, is a genetic disorder that affects muscles and has different names like myopathy with fiber-type disproportion, CFTD, nemaline myopathy 1 (NEM1), and cap myopathy 1 (CAPM1). It is an autosomal dominant disease that causes muscle weakness in infancy or childhood. The severity and type of weakness can vary, but most people with this condition have delayed motor development and weak muscles, especially in the limbs and neck, making it hard to run and causing fatigue easily. Many also have breathing problems, facial muscle weakness, high arched palate, myasthenia, scapular winging, and scoliosis. Muscle biopsies can show different findings, like nemaline rods and cap structures. CMYP4A is caused by a mutation in the TPM3 gene, which plays an essential role in muscle contraction regulation.

MYO189, born in 1998 in Göreme, had trouble holding his head up as a newborn. At the age of 1, he began walking but experienced frequent falls. Climbing stairs posed difficulties, and trouble getting up from a seated position without support has been reported. The physician's physical examination indicated upper and lower extremity involvement; autosomal dominant congenital myopathy was considered as the preliminary diagnosis. WES analysis revealed that he carries a variant in the TPM3 gene, p. Arg168Cys, in a heterozygous state.

In the literature, an adult patient with a mixed morphological phenotype characterized by a combination of cap structures and nemaline bodies has been reported. The presence of these two distinct lesions (caps and rods) was confirmed by both light and electron microscopy. Exome sequencing of the patient revealed that the disease-causing mutation was p. Arg168Cys. This alteration was confirmed by Sanger sequencing and was not present in the parents, indicating that the mutation had occurred de novo. The clinical course of the disease was generally stable until the age of 32, when progressive exertional dyspnea associated with severe fatigue and decreased walking endurance developed. The residue 168 is considered a hotspot by Yuen et al., 2015) as different mutations in the codon (e.g., p.Arg168Gly and p.Arg168His) have also been reported to cause different diseases. Even the same mutation can lead to different phenotypes in the same family, as indicated in Table 2. Here, we see that genetic analysis alone does not always yield 100% results for differential diagnosis; it is a complex process, and different approaches are important for accurate diagnosis and treatment.

Table 6.2 *TPM3* gene mutations Arg168 residue. The table was adapted by Yuen et al., 2015. MYO189 added.

Patient	Mutation	Disease	Age of Onset
4	R168G	CTFD	10
5	R168H	CTFD	40
6a	R168H	NM	20
6b	R168H	CTFD	56
7	R168C	Cap	3
8	R168C	CTFD	19
10	R168H	NM	53
MYO189	**R168C**	**?**	**1**

6.1.1.2. Genes in LGMD

COL6A2

Bethlem myopathy-1 (BTHLM1) is a congenital muscular dystrophy characterized by distal joint laxity and a combination of distal and proximal joint contractures. Weakness usually begins in middle childhood or adolescence, but progression is slow, and walking is usually preserved into adulthood. BTHLM1B is caused by a heterozygous mutation in the *COL6A2* gene. This gene encodes the protein collagen VI alpha 2, a beaded filamentous collagen found in most connective tissues. The product of this gene has been shown to bind to extracellular matrix proteins. This interaction explains the importance of collagen in the regulation of matrix components.

LGMD67, born in 1989, suffered from progressive muscle weakness since childhood. CK is elevated. He had a sibling who was affected and had passed away in 2009. EMG showed primary muscle fiber involvement in the proximal muscles. WES analysis revealed that the *COL6A2* gene carries a novel variant in a homozygous state (p. Gly445Glu: c.1334G>A). The patient's symptoms were found to be compatible with Bethlem myopathy by his physician.

EMD

Emery-Dreifuss muscular dystrophy (EDMD1) is a degenerative myopathy characterized by muscle weakness and atrophy without affecting the nervous system. Flexion deformities of the elbows from early childhood, mild pectus excavatum, signs of cardiac involvement and

absence of muscle pseudohypertrophy, involvement of forearm muscles, and mental retardation are observed. Autosomal dominant and recessive Emery-Dreifuss muscular dystrophy are caused by mutations in the Lamin A/C gene whereas X-linked Emery-Dreifuss muscular dystrophy-1 (EDMD1) is caused by emerin. The protein, encoded by the *EMD* gene on chromosome Xq28, plays a crucial role in maintaining the structural integrity of the nucleus. It is located in the inner nuclear membrane and interacts with other nuclear envelope proteins, as well as components of the cytoskeleton. Emerin contains a LEM (LAP2, emerin, and MAN1) domain, which mediates its binding to the chromatin-associated protein called Barrier-to-Autointegration Factor (BAF). BAF is involved in various nuclear processes, including chromatin organization, nuclear assembly, and cell division. By binding to BAF, emerin contributes to the regulation of higher-order chromatin structure and nuclear membrane integrity.

MYO211, a male born in 1994, initially presented with complaints of easy fatigue during childhood, which progressed to difficulty walking around the age of 12. He has also experienced weakness in his arms for the past 3-4 years and exhibits a foot deformity. Muscle biopsy findings indicate hypertrophic-scattered atrophic fibers, suggestive of non-specific, non-inflammatory myopathy. Neurological examination revealed prominent weakness, particularly in the right upper and lower limbs, with no sensory deficits noted. Deep tendon reflexes (DTR) were found to be normative. The patient underwent pacemaker implantation six years ago. There is no reported history of similar diseases in his family. However, it's noteworthy that three older brothers and one older sister all died at the age of two for unknown reasons, indicating a potential genetic component. WES identified a hemizygous variant in the *EMD* gene: c.188-6A>G. This variant, located at the intron2/exon3 boundary (Figure 1), is predicted to create a new splicing acceptor site, likely disrupting normal gene function.

A similar variant in the *EMD* gene was reported by Calame et al. in a 19-year-old male patient with muscular dystrophy. This patient exhibited comparable clinical features, including frequent falls, easy fatigue, joint stiffness, and motor difficulties progressing with age. Both cases also presented with bilateral ankle and elbow contractures, along with weakness in various muscle groups. While specific information about cardiac issues in Calame et al.'s patients is lacking, it is noted that cardiac problems may occur in EMD patients, supported by MYO211's history of pacemaker implantation. Based on the clinical presentation, family history, genetic analysis, and comparison with a previously reported case, this patient is diagnosed with Emery-Dreifuss muscular dystrophy type1 (EDMD1).

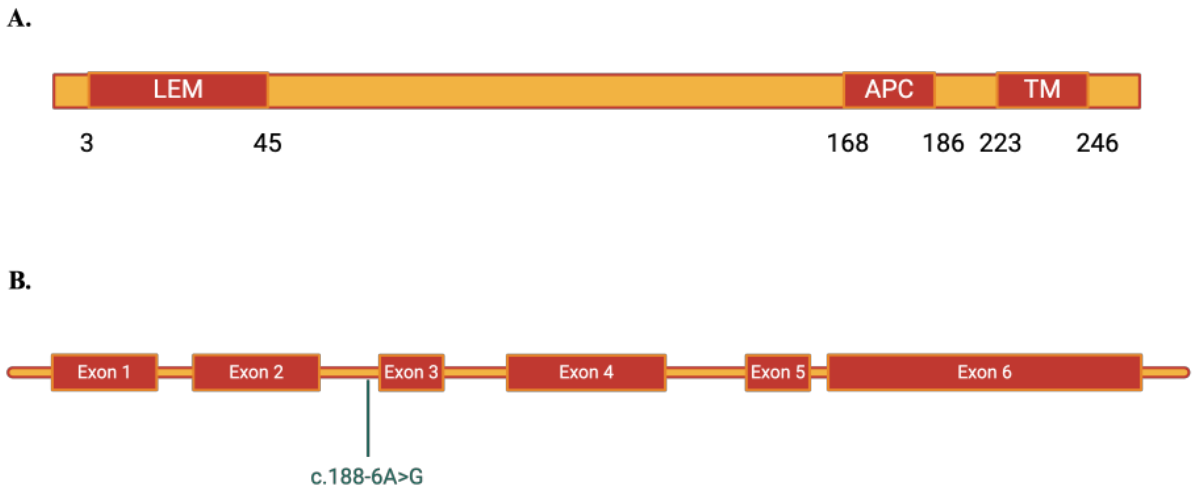


Figure 6.1 A. Functional domains of the emerin protein. **B.** Exons of the *EMD* gene. The mutation is a splice donor region variant which creates a new splicing acceptor site.

ANO5

Anoctamin5 (ANO5) is a 913 amino acid, 107 kD protein belonging to the anoctamin family. Encoded by the *ANO5* gene, this family member comprises 22 exons, of which exons 5 and 20 are hotspot regions (Soontrapa & Liewluck, 2022); (Savarese et al., 2015). ANO5 is characterized by eight transmembrane domains, typical of the anoctamin protein family (Stehlíková et al., 2014). The gene is specifically associated with the regulation of calcium transport within muscle tissue. The ANO5 gene encodes a protein crucial for muscle contraction by regulating calcium transport. Mutations in ANO5 can compromise its function, especially by affecting calcium channels in the cell membrane. Disruptions in this regulatory process can lead to diverse LGMDR12 and MMD3 (Soontrapa & Liewluck, 2022). The prevalent ANO5 mutations include c.191dupA (p. N64KfsX15) in exon 5, c.1898+1G > A in intron 17, c.2272C > T (p. Arg758Cys) in exon 20, and c.692G > T (p. Gly231Val) in exon 8.

In this thesis, two distinct families with LGMD phenotypes were shown to have biallelic variants in the ANO5 gene: one homozygous non-sense in LGMD31 and a compound heterozygous mutation in MYO141. Although the mutation in LGMD31, p. Glu419Ter (c.1255G>T), is novel, its scores in prediction tools are appropriate for being pathogenic. MYO141 had a pathogenic mutation, p. Asn64Lys*fs15 (c191dupA), that was already widely known as pathogenic in the literature. He additionally carries the p. Arg758His (c.2273G>A) variant, which is novel. One of the common mutations, (p. Arg758Cys) c.2272C>T, affects the same codon (Figure 2).

In different studies, the changes p. Asn64Lys*fs15 (c191dupA), and p. Arg758Cys (c.2272C>T) were reported in compound heterozygosity. Hicks et.al, 2010 identified these two variants in a German patient diagnosed with LGMD at the age of 35. The age of disease onset of this patient was described as late adolescence, and a decrease in sports performance and severe atrophy of the quadriceps were observed. CK level was reported as high. Our patient, MYO141, was born in 1974, and his age of onset is 46. His complaints are muscle weakness in the left leg after heavy exercise. During examination, atrophy was observed in the left leg. An increase in CK level was also observed. MYO141, who is a farmer, can still perform his daily work.

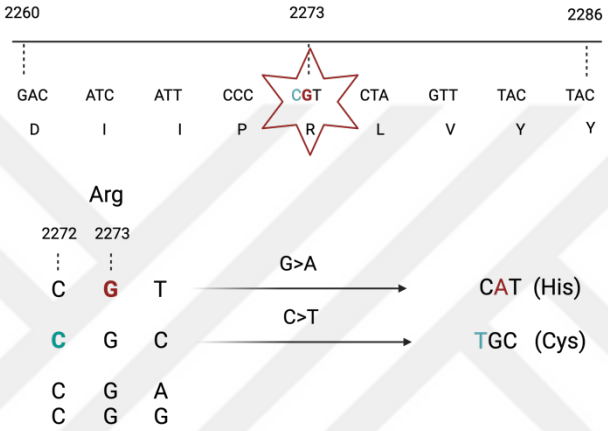


Figure 6.2 Single nucleotide changes of Arg to His or Cys.

CAPN3

Limb-girdle muscular dystrophy type2A (LGMD2A) is indeed the most common form of autosomal recessive LGMDs type 2. It is characterized by progressive weakness and wasting of muscles, particularly those around the shoulders and hips. LGMD2A is caused by mutations in the *CAPN3* gene, which is located on chromosome 15q15.1q21.1. This gene encodes the protein calpain-3, which is a proteolytic enzyme belonging to the calpain superfamily. Calpains are calcium-dependent cysteine proteases involved in various cellular processes, including signal transduction, cytoskeletal remodeling, and apoptosis. The *CAPN3* gene consists of 24 exons, and its protein product, calpain-3, has a molecular weight of approximately 94kDa. Calpain-3 plays a crucial role in muscle maintenance and repair, likely by regulating the turnover of proteins involved in muscle structure and function. Mutations in the *CAPN3* gene can disrupt the normal function of calpain-3, leading to muscle degeneration and the symptoms observed in LGMD2A patients (Chen et al., 2021).

According to a study conducted in 2007, it was found that one of the patients diagnosed with LGMD carried the c.550delA p. T184RfsX36 and c.598-612del p.F200_L204del variants in compound heterozygous state. The patient's age of onset was reported to be five years. The patient, who was seven years old in 2007, suffered from mild proximal muscle weakness in the upper and lower extremities (Stehlíková et al., 2014). Our patient, MYO212, carries this mutation homozygously and currently appears to be the first homozygous patient in the literature. Clinical symptoms started at the age of 3 with the inability to run or walk. Over time, weakness in the upper extremities was also added. There is no cranial involvement. There is no difficulty in swallowing. It has been reported that he cannot sleep due to sleep disturbance and shortness of breath, but respiratory arrest is mild. The disease was also reported in an older brother and a younger sister. In 2024, MYO212 had difficulty to come to the neurologic examination. It was reported that his sister's condition was more severe, however still with no requirement for non-invasive ventilation. Since we do not have her blood sample, we have not confirmed this variant in this patient yet. If we compare the two sibs, their ages of onset are close to each other, but the symptoms of MYO212 begin earlier. Myasthenia gravis has been excluded from this family.

DYSF

Miyoshi myopathy type 1 (MMD1), Limb Girdle Muscular Dystrophy Recessive 2 (LGMDR2), and anterior tibial onset distal myopathy are linked to the *DYSF* gene. *DYSF* encodes dysferlin, a protein essential for various muscle functions, and mutations in this gene lead to dysferlin deficiency (dysferlinopathy). These conditions typically emerge in adolescence or early adulthood, presenting symptoms such as loss of Achilles tendon reflexes, difficulty in climbing stairs, tiptoeing while standing, and a gradual, progressive weakening and atrophy of limb muscles. According to Bouchard and Tremblay, 72% of patients predominantly experience lower extremity weakness, with 15% affecting the proximal, 32% the distal, and 25% both simultaneously. Atrophy (27%), pain, stiffness, and cramps (13%) are commonly reported, along with significantly elevated CK levels, approximately 54 times higher than normal. Dysferlin, primarily a membrane protein in the sarcolemma, exhibits expression in various tissues, predominantly in skeletal muscles. It plays a pivotal role in functions such as membrane repair, vesicle fusion, and the regulation of molecules, including Ca2+ signaling. Although dysferlin is also expressed in the heart muscle, mutations in dysferlin have no observable impact on the cardiac phenotype.

The *DYSF* gene, encompassing 55 exons, codes for Dysferlin, a 237 kDa protein with a distinctive domain structure, including seven C2 (C2A2-C2G), three Fer (FERA, FERB, FERC), two nested DysF domains, and a C-terminal transmembrane domain. Presently, more than 700 mutations associated with dysferlinopathy are known, with a majority being missense and/or pathogenic mutations. Pathogenic variants in *DYSF* are distributed throughout the protein, but a notable concentration is observed within the DysF domain, with p. Trp999Cys being the most prevalent pathogenic variant.

This thesis presents findings indicating that two families, initially diagnosed with an LGMD phenotype, exhibit distinct homozygous variants in the *DYSF* gene. Patient LGMD30 carries an in-frame deletion (p. Lys1050del, c.3149_3151del), identified as a novel mutation with an LP score in prediction tools. On the other hand, LGMD55 and her sister LGMD56 have a missense mutation, p. Arg573Trp (c.1717C>T), previously reported as pathogenic in the literature (also known as p. Arg555Trp (c.1663C>T) with a different transcript number (NM_003494.4)). Family segregation analysis, aligned with clinical presentations compatible with DYSF-associated LGMDR2, confirmed these variants as causative. The onset of symptoms for LGMD30 occurred at 27 years, while LGMD55 experienced symptoms at 25 years, consistent with the reported age range for DYSF-related diseases.

In the literature, p. Arg573Trp, previously identified as pathogenic through a gene panel, was reported in a Korean male patient as one of the compound heterozygous variants, with the other allelic mutation being the common p. Trp999Cys. This patient had an asymptomatic childhood and adolescence, serving in the army. However, in his late 20s, he reported falling quickly and difficulty climbing stairs, leading to a diagnosis of "some type of myopathy" at 28. Symptoms progressed, and by age 55 he required a wheelchair.

SGCA

Sarcoglycanopathies (SGP) are a group of muscular dystrophy-type muscle disorders caused by mutations in SG genes, which encode sarcoglycans (SGs). One specific type, known as LGMD2D, occurs due to mutations in the α-SG gene (SGCA). *SGCA* mutations cause various symptoms, including progressive weakness and wasting of muscles around the shoulders and hips, frequent calf enlargement, respiratory insufficiency, and occasionally, heart complications. These genetic abnormalities lead to abnormal restructuring of the skeletal muscle and an elevation in serum creatine kinase levels. The clinical manifestations associated with mutations in SG genes, particularly α-SG, exhibit considerable heterogeneity even within affected families, with differences in age of onset and severity. Sarcoglycans are glycosylated

single-pass transmembrane proteins, with α-SG being a type I transmembrane protein, consisting of a short intracellular tail and a long extracellular domain. These proteins play a crucial role in maintaining the structural integrity of muscle fibers. Mutations in SG genes lead to the loss of this function, weakening muscle fibers and contributing to the development of sarcoglycanopathies.

LGMD52, born in 1989, suffered from lower motor neuron involvement that began at ages seven/ eight. Her symptoms were found to be compatible with LGMD by her physician. WES analysis revealed that the *SGCA* gene carries a variant in homozygous state p. Glu137Lys (c.409G>A) which has previously been reported as pathogenic. In 2002, in the article of Mognini et al. entitled "Alpha-Sarcoglycan Deficiency Featuring Exercise Intolerance and Myoglobinuria," an eight-year-old male patient complained of easy fatigue, HyperCchememia, and mild scapular winging. Muscle biopsy of the quadriceps muscle showed slight fiber size variability. Dystrophin was distributed normally, some enzymes had normal activities. They reported that the patient developed exercise intolerance and myoglobinuria in the following years. They reported that the patient carried compound heterozygous variants p. Arg284Cys and p. Glu137Lys in the SGCA gene. The study suggested that exercise intolerance and muscle breakdown could be common symptoms as the patient gets older, but they noted that this case was not severe. Another study by Soheili et al. in 2011 examined different SGCA mutations. They found that the p.E137K variant, similar like what the patient had, caused severe symptoms in 14 people because it altered the structure of the protein it codes for. In our patient, although the lower motor neuron involvement, which the doctor specifically noted, was suggestive of ALS, it solved p. Glu137Lys (c.409G>A) in *SGCA* homozygously.

6.1.1.3. Genes in Metabolic Myopathy

PYGM

Glycogen storage disease type V (McArdle Disease), a type of metabolic myopathy, is a disease characterized by a deficiency in muscle myophosphorylase activity. The myophosphorylase enzyme is encoded by the *PYGM* gene. *PYGM* is a gene consisting of 20 exons and 842 amino acids, and homozygous or compound heterozygous pathogenic variants in this gene cause McArdle disease. The disease usually presents with symptoms that vary from childhood to adulthood, including exercise intolerance, fatigue, myalgia, and muscle cramps. After exercise, there is often a marked increase in serum creatine kinase (CK) activity, and in severe cases, strenuous exercise leads to rhabdomyolysis. However, comorbidities with myoglobinuria (resulting in "dark urine") may also be observed.

MYO149, born in 1996, has parents who are bilaterally consanguineous and of Kurdish origin. She had complaints of muscle weakness and myalgia since childhood. She started walking when she was 18 months old and recalls falling behind her peers and getting tired easily at the age of 5-6. The patient, whose muscle enzyme levels increased, was diagnosed with rhabdomyolysis in 2014. WES analysis revealed homozygous p. Phe710del c.2128_2130del variant in the *PYGM* gene. This variant was previously reported as pathogenic in a study conducted in Turkey in 2017 (Inal-Gültekin et al., 2017). In this study, the P170del mutation, previously known as a benign polymorphism, was detected homozygously in a family from Southeastern Anatolia with a preliminary diagnosis of rhabdomyolysis, and it was demonstrated for the first time that this variant was pathogenic. In the Inal-Gultekin study, through intrafamilial segregation analysis, the homozygous state of the mutation was confirmed in the patient and her affected sister, while their healthy mother was identified as a heterozygous carrier for this variant. Since we did not have blood samples from MYO149's family members, we could not perform segregation analysis. However, the clinical symptoms of the two patients were found to be compatible with McArdle Disease.

MYO2010 was born in Kayseri in 2002. At the age of 21, he was hospitalized in the internal medicine ward due to urinal bleeding, and his CK was found to be around 37,000. As a result of interventions, his CK level decreased to 12,000, but it still remained high, prompting referral to a neurology service. As reported by his neurologist, MYO2010 has been experiencing significant fatigue since childhood and occasional cramps and persistent pain, especially in his legs. There is essential tremor in his left hand, and his sleep pattern is irregular. Deep tendon reflexes decreased only in his right lower extremity. The preliminary diagnosis provided to us was rhabdomyolysis and myopathy. Following WES analysis, the p. Arg50Ter, c.148C>T variant in the *PYGM* gene, previously reported as pathogenic in many publications, was homozygously detected in the patient. McArdle Disease, a common metabolic myopathy with a prevalence of 1 in 139,000 in Caucasians, is associated with the most common mutation (approximately 50%) in the same population, the p.R50X (c.148C>T). Mutations such as R50X introduce premature stop codons (UAA, UAG, and UGA) into the protein-coding gene sequences, resulting in premature arrest of translation and production of shortened proteins (Tarrasó et al., 2020). In the study by Inal-Gultekin et al., the R50X variant was noted for the first time in a Turkish patient. “Interestingly, p. Arg50*, the most common *PYGM* mutation reported in Caucasians of European descent, was seen in only one patient in our study group.” Again, due to the unavailability of blood samples from family members, intra-family

segregation analysis was not conducted. It is noteworthy that one out of every two McArdle disease patients carries this mutation, and despite our small sample size.

GNE

GNE myopathy is a rare genetic muscle disease characterized by progressive skeletal muscle atrophy due to mutations in *GNE*, the gene encoding the bifunctional UDP-N-acetylglucosamine (GlcNAc) 2-epimerase/N-acetylmannosamine (ManNAc) kinase enzyme. Although initially described in Japanese and Middle Eastern populations, patients with GNE myopathy have been reported worldwide. The prevalence of GNE myopathy is estimated to be approximately 1 to 9 per 1,000,000.

Patients with GNE myopathy typically present in early adulthood, between the ages of 20 and 40, with symptoms of anterior tibialis weakness, such as tripping, gait disturbance, inability to lift toes, or foot drop. GNE myopathy progresses slowly, often taking decades for patients to progress from the early to late stages of the disease GNE myopathy initially affects the distal muscles of the lower extremities, followed by the thigh muscles, with the quadriceps relatively spared. Gait is increasingly affected, resulting in a striding gait, increased risk of falls, and decreased balance. It is estimated that patients will require the use of a wheelchair approximately 10 to 20 years after the onset of symptoms. The upper extremities are affected 5-10 years after symptom onset and do not necessarily follow the distal-to-proximal progression seen in the lower extremities. In advanced stages of the disease, neck muscles may also be affected. Ultimately, disease progression can lead to complete loss of skeletal muscle function and dependence on caregivers. The disease does not affect swallowing or facial muscles and does not impair cognition, although respiratory muscle involvement has been described in the late stages of the disease.

The *GNE* gene encodes UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase (GNE), a bifunctional and rate-limiting enzyme in sialic acid biosynthesis and a regulator of cell surface sialylation. The activities of GNE enzymatic domains are interrelated; mutations in one domain also affect the enzyme activity of the other domain. The function of *GNE* appears to be critical during development. GNE is present in the nucleus where it may play a role in muscle regeneration after injury. *GNE* protein interacts with proteins involved in cytoskeletal organization and development and has been shown to play a role in survival apoptosis signaling proliferation, gene expression, and oxidative stress.

GNE myopathy is an autosomal recessive hereditary disease. There are ethnic GNE founder mutations found in Middle Eastern (Met743Thr), Japanese (Cys44Ser, Asp207Val, and

Val603Leu), Romani Bulgarian (Ile618Thr), and Indian/Asian (Val727Met) populations (Mullen et al., 2022). Besides founder mutations, more than 200 *GNE* gene mutations in the epimerase or kinase-encoding domains of the enzyme have been reported in more than 950 *GNE* myopathy patients worldwide. Interestingly, no patient has been reported to have two null mutations, supporting further evidence that the absence of GNE function is incompatible with life. Since only approximately 950 individuals have been reported, it is estimated that many patients with the disease are misdiagnosed or undiagnosed.

MYO139 was born in 1967. He consulted a doctor with a complaint of gait disturbance that started at the age of 40 and has been progressing for 15 years. According to his physician's report, the patient has lower extremity involvement. A similar disease was described in his sister. With WES analysis, we first identified a heterozygous and previously reported c.760A>G, p. Met254Val variant in the *FUS* gene, which is associated with both ALS with or without FTLD and Tremor Hereditary Essential, 4, inherited as an autosomal dominant in MYO139. When we performed intrafamilial segregation analysis, the index individual, and his affected sister (MYO146) carried the variant as heterozygous, while his healthy mother, brother, and sister were WT for the variant. However, as both patients' phenotypes were not suitable, the WES data was re-examined, and we determined that he carried a novel p. Tyr621His c.1861T>C variant, associated with GNE myopathy, in homozygous state. Later, family segregation analysis, it revealed that his sister was also homozygous for this variant. Final opinions for MYO139 and MYO146 were received from their doctors, and the cases were resolved. (Because it is related to metabolic activities, GNE myopathy is placed in the Metabolic myopathies class.)

6.1.1.4. Genes in Channelopathies

CLCN1

Myotonia congenita (MC), the most common form of NDM, can be inherited in either autosomal dominant (Thomsen disease) or recessive (Becker) patterns. It is characterized by muscle fiber hyperexcitability, stiffness, and hypertrophy. Becker type myotonia, usually more severe, is more prevalent. The two forms of MC differ in clinical features such as age of onset, spreading of myotonia, and the presence of transient muscular weakness, which is typical only in the recessive trait.

MYO200, born in 1996, has had problems with muscle relaxation since his childhood. The doctor gave the preliminary diagnosis as Thomsen-Becker myotonia. A similar disease was reported in his sister born in 1998. WES detected in the CLCN1 gene, a previously reported

pathogenic variant in Exon 15 p.Val536Ile c.1606G>A in homozygous state(Al-Ghamdi et al., 2017).

MYO207, born in 1984, has had problems with muscle relaxation that started in 1991 and has continued since his childhood. The doctor gave the preliminary diagnosis as Thomsen, Becker. A similar disease was reported in his son. With WES, three different heterozygous variants were detected in the CLCN1 gene. These are=Exon 3 p. Arg105Cys c.313C>T, Exon 4 p. Phe167Leu c. 501C>G, and Exon 22 Gly845Ser c.2533G>A. All three variants have been previously reported as pathogenic. It was reported that the R105C variant leads to recessive myotonia congenita, the P167L variant can give rise to both recessive and dominant disease, and the G854s variant is not found to be disease-causing in the heterozygous state, (Ulzi et al., 2012). The P167L variant was seen as heterozygous in one family, and the same variant was also seen as heterozygous in the asymptomatic mother and daughter of the index individual. p.F167L may show incomplete penetrance in most families but may predominate in some families. However, the evidence so far suggests that this is more likely to be a recessive mutation. For this reason, they also reported that the index individual may carry another variant, but they could not find it. Additionally, the P167L variant and the G845S variant were also published in a patient who was compound heterozygous for these two variants. According to the report published by Vindas-Smith et al. in 2016, the R105C mutation in exon 3 was observed together with the P167L variant in one of their patients. Brugnoni et al. (2013) reported that two siblings with p.F167L and p.R105C mutations were associated with a third CLCN1 mutation (Q812X). The combination of both mutations has previously been seen in different Italian families. In line with this information, intra-family segregation analysis was performed on MYO207. MYO207's healthy first-degree relative, MYO209, was WT for the R105C and P167L variants while carrying the G845S variant as heterozygous. MYO207's affected son, MYO208, unlike his father, carried only the G845S variant hemizygously. Interestingly, MYO218, the reported healthy daughter of MYO207, is a heterozygous carrier for the R105C, P167L and G845S variants. However, she does not show any symptoms of the disease yet. The phenotypes and location of the mutations of all patients discussed above are shown in Figure 3 and Table 3.

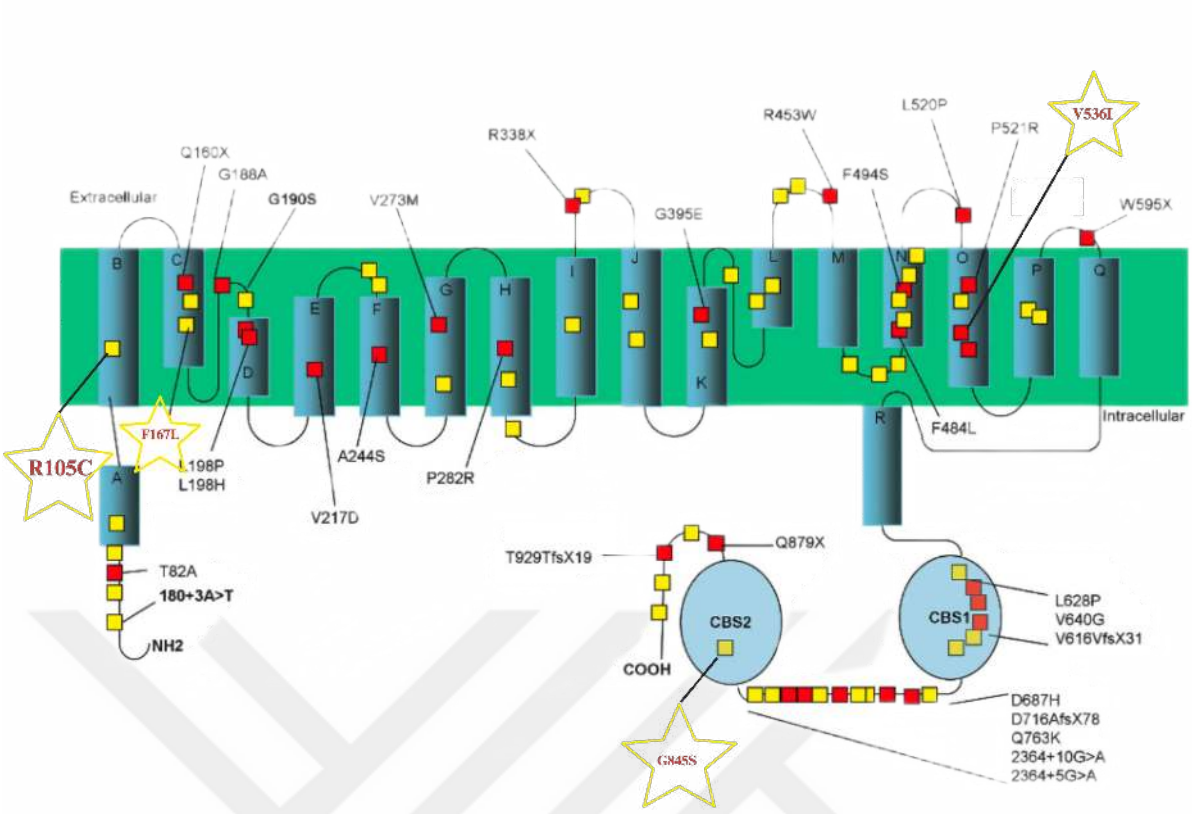


Figure 6.3 Localization on the CLC-1 protein mutations. Mutations enclosed in asterisks and written in red are mutations belonging to MYO200 and MYO207. The F167L mutation is among the top three most common mutations. Inside of stars, red variants are our patient's carrier variants. This figure adapted by (Brugnoni et al., 2013).

Table 6.3 Comparison of the clinic of MYO200 and MYO207 with the reported cases.

Family Number	Patient Number	Index/ Family Member	Mutation(s)	Sex	Age of Onset	Clinical Information
1	T3	index	V536I (hom)	F	3 months	T3, whose family members have previously been described with a similar disease, complains of difficulty in swallowing. T3 exhibits the warm-up phenomenon without muscle hypertrophy and sensitivity to cold. However, she did not respond to medication.
2	**MYO200**	index	V536I (hom)	M	Childhood	He experienced difficulty in muscle relaxation.
3	CR2	asymptomatic mother	F167L (het)	F	-	Healthy
	CR3	index	F167L hetF167L (het)	M	Childhood	The patient exhibits bulky muscles without a massive appearance, with soft muscles on palpation. There is no evidence of muscle cleamyotrophy, pain, or weakness. Positive findings for myotonia are noted, including myotonia with percussion of hand muscles, eyelids, and hands. The patient also experiences muscle cramps, symmetric myotatic reflexes, bradykinesia, speech difficulty, and normal sensitivity in the upper extremities. Distal hypoesthesia in the lower extremities is observed. Furthermore, the patient demonstrates muscular stiffness, particularly notable with a warm-up phenomenon.
	CR4	asymptomatic daughter	F167L (het)	F	-	Healthy
4	CR5	index	R105C (het) & F167L (het)	F	Childhood	The patient's muscles appear voluminous but not excessively large. Muscle tissues feel soft to the touch. There is no evidence of cleamyotrophy in the muscles, and there is no significant muscle pain or weakness. Positive findings for myotonia are present (percussion of eye lids and hands). Additionally, the patient experiences muscle cramps, symmetric myotatic reflexes, bradykinesia, speech difficulties, normal sensitivity in the upper extremities, and distal hypoesthesia in the lower extremities. Furthermore, the patient exhibits the phenomenon of warmth. This phenomenon is characterized by a sensation of stiffness or contraction in the muscles, which typically alleviates or diminishes in a warm environment
	CR6	asymptomatic sister	R105C (het) & F167L (het)	F	-	Healthy

Table 6.3 cont. Comparison of the clinic of MYO200 and MYO207 with the reported cases.

Family Number	Patient Number	Index/ Family Member	Mutation(s)	Sex	Age of Onset	Clinical Information
5	P8	index	F167L (het) & G845S (het)	F	Third decade	The typical symptoms comprise stiffness in the lower extremities and muscle pain. The patient exhibits notable myotonia, particularly in the hands and legs. Additionally, signs of muscle weakness are evident, exacerbated by rest. Nonetheless, the patient responded favorably to treatment, achieving optimal symptom management. Yet, it's essential to consider supplementary clinical observations such as depression
6	**MYO207**	index	R105C (het) & F167L (het) & G845S (het)	M	Childhood	He experienced difficulty in muscle relaxation
	MYO208	affected son	G845S (hom)	M	Childhood	He experienced difficulty in muscle relaxation
	MYO209	asymptomatic wife	G845S (het)	F	-	Healthy
	MYO218	asymptomatic daughter	R105C (het) & F167L (het) & G845S (het)	F	-	Healthy

6.1.1.5. Others

AARS1

Charcot–Marie–Tooth disease (CMT) is the most common inherited neuropathy with a heterogeneous clinical presentation and a complex genetic background. The axonal form (CMT2) is characterized by decreased action potentials, indicating primary axonal damage. ARSs are important enzymes found everywhere in cells, both in the cytoplasm and mitochondria. They help connect amino acids to their matching tRNA molecules. Mutations in four genes that make aminoacyl-tRNA synthetases (called ARS) have been linked to CMT disease, where nerves are affected. One of these genes, alanyl-tRNA synthetase (AARS), is involved in a type of CMT called 2N. The symptoms can vary, including muscle weakness, reduced reflexes, numbness in arms and legs, high arches in feet, and a specific walking pattern.

LGMD64, born in 1986, has suffered from progressive muscle weakness since the age of 16. Initially diagnosed with LGMD, WES revealed a heterozygous variant in the AARS gene: p. Asn71Ser c.212A>G. In a 2017 study by Dohrn et al., this variant was described for the first time in a patient with neuropathy. The amino acid affected by this variant is a crucial part of the protein and is conserved across species; there are also other known harmful mutations in the same region (Figure 4). When the patient's clinician was consulted, he mentioned his father's unclear cause of death but also noted symptoms related to the peripheral nervous system in the father. Since LGMD and neuropathy may share a few symptoms, genetic testing is crucial for accurate diagnosis.

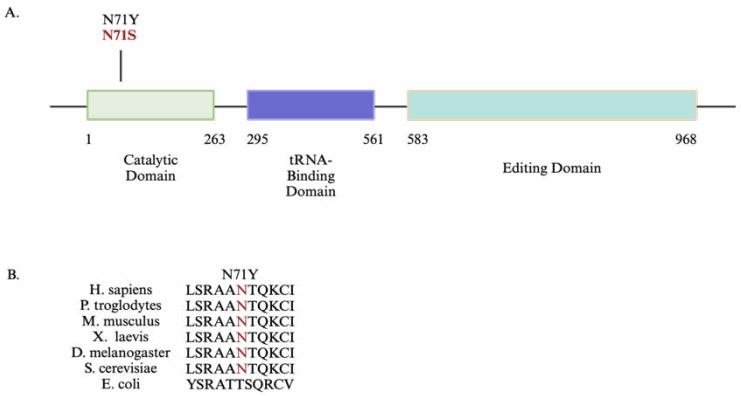


Figure 6.4 Localization and conservation of AARS variant **A.** Lolliplot of *AARS1* gene. LGMD64 variant is shown in red. **B.** In evolutionarily different species the conservation of the affected amino acid position. (adapted from McLaughlin et al.,2012).

6.2. Genes in Congenital Myasthenic Syndrome

Congenital myasthenic syndrome (CMS) is a heterogeneous disorder associated with mutations that cause muscle weakness resulting from improper neuromuscular transmission (pre-synaptic, synaptic, and post-synaptic protein defects). To date, CMS has been associated with more than thirty genes. In particular, *CHRNE*, associated with acetylcholine receptors; *COLQ,* associated with acetylcholine esterase enzyme; and *CHAT,* associated with choline acetyltransferase enzyme, are the most common genes. All three genes, when mutated, jointly cause impaired nerve conduction and, thus, persistent, or fluctuating weakness in the muscles of the extremities and extraocular muscles, facial, bulbar, and respiratory muscles, with an underlying feeling of abnormal fatigue. In addition, gene defects can cause hypotonia and developmental delay. Age of onset, onset of symptoms, and response to treatment vary depending on the molecular mechanism resulting from the underlying genetic defects. (Özsoy et al., 2023)

The acetylcholine receptor protein is a multi-subunit transmembrane protein of approximately 250kDa (Shen et al., 2017). It is encoded by the CHRN family and includes *CHRNA1, CHRNB, CHRND*, and *CHRNE*. Mutations in the *CHRND* gene are incompatible with life (Engel et al., 2015). Among the CHRN gene family associated with CMS, the CHRNE gene encodes the epsilon subunit of the acetylcholine receptor, and approximately 30-50% of CMS is caused by mutations in this gene (Chan et al., 2023). Mutations in the CHRNE gene lead to;

i. Myasthenic syndrome 4a, slow channel syndrome, is caused by a kinetic defect in the acetylcholine receptor and is inherited in AD and AR fashions. This syndrome causes prolongation of synaptic currents and action potentials.
ii. Myasthenic syndrome 4B, a fast channel inherited from AR, causes the shortening of synaptic currents and the shortening of the amplitude of action potentials.
iii. AR inherited syndrome, congenital, 4C, associated with acetylcholine receptor deficiency. The age of onset of the first of these 3 groups varies, while the other two are in infancy.

The *CHAT* gene encodes the enzyme choline acetyltransferase. This enzyme plays an important role in the synthesis of acetylcholine. Acetylcholine is a neurotransmitter

used by nerve endings in neuromuscular connections. Mutations in the *CHAT* gene can reduce acetylcholine production by disrupting the normal function of choline acetyltransferase. In this case, it is associated with AchRs due to a lack of molecules to interact with, rather than numerical or slow-fast permeability, and is also associated with CMS due to a disruption in neurotransmission. In this thesis, four different families with a preliminary diagnosis of congenital myasthenic syndrome and two patients with myasthenia gravis were analyzed.

MYO198, an offspring of a first-degree consanguineous marriage, was taken to the doctor by his family at the age of 16 months due to walking difficulties: the doctor's preliminary diagnosis was CMS. He was found to be homozygous for the pathogenic *CHAT*-variant p. Ile620Leu (c.1858A>C) in our laboratory.

MYAS16, also an offspring of a cousin's marriage, was taken to the doctor by his family when he was four years old because he had droopy eyelids and speech problems. The doctor's preliminary diagnosis was CMS. In 2018, Yang et al. published a paper in which they confirmed that their patient was compound heterozygously carried the pathogenic p. Cys148Ser (c.442T>A) and p. Arg99Ter (c.295C>T) variants in the *CHRNE* gene.

MYAS18, with unrelated parents, was taken to the doctor with complaints of bilateral ptosis and difficulty in walking that started at the age of two and a half years. Congenital myasthenic syndrome, which was the preliminary diagnosis, was described in a patient in a study previously conducted by Durmuş et al. in a Turkish cohort. The patient was shown to carry the pathogenically reported *CHRNE* p. Glu204Lys (c.610G>A) and p. Glu227ArgfsTer73 (c.675del) variants, not previously described in the literature in a compound heterozygous state. Durmuş et al. reported that the patient was unaffected in most daily living activities, except for easy fatigue, and improved significantly with pyridostigmine administration.

MYO156, our last patient with a pre-diagnosis of CMS, is the son of first cousins., He suffered from droopy eyelids at birth and had complaints of excessive fatigue in childhood: when he was examined by WES, in the *CHRNE*, c.1219+2T>G change, which was previously stated as "the most common mutation in the Turkish population" in the article published by Durmuş et al. in 2019, was detected. The step-nieces of the index case, showing similar symptoms, were also found to be homozygous for the same variant in *CHRNE* as shown by segregation analysis with Sanger sequencing.

For the other two patients referred to us with a diagnosis of myasthenia gravis, a disease-causing gene variant could not be identified through WES analysis.

6.3. Genotype-Phenotype Association with Reported/Novel Variant Throughout This Thesis

Next-generation sequencing technologies have significantly contributed to the number of genes, associated with neuromuscular diseases such as myopathies, subgroups of myopathies, and congenital myasthenic syndromes, significantly blurring the line between these diseases.

Our WES- based study identified 19 previously reported and eight new variants in 30 Turkish families, thus consolidating the association between known myopathy and congenital myasthenic syndrome genes, but also revealing new variants, thus novel genotype-phenotype associations.

6.4. The Importance of Variants of Uncertain Significance

The significant role of NGS in disease diagnosis is beyond dispute. The use of WES in clinical practice has led to an increase in the number of Variants of Uncertain Significance, indicating uncertainty about the effects of these variants on health. At this point, highlighting genotype-phenotype correlations and family studies in the interpretation of genetic variants is crucial to understanding the molecular bases and mechanisms of diseases. Assessing whether genetic variants are associated with disorders, relies on understanding the genotype-phenotype relationships. In-depth examination of the patient's clinical features (deep phenotyping) and approaches that investigate relevant clinical characteristics, starting from genetic information (reverse phenotyping) play a significant role in understanding the effects of genetic variants. These detailed evaluations can help to better understand the clinical significance of Variants of Uncertain Significance.

In conclusion, the multidimensional approach in the process of evaluating genetic variants is a vital tool to make informed clinical decisions on one hand and provide personalized genetic counseling on the other. This will enable more effective development of treatment and management plans for patients, accurate provision of genetic information to families, and the establishment of stronger foundations for genetic counseling practices.

In the framework of this study, seven out of 22 variants were determined and presented with VUS or VUS-LP criteria according to Varsome (Table 5.3). Eight of these variants were novel. VUS variants were comprehensively discussed with the relevant expert clinicians to make an accurate final diagnosis in which also family segregation analyses were instrumental.

6.5. Limitation of WES

In this thesis, WES achieved a diagnostic yield of 78%. In the remaining 22% of cases, WES was unsuccessful in identifying mutations in disease-associated genes. In addition to index cases, sequencing other members of the families is also important. On the other hand, it is also possible that the patient could not be diagnosed despite WES, which can be due to the technical limitations of WES itself rather than the lack of sequencing of family members. These limitations are considered as follows:

- Lack of non-coding regions: WES is limited by not covering the non-coding regions of the genome, which may cause it to overlook mutations in intronic regions that can cause disease.

- Limited Detection of Structural Variations: WES cannot detect structural variations and repeat expansions. Due to read length limitations, structural variants can be challenging to detect.

- False Positivity: The problem of false positives in WES should not be underestimated. Biases in library construction, faulty polymerase reactions, and other technical reasons can lead to false positives.

- Read Length Limitations: WES may not effectively detect structural variants due to read length limitations. This limitation can be overcome as read length increases with new technologies.

- Low Gene Coverage: Low gene coverage in some regions may result in low coverage for a heterozygous variant.

- Failure to Sequence the Entire Genome: WES cannot sequence the entire genome. Therefore, new strategies may be needed to discover new genes that may cause disease.

- Bioinformatics Challenges: WGS (Whole Genome Sequencing) can provide a more comprehensive analysis than WES data. However, WGS data is challenging to

interpret and may require more sophisticated bioinformatics tools, which may bring additional and significant computational challenges.

- Cost and Time Factors: WES can be cost-effective and timesaving but is less comprehensive than WGS. Until WGS becomes more cost-effective and timesaving, one solution may be to expand the targeted regions of exome capture kits.

Other techniques can be performed to increase the diagnostic rate based on the probable genetic reason. For instance, if i) the case is a candidate to be solved with copy number variations, MLPA can be applied, ii) repeat expansions are suspected, repeat-primed PCR can be planned, iii) mitochondrial genes are thought to be the cause, different NGS techniques can be used in a targeted mitochondrial gene panel (Efthymiou et al., 2016). Over time, the number of known genes will increase, and some unsolved cases can be solved with new genes.

Importantly, the unsolved cases can be attributed to the limitations of WES, however our unsolved families may also not be obeying to Mendelian genetics, but a more complex inheritance pattern.

Chapter 7: CONCLUSION

Myopathies are muscle disorders generally characterized by muscle weakness and fatigue. Congenital myasthenic syndromes are neuromuscular disorders characterized by easy fatiguability, muscle weakness, and ptosis. Although extensive research has been conducted, the complex mechanisms are still required to be enlightened.

This thesis aims to comprehend the genetic findings in Turkish Myopathy and CMS patients by identifying variants using WES, bioinformatic analysis and wet lab approaches. In 30 index patients, 23 cases were solved. Patients with recessive inheritance were more common than those with dominant inheritance, due to the relatively high consanguinity rate in the Turkish cohort under study. With this thesis, the genetic causes of myopathies in the cohort were partly identified, leading to a firm differential diagnosis in the affected families.

WES is the current gold standard for the genetic investigation of heterogeneous inherited disorders. The results of this thesis can hopefully lead to a better understanding of the clinical and genetic picture of Turkish myopathy and CMS patients. As future therapies will be based on genetic findings, molecular analysis is paramount. The chances of a promising future are high in the genetic era.

BIBLIOGRAPHY

Akat, A., & Karaöz, E. (2023). Cell Therapy Strategies on Duchenne Muscular Dystrophy: A Systematic Review of Clinical Applications. *Stem Cell Reviews and Reports*. https://doi.org/10.1007/s12015-023-10653-8

Al-Ghamdi, F., Darras, B. T., & Ghosh, P. S. (2017). Spectrum of Nondystrophic Skeletal Muscle Channelopathies in Children. *Pediatric Neurology*, *70*, 26–33. https://doi.org/10.1016/j.pediatrneurol.2017.02.006

Bitoun, M., Maugenre, S., Jeannet, P.-Y., Lacène, E., Ferrer, X., Laforêt, P., Martin, J.-J., Laporte, J., Lochmüller, H., Beggs, A. H., Fardeau, M., Eymard, B., Romero, N. B., & Guicheney, P. (2005). Mutations in dynamin 2 cause dominant centronuclear myopathy. *Nature Genetics*, *37*(11), Article 11. https://doi.org/10.1038/ng1657

Brisset, M., Ben Yaou, R., Carlier, R.-Y., Chanut, A., Nicolas, G., Romero, N. B., Wahbi, K., Decrocq, C., Leturcq, F., Laforêt, P., & Malfatti, E. (2019). X-linked Emery–Dreifuss muscular dystrophy manifesting with adult onset axial weakness, camptocormia, and minimal joint contractures. *Neuromuscular Disorders*, *29*(9), 678–683. https://doi.org/10.1016/j.nmd.2019.06.009

Brugnoni, R., Kapetis, D., Imbrici, P., Pessia, M., Canioni, E., Colleoni, L., De Rosbo, N. K., Morandi, L., Cudia, P., Gashemi, N., Bernasconi, P., Desaphy, J.-F., Conte, D., & Mantegazza, R. (2013). A large cohort of myotonia congenita probands: Novel mutations and a high-frequency mutation region in exons 4 and 5 of the CLCN1 gene. *Journal of Human Genetics*, *58*(9), 581–587. https://doi.org/10.1038/jhg.2013.58

Cassandrini, D., Trovato, R., Rubegni, A., Lenzi, S., Fiorillo, C., Baldacci, J., Minetti, C., Astrea, G., Bruno, C., & Santorelli, F. M. (2017). Congenital myopathies: Clinical phenotypes and new diagnostic tools. *Italian Journal of Pediatrics*, *43*, 101. https://doi.org/10.1186/s13052-017-0419-z

Chen, L., Tang, F., Gao, H., Zhang, X., Li, X., & Xiao, D. (2021). CAPN3: A muscle-specific calpain with an important role in the pathogenesis of diseases (Review). *International Journal of Molecular Medicine*, *48*(5), 203. https://doi.org/10.3892/ijmm.2021.5036

Deymeer, F. (2020). History of Myasthenia Gravis Revisited. *Archives of Neuropsychiatry*, *58*(2), 154–162. https://doi.org/10.29399/npa.27315

Dubowitz, V., & Everson Pearse, A. G. (1960). OXIDATIVE ENZYMES AND PHOSPHORYLASE IN CENTRAL-CORE DISEASE OF MUSCLE. *The Lancet*, *276*(7140), 23–24. https://doi.org/10.1016/S0140-6736(60)92665-9

Engel, A. G. (2020). Chapter 32—Congenital myasthenic syndromes. In R. N. Rosenberg & J. M. Pascual (Eds.), *Rosenberg's Molecular and Genetic Basis of Neurological and Psychiatric Disease (Sixth Edition)* (pp. 539–558). Academic Press. https://doi.org/10.1016/B978-0-12-813866-3.00032-1

Ghaoui, R., Cooper, S. T., Lek, M., Jones, K., Corbett, A., Reddel, S. W., Needham, M., Liang, C., Waddell, L. B., Nicholson, G., O'Grady, G., Kaur, S., Ong, R., Davis, M., Sue, C. M., Laing, N. G., North, K. N., MacArthur, D. G., & Clarke, N. F. (2015). Use of Whole-Exome Sequencing for Diagnosis of Limb-Girdle Muscular Dystrophy: Outcomes and Lessons Learned. *JAMA Neurology*, *72*(12), 1424. https://doi.org/10.1001/jamaneurol.2015.2274

Gilhus, N. E., Tzartos, S., Evoli, A., Palace, J., Burns, T. M., & Verschuuren, J. J. G. M. (2019). Myasthenia gravis. *Nature Reviews Disease Primers*, *5*(1), 30.

https://doi.org/10.1038/s41572-019-0079-y

González-Jamett, A. M., Bevilacqua, J. A., & Díaz, A. M. C. (2018). Hereditary Myopathies. In K. Sakuma (Ed.), *Muscle Cell and Tissue—Current Status of Research Field*. InTech. https://doi.org/10.5772/intechopen.76076

Greenfield, J. G., Cornman, T., & Shy, G. M. (1958). THE PROGNOSTIC VALUE OF THE MUSCLE BIOPSY IN THE "FLOPPY INFANT". *Brain*, *81*(4), 461–484. https://doi.org/10.1093/brain/81.4.461

Helbling-Leclerc, A., Bonne, G., & Schwartz, K. (2002). Emery-Dreifuss muscular dystrophy. *European Journal of Human Genetics*, *10*(3), Article 3. https://doi.org/10.1038/sj.ejhg.5200744

Kim, J.-B. (2014). Channelopathies. *Korean Journal of Pediatrics*, *57*(1), 1. https://doi.org/10.3345/kjp.2014.57.1.1

Krenn, M., Wagner, M., Zulehner, G., Weng, R., Jäger, F., Keritam, O., Sener, M., Brücke, C., Milenkovic, I., Langer, A., Buchinger, D., Habersam, R., Mayerhanser, K., Brugger, M., Brunet, T., Jacob, M., Graf, E., Berutti, R., Cetin, H., … Rath, J. (2023). Next-generation sequencing and comprehensive data reassessment in 263 adult patients with neuromuscular disorders: Insights into the gray zone of molecular diagnoses. *Journal of Neurology*. https://doi.org/10.1007/s00415-023-12101-6

Laing, N. G., Wilton, S. D., Akkari, P. A., Dorosz, S., Boundy, K., Kneebone, C., Blumbergs, P., White, S., Watkins, H., Love, D. R., & Haan, E. (1995). A mutation in the α tropomyosin gene TPM3 associated with autosomal dominant nemaline myopathy. *Nature Genetics*, *9*(1), Article 1. https://doi.org/10.1038/ng0195-75

Laitila, J., & Wallgren-Pettersson, C. (2021). Recent advances in nemaline myopathy. *Neuromuscular Disorders*, *31*(10), 955–967. https://doi.org/10.1016/j.nmd.2021.07.012

Laval, S. H., & Bushby, K. M. D. (2004). Limb-girdle muscular dystrophies—From genetics to molecular pathology. *Neuropathology and Applied Neurobiology*, *30*(2), 91–105. https://doi.org/10.1111/j.1365-2990.2004.00555.x

Lehtokari, V.-L., Pelin, K., Donner, K., Voit, T., Rudnik-Schöneborn, S., Stoetter, M., Talim, B., Topaloglu, H., Laing, N. G., & Wallgren-Pettersson, C. (2008). Identification of a founder mutation in TPM3 in nemaline myopathy patients of Turkish origin. *European Journal of Human Genetics*, *16*(9), Article 9. https://doi.org/10.1038/ejhg.2008.60

Liang, W.-C., & Nishino, I. (2011). Lipid Storage Myopathy. *Current Neurology and Neuroscience Reports*, *11*(1), 97–103. https://doi.org/10.1007/s11910-010-0154-y

Mammen, A. L., Roda, R. H., & Leung, D. G. (2018). Myopathy: Recent Progress, Current Therapies, and Future Directions. *Neurotherapeutics*, *15*(4), 837–839. https://doi.org/10.1007/s13311-018-00688-y

Mercuri, E., & Muntoni, F. (2013). Muscular dystrophies. *The Lancet*, *381*(9869), 845–860. https://doi.org/10.1016/S0140-6736(12)61897-2

Milone, M., & Wong, L.-J. (2013). Diagnosis of mitochondrial myopathies. *Molecular Genetics and Metabolism*, *110*(1–2), 35–41. https://doi.org/10.1016/j.ymgme.2013.07.007

Mohassel, P., & Bönnemann, C. G. (2015). Limb-girdle Muscular Dystrophies. In *Neuromuscular Disorders of Infancy, Childhood, and Adolescence* (pp. 635–666). Elsevier. https://doi.org/10.1016/B978-0-12-417044-5.00034-2

Mullen, J., Alrasheed, K., & Mozaffar, T. (2022). GNE myopathy: History, etiology, and treatment trials. *Frontiers in Neurology*, *13*, 1002310. https://doi.org/10.3389/fneur.2022.1002310

Nagy, H., & Veerapaneni, K. D. (2023). Myopathy. In *StatPearls*. StatPearls

Publishing. http://www.ncbi.nlm.nih.gov/books/NBK562290/

Navarro-Martínez, A., Vicente-García, C., & Carvajal, J. J. (2023). NMJ-related diseases beyond the congenital myasthenic syndromes. *Frontiers in Cell and Developmental Biology*, *11*, 1216726. https://doi.org/10.3389/fcell.2023.1216726

Nicot, A.-S., Toussaint, A., Tosch, V., Kretz, C., Wallgren-Pettersson, C., Iwarsson, E., Kingston, H., Garnier, J.-M., Biancalana, V., Oldfors, A., Mandel, J.-L., & Laporte, J. (2007). Mutations in amphiphysin 2 (BIN1) disrupt interaction with dynamin 2 and cause autosomal recessive centronuclear myopathy. *Nature Genetics*, *39*(9), Article 9. https://doi.org/10.1038/ng2086

Nurk, S., Koren, S., Rhie, A., Rautiainen, M., Bzikadze, A. V., Mikheenko, A., Vollger, M. R., Altemose, N., Uralsky, L., Gershman, A., Aganezov, S., Hoyt, S. J., Diekhans, M., Logsdon, G. A., Alonge, M., Antonarakis, S. E., Borchers, M., Bouffard, G. G., Brooks, S. Y., … Phillippy, A. M. (2022). *The complete sequence of a human genome*.

Oflazer, P., Durmuş-Tekçe, H., & Deymeer, F. (n.d.). *KAS VE NÖROMÜSKÜLER KAVŞAK HASTALIKLARI*.

Ogasawara, M., & Nishino, I. (2021). A review of core myopathy: Central core disease, multiminicore disease, dusty core disease, and core-rod myopathy. *Neuromuscular Disorders: NMD*, *31*(10), 968–977. https://doi.org/10.1016/j.nmd.2021.08.015

Ogasawara, M., & Nishino, I. (2023a). A review of major causative genes in congenital myopathies. *Journal of Human Genetics*, *68*(3), Article 3. https://doi.org/10.1038/s10038-022-01045-w

Ogasawara, M., & Nishino, I. (2023b). A review of major causative genes in congenital myopathies. *Journal of Human Genetics*, *68*(3), Article 3. https://doi.org/10.1038/s10038-022-01045-w

Ohno, K., Ohkawara, B., Shen, X.-M., Selcen, D., & Engel, A. G. (2023). Clinical and Pathologic Features of Congenital Myasthenic Syndromes Caused by 35 Genes-A Comprehensive Review. *International Journal of Molecular Sciences*, *24*(4), 3730. https://doi.org/10.3390/ijms24043730

Özsoy, Ö., Cinleti, T., Günay, Ç., Sarıkaya Uzan, G., Giray Bozkaya, Ö., Çağlayan, A. O., Hız Kurul, S., & Yiş, U. (2023). Genetic, serological and clinical evaluation of childhood myasthenia syndromes- single center subgroup analysis experience in Turkey. *Acta Neurologica Belgica*. https://doi.org/10.1007/s13760-023-02370-3

Parsons, L. N. (2014). Mitochondrial Myopathies. In *Pathobiology of Human Disease* (p. 188). Elsevier. https://doi.org/10.1016/B978-0-12-386456-7.01508-2

Phillips, L., & Trivedi, J. R. (2018). Skeletal Muscle Channelopathies. *Neurotherapeutics*, *15*(4), 954–965. https://doi.org/10.1007/s13311-018-00678-0

Rafay, M. F., Halliday, W., & Bril, V. (2005). Hyaline Body Myopathy: Adulthood Manifestations. *Canadian Journal of Neurological Sciences / Journal Canadien Des Sciences Neurologiques*, *32*(2), 253–256. https://doi.org/10.1017/S0317167100004078

Romero, N. B., & Clarke, N. F. (2013). Congenital myopathies. In *Handbook of Clinical Neurology* (Vol. 113, pp. 1321–1336). Elsevier. https://doi.org/10.1016/B978-0-444-59565-2.00004-6

Satam, H., Joshi, K., Mangrolia, U., Waghoo, S., Zaidi, G., Rawool, S., Thakare, R. P., Banday, S., Mishra, A. K., Das, G., & Malonia, S. K. (2023). Next-Generation Sequencing Technology: Current Trends and Advancements. *Biology*, *12*(7), 997. https://doi.org/10.3390/biology12070997

Savarese, M., Di Fruscio, G., Tasca, G., Ruggiero, L., Janssens, S., De Bleecker, J., Delpech, M., Musumeci, O., Toscano, A., Angelini, C., Sacconi, S., Santoro, L., Ricci,

E., Claes, K., Politano, L., & Nigro, V. (2015). Next generation sequencing on patients with LGMD and nonspecific myopathies: Findings associated with ANO5 mutations. *Neuromuscular Disorders*, *25*(7), 533–541. https://doi.org/10.1016/j.nmd.2015.03.011

Schätzl, T., Kaiser, L., & Deigner, H.-P. (2021). Facioscapulohumeral muscular dystrophy: Genetics, gene activation and downstream signalling with regard to recent therapeutic approaches: an update. *Orphanet Journal of Rare Diseases*, *16*(1), 129. https://doi.org/10.1186/s13023-021-01760-1

Schorling, D., Kirschner, J., & Bönnemann, C. (2017). Congenital Muscular Dystrophies and Myopathies: An Overview and Update. *Neuropediatrics*, *48*(04), 247–261. https://doi.org/10.1055/s-0037-1604154

Shingde, M. V., Spring, P. J., Maxwell, A., Wills, E. J., Harper, C. G., Dye, D. E., Laing, N. G., & North, K. N. (2006). Myosin storage (hyaline body) myopathy: A case report. *Neuromuscular Disorders*, *16*(12), 882–886. https://doi.org/10.1016/j.nmd.2006.09.001

Shy, G. M., Engel, W. K., Somers, J. E., & Wanko, T. (1963). NEMALINE MYOPATHY: A NEW CONGENITAL MYOPATHY. *Brain*, *86*(4), 793–810. https://doi.org/10.1093/brain/86.4.793

SHY, G. M., & MAGEE, K. R. (1956). A NEW CONGENITAL NON-PROGRESSIVE MYOPATHY. *Brain*, *79*(4), 610–621. https://doi.org/10.1093/brain/79.4.610

Soontrapa, P., & Liewluck, T. (2022). Anoctamin 5 (ANO5) Muscle Disorders: A Narrative Review. *Genes*, *13*(10), 1736. https://doi.org/10.3390/genes13101736

Srinivasan, J., & Amato, A. A. (2003). Myopathies. *Physical Medicine and Rehabilitation Clinics of North America*, *14*(2), 403–434. https://doi.org/10.1016/S1047-9651(02)00128-6

Statland, J. M., & Barohn, R. J. (2013). Muscle Channelopathies: The Nondystrophic Myotonias and Periodic Paralyses. *CONTINUUM: Lifelong Learning in Neurology*, *19*, 1598–1614. https://doi.org/10.1212/01.CON.0000440661.49298.c8

Stehlíková, K., Skálová, D., Zídková, J., Mrázová, L., Vondráček, P., Mazanec, R., Voháňka, S., Haberlová, J., Hermanová, M., Zámečník, J., Souček, O., Ošlejšková, H., Dvořáčková, N., Solařová, P., & Fajkusová, L. (2014). Autosomal recessive limb-girdle muscular dystrophies in the Czech Republic. *BMC Neurology*, *14*(1), 154. https://doi.org/10.1186/s12883-014-0154-7

Tavtigian, S. V., Greenblatt, M. S., Harrison, S. M., Nussbaum, R. L., Prabhu, S. A., Boucher, K. M., & Biesecker, L. G. (2018). Modeling the ACMG/AMP Variant Classification Guidelines as a Bayesian Classification Framework. *Genetics in Medicine : Official Journal of the American College of Medical Genetics*, *20*(9), 1054–1060. https://doi.org/10.1038/gim.2017.210

Todd, P. K., & Paulson, H. L. (2010). RNA-mediated neurodegeneration in repeat expansion disorders. *Annals of Neurology*, *67*(3), 291–300. https://doi.org/10.1002/ana.21948

Topaloglu, H. (2020). Core myopathies—A short review. *Acta Myologica: Myopathies and Cardiomyopathies: Official Journal of the Mediterranean Society of Myology*, *39*(4), 266–273. https://doi.org/10.36185/2532-1900-029

Urtizberea, J. A., Severa, G., & Malfatti, E. (2023). Metabolic Myopathies in the Era of Next-Generation Sequencing. *Genes*, *14*(5), 954. https://doi.org/10.3390/genes14050954

Wiendl, H., Abicht, A., Chan, A., Della Marina, A., Hagenacker, T., Hekmat, K., Hoffmann, S., Hoffmann, H.-S., Jander, S., Keller, C., Marx, A., Melms, A., Melzer, N., Müller-Felber, W., Pawlitzki, M., Rückert, J.-C., Schneider-Gold, C., Schoser, B.,

Schreiner, B., … Meisel, A. (2023). Guideline for the management of myasthenic syndromes. *Therapeutic Advances in Neurological Disorders*, *16*, 17562864231213240. https://doi.org/10.1177/17562864231213240

Yuen, M., Cooper, S. T., Marston, S. B., Nowak, K. J., McNamara, E., Mokbel, N., Ilkovski, B., Ravenscroft, G., Rendu, J., De Winter, J. M., Klinge, L., Beggs, A. H., North, K. N., Ottenheijm, C. A. C., & Clarke, N. F. (2015). Muscle weakness in *TPM3* -myopathy is due to reduced Ca $^{2+}$ -sensitivity and impaired acto-myosin cross-bridge cycling in slow fibres. *Human Molecular Genetics*, *24*(22), 6278–6292. https://doi.org/10.1093/hmg/ddv334

Yunisova, G., Ceylaner, S., Oflazer, P., Deymeer, F., Parman, Y. G., & Durmus, H. (2022). Clinical and genetic characteristics of Emery-Dreifuss muscular dystrophy patients from Turkey: 30 years longitudinal follow-up study. *Neuromuscular Disorders*, *32*(9), 718–727. https://doi.org/10.1016/j.nmd.2022.07.397