A Clinical and Genetic Study of the Skeletal Muscle Channelopathies

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I, Dr Dipa Lakshmi Raja Rayan, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the work.

ABSTRACT

The skeletal muscle channelopathies are a group of inherited muscle diseases characterised by the abnormal functioning of voltage-gated ion channels expressed in skeletal muscle. They manifest as the non-dystrophic myotonias and the periodic paralyses.

This thesis increases the current understanding of the clinical and genetic basis of this group of diseases. It identifies the overall prevalence in England as 1.12/100,000 and determines the individual minimum prevalence of each disease, which has not previously been documented. It presents a detailed phenotype study of periodic paralysis (PP), paramyotonia congenita (PMC) and sodium channel myotonia (SCM), which is the first comparative study of these diseases. In the process it uncovers the marked similarity between PMC and SCM and suggests that these may be a spectrum of one disease, rather than two distinct diseases as traditionally thought. It provides the first systematic study of pregnancy and anaesthetics in a large number of channelopathy patients, identifying a marked increase in severity of symptoms during pregnancy that has not previously been documented.

To widen the spectrum of genetic diagnosis and techniques in this group of diseases, this thesis describes the first cases of large scale rearrangements in CLCN1 causing myotonia congenita. It demonstrates how, using whole exome sequencing, the genetic diagnosis rate can be improved and illustrates two cases that may be explained by variations in RYR1 and another case in which a genetic diagnosis of Liddle's syndrome may underlie secondary PP. This suggests that RYR1 variations may account for some unconfirmed cases and others may be explained by genetic causes of secondary PP.

Finally this thesis presents convincing evidence of the efficacy of mexiletine in non-dystrophic myotonia in a double-blind placebo-controlled trial. It demonstrates improvement of the primary outcome measure of patient-reported stiffness and the majority of secondary outcome measures assessed.

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ABBREVIATIONS

3T - 3 Tesla

ABI - Applied Biosystems

aCGH - Array-based comparative genomic hybridisation

ADM - Adductor digiti minimi

ADR - Arrested development of righting response

ATS - Andersen-Tawil Syndrome

bp - Base pair

BWA - Burrows-Wheeler Aligner

CBS - Cystathionine-β-synthase

CI - Confidence interval

CINCH - Clinical Investigation of Neurological Channelopathies

CK - Creatine kinase

CMAP - Compound muscle action potential

CNV - Copy number variation

COX - Cytochrome oxidase

Cy - Cyanine

DM1 - Myotonic dystrophy type 1

DM2 - Myotonic dystrophy type 2

DNA- Deoxyribonucleic acid

ECG - Electrocardiogram

EMG - Electromyography

FDA - Food and Drug Administration

FISH - Fluorescence in situ hybridisation

FVC - Forced vital capacity

HyperPP - Hyperkalaemic periodic paralysis

HypoPP - Hypokalaemic periodic paralysis

HypoPP1 - Hypokalaemic periodic paralysis type 1

HypoPP2 - Hypokalaemic periodic paralysis type 2

IVR - Interactive voice response

LGMD - Limb Girdle Muscular Dystrophy

MC - Myotonia congenita

MH - Malignant hyperthermia

MHRA - Medicines and Healthcare products Regulatory Agency

MLPA - Multiplex Ligation-dependent Probe Amplification

MRC - Medical Research Council

MRI - Magnetic Resonance Imaging

mRNA - messenger Ribonucleic Acid

NCBI - National Centre for Biotechnology Information

NDM - Non-dystrophic myotonia

NHNN - National Hospital for Neurology and Neurosurgery

PAM - Potassium-aggravated myotonia

PCR - Polymerase chain reaction

PIP_2 - Phosphatidylinositol-3,4-bisphosphate

PMC - Paramyotonia congenita

PP - Periodic Paralysis

QOL - Quality of life

RCT - Randomised controlled trial

SCA - Spinocerebellar ataxia

SCM - Sodium channel myotonia

SD - Standard deviation

SNAP - Sensory nerve action potential

SNP - Single nucleotide polymorphism

SNV - Single nucleotide variation

STIR - Short tau inversion recovery

UCLH - University College London Hospital

UCSC - University of California, Santa Cruz

WT - Wild-type

Chapter 1

INTRODUCTION

1.1 Skeletal Muscle Channelopathies

The skeletal muscle channelopathies are a heterogeneous group of inherited muscle diseases. They are characterised by genetic variations that result in abnormal functioning of voltage-gated ion channels expressed in skeletal muscle. This results in an alteration in muscle membrane excitability which manifests either as episodic muscle weakness or as stiffness. They are considered in two main groups: periodic paralysis (PP), in which the muscle membrane is rendered inexcitable resulting in episodes of paralysis and non-dystrophic myotonia (NDM) in which the muscle membrane becomes hyperexcitable resulting in myotonia and stiffness.

Voltage-gated ion channels play a vital role throughout the nervous system and dysfunction of these proteins are thought to be important in both rare Mendelian diseases, such as the skeletal muscle channelopathies, and common paroxysmal diseases, such as migraine and epilepsy. In these common diseases the precise genetic mechanisms that relate the ion channel dysfunction to the clinical phenotype is complex and multi-factorial and therefore remains poorly understood. The rarer Mendelian disorders, however, are better understood and at present have a greater scope for in-depth investigation as the underlying genetic defects are more clearly defined. Improving our knowledge of these rare disorders, therefore forms a platform on which to further our understanding of the more common disorders.

1.2 Scope of thesis

This thesis aims to increase the understanding of the skeletal muscle channelopathies with specific emphasis on non-dystrophic myotonia and periodic paralysis. It specifically investigates the prevalence of these conditions in the UK. It also increases our understanding of the phenotypes of these diseases and how they affect pregnancy and anaesthetic use. It expands the current knowledge of genetic diagnosis and investigates the efficacy of treatment in this group of diseases.

Chapter 2

BACKGROUND

2.1 Ion Channels

Ion channels are essential membrane-spanning glycoproteins that allow the movement of ions across a cell membrane. They may be categorised as voltage-gated, ligand-gated or sensory transducers. Voltage-gated ion channels have a highly voltage-dependent open probability. They contain charged amino acid residues that sense a change in the electrical field across the membrane, triggering channel opening. Ligand-gated ion channels depend on the binding of a ligand to initiate channel opening. Sensory transducers convert a sensory signal into an electrical signal, transforming environmental properties into electrical potentials (Hille, 2001).

2.1.1 Voltage-gated Ion Channels

Voltage-gated ion channels play a crucial role in the generation and propagation of electrical signals. They consist of a combination of principal pore-forming subunits, which determine the primary channel properties and secondary auxiliary subunits, which modify channel function. Ion selectivity in these channels is determined by the selectivity filter which consists of hydrophilic loops located within the pore. Gating is voltage-sensitive and is therefore coupled to a sensor that detects changes in transmembrane voltage. In the majority of channels this voltage sensor originates from a membrane-spanning α-helical segment with positively charged amino acids known as the S4 segment. Membrane depolarisation causes this segment to move outwards, altering the channel conformation and increasing the open probability (Hille, 2001) (Figure 2.1).

The main voltage-gated ion channels in skeletal muscle are the chloride, potassium,

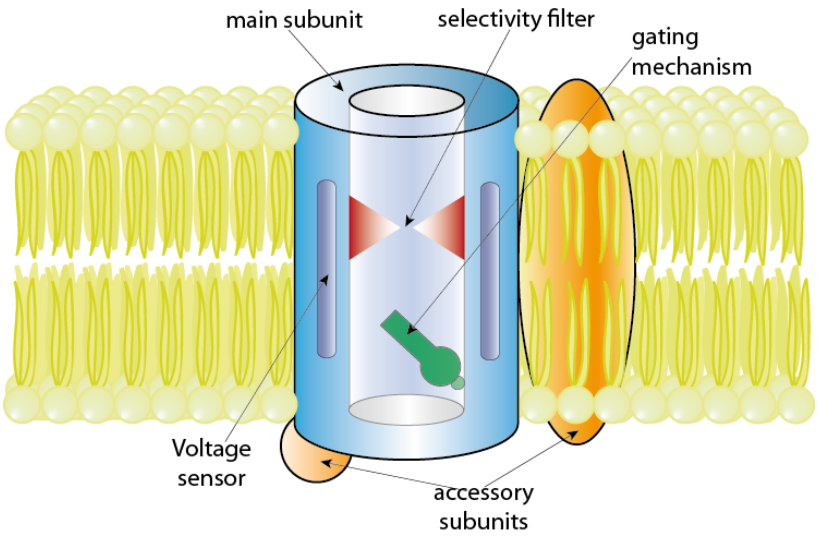


Figure 2.1: Schematic diagram representing the main components of a voltage-gated ion channel

sodium and calcium channels. The chloride channels are markedly different from the others, not having an S4 segment for voltage sensing and being made up of a homodimer with two distinct pores. The voltage-gated potassium, sodium and calcium channels are all derived from the same gene superfamily with marked sequence homology. Diseases of voltage-gated ion channels are caused by mutations that affect the key aspects of channel function: gating, voltage-sensing, permeation, assembly and trafficking. The clinical manifestations of these diseases, however, are mainly dictated by the tissue distribution of the particular channel.

2.1.1.1 Voltage-gated Potassium Channels

Potassium channels play a role in stabilising the membrane potential. When these channels open, potassium ions flow outward making the cell less depolarised and less excitable. They consist of three main families: the six transmembrane domain family, the four transmembrane two-P (leak) potassium channels and the two transmembrane domain inward rectifying potassium channels (Figure 2.2). To date over 90 genes have been identified for potassium-sensitive channels (Seal et al., 2011), many with alternative splicing leading to the production of multiple isoforms.

2.1.1.2 Inward Rectifying Potassium Channels

Although inward rectifying potassium (Kir) channels are not true voltage-gated channels with a distinct S4 segment, they are still voltage-sensitive and mutations in these channels have been shown to cause muscle channelopathies. They aid in maintaining the resting potential,

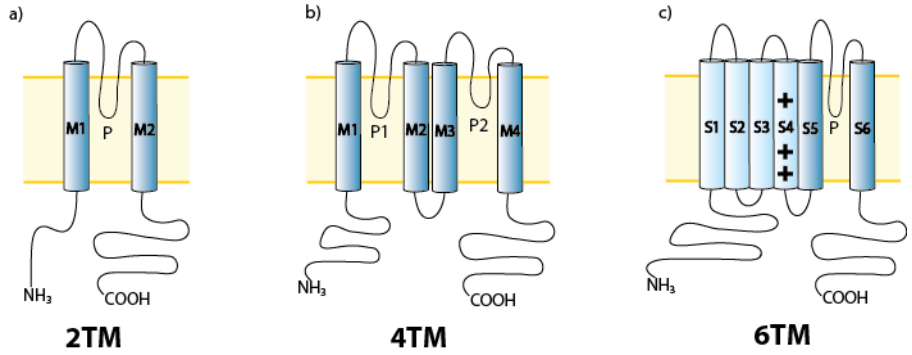


Figure 2.2: Topology of the 3 different classes of potassium channel. a) 2 transmembrane domain family, inward-rectifying potassium channels; b) 4 transmembrane domain family, two-pore potassium channels; c) 6 transmembrane domain potassium channels. (P = pore-loops)

acting as a one-way valve by allowing potassium ions to enter during hyperpolarisation but not to exit during depolarisation. Their inward rectifying properties are derived from the voltage-dependent binding of intracellular polyvalent ions that block the channel (Bichet et al., 2003). These channels consist of two transmembrane domains linked by a pore-forming region which contains the selectivity filter (Figure 2.2a). Four subunits come together to form a tetramer resulting in a functional channel (Glowatzki et al., 1995).

The main Kir channels found in skeletal muscle are the Kir2.x family and the ATP-sensitive potassium channels, Kir6.x (Table 2.1). The Kir2.x channels are constitutively active with steep inward rectification which is regulated by magnesium and polyamines (Lopatin et al., 1994). They are thought to be important in setting the resting membrane potential and maintaining membrane stability. Kir2.1 is expressed in skeletal, smooth and cardiac muscle as well as the brain, eye and kidney (de Boer et al., 2010). Mutations in this channel are known to cause Andersen-Tawil Syndrome (ATS) (Plaster et al., 2001). The Kir2.6 channel was recently discovered with 98% sequence identity with Kir2.2 and is also expressed in skeletal muscle. Mutations in this channel are associated with susceptibility to thyrotoxic periodic paralysis (Ryan et al., 2010).

2.1.1.3 Voltage-gated Sodium Channels

The voltage-gated sodium channels play a crucial role in the generation and propagation of action potentials in myocytes and neurons (Hodgkin and Huxley, 1952). They are selective ion channels that activate following initial membrane depolarisation and then rapidly inactivate. They consist of a complex of an α-subunit that associates with a number of auxiliary β-subunits. Each α-subunit consists of four domains (I-IV), each made up of six

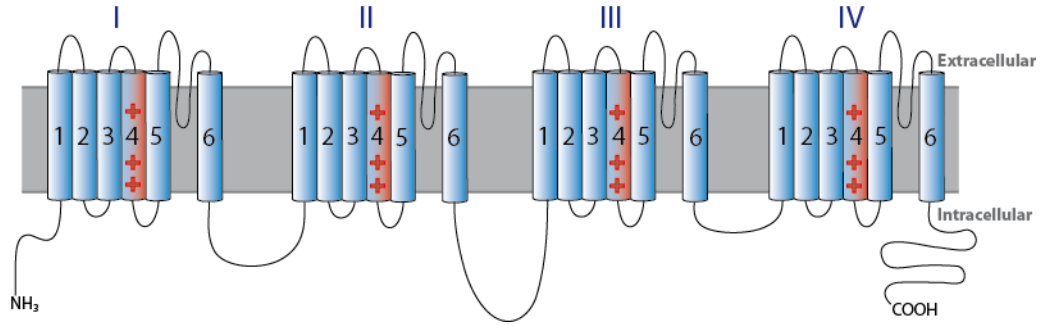


Figure 2.3: Membrane topology of the voltage-gated sodium and calcium channel. (The location of the S4 segments are indicated with + signs)

transmembrane sections (Figure 2.3). Each domain has an S4 voltage-sensing domain with positively charged arginine residues at every third position. X-ray crystallography of NavAb in *Arcobacter butzleri* suggests that the ion selectivity is determined by glutamate side chains in the narrowest part of the pore and that the combination of the movement of the S4 segments and the S4-S5 linker, open out to dilate the central pore allowing partially hydrated sodium ions into the water-filled central pore (Payandeh et al., 2011). Inactivation is thought to occur via the binding of the intracellular loop between domains III and IV to the intracellular part of the pore like a hinged lid (Vassilev et al., 1988, 1989).

To date nine distinct α-subunits (NaV1.1-1.9) have been identified but only NaV1.4 and 1.6 are expressed in skeletal muscle (Table 2.1). NaV1.4 is highly expressed in skeletal muscle (Bailey et al., 2003) and is co-expressed with the β1-subunit which increases the current through the channel. The importance of its action in skeletal muscle is evidenced by mutations in the channel causing NDM and PP. NaV1.6 is primarily expressed in denervated muscle. The β3-subunit is also expressed in skeletal muscle and acts by facilitating fast gating so may play a role in modulating the muscle action potential (Jurkat-Rott et al., 2006).

2.1.1.4 Voltage-gated Calcium Channels

The voltage-gated calcium channels aid in regulating neurotransmission, contraction and secretion as well as shaping action potentials. They act as a coupling mechanism between electrical signals and the physiological events in cells. They consist of four or five different subunits. The primary subunit, α1-subunit, has a similar arrangement to the sodium channel as it is made up of four domains (I-IV) each with six transmembrane segments. The S4 segment confers voltage sensitivity and the loop between S5 to S6 determines ion selectivity. In this loop, three amino acids in domains I, III and IV confer calcium ion selectivity.

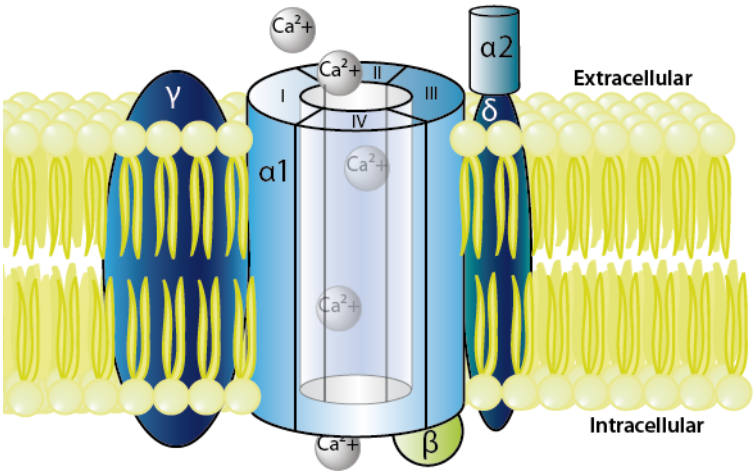


Figure 2.4: Representation of the P/Q-type calcium channel with the central pore-forming subunit (α1) and its auxiliary subunits (α2, β,γ & δ).

The auxiliary subunits modify the properties of the channel. Most calcium channels have an intracellular β-subunit, a disulphide-linked $\alpha 2\delta$-subunit and, in skeletal muscle cells, a γ-subunit1 (Figure 2.4).

26 different mammalian calcium channel subunit genes have been identified to date (Seal et al., 2011), 10 of these encode the α1-subunit (Table 2.1). The L-type CaV1.1 (CACNA1S) is the one most predominantly expressed in skeletal muscle. The β-subunits are known to accelerate current activation kinetics (Lacerda et al., 1991) and γ1-subunit can increase the inactivation rate (Ursu et al., 2004). This channel is also known to interact with the ryanodine receptor (RYR1) via the interlinker between domains II and III, helping to mediate excitation-contraction coupling and activate RYR1 (Paolini et al., 2004). Its vital role in skeletal muscle is illustrated by mutations in the gene causing hypokalaemic periodic paralysis and susceptibility to malignant hyperthermia.

2.1.1.5 Chloride Channels

Chloride channels play a number of important roles throughout the mammalian system. They stabilise membrane potential, regulate cell volume and intracellular pH, modulate neuronal excitability and are vital in signal transduction and epithelial salt transport.

The only group of chloride channels that are voltage-gated belong to the ClC family (Table 2.1). This family consists of nine genes, four of which encode voltage-gated chloride channels and the other five encode chloride/hydrogen ion exchangers. The mammalian voltage-gated chloride channels are activated on depolarisation and slowly inactivate with

hyperpolarising steps. They are distinct from the other voltage-gated channels as they form a homodimer with two separate pores (protochannels). A key characteristic is that each of the protochannels have individual voltage-sensors, selectivity filters and are independently gated (Miller and White, 1984) with a "fast" gate with independent opening and a "slow" gate which can close both pores simultaneously. Both gates are sensitive to changes in voltage, chloride ion concentration and pH (Pusch, 2004) (Figure 2.5b).

ClC channels consist of 18 transmembrane domains per subunit with a cytoplasmic amino-terminus and two cystathionine β-synthase (CBS) domains in the cytoplasmic C-terminus (Dutzler et al., 2002) (Figure 2.5a). The ClC-1 channel is the main isoform expressed in skeletal muscle (Steinmeyer et al., 1991). It is important for maintaining the resting membrane potential and repolarisation, accounting for 85% of the resting membrane conductance (Bryant and Morales-Aguilera, 1971; Palade and Barchi, 1977). The importance of this channel in skeletal muscle is illustrated by mutations causing myotonia congenita (MC). In this disease patients have increased membrane excitability which triggers repetitive sodium channel opening resulting in stiffness.

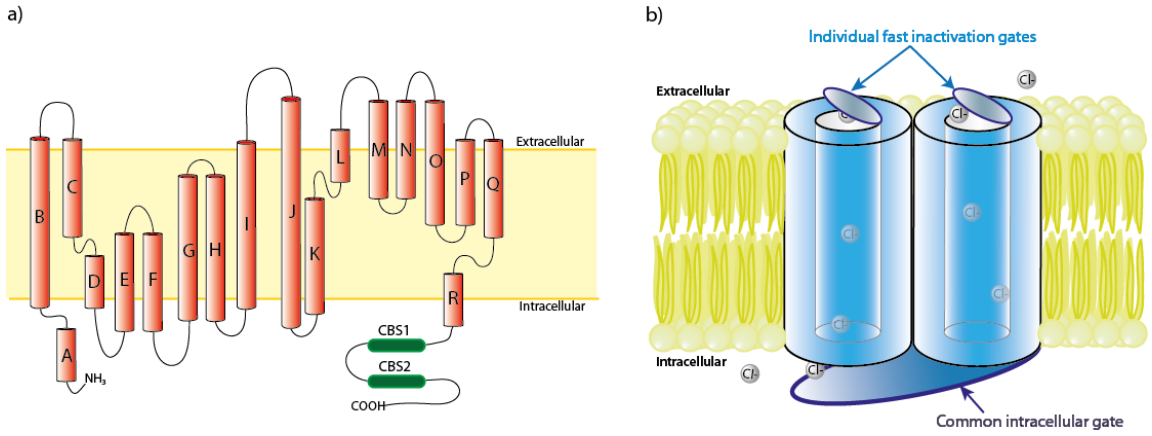


Figure 2.5: Chloride channel, ClC-1 (a) Representation of the membrane topology of the chloride channel (b) Schematic diagram of the double-barrelled ClC-1 channel. This shows two separate pores formed by each ClC-1 subunit, the common slow intracellular gate and the individual fast inactivation gates.

Table 2.1: Table of voltage-gated ion channels and corresponding genes

	Ion channels	Gene
Inward rectifying potassium channels		
	$K_{ir}1.1$	*KCNJ1*
	$K_{ir}2.1$	*KCNJ2*
	$K_{ir}2.2$	*KCNJ12*
	$K_{ir}2.3$	*KCNJ4*
	$K_{ir}2.4$	*KCNJ14*
	$K_{ir}3.1$	*KCNJ3*
	$K_{ir}3.2$	*KCNJ6*
	$K_{ir}3.3$	*KCNJ9*
	$K_{ir}3.4$	*KCNJ5*
	$K_{ir}4.1$	*KCNJ10*
	$K_{ir}4.2$	*KCNJ15*
	$K_{ir}5.1$	*KCNJ16*
	$K_{ir}6.1$	*KCNJ8*
	$K_{ir}6.2$	*KCNJ11*
	$K_{ir}7.1$	*KCNJ13*
Voltage-gated sodium channels		
	$Na_v1.1$	SCN1A
	$Na_v1.2$	SCN2A
	$Na_v1.3$	SCN3A
	$Na_v1.4$	SCN4A
	$Na_v1.5$	SCN5A
	$Na_v1.6$	SCN8A
	$Na_v1.7$	SCN9A
	$Na_v1.8$	SCN10A
	$Na_v1.9$	SCN11A
Voltage-gated calcium channels		
	$Ca_v1.1$	*CACNA1S*
	$Ca_v1.2$	*CACNA1C*
	$Ca_v1.3$	*CACNA1D*
	$Ca_v1.4$	*CACNA1F*
	$Ca_v2.1$	*CACNA1A*
	$Ca_v2.2$	*CACNA1B*
	$Ca_v2.3$	*CACNA1E*
	$Ca_v3.1$	*CACNA1G*
	$Ca_v3.2$	*CACNA1H*
	$Ca_v3.3$	*CACNA1I*
Voltage-gated chloride channels		
	ClC-1	*CLCN1*
	ClC-2	*CLCN2*
	ClC-Ka	*CLCNKA*
	ClC-Kb	*CLCNKB*
	ClC-3	*CLCN3*
	ClC-4	*CLCN4*
	ClC-5	*CLCN5*
	ClC-6	*CLCN6*
	ClC-7	*CLCN7*

2.2 Skeletal Muscle Channelopathies

As described, the skeletal muscle channelopathies are associated with variations in genes encoding key voltage-gated ion channels expressed in skeletal muscle. They form two distinct groups, the NDMs and the PPs.

The NDMs are a group of conditions characterised by muscle stiffness on voluntary movement due to delayed relaxation of the skeletal muscle. This group consists of MC, paramyotonia congenita (PMC) and sodium channel myotonia (SCM) (Figure 2.6). They are caused by mutations in either the chloride channel (CLCN1) or sodium channel (SCN4A) genes and are clinically distinct from the dystrophic myotonias, myotonic dystrophy type 1 (DM1) and type 2 (DM2), by the absence of systemic symptoms and progressive weakness.

The PPs are characterised by episodic muscle weakness often related to potassium levels. They consist of hyperkalaemic periodic paralysis (HyperPP), hypokalaemic periodic paralysis (HypoPP) and ATS, caused by mutations in the calcium (CACNA1S), sodium (SCN4A) and potassium channel (KCNJ2) genes respectively (Figure 2.6).

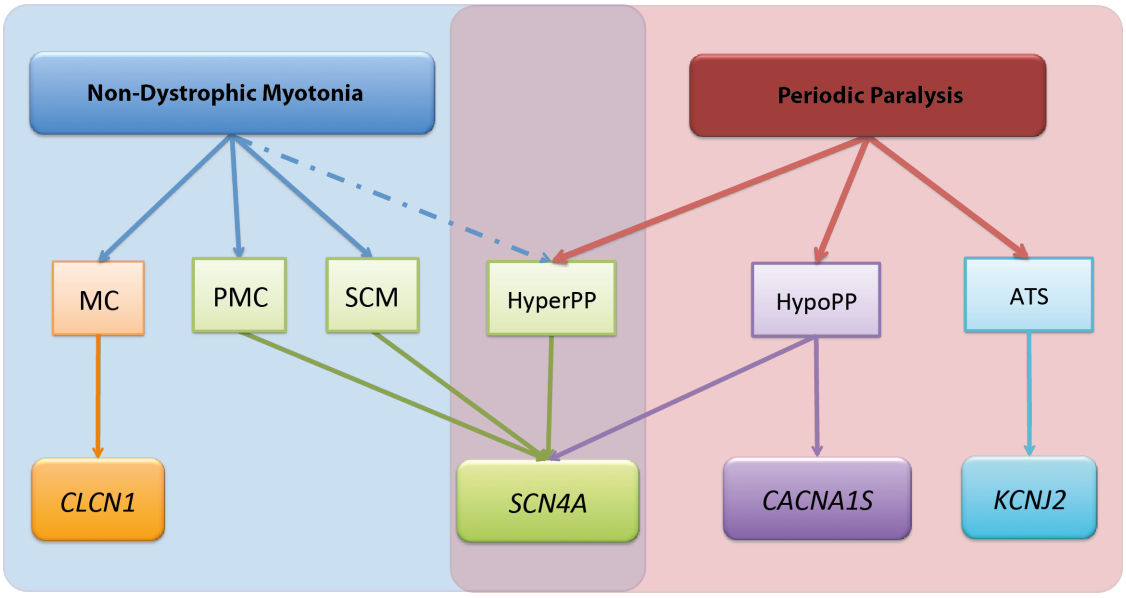


Figure 2.6: Flow chart of the different diseases associated with non-dystrophic myotonia and periodic paralysis and the associated genes.

2.3 Prevalence of Skeletal Muscle Channelopathies

Prevalence estimates are an important aspect of healthcare. They aid in estimating the burden of disease which aids in planning healthcare. They give an indication to patients of the rarity of a disease and are also useful in determining key patterns in the distribution of the disease, thereby offering insights into disease mechanisms. Performing prevalence studies also aids in establishing databases of affected patients which further aids research and clinical trials.

2.3.1 Current Prevalence Data

Estimates of the prevalence of skeletal muscle channelopathies vary widely depending on region. Published studies suggest a prevalence range from 1 to 10/100,000 (Jurkat-Rott et al., 2010), but worldwide the prevalence has not been accurately determined and the majority of studies pre-date genetic diagnosis which may compromise their accuracy.

2.3.1.1 UK Prevalence

To date there has only been one study looking at the prevalence of channelopathies in the UK. This study was conducted in 1954 and only investigated the prevalence of MC in North East England (Walton and Nattrass, 1954), estimating it at 0.3/100,000. A prevalence study in the UK is therefore timely.

2.3.1.2 Worldwide Prevalence

Prevalence studies in the rest of the world vary widely, from 0.4/100,000 in Switzerland (Emery, 1991) to 9.0/100,000 in Northern Norway (Sun et al., 2001). There are pockets of high prevalence, specifically of MC in the Scandinavian countries due to geographical location and the founder effect. The results of all of these studies are detailed in Table 2.2.

Previous studies have been very limited, only covering the more common diseases: MC, HypoPP and PMC. None of the published studies investigate the individual prevalence of HyperPP, ATS or SCM. These studies have also primarily focused on prevalence within small population groups making it difficult to be certain that they are true estimates over a larger region. The majority of studies have been conducted in the Scandinavian countries, which seem to have a much higher prevalence than other countries worldwide and therefore cannot be extrapolated to give an accurate estimate of prevalence in the UK. This lack of data further suggests that a comprehensive study of the prevalence of all the skeletal channelopathies is necessary and therefore this thesis investigates the prevalence in England.

Table 2.2: Studies evaluating the prevalence of non-dystrophic myotonias and periodic paralyses

Date	Reference	Geographical area	n	Total population	Prevalence (per 100,000)
Myotonia congenita					
1954	Walton et al, 1954	North East England[a]	6	2,262,292	0.3
1954	Kurland, 1958	Rochester, USA	1	29,885	3.3
1956	Emery, 1991	Switzerland*	18	4,714,992	0.4
1960	Becker, 1977	West Germany*	284	55,958,000	0.5
1963	Gudmundsson, 1968	Iceland	2	187,200	1.1
1979	Pinessi et al, 1982	Turin, Italy*	10	1,160,686	0.9
1988	Ahlström et al, 1993	Örebro, Sweden	3	269,341	1.1
1989	Ferrero et al, 1991	Central Spain[b]	11	235,976	4.7
1989	Al Rajeh et al, 1993	Thugbah, Saudi Arabia	2	23,227	8.6
1990	Emery, 1991	Ljubljana, Slovenia	10	1,996,377	0.5
1994	Hughes et al, 1996	Northern Ireland[c]	17	1,573,282	1.1
1995	Darin et al, 2000	Western Sweden[d]	3	359,676	0.8
1997	El-Tallawy et al, 2005	Assiut, Egypt	2	52,203	3.8
1998	Baumann et al, 1998	Northern Finland*	54	732,000	7.3
2001	Sun et al, 2001	Northern Norway*	45	500,000	9.0
2001	Chung et al, 2003	Hong Kong, China[e]	7	1,335,469	0.5
Average estimated prevalence[#]			*475*	*71,390,606*	***0.67***
Paramyotonia congenita					
1965	Emery, 1991	West Germany*	160	57,000,000	0.3
1983	Tangsrud et al, 1988	Southern Norway[f]	1	573,762	0.2
1995	Darin et al, 2000	Western Sweden[d]	4	359,676	1.1
Average estimated prevalence[#]			*165*	*57,933,438*	***0.28***
Hypokalemic periodic paralysis					
1978	Johnsen et al, 1981	Denmark*[g]	66	5,096,959	1.3
1983	Araki et al, 1987	Kumamoto, Japan	18	1,800,000	1.0
1983	Tangsrud et al, 1988	Southern Norway[f]	3	573,762	0.5
1989	Ferrero et al, 1991	Central Spain[b]	4	235,976	1.7
1992	Kantola et al, 1992	Finland*[h]	21	4,998,478	0.4
Average estimated prevalence[#]			*112*	*12,705,175*	***0.88***

*Studies specifically conducted to evaluate the prevalence of the periodic paralyses or the non–dystrophic myotonias. [#]Average estimated prevalence based on the total number of patients identified in the studies/total population. [a]Total population from 1951 Census of England and Wales. [b]Prevalence among 18 year–old males. [c]Prevalence of non–dystrophic myotonia. [d]Prevalence among children <16 years. [e]Prevalence among children <19 years. [f]Prevalence among children <18 years. [g]Only familial cases; total population data from Statistics Denmark. [h]Total population data from Statistics Finland.

2.4 Clinical Phenotypes of Skeletal Muscle Channelopathies

The skeletal muscle channelopathies form a spectrum of disorders with significant phenotypic overlap, which often creates difficulties in diagnosis. Overall they are characterised as conditions that cause episodic symptoms marked by either stiffness and/or weakness depending on the channel defect and its effect on muscle membrane excitability. Given the phenotypic and genotypic heterogeneity, phenotyping these patients is not only important for determining the diagnosis but also for informing management and understanding the natural history of the disease.

2.4.1 Phenotype of Non-Dystrophic Myotonia

NDM is a term used to describe the group of diseases characterised by predominant clinical and electrical myotonia. Myotonia is caused by membrane hyperexcitability, which triggers repetitive sodium channel opening, manifesting symptomatically as stiffness. The NDMs were traditionally distinguished from the "dystrophic" myotonias, DM1 and DM2, because patients with the condition did not demonstrate muscle degeneration on muscle biopsy. It is now known that this is not always the case, however genetically these are very distinct diseases. The myotonic dystrophies are multi-system disorders related to abnormal splicing of multiple genes whereas NDM is related to the abnormal function of a single ion channel gene only affecting skeletal muscle.

Prior to the advent of genetic testing the NDMs were divided according to inheritance and phenotype. Since the discovery of the causative genes they are now divided into two main groups, the chloride channelopathies (recessive and dominant MC) and the sodium channelopathies (PMC and SCM).

2.4.1.1 Chloride Channelopathies

The chloride channelopathies are made up of recessive MC and dominant MC and are characterised by impaired relaxation of skeletal muscle after voluntary contraction following a period of rest. The myotonia classically improves after repeated exertion, known as the "warm-up" phenomenon (Becker et al., 1977). Age of onset is approximately 10 years old (Trip et al., 2009b; Dupre et al., 2009; Trivedi et al., 2013), and patients primarily experience stiffness in the legs (Fialho et al., 2007; Trivedi et al., 2013). Some patients also report worsening in the cold (50-60%) and suffer with pain (approximately 30-50%) (Thomsen, 1876; Trivedi et al., 2013; Fialho et al., 2007; Trip et al., 2009b; Dupre et al., 2009).

Dominant versus Recessive Myotonia Congenita

MC has traditionally been subdivided according to inheritance into dominant MC (Thomsen's disease) (Thomsen, 1876) and recessive MC (Becker's myotonia) (Becker et al., 1977). Although these two subgroups have marked overlap in features, both having warm-up and varying degrees of muscle hypertrophy, some distinguishing clinical features have been reported (Fialho et al., 2007) (Table 2.3). Originally Becker reported dominant MC as having an earlier onset of symptoms, often being noticed in the first few of years of life, but more recent studies have illustrated that recessive MC has a younger age of onset which mirrors its increased severity (Trivedi et al., 2013; Fialho et al., 2007; Dupre et al., 2009). In dominant MC, symptoms are milder and more commonly seen in the upper limbs (Becker et al., 1977). Recessive MC, however, is associated with more severe myotonia, often with episodes of transient weakness on initiation of movement. It is also more commonly associated with generalised muscle hypertrophy (Trivedi et al., 2013; Fialho et al., 2007; Dupre et al., 2009). Of the two conditions, recessive MC is thought to have a higher prevalence (Becker et al., 1977; Baumann et al., 1998).

Much of the original phenotypic information on MC is derived from the original papers published by Thomsen and Becker before genetic testing was available. More recently however, there have been a number of comprehensive analyses of genetically confirmed groups of patients expanding the knowledge available on the phenotype of MC and looking closely at phenotype-genotype correlations, which is summarised in Table 2.3.

2.4.1.2 Sodium Channelopathies

The sodium channelopathies can be divided into PMC and SCM. The two types are distinguished clinically by the presence or absence of weakness, with patients with PMC having clear episodes of weakness, often with accompanying paramyotonia (worsening of myotonia with exercise) and cold sensitivity. SCM is the term now commonly used to encompass the traditional group of "pure myotonic" syndromes including the potassium aggravated myotonias (PAM) and cold-sensitive pure myotonic syndromes (Fournier et al., 2004, 2006; Matthews et al., 2010). The sodium channelopathies tend to have a younger age of onset than the chloride channelopathies, usually beginning between 3-5 years old (Trivedi et al., 2013; Trip et al., 2009b; Miller et al., 2004). Recent data suggests that cold is a common trigger (90-100%) (Trivedi et al., 2013; Matthews et al., 2010; Miller et al., 2004).

The sodium channelopathies are best distinguished clinically from MC by the presence of myotonia of face, eyes and tongue which, although may not be sensitive, is very specific to the sodium channelopathies (Matthews et al., 2010; Trip et al., 2009b) (Table 2.3).

Table 2.3: Clinical phenotype of the non-dystrophic myotonias

	Dominant MC	Recessive MC	PMC	SCM
Clinical Features: Onset:	Later onset (7-16yrs)	Early onset (4-12yrs)	Early onset (3-5yrs)	Early onset (3-5yrs)
Distribution:	Upper limbs > lower	Lower limbs > upper	Face & eyes	Face & eyes
Muscle Weakness:	No weakness	Transient weakness	Muscle weakness, may be prolonged	No weakness
Other findings:	Mild symptoms	Muscle hypertrophy Severe symptoms	Paramyotonia	Painful myotonia
Precipitants	Improves with exercise - "warm up" phenomenon		Cold & exertion	Potassium ingestion, cold, exertion
Electrophysiology Short Ex Test:	No change but fall in CMAP with cooling	Early fall in CMAP with early recovery with exercise	Gradual decrease in CMAP with further exercise, Marked fall with cold, does not recover with rewarming	No change even after cooling
EMG:	Myotonic potentials	Myotonic potentials	Myotonic potentials	Myotonic potentials
Fournier pattern:	Type III	Type II	Type I	Type III
Muscle Biopsy	Normal / absence of type 2b fibres		Normal/may have myopathic changes, large vacuoles & tubular aggregates	

Distinguishing Paramyotonia Congenita and Sodium Channel Myotonia

PMC was first described in 1886 by Eulenburg as episodic muscle cramps and paralysis, markedly worsened by exercise and cold (Eulenburg, 1886). This weakness may typically persist for hours or even days (Miller et al., 2004). It was considered distinct from MC as patients with PMC worsened rather than improved with exercise. A century later three other types of myotonia, characterised by lack of cold-sensitivity and episodic weakness were described which were felt to be distinct from PMC and MC. In 1987 Trudell *et al* identified a kindred with painful myotonia worsened by potassium and improved by acetazolamide that they termed Acetazolamide-responsive myotonia congenita (Trudell et al., 1987). In 1990, Ricker *et al* described a fluctuating type of myotonia with no weakness but worsening myotonia with delayed onset after exercise or ingestion of potassium and termed it myotonia fluctuans (Ricker et al., 1990). Finally a more severe type of myotonia fluctuans, myotonia permanens was described in patients with ventilation impairment from myotonia of the respiratory muscles (Lerche et al., 1993). These three conditions were grouped together

and called the "potassium-aggravated myotonias". Further cold-sensitive, pure myotonic phenotypes were also described that were thought to be clinically distinct from the PAMs as they were insensitive to potassium ingestion (Heine et al., 1993; Koch et al., 1995; Wu et al., 2001). Genetic testing unified these diseases as they and PMC were all found to be caused by mutations in the sodium channel SCN4A (Koch et al., 1991a; McClatchey et al., 1992a,b; Lerche et al., 1993; Ptacek et al., 1991, 1993, 1994; Rüdel et al., 1993). Following this discovery the term "sodium channel myotonia" began to be used to describe the PAMs and pure myotonic phenotypes associated with SCN4A mutations (Rüdel et al., 1993; Lehmann-Horn et al., 1993; Fournier et al., 2004, 2006; Rüdel and Lehmann-Horn, 1997; Matthews et al., 2010). They were always felt to be distinct from PMC because of the absence of episodes of weakness and this formed the basis for the definition of these two diseases for the last 30 years.

SCM is characterised by pure myotonia of varying severity without weakness. Some patients may show marked worsening with potassium but are often unaffected by cold. Out of all the NDMs they tend to show the most marked painful myotonic episodes although pain often occurs in both subtypes (Ptacek et al., 1994). There is very little data comparing the phenotypes of PMC and SCM as much of the data focusses on the characteristics of all of the sodium channelopathies. The data that does exist, suggests that SCM patients often have warm-up and tend to be less cold sensitive making them appear clinically very similar to dominant MC and are often very difficult to distinguish (Trip et al., 2009b). There is therefore a need for more research looking into the key differences between these groups of patients.

Neurophysiology techniques also emerged to further aid diagnosis of patients with NDM. Fournier *et al* described the use of short and long exercise testing at room temperature and cooling to help differentiate between PMC and SCM (Fournier et al., 2004, 2006). They identified that patients with PMC commonly had a type I pattern with progressive decrease in compound muscle action potentials (CMAP) with subsequent trials of exercise and a decrease on long exercise testing. SCM patients had a type III pattern with no decrement on short or long exercise testing. As a result in current clinical practice, patients are diagnosed as PMC or SCM based on a combination of the presence or absence of weakness and their pattern of decrement on neurophysiology exercise testing. However, as groups have begun to correlate phenotype and genotype in larger numbers of patients with specific SCN4A

mutations it has become apparent that certain mutations can be associated with both PMC and SCM phenotypes and that neurophysiology types may not be as clearly differentiating as first thought (Lerche et al., 1993; Ferriby et al., 2006; Matthews et al., 2008b; Trivedi et al., 2013). This has led to questions about how a specific mutation could cause two distinct diseases and about how best to differentiate between PMC and SCM given the genotypic heterogeneity.

2.4.2 Phenotype of Periodic Paralysis

The primary periodic paralyses can be subdivided into HypoPP, HyperPP and ATS. They are characterised by episodes of weakness often associated with a change in serum potassium concentration. Secondary PP has also been documented when patients develop hypo or hyperkalaemia as a result of a secondary condition (Table 2.4). More recently, a fourth type of PP, thyrotoxic PP, causing episodes of paralysis in the context of thyrotoxicosis, has been discovered to have an underlying genetic basis rather than being a secondary type of PP.

Table 2.4: Common causes of secondary periodic paralysis

Secondary Hypokalaemia	Secondary Hyperkalaemia
• Thyroxicosis/ hypothyroidism	• Hyporeninemic hypoaldosteronism (secondary to chronic kidney disease)
• Drugs - Thiazide/Loop diuretic - Liquorice - Steroids - Alcohol, cocaine	• Drugs - Potassium-sparing diuretics
• Hyperaldosteronism	• Metabolic acidosis
• Renal Tubular Acidosis	• Type IV renal tubular acidosis
• Gastro-intestinal potassium loss	• Rhabdomyolysis

2.4.2.1 Hypokalaemic Periodic Paralysis

HypoPP is characterised by attacks of weakness associated with an initial low serum potassium, beginning in the first or second decade of life. Attacks most commonly occur in the night or early morning and may last from hours to days. They are often precipitated by a carbohydrate load or rest after exercise and they may be focal or more generalised and involve all four limbs and neck, but usually sparing facial and respiratory muscles. In rare cases, respiratory compromise has been noted (Miller et al., 2004; Kil and Kim, 2010). Approximately 72% of patients develop a persistent proximal myopathy that in some cases

may become disabling over time (Miller et al., 2004; Links et al., 1990).

HypoPP1 versus HypoPP2

There are two forms of HypoPP, one caused by calcium channel mutations (HypoPP1) and the other caused by sodium channel mutations (HypoPP2). Clinical differentiation between these subtypes of HypoPP is difficult given the sparsity of evidence available. The few studies that exist suggest that those with calcium channel mutations may have an earlier age of onset, usually around 10 years compared to 16 years for HypoPP2. HypoPP1 is thought to have a higher incidence of fixed proximal weakness and longer duration of episodes (20 hours versus 1 hour) (Miller et al., 2004) (Table 2.5). Further studies are needed in this area to identify differences between these two groups and to further delineate genetically confirmed cases.

2.4.2.2 Hyperkalaemic Periodic Paralysis

HyperPP manifests earlier than HypoPP, often in the first decade, although in a recent study 25% of patients manifested in the second decade (Charles et al., 2013). Attacks tend to be worst in adolescence and early adulthood and decrease in frequency and severity later in life (Charles et al., 2013; Miller et al., 2004). Attacks are thought to be brief, lasting around one to two hours, although there have been reports of longer attacks lasting several days (Charles et al., 2013; Miller et al., 2004). Attacks primarily occur in early morning or during sleep. Patients are typically potassium sensitive and may have a high potassium at the start of the attack. A separate term, normokalaemic periodic paralysis, was coined to describe those with normal potassium levels during an attack. Initially this was considered a separate intermediate disease but following genotyping, all patients had typical HyperPP mutations, suggesting it is a phenotypic variant of HyperPP (Chinnery et al., 2002). Other attack precipitants include rest after exercise, cold exposure and stress. Patients may develop a fixed proximal myopathy but this is less common than in HypoPP (Miller et al., 2004) (Table 2.5).

A key distinguishing feature from HypoPP is the presence of electrical myotonia which is not found in either type of HypoPP (Sugiura et al., 2000). Around 75% of patients may have clinical or electrical myotonia (Charles et al., 2013; Miller et al., 2004). Due to the presence of myotonia, some patients may have overlapping clinical phenotypes with PMC and it can be difficult to distinguish between the two. Charles *et al* suggested a distinction between types of HyperPP as: (i) those without myotonia, (ii) those with clinical or electrical myotonia and (iii) those with a PMC phenotype (Charles et al., 2013). This clinically allows

Table 2.5: Clinical phenotype of the primary periodic paralyses

	HyperPP	HypoPP1	HypoPP2	ATS	Thyrotoxic PP
Clinical Features					
Onset	1st decade (25% 2nd decade)	1st/2nd decade	Later onset (2nd decade)	1st/2nd decade	Very late onset, 20-40yrs
Attacks:	Short episodes (1-4hrs) Focal attacks Attacks reduce in severity over time	Long episodes (hrs-days) Often generalised attacks	Short episodes (hrs)	Attacks may follow any of the PP patterns	Short attacks (6-8hrs)
Ictal potassium levels	High or normal	Low	Low	Low, high or normal	Low potassium
Weakness:	May develop fixed proximal weakness	Fixed proximal weakness	Less likely to develop fixed weakness		
Precipitants	Potassium loading, Rest after exercise	Carbohydrate load, Rest after exercise	Rest after exercise Acetazolamide	Prolonged rest, Rest after exercise	Thyrotoxicosis Carbohydrates, rest after exercise
Electrophysiology					
Short Ex Test	Rise in CMAP	No change	Rise/ no change	Show pattern appropriate for type of paralysis attacks	
:Long Ex test:	Initial rise in CMAP then fall in CMAP	Initial rise in CMAP	Initial rise and may see fall in CMAP		
EMG	May have myotonia	No myotonia	No myotonia	No myotonia	
Fournier pattern:	Type IV	Type V	Type IV or V	Type IV or V	
Muscle Biopsy	Normal/ vacuolar myopathy	Normal/ vacuoles or myopathic changes	Normal/ tubular aggregates or vacuoles	Normal/ tubular aggregates	Normal/vacuoles and myopathic changes
Genotype	SCN4A	CACN1S	SCN4A	KCNJ2	KCNJ18

a useful differentiation of where these patients lie on the spectrum of PP and NDM.

The recent trial by Charles *et al* has aided in further phenotyping HyperPP (Charles et al., 2013) but this study demonstrated significant differences with the earlier literature and included patients with mutations associated with PMC and SCM. The data therefore has to be regarded with a little caution and further evidence would be useful to clarify the phenotypes in this group of patients.

2.4.2.3 Andersen Tawil Syndrome

ATS is a distinct type of PP, marked by the fact that it affects multiple organs. It is characterised by the triad of episodes of paralysis, cardiac abnormalities and distinctive facial and skeletal features. The episodes of paralysis begin in the first or second decade and are exacerbated by prolonged rest or rest following exertion. The attacks are most commonly associated with hypokalaemia, but may also be associated with hyperkalaemia or normokalaemia (Andersen et al., 1971; Tawil et al., 1994) (Table 2.5).

The distinctive features seen include micrognathia, low set ears, hypertelorism and skeletal abnormalities, such as clinodactyly and syndactyly (Tawil et al., 1994; Tristani-Firouzi et al., 2002) (Figure 2.7). Cardiac arrhythmias are commonly seen, the most frequent ECG finding being long QT, but a range of ECG changes are described including premature ventricular contractions, ventricular arrhythmias, ventricular bigeminy and prominent U waves even in the presence of a normal potassium. Bidirectional ventricular tachycardia may also occur. Fatal arrhythmias are reported, but are rare compared with other genetically-determined long QT syndromes (Haruna et al., 2007).

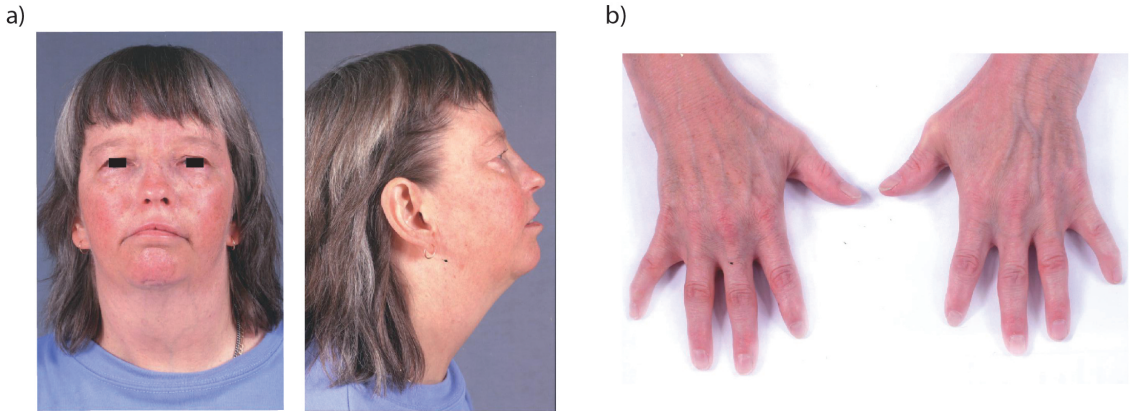


Figure 2.7: Images of a patient with ATS. (a) Face demonstrates micrognathia, low set ears and hypertelorism. (b) Small hands with clinodactyly and syndactyly

In a recent study investigating 45 genetically confirmed KCNJ2 patients with a large

variety of phenotypes only 13% had all three features of the triad, 67% had arrhythmias, 40% had periodic paralysis and 36% had dysmorphisms. 88% however, had abnormal U waves on ECG. This suggests that the ATS phenotype does not require all three components of the triad as suggested previously (Kimura et al., 2012). Very little data has been collected comparing patients with ATS with those with other types of PP and a comparative study may further the understanding of this disease.

2.4.2.4 Thyrotoxic Periodic Paralysis

Secondary HypoPP has always been considered as a separate entity to inherited primary PP. More recently however, there has been the discovery of a susceptibility gene for thyrotoxic PP, suggesting this acquired disease may have a genetic basis (Ryan et al., 2010).

Thyrotoxic PP was thought to be an acquired muscle disorder, characterised by typical HypoPP attacks in the context of thyrotoxicosis, which resolves with the treatment of the thyrotoxicosis. Clinically it is indistinguishable from familial HypoPP except for the presence of thyrotoxicosis and the lack of family history. It occurs in approximately 2% of thyrotoxicosis cases in East Asians and Hispanics (Okinaka et al., 1957) and around 0.1-0.2% of non-Hispanic Caucasians (Kelley et al., 1989). It is more common in males and occurs at around 20-40 years. Attacks predominantly occur at night following triggers such as a carbohydrate load, alcohol or strenuous activity (Kung, 2006); (Falhammar et al., 2013). Mutations in KCNJ18 have now been reported in both thyrotoxic PP cases and some sporadic PP cases (Ryan et al., 2010; Cheng et al., 2011) (Table 2.5).

2.4.3 Anaesthetics in Skeletal Muscle Channelopathies

There is very little data available on the effect of anaesthetics in patients with muscle channelopathies. Much of the current knowledge is based on case reports. MC has traditionally been associated with the risk of malignant hyperthermia (MH)-like episodes. This is based on a handful of case reports that pre-date genetic testing. In one report a boy with a MC phenotype developed a hyperthermia-like episode following administration of a non-triggering anaesthetic and subsequently died despite pretreatment with dantrolene, but this child developed a very high creatine kinase (CK) and other complications (Haberer et al., 1989). In another, a girl with possible MC was given a depolarising anaesthetic and died after a typical MH reaction (Saidman et al., 1964). The only other case studies report severe myotonia after the use of depolarising muscle relaxants such as suxamethonium

(Heiman-Patterson et al., 1988; Farbu et al., 2003). Given that there are no further case reports of MH-like reactions since genetic diagnosis was developed it does suggest that these reactions could be due to other causes. There is however the suggestion that myotonia may be worsened by depolarising muscle relaxants and therefore these are commonly avoided.

In PMC and SCM there are no reports of MH with general anaesthetic. In HyperPP there are a few reports of potassium-releasing drugs such as succinylcholine inducing weakness (Weller et al., 2002) or spasm (Paterson, 1962; Cody, 1968). There have been no reports of problems with non-depolarising anaesthetic. In patient surveys 20-30% of patients reported developing an attack of weakness following general anaesthesia although the type of anaesthetic was not specified (Miller et al., 2004; Charles et al., 2013).

In HypoPP there have been case reports of MH in patients, however the only patient that was genetically confirmed had an RYR1 mutation, which is known to be associated with MH (Marchant et al., 2004). In a survey of patients, very few reported weakness following anaesthetic (Miller et al., 2004). There has, however, been an association with a mutation in the CACNA1S gene that confers susceptibility to MH and therefore caution is exercised when giving these patients anaesthetic (Monnier et al., 1997). In ATS no data was available on responses to anaesthesia.

2.4.4 Pregnancy in Skeletal Muscle Channelopathies

There is even less information regarding pregnancy in muscle channelopathies than for the use of anaesthetics in this cohort. Data suggests that pregnancy and menstruation may worsen symptoms in 25% of female MC patients and 44% of female sodium channelopathy patients (Trivedi et al., 2013). In HyperPP 92% of women reported an increase in attack frequency although 80% thought their attacks were less severe. 75% had worsening stiffness and 25% had an improvement in stiffness (Charles et al., 2013). In ATS no data was available in pregnancy.

2.4.5 Investigating Clinical Phenotypes

Prior to the advent of genetic testing in muscle channelopathies, extensive phenotyping of patients was performed to try and understand and categorise these diseases. With the identification of causative mutations, phenotyping has become more complex and large studies investigating patient phenotype have become more sparse. Much of the current wisdom is still based on the original work pre-dating genetic testing and therefore may be

inaccurate. It is therefore timely that a large scale study of clinical phenotypes is done to better understand the natural history and prognosis of these diseases and better inform patients and direct treatment trials.

In the UK, extensive phenotyping data has already been published for the MC cohort by Fialho *et al* (Fialho et al., 2007) and therefore this thesis will concentrate on the phenotyping of the other muscle channelopathies. Very little data exists on the effect of pregnancy and anaesthetics in all channelopathy patients and therefore this is another aspect investigated in this thesis.

2.5 Genetic Diagnosis in Skeletal Muscle Channelopathies

2.5.1 Genotypes of Skeletal Muscle Channelopathies

Across the two main groups of skeletal muscle channelopathies, PP and NDM, a number of different genes are known to be responsible for disease, although there is extensive genotypic heterogeneity (Figure 2.6).

2.5.1.1 Hypokalaemic Periodic Paralysis

HypoPP is associated with variations in the calcium channel gene, CACNA1S, causing HypoPP type 1 (HypoPP1) (Fontaine et al., 1994) and more rarely variations in the sodium channel gene, SCN4A, causing HypoPP type 2 (HypoPP2) (Bulman et al., 1999). HypoPP1 was first associated with mutations in CACNA1S after linkage mapped the locus to chromosome 1 (Fontaine et al., 1994). 90% of mutations affect key arginine residues in the S4 segments causing loss of the positive charge. The majority of patients have mutations that affect the Arg528 or Arg1239 positions in domains II and IV (Matthews et al., 2009) (Figure 2.8). Mutations in SCN4A that are associated with HypoPP2 are also located at arginine residues of the S4 segments, highlighting the importance of the voltage-sensing regions of these channels in causing disease (Matthews et al., 2009) (Figure 2.9).

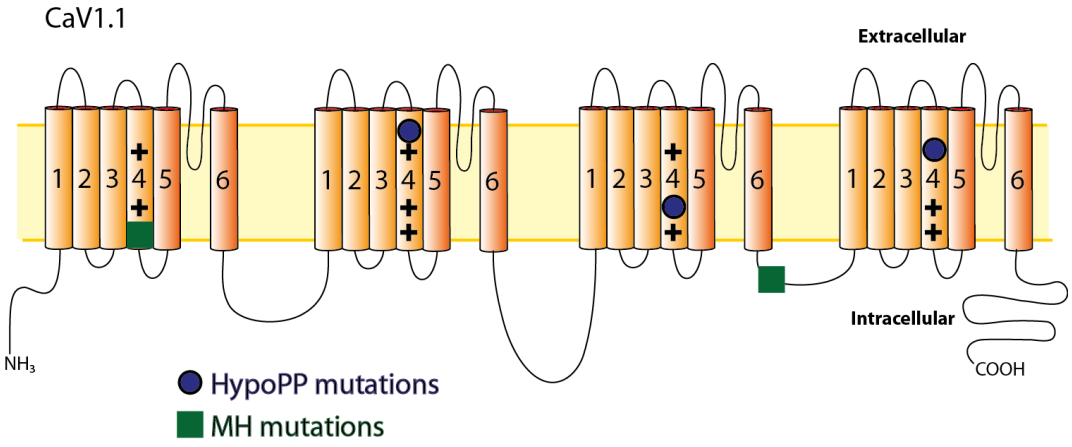


Figure 2.8: Variations in CaV1.1 known to be associated with HypoPP and malignant hyperthermia

Mutations in CACNA1S have also been associated with a small number of cases of MH. MH occurs when there is increased release of calcium into the myoplasm under certain conditions (exposure to inhaled anaesthetics and suxamethonium), causing increased muscle metabolism and leading to hypoxia, metabolic acidosis and rhabdomyolysis. It is thought to be due to uncoupling of the ryanodine receptor, RyR1, from the calcium

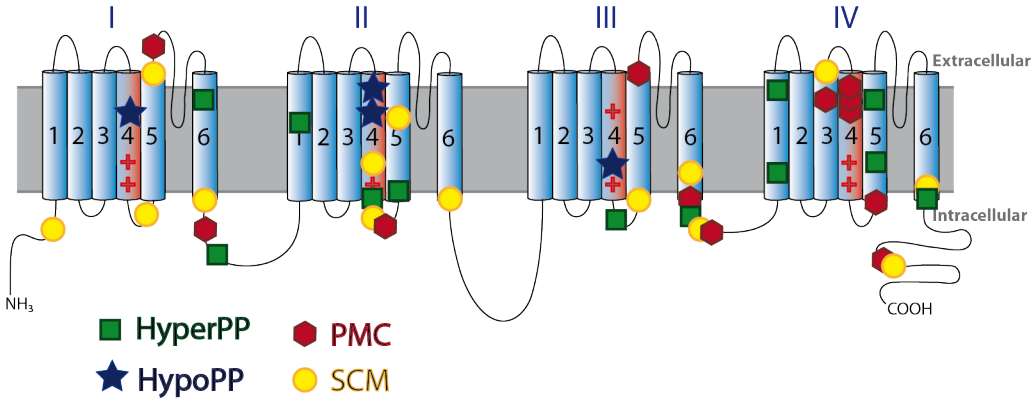


Figure 2.9: Known NaV1.4 mutations associated with HyperPP, HypoPP, PMC and SCM.

channel, CaV1.1 (encoded by CACNA1S) (Weiss et al., 2004). The majority of cases are due to mutations in the RYR1 gene but a small number of cases have been identified with mutations in CACNA1S (Monnier et al., 1997; Stewart et al., 2001; Carpenter et al., 2009) (Figure 2.8). Conversely there has also been a case of atypical periodic paralysis with multi-minicore disease associated with a variation in RYR1 which suggests that disruption of excitation-contraction coupling may also lead to episodic attacks of paralysis (Zhou et al., 2010).

Recently it was discovered that mutations in KCNJ18 predispose individuals to thyrotoxic PP (Ryan et al., 2010). This gene is expressed in muscle and transcriptionally regulated by the thyroid hormone. However, mutations in this gene were only identified in 33% of patients with this phenotype, suggesting it may be a susceptibility factor. With such a large diversity of potassium channels in the human genome it also leads to the suspicion that other genes expressed in skeletal muscle may be responsible for undiagnosed cases of PP.

2.5.1.2 Hyperkalaemic Periodic Paralysis

HyperPP (including normokalaemic PP) is also associated with variations in SCN4A (Fontaine et al., 1990; Koch et al., 1991b). Approximately 60% of mutations are located in exons 13 and 24, with the commonest variations being Thr704Met and Met1592Val (Jurkat-Rott and Lehmann-Horn, 2007). The remaining variations are spread throughout the gene (Figure 2.9).

2.5.1.3 Andersen Tawil Syndrome

ATS is associated with variations in the potassium channel gene, KCNJ2 which encodes Kir2.1, an inward-rectifying potassium channel (Plaster et al., 2001). To date over 30

different missense mutations or small in-frame deletions have been described throughout the gene, with many families having private mutations. Variations in this gene, however, only account for 60% of cases and the original case described by Tawil remains undiagnosed making it extremely likely that there is genetic heterogeneity in this disease (Donaldson et al., 2004). This has been illustrated by the recent finding of a variation in KCNJ5 (encoding the Kir3.4 channel), implicated in a case of ATS with episodic weakness and arrhythmias without dysmorphic features. The mutated Kir3.4 was shown to reduce potassium current when co-expressed with Kir2.1 (Kokunai et al., 2014).

2.5.1.4 Paramyotonia Congenita and Sodium Channel Myotonia

Variations in SCN4A are also associated with PMC and SCM (McClatchey et al., 1992a; Koch et al., 1991a). At present, 16 mutations in SCN4A have been documented as causing a PMC phenotype (Matthews et al., 2010). The majority of mutations lie in exons 22 and 24 (90% in UK cohort and 100% in Dutch cohort) (Trip et al., 2008; Matthews et al., 2008b), the commonest being the Thr1313Met mutation and those at the Arg1448 position (Figure 2.9).

Over 30 mutations in SCN4A have been associated with the SCM phenotype (Matthews et al., 2010) but unlike PMC they are spread throughout the sodium channel. The commonest variations, however are still in exons 22 and 24 and are associated with Val1589Met and those at position Gly1306 (Vicart et al., 2005) (Figure 2.9). Although SCM is thought to be clinically distinct from PMC, there is significant heterogeneity, with many of the same variations causing either SCM or PMC in both different pedigrees and within the same pedigree (Matthews et al., 2008b; Vicart et al., 2005). It is unclear why this phenotypic variability occurs for the same mutations.

2.5.1.5 Myotonia Congentia

MC is associated with variations in the chloride channel gene, CLCN1, resulting in dominant or recessive MC (Koch et al., 1992). This gene has 23 exons and is composed of 18 transmembrane domains. To date over 120 mutations have been published in the literature with further variations still being identified (Matthews et al., 2010) (Figure 2.10). Variations associated with dominant MC primarily cluster around exon 8 whereas recessively inherited mutations may occur anywhere in the channel and in many cases are specific to individual pedigrees (Fialho et al., 2007).

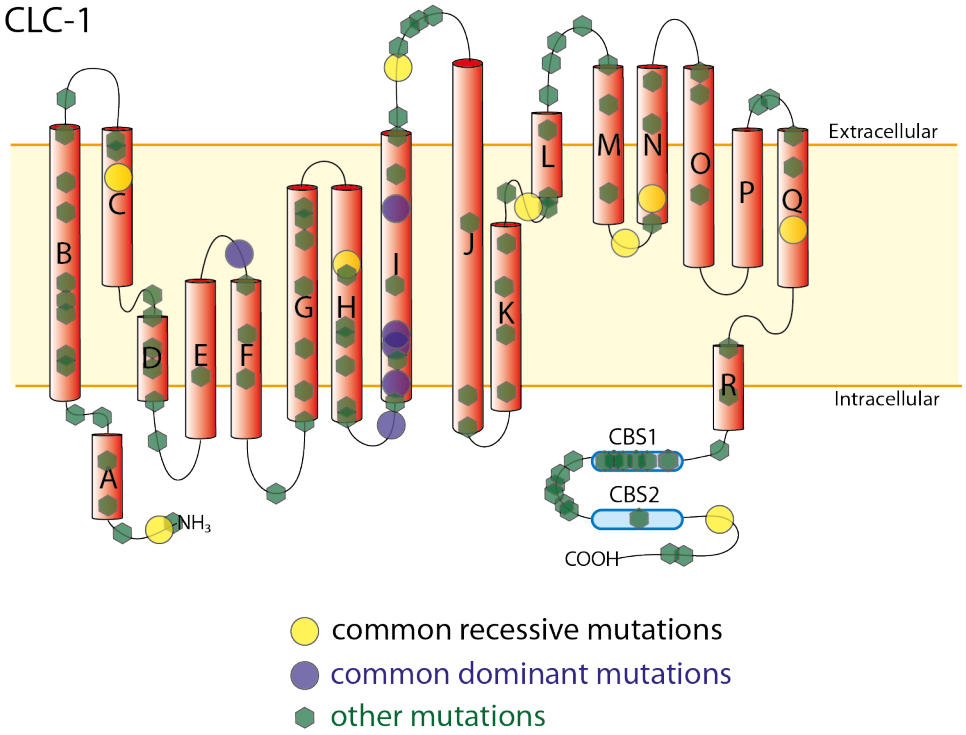


Figure 2.10: Known pathogenic ClC-1 mutations

2.5.2 Making a Genetic Diagnosis in Skeletal Muscle Channelopathies

2.5.2.1 Current Diagnostic Methods

In current clinical practice, patients are diagnosed following detailed phenotype analysis to determine the appropriate genetic test. This allows classification of patients into PP or NDM and then further disease-specific classification to direct testing. Patients then typically have Sanger sequencing of key genes, directed by the most likely diagnosis (Figure 2.11) (Raja Rayan and Hanna, 2010).

Genetic Diagnosis in Periodic Paralysis

Patients with probable PP are split into HyperPP, HypoPP or ATS phenotypes. HyperPP patients have sequencing of exons 13 and 24b of SCN4A. Patients with a HypoPP phenotype have exons 11 and 30 of CACNA1S sequenced and patients with probable ATS have the coding exon of KCNJ2 sequenced. This strategy was developed on the basis of the location of the common mutations in each of these diseases. In HyperPP this strategy will cover the mutations Thr704Met and Met1592Val, which account for approximately 30% and 13% of known mutations respectively (Jurkat-Rott and Lehmann-Horn, 2007). In HypoPP, mutations at positions 528 and 1239 (in exons 11 and 30) account for approximately 55-70% of mutations (Sternberg et al., 1993). In ATS the mutations are spread throughout the coding

exon and so the whole exon is sequenced but this still only identifies approximately 60% of cases (Donaldson et al., 2003) (Figure 2.11).

If patients do not have a causative mutation identified using the initial strategy then second level testing is initiated which involves expanding the number of exons sequenced (Figure 2.11). In HypoPP, second level testing involves sequencing the voltage-sensing S4 segments of CACNA1S and SCN4A. These are located in exons 4,11,12,21 and 30 of CACNA1S and exons 5,12,13,18 and 24 in SCN4A. Evidence suggests that 90% of known HypoPP mutations are located within these regions. These S4 segments include the key arginine residues that, when substituted, lead to the formation of a gating pore current which is thought underlie the episodes of paralysis (Matthews et al., 2009).

As the mechanism behind HyperPP is different and does not specifically involve S4 segments, mutations may occur throughout the gene. In these cases, second level testing therefore involves sequencing the whole of SCN4A if initial targeted sequencing is negative (Figure 2.11).

Genetic Diagnosis in Non-dystrophic Myotonia

Prior to testing, patients with NDM are split into either sodium channel (PMC/SCM) or chloride channel (MC) phenotypes. If PMC/SCM is suspected, patients have sequencing of exons 22 and 24 of SCN4A, which identifies approximately 69% of mutations (Trip et al., 2008; Matthews et al., 2008b). If no mutations are found then the remaining exons of SCN4A are sequenced and this typically gives a diagnostic rate of approximately 93% (Trip et al., 2008) (Figure 2.11).

If MC is suspected then patients have all 23 exons of CLCN1 sequenced. This is because many patients have private mutations that are spread throughout the gene (Fialho et al., 2007) (Figure 2.11).

There are a number of patients with MC that only have one mutation identified after sequencing all 23 CLCN1 exons. In the majority of cases these patients have a dominant pedigree and a phenotype consistent with Thomsen's disease (Koch et al., 1992). In those cases, the mutations cause dominant-negative inhibition of the homodimer leading to disease. This can be illustrated by whole cell patch clamping of *Xenopus* oocytes that co-express the mutated and a normal ClC-1 channel and demonstrating a drop in current in heterozygous oocytes compared with wild-type (WT) (Pusch et al., 1995). However, there are a number of cases where only one mutation is identified but the patient has a recessive pedigree, a

recessive MC phenotype and a mutation that only causes a drop in current significant enough to cause myotonia when homozygously expressed (Trip et al., 2008). In these cases it is hard to explain the patients' phenotype with a single loss of function mutation as clinical myotonia occurs when there is a minimum of 60% drop in chloride conductance (Barchi, 1975; Kwiecinski et al., 1988) and a single loss of function mutation would not be sufficient to cause this degree of drop. These single mutations in recessive MC cases are found in approximately 12-14% of MC cases (Trip et al., 2008; Fialho et al., 2007) and therefore these cases need further investigation.

2.5.2.2 Rate of Genetic Diagnosis in Clinical Practice

Using the current schema the rate of genetic diagnosis in clinical practice is approximately 70-80% and in ATS may be as low as 60% (Donaldson et al., 2003). In PP the rate is 80% when key exons and S4 segments are sequenced (Sternberg et al., 1993; Jurkat-Rott and Lehmann-Horn, 1993). In NDM it is as high as 93% when the whole of CLCN1 and SCN4A are sequenced (Trip et al., 2008), however this does include the 12% of patients with recessive MC that only have a single mutation identified.

2.5.3 Possible Explanations for Undiagnosed Patients

20-40% of patients remain undiagnosed despite extensive Sanger sequencing of the known genes. This suggests that there are novel variations that remain to be identified in these patients. There are a number of possible reasons for this including limitations of the current techniques preventing discovery of the variations and the possibility of novel genes underlying some of these cases.

2.5.3.1 Limitations of Current Techniques

Sanger sequencing has a number of limitations which may account for some of the undiagnosed patients. Firstly, current sequencing utilises primers that only cover the coding exons of genes with a small overlap of 100-150 base pairs of the intron at the intron-exon and exon-intron boundaries. This means that any pathogenic variations that lie deep within the introns would not be detected. Secondly, although Sanger sequencing can detect small insertions or deletions, it may miss any whole exon or group of exon deletions as it is unable to accurately detect a reduction in the volume of sequence data. This means it would not identify large scale rearrangements in patient DNA. Finally Sanger sequencing has a false negative rate which is caused by a combination of operator error, the presence of variations

within the PCR or sequencing primer binding site and tissue mosaicism.

Intronic Variations

Intronic variations may underlie the diagnosis in some of the undiagnosed cases. Typically intronic mutations have been associated with disease in recessive MC where they affect the splicing of CLCN1 exons (Chen et al., 2004; Brugnoni et al., 1999; Meyer-Kleine et al., 1995). However, the majority of intronic mutations discovered in MC were identified during routine sequencing of the exons as they are located near to intron-exon boundaries affecting conserved splice sites. Traditionally it has been difficult to prove pathogenicity of these variations without tissue samples but the recent development of a mini-gene assay has established an effective method of determining pathogenicity of these variations (Ulzi et al., 2012).

Deeper intronic variations were traditionally felt to be unlikely to cause disease but numerous reports now exist of variations that create cryptic splice donor and acceptor sites (Dobkin et al., 1983). These sites cause the creation of a pseudoexon that lies out-of-frame causing a frameshift of the coding exon and resulting in nonsense mediated decay of the transcript. Confirmation of the pathogenicity of these variations requires a tissue sample making it difficult (Tuffery-Giraud et al., 2003).

As intronic mutations typically cause disease by altering splicing and ultimately triggering nonsense-mediated decay of the transcript, they would be most likely to cause disease in recessive MC. It was thought that they would be unlikely to cause disease in dominant MC, PMC, SCM and PP as these diseases are all caused by gain of function mutations that alter channel properties and therefore are typically caused by an exonic change. However, there is one case of an intronic single nucleotide insertion with a five nucleotide deletion in SCN4A which caused a gain of function. This combination of insertion and deletion caused aberrant splicing with the insertion of 35 amino acids into a cytoplasmic loop which allowed the formation of a viable abnormal sodium channel with impaired fast inactivation (Kubota et al., 2011). This implies that it may be the mechanism for disease in all types of skeletal muscle channelopathy.

Large Scale Rearrangements

Large deletions and duplications account for 7-10% of all reported mutations in the human genome (Stenson et al., 2009) and it therefore follows that it may be an important mechanism in skeletal muscle channelopathies. As with intronic mutations it would be more likely to

cause disease by loss of function as the loss or gain of a number of exons would most commonly produce significantly abnormal mRNA (messenger ribonucleic acid) which would be targeted for nonsense mediated decay. It would therefore be most likely to underlie disease in patients with recessive MC.

It is theoretically possible that it could cause disease in dominantly inherited skeletal muscle channelopathies as there have been cases of small deletions associated with these diseases. In PMC the case above of the small intronic insertion and deletion in SCN4A associated with impaired inactivation of the channel is one example (Kubota et al., 2011), however it seems unlikely that a large exonic deletion or duplication would have a similar effect. In ATS there have been reports of loss of function variations causing a mild form of primarily cardiac disease, one of which included an in-frame deletion (Fodstad et al., 2004). There has also recently been a case of a whole KCNJ2 gene deletion identified using array comparative genomic hybridisation resulting in a pure cardiac phenotype ATS without the episodes of paralysis and dysmorphic features (Marquis-Nicholson et al., 2014), suggesting that large deletions and duplications could underlie some cases of ATS.

2.5.3.2 Novel Genes and Atypical Diagnoses

Aside from the limitations of Sanger sequencing, there are other possible factors that may contribute to patients remaining undiagnosed. Firstly there may be as yet undiscovered genes that are responsible for the disease. A second possibility is that these patients have an atypical presentation of another known disease. Finally, in those patients with only a single loss of function mutation in CLCN1 the second mutation may be in a different gene causing "double trouble".

Novel Genes

Currently, patients are diagnosed by sequencing genes known to be associated with skeletal muscle channelopathies as this is the quickest and most efficient process (Figure 2.11). However, as the rate of diagnosis is only 70-80% there is a high likelihood that there may be other genes responsible for a small number of cases, especially in patients with an atypical presentation of the disease. This is illustrated by the recent discovery of a number of patients with thyrotoxic HypoPP who have variations in KCNJ18 (Ryan et al., 2010). Variations in this gene were identified in 33% of patients with this phenotype, all of which alter muscle membrane excitability. There has also been the recent discovery of a variation in

KCNJ5 associated with a case of atypical ATS without facial dysmorphism. The mutated channel when co-expressed with WT KCNJ2 resulted in reduced potassium currents across the membrane and suggests it has a modifying effect (Kokunai et al., 2014). With such a large diversity of ion channels in the human genome, it leads to the suspicion that other ion channel genes expressed in skeletal muscle may be responsible for the undiagnosed cases.

Atypical Phenotypes

Another possibility is that patients with a channelopathy may have an unusual presentation of another known muscle disease. The key feature of channelopathies compared with other conditions is the paroxysmal nature of the condition and it is this that often directs the diagnosis towards a channelopathy. It may be the case, however, in some diseases that early on symptoms may appear episodic progressing later on in the disease to more typical features.

In one example of an unusual presentation, the patient had signs and symptoms of a congenital myopathy at birth accompanied by atypical normokalaemic attacks of periodic paralysis. Her histopathology and limb magnetic resonance imaging (MRI) was suggestive of multi-minicore disease. She had normal sequencing of the channel genes but was compound heterozygote for mutations in RYR1 which is associated with multi-minicore disease. The RYR1 gene encodes the main ryanodine receptor in skeletal muscle sarcoplasmic reticulum which has a vital role in excitation-contraction coupling via its interaction with the CaV1.1 channel. Variations in the gene have been associated with the autosomal dominant diseases, malignant hyperthermia susceptibility and central core disease, and autosomal recessive diseases, multi-minicore disease and congenital fibre-type disproportion. In this particular patient, the long exercise test was negative but she had cold-induced excitation-contraction uncoupling on electromyography (EMG). As RYR1 is coupled to CaV1.1 this suggests that disruption of the excitation-contraction coupling may lead to PP-type attacks (Zhou et al., 2010).

There has also been a recent report of a patient with myalgia, muscle stiffness and myotonia on EMG with normal sequencing of CLCN1, SCN4A and testing for DM1 and DM2 who had a mutation in the caveolin-3 gene, which is normally associated with rippling muscle disease and limb-girdle muscular dystrophy (LGMD) (Milone et al., 2012). These examples suggest that mutations in known disease-causing genes may be important in explaining some undiagnosed cases.

"Double Trouble" - Variations in Multiple Genes

Patients with only one loss of function mutation in CLCN1 and a recessive pedigree do not have a complete diagnosis. In these cases the undiscovered "second mutation" may either be missed by Sanger sequencing or alternatively may be in a second related gene whose protein interacts with CLCN1 or contributes to the muscle membrane excitability. There are a number of cases of patients with both a single CLCN1 mutation and expansions in ZNF9 causing DM2 which underlies the patient's diagnosis (Suominen et al., 2008; Lamont, 2004; Cardani et al., 2012) and it therefore follows that some cases may be explained by mutations in ZNF9 or other related genes such as DMPK or SCN4A. In these cases it is anticipated that patients may have a more severe phenotype or atypical presentation due to the effect of the mutations in both genes. It also follows that patients with a more severe phenotype that were considered fully diagnosed, could potentially have another mutation in a second gene to account for their increased severity.

2.5.4 Investigating Undiagnosed Patients

The likely explanation for many of the genetically unconfirmed cases either lies in missing the variation using standard Sanger sequencing of known genes or identifying novel variations in novel or different genes. The best way to investigate these unconfirmed cases would be to systematically address these two aspects.

2.5.4.1 Addressing Limitations in Current Techniques

There are a number of ways that the limitations of Sanger sequencing may be addressed. The false negative rate can be minimised by firstly repeating all negative samples with fresh samples to check there has not been operator or machine error when extracting and sequencing the deoxyribonucleic acid (DNA). This would also reduce the error related to tissue mosaicism. Any exons that failed analysis could be repeated with different primers to account for the presence of variations located in the primer binding sites.

Identifying Intronic Variations

Intronic mutations can be identified by Sanger sequencing if primers are added to cover the introns as well. The introns, however, are very large and therefore would require a large volume of sequencing. Alternatively, high throughput methods could be used to sequence the intronic regions, which would be faster and allow simultaneous analysis of other genes.

Identifying Deletions and Duplications

There are a number of ways that exonic deletions and duplications can be identified. For a small number of genes, Mulitplex Ligation-dependent Probe Amplification (MLPA) can be used to detect copy number variations (CNVs) and large scale rearrangements. This technique requires only a small quantity of DNA and can detect a single nucleotide deletion or insertion as well as whole exon deletions (Schouten et al., 2002).

Another traditionally used technique is Fluorescence In Situ Hybridisation (FISH) which is used to identify large deletions, duplications or rearrangements (Landegent et al., 1985). This technique can only detect deletions and duplications of around 10kbp and therefore will miss small variations. It can, however, be used to look at the whole genome rather than a small number of genes.

A variation on FISH is array-based comparative genomic hybridisation (aCGH) which detects loss or gain of chromosomal material throughout the whole genome without needing to look directly at the chromosomes (Pinkel et al., 1998). This technique is now advanced enough to pick up CNVs of a few exons in a gene and therefore is a more efficient tool for screening the entire genome. It is therefore a powerful technique for investigating the DNA of syndromic patients that may have complex rearrangements whilst also picking up smaller CNVs in a single gene.

2.5.4.2 Identifying Novel Genes

In some patients, variations in novel genes, or in known genes with a novel phenotype, may be the cause of their disease. In patients with large families of affected members it is possible to identify the causative gene using linkage studies. However, in many of the channelopathy cases there is no family history, suggesting sporadic mutations, or only a small number of affected family members because of smaller family units. Trying to identify the causative gene with Sanger sequencing without any candidate genes is a time consuming and complex job with low yield. Therefore a different process is needed to identify causative genes.

Next-generation Sequencing

In the past decade, next-generation sequencing has become a viable method (Metzker, 2010). This is a high throughput automated screening technique that can be used to sequence an individual's entire genome, exome or targeted genes in a short period of time. It is therefore a more efficient way of identifying mutations in all of the patient's known genes and could

be used to identify the causative gene in an undiagnosed patient.

The utility of next-generation sequencing has been shown in a proof of concept experiment to identify the known causal gene in 12 unrelated patients with a rare genetic disorder (Ng et al., 2009). It was later used to identify the novel gene responsible for a previously uncharacterised rare Mendelian disorder in four affected individuals from three independent kindreds (Ng et al., 2010). It is therefore a proven technique for identifying unknown disease genes in groups of phenotypically similar patients and can be targeted to specific genes, the whole exome or the entire genome of a patient. This could therefore be a useful technique for identifying mutations in novel genes and intronic regions.

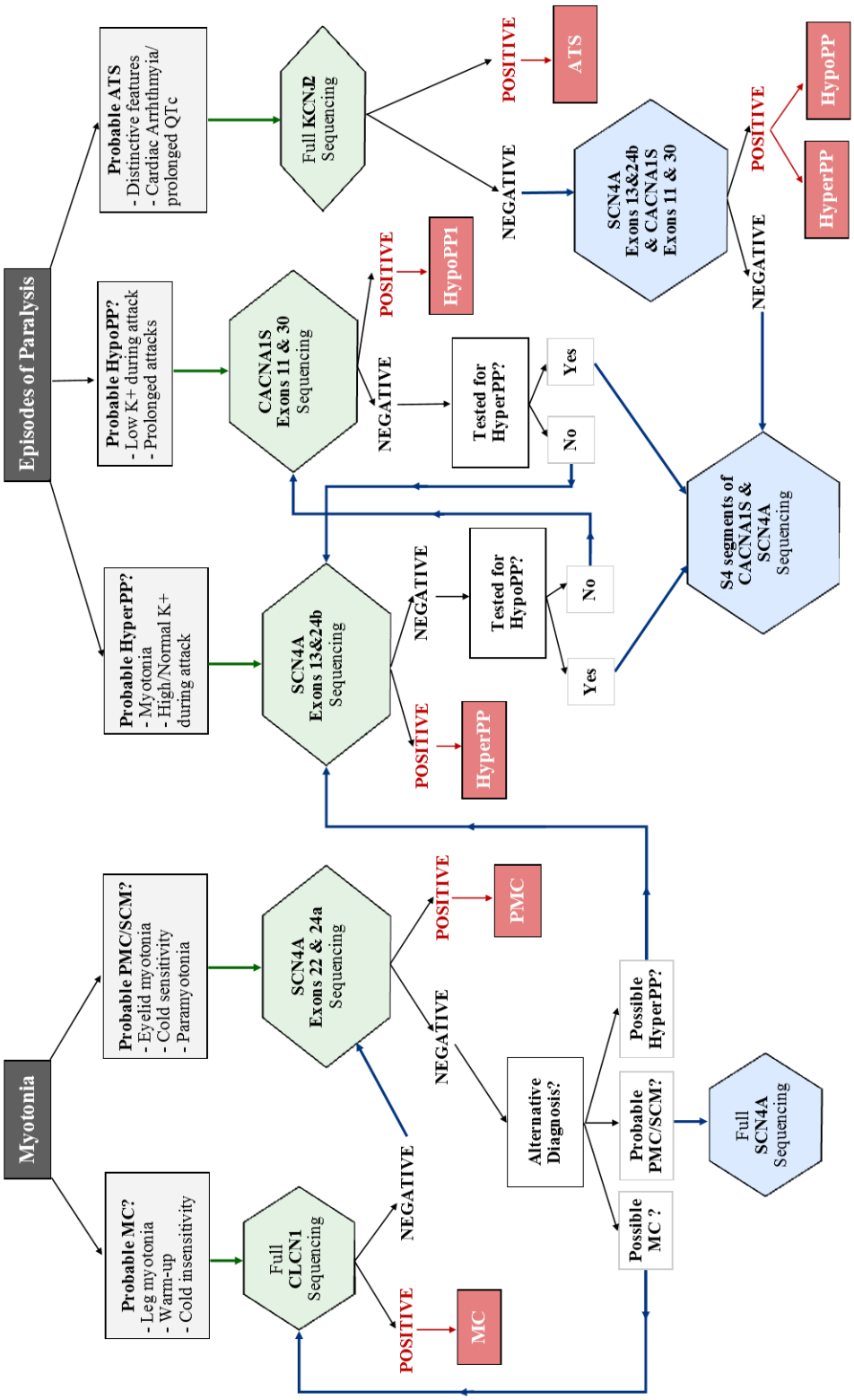


Figure 2.11: Diagnostic genetic testing algorithm for non-dystrophic myotonia and periodic paralysis (1st line testing in green, 2nd line testing in blue)

2.6 Investigating the Skeletal Muscle Channelopathies

A combination of clinical phenotyping and investigations are currently the most effective way of guiding genetic analysis. Traditionally clinical electrophysiology has played a large part in identifying and defining these disorders. Currently emerging work indicates that muscle magnetic resonance techniques and ultrasound may also have a role in identifying muscle damage and in disease monitoring.

2.6.1 Clinical Electrophysiology

Needle EMG is used to identify the presence of electrical myotonia. Myotonia is caused by membrane hyperexcitability, allowing mild depolarisation of the muscle membrane and resulting in spontaneous self-sustained runs of muscle fibre action potentials. In PP and PMC the sarcolemmal depolarisation becomes severe enough to make the muscle fibre inexcitable resulting in weakness. Exercise can act as a trigger to the sarcolemmal depolarisation and therefore specialised short and long exercise testing can be used to help to differentiate between the types of channelopathy (McManis et al., 1986; Fournier et al., 2004, 2006).

2.6.1.1 Needle EMG

Needle EMG can be used in NDM to demonstrate the presence of myotonia. Key characteristics of the myotonia can also vary between the different diseases and aids in narrowing the diagnosis.

In recessive MC, needle EMG shows extensive myotonic potentials and post-exercise myotonic potentials (Fournier et al., 2004). In dominant MC, EMG may only show scanty discharges (Tan et al., 2011).

PMC patients may also demonstrate post exercise myotonic potentials following cooling (Fournier et al., 2006). In some patients with PMC, higher frequency "musical" discharges are seen as well as lower frequency myotonic discharges (Tan et al., 2011).

HyperPP patients may have myotonia on their EMG. HypoPP and ATS patients do not have myotonia on EMG but may have a myopathy (Fournier et al., 2004; Katz et al., 1999).

2.6.1.2 Short Exercise Test

The short exercise test monitors the CMAP amplitude following three short bursts of isometric exercise. The CMAP amplitude is an indirect measure of sarcolemmal excitability, giving an indication of the ability of active fibres to depolarise and repolarise (Streib, 1987a).

This test shows distinct patterns depending on the channel defect and therefore can be helpful in guiding diagnosis and genetic testing.

Patients with recessive MC typically show a Fournier type II pattern of neurophysiological changes as described by Fournier *et al* (Fournier et al., 2004). This is a transient fall in CMAP amplitude after short exercise testing with recovery following subsequent trials of exercise. In some cases this pattern is only seen in patients after cooling and rewarming (Tan et al., 2011), although cooling on its own does not usually alter the CMAP amplitude. Occasionally this pattern is seen in dominant MC and can be seen in DM1 and DM2 as these diseases also reduce chloride conductance (Charlet et al., 2002) (Table 2.3).

In the majority of dominant MC patients there is no fall in CMAP (Fournier type III pattern), a pattern also seen in SCM and controls. However, when the test is repeated with cooling they may demonstrate the typical fall in CMAP and improvement following exercise that is normally seen in recessive MC (Fournier et al., 2006) (Table 2.3).

Patients with PMC typically show a Fournier type I pattern of electrophysiology, with a prolonged decrement in CMAP after short exercise testing and gradual progression with further trials of exercise. This decrease in CMAP is exacerbated by cooling and in some cases only apparent after cooling (Fournier et al., 2004, 2006). The SCMs typically have normal responses to short exercise testing (Fournier type III) even after cooling and their only finding may be myotonia on EMG (Fournier et al., 2004, 2006) (Table 2.3).

HyperPP patients have a Fournier type IV pattern on exercise testing. They may have an increase in CMAP after short exercise testing. HypoPP1, patients have no change in CMAP following short exercise testing (Fournier et al., 2004). ATS patients tend to show the pattern appropriate for their type of episodes of paralysis (Katz et al., 1999) (Table 2.5).

Short exercise testing can therefore be extremely useful in diagnosing the different types of channelopathy. Tan *et al* suggest that a combination of short exercise testing and clinical characterisation into those with eye closure myotonia versus those with prominent falls can increase the diagnostic accuracy in determining the appropriate genetic test (Tan et al., 2011).

2.6.1.3 Long Exercise Test

The long exercise test (McManis test) involves repeated exercise over five minutes interspersed with rest with regular CMAP measurements for 50 minutes afterwards. This test is thought to identify reduced muscle membrane excitability triggered by vigorous exercise (McManis et al., 1986).

The long exercise test is most useful in patients with periodic paralysis. A decrement of CMAP greater than 40% from maximum during the 50 minute period after exercise is considered significant. In HypoPP1, patients may have a significant prolonged decrement of CMAP area and amplitude at around 10 to 20 minutes after exercise. In HypoPP2, however they may show a late decrement (Fournier et al., 2004) (Table 2.5).

In HyperPP, patients may have an initial increase in CMAP followed by a late decrement (Fournier et al., 2004). In ATS, patients may have an abnormal amplitude decrement but more frequently have an abnormal CMAP area decrement (>40%) which may be a more sensitive marker in this group of patients (Tan et al., 2011) (Table 2.5).

The long exercise test is also occasionally positive in NDM patients. A small number of MC patients have positive tests with the decrement commonly occurring during the five minutes of exercise (Tan et al., 2011; Kuntzer et al., 2000). In PMC the long exercise test is frequently abnormal with progressive decrement during exercise and slow recovery in the rest period. In patients with SCM the long exercise test is normal (Fournier et al., 2004; Tan et al., 2011).

Although the long exercise test is a useful positive in PP it is not always abnormal, especially when patients are on treatment. However, it can also give false positives, particularly amongst the Afro-Caribbean population as normal controls from this group have been shown to have a decrement of greater than 40%. In this population a positive result must be treated with caution and a decrement greater than 54% may be necessary to be sure of a true positive test (Tan et al., 2011).

2.6.1.4 Quantitative Muscle Testing

Although neurophysiology is helpful in aiding diagnosis, it does not provide a sensitive outcome measure to monitor change and is often variable between visits. Semi-quantitative functional measures of clinical myotonia such as grip and eyelid relaxation time following maximum voluntary contraction are useful bedside tests but are relatively insensitive measures (Kwiecinski et al., 1992).

A more sensitive and objective quantitative muscle analysis system has therefore been devised for measuring myotonia in DM1 and NDM. This quantitative hand grip system uses a strain gauge to measure the force of contraction on the handle of a grip ergometer and plots this over time. The decrease in force over time (from maximum voluntary isometric contraction) from when the subject is asked to relax their grip is called the relaxation time. In

DM1 this is significantly increased compared to controls and is proportional to the severity of myotonia. It can be quantified by measuring the time taken to drop from 90% to 5% of maximum force (Logigian et al., 2005; Moxley R. T. et al., 2007). This has been successfully used as a sensitive outcome measure in a drug treatment trial of mexiletine in DM1 (Logigian et al., 2010). We have also recently published a study of 91 patients with NDM where it aided in differentiating between sodium and chloride channel mutation patients and may also provide a useful outcome measure for clinical trials in NDM (Statland et al., 2012).

2.6.1.5 Muscle Biopsy

Muscle biopsy is a useful tool in many of the genetic muscle diseases, however in NDM the findings are non-specific. Some MC patients may have an absence of type 2b fibres (Crews et al., 1976), whereas in PMC, myopathic changes, large vacuoles and tubular aggregates may be seen (Table 2.3).

In PP, more extensive but often non-specific changes may be seen including vacuolar myopathy, tubular aggregates and myopathic changes (Miller et al., 2004). In ATS, tubular aggregates are more frequently seen (Table 2.5). As the changes in all these conditions are non-specific, muscle biopsy is no longer commonly done.

2.6.1.6 Muscle MRI

Muscle MRI in skeletal muscle channelopathies is an evolving technique that may have potential in a clinical setting. For example, detecting patterns of fatty infiltration and muscle atrophy on limb MRI may aid in diagnosis as has been done in other genetic diseases (Wattjes et al., 2010). Studies using MRI in muscle channelopathies is just starting to emerge. One recent study involved three patients with MC and used whole-body 3 Tesla (3T) MRI images with T1-weighted and T2-weighted turbo spin echo to evaluate changes in eight muscles (Kornblum et al., 2010). They did not show any abnormalities in these patients, but this was a small study in young patients with no corresponding muscle strength recorded.

MR spectroscopy may be a useful tool in understanding the disease mechanism in channelopathies. It was shown that MRI with ultra-short echo times detected an accumulation of intramuscular ^{23}Na and 'oedema-like' changes in patients with PMC following cold-induced weakness and in patients with HyperPP after cold-induced and exercise-induced weakness (Weber et al., 2006). These changes were not seen in SCM patients with a PAM phenotype. In a more recent study, MR spectroscopy was employed

in HypoPP patients. 21/25 patients with permanent weakness had fatty muscle degeneration and 18/25 had oedema on ^{1}H-MRI of the lower limbs. They also noted that patients' weaker muscles had a higher sodium signal at rest and this signal increased with weakness induced by cooling. Interestingly, treatment of the severely affected patients with acetazolamide and potassium for 4 weeks showed a reduction in sodium signal that matched the increased muscle strength (Jurkat-Rott et al., 2009). These studies illustrate the potential value of ^{23}Na-MRI for diagnosis and monitoring of treatment in particular muscle channelopathies.

As quantitative MRI techniques become more readily available they may also provide us with non-invasive outcome measures in clinical trials.

2.7 Treatment of the Skeletal Muscle Channelopathies

Current management of the skeletal muscle channelopathies is primarily symptomatic and based on small case studies done in the 1970s and 80s. There is therefore much scope for identifying effective treatments using high quality evidence.

2.7.1 Current Treatments

At present, there is little randomised control trial (RCT) level evidence for treatments in NDM or PP.

2.7.1.1 Treatment of Non-dystrophic Myotonia

In cases of NDM with mild myotonia, patients are advised to avoid triggers such as cold or strenuous exercise. In patients with more severe myotonia, a number of different treatments have been tried. Most of the drug treatments used for myotonia in the clinical setting are sodium channel blockers as they reduce the open probability of the sodium channel responsible for the membrane hyperexcitability that underlies myotonia. At present, only the sodium channel has been targeted as there are no safe ClC-1-specific drugs available (Verkman and Galietta, 2009).

The main drugs that have been used to treat myotonia are: quinine (Leyburn and Walton, 1959), procainamide (Leyburn and Walton, 1959; Griggs et al., 1975), phenytoin (Griggs et al., 1975; Ricker et al., 1978); (Fouad et al., 1997), carbamazepine (Ricker et al., 1978), tocainide (Streib, 1987b), mexiletine (Kwiecinski et al., 1992) and flecainide (Rosenfeld et al., 1997). Low level evidence from an uncontrolled trial suggested that tocainide and mexiletine were effective in NDM (Kwiecinski et al., 1992). Although tocainide was shown to be as effective as mexiletine, it has been withdrawn due to the risk of agranulocytosis. Procainamide and prednisolone were shown to be effective in a small double-blind RCT in patients with DM1 or MC but were not recommended due to their poor side effect profile. Quinine, although it has a better side effect profile, was shown to be relatively ineffective (Leyburn and Walton, 1959). Overall the 2006 Cochrane review, which was updated in 2009, concluded that there was insufficient data to consider any of the published treatments safe and effective for myotonia (Trip et al., 2006).

More recently a randomised controlled crossover study showed mexiletine to be safe and effective for reducing myotonia in patients with DM1 (Logigian et al., 2010). It was however unclear if there was any improvement in quality of life for these patients, especially since in

DM1 hand grip myotonia only rarely affects quality of life. There is no good RCT evidence for the efficacy of mexiletine in NDM although it is commonly used in clinical practice.

2.7.1.2 Treatment of Periodic Paralysis

One of the key aspects of treatment in PP is the avoidance of precipitants. In HyperPP, this includes avoidance of potassium-rich foods and prolonged fasting. Treatment options during an attack include inhalation of salbutamol, glucose/insulin therapy or even mild exercise to help shorten the episode (Ricker et al., 1989).

In HypoPP, patients are advised to eat small, regular meals and avoid carbohydrate loads and alcohol. During an attack, oral potassium can be prescribed to alleviate or shorten attacks (Cleland and Griggs, 2008). The evidence for all of these recommendations however, are based on guidelines and small epidemiological studies.

Some studies have been conducted investigating the prevention of attacks with acetazolamide in HyperPP and HypoPP. It was shown to be effective in improving inter-attack strength in an observational study (Griggs et al., 1970) and anecdotally in reducing attack frequency, but at present there is no RCT evidence. There is however, case study evidence that acetazolamide may worsen attacks in genetically defined HypoPP2, (Sternberg et al., 2001), although there are also reports of some patients benefiting (Davies et al., 2001; Venance et al., 2004; Vicart et al., 2004).

Dichlorphenamide was shown in a double-blinded placebo-controlled trial to reduce attack frequency in both clinically defined HyperPP and HypoPP (Tawil et al., 2000). The mechanism of action of the carbonic anhydrase inhibitors is unclear; they may act by opening calcium-activated potassium channels located in the muscle or by acidifying and stabilising intracellular potassium levels (Camerino et al., 2007; Tricarico et al., 2006).

In ATS anecdotal evidence suggests that carbonic anhydrase inhibitors may help to prevent attacks of paralysis. From a cardiac perspective, patients are often put on beta blockers for ventricular tachycardia, although there is little evidence that they alter its frequency. Those with tachycardia-induced syncope may benefit from an implantable cardioverter-defibrillator (Chun et al., 2004), and a single case study has reported that flecainide may be effective in suppressing bidirectional ventricular tachycardia (Pellizzon et al., 2008). However, further research is required to determine the optimum management of ATS.

2.7.2 Mexiletine in the Treatment of NDM

As highlighted in the Cochrane review, there is currently insufficient level I evidence for the treatment of myotonia (Trip et al., 2006). This is primarily because of the difficulty in recruiting a sufficient number of patients to demonstrate a treatment effect in this rare disease. This causes problems for the care of patients with NDM as no licensed treatments exist and many of the treatments are difficult to source. The current evidence for the efficacy of mexiletine is a combination of anecdotal evidence (Pouget and Serratrice, 1983; Leheup et al., 1986; Kwiecinski et al., 1992; Jackson et al., 1994) and convincing *in vitro* and *in vivo* evidence.

In vitro data suggests that mutations seen in NDM cause a persistent inward current, depolarizing the sarcolemmal membrane, resulting in myotonia. Mexiletine causes a tonic and use-dependent block of muscle sodium channels, correcting the increased excitability caused by the persistent inward current (Catterall, 1987). In mutated human sodium channel constructs, mexiletine has been shown to correct the delay in inactivation (Mohammadi et al., 2005; Wang et al., 2004).

In the animal model of recessive MC, the arrested development of righting response (ADR) mouse, mexiletine was shown to significantly reduce the prolonged righting reflex time (De Luca et al., 2004). In 1994, Jackson and Barohn demonstrated the efficacy of mexiletine in a patient with a missense mutation on chromosome 17q in the SCN4A gene (Jackson et al., 1994). They demonstrated improvement of the short exercise test, with increased CMAPs following mexiletine therapy. In a study that included an evaluation of 9 myotonic dystrophy patients along with 9 patients with dominant MC, and 12 patients with recessive MC (Kwiecinski et al., 1992) the investigators compared the efficacy of phenytoin, disopyramide, mexiletine (200mg TDS), and tocainide to placebo. Based upon their analysis, these researchers concluded that mexiletine and tocainide were the most potent anti-myotonic agents, showing a decrease in time to open eyes after forced eye closure, to open the hand after forced hand grip and to climb ten steps as well as decreased relaxation times on EMG.

More recently investigators at the University of Rochester studied the effectiveness of mexiletine in reducing myotonia in a placebo-controlled crossover trial in DM1. Mexiletine was shown to be superior to placebo in improving a quantitative measure of hand grip myotonia by reducing relaxation times following forced hand grip (Logigian et al., 2010). Given this evidence mexiletine appears to be an appropriate choice for further investigation.

Chapter 3

AIMS AND OBJECTIVES

This thesis aims to increase the understanding of the phenotype, genotype and management of skeletal muscle channelopathies. To achieve this I have the following objectives:

1. **To determine the prevalence of skeletal muscle channelopathies in England.**
2. **To investigate the key phenotypic characteristics of patients with PMC, SCM, HypoPP, HyperPP and ATS, including:**
   (a) identifying key differentiating features between the conditions
   (b) determining genotype-phenotype correlations.
   (c) understanding the main problems during pregnancy and with anaesthetics in patients with skeletal muscle channelopathies.
3. **To identify the novel variations in a cohort of genetically undiagnosed patients with skeletal muscle channelopathies, including:**
   (a) identifying novel genes in patients with a NDM or PP phenotype
   (b) identifying the "second mutation" in recessive MC patients with only a single loss of function mutation.
4. **To ascertain the efficacy of mexiletine in the treatment of NDM in a double-blind randomised control study.**

Chapter 4

METHODS

4.1 Standard Protocol Approvals, Registration and Patient Consents

Ethical approval was obtained from the University College London Hospital (UCLH) ethics committee for all the research done in this thesis. All patients gave written informed consent for genetic and clinical testing.

4.2 Prevalence Study

4.2.1 Patient Selection

Patients for the prevalence study were selected from those referred by neurologists or other specialists to the MRC Centre for Neuromuscular Diseases, London, between April 1997 to April 2011 for further clinical or genetic assessment for possible NDM or PP.

Inclusion criteria were individuals of any age, living in the UK with clinical and neurophysiological features of NDM or PP and confirmed mutations in genes encoding the skeletal muscle voltage-gated potassium (KCNJ2), calcium (CACNA1S), sodium (SCN4A) or chloride (CLCN1) channels. Genetically unconfirmed or uncertain cases were excluded. Alternative causes, such as thyrotoxic PP or DM1 or DM2, were ruled out by appropriate tests when indicated. Patients living outside the UK were excluded.

4.2.2 Data Collection

Demographic, clinical, electrophysiological and genetic data from patients was prospectively collected in a database. Genetic analysis was performed by the National Hospital for Neurology and Neurosurgery (NHNN) neurogenetics laboratory by Sanger sequencing as

outlined below. All NDM patients had sequencing of all exons of CLCN1 and exons 22 and 24 of SCN4A. If appropriate this was extended to all exons of SCN4A and MLPA of CLCN1 to ensure a full genetic diagnosis was made. All PP patients had sequencing of exons 11 & 30 CACNA1S, exons 13, 22 & 24 of SCN4A and the coding exon of KCNJ2. If negative this was extended to S4 segments of CACNA1S and all exons of SCN4A to ensure that the maximum possible number of genetically confirmed cases were included in the study.

4.2.3 Data Analysis

Point prevalence rates were calculated as the number of patients in the study area on the prevalence day divided by the total number of individuals who were at risk at that time. December 31st 2011 was chosen as the prevalence day.

England was selected as the geographical area for the prevalence analysis, with a total population of 53,012,500 according to the latest available data (UK National Statistics;www.statistics.gov.uk). All patients in England can be referred to the National Commissioning Group funded service at the MRC Centre for Neuromuscular Diseases for genetic diagnosis making it likely that we would have a good ascertainment in this area. Patients from Scotland, Wales and Northern Ireland were not included in the calculation because these areas have alternative centres to which they may send DNA and therefore complete ascertainment of cases was less likely. Prevalence rates were expressed as cases/100,000, and 95% confidence intervals (CI) were calculated using the method proposed by Schoenberg (Schoenberg, 1983), based on the Poisson distribution.

The frequency of mutations in each gene was also determined. For this analysis, all patients from any UK location were included. Descriptive statistics were performed using IBM SPSS Statistics v20.

All patient selection and data collection was performed by myself. Data analysis was done with the help of Dr Alejandro Horga.

4.3 Clinical Phenotype Study

4.3.1 Patient Selction

Clinical phenotype data was collected from a comprehensive clinical database of all channelopathy patients seen or assessed at the MRC Centre for Neuromuscular Diseases. Further information was also gathered from patient notes and letters from referring clinicians if data was incomplete. Patients from the natural history study in NDM conducted as part of the Clinical Investigation of Neurological Channelopathies (CINCH) consortium were also included. The data for those patients was collected by the specialist muscle clinicians at the individual centres.

The inclusion criteria was all patients with genetic confirmation of a skeletal muscle channelopathy with symptoms and signs of disease. Patients with MC and mutations in CLCN1 were excluded from phenotype analysis as they had been incorporated in a previous published study.

4.3.2 Data Collection

Patients were assessed for severity of myotonia, pain and attacks of weakness, based on their symptoms and clinical examination. Severity of myotonia and weakness were graded on a scale of 1-4 with 1 being mild and 4 being severe. Pain was graded on a scale of 1-3 for mild, moderate and severe, respectively. Functional status was graded from 0-4 with 0-normal walking, 1-difficulty with stairs, 2- mobilising with one stick, 3-mobilising with two sticks or frame and 4-wheelchair bound. Muscle strength was assessed using the standard Medical Research Council (MRC) score from 0-5, with 5 being normal strength and 0 being no muscle movement (Medical Research Council, 1976).

Neurophysiology testing was performed on 86 patients and included short exercise testing as described by Fournier *et al* (Fournier et al., 2004), needle electromyography (EMG) and long exercise testing as described by McManis (McManis et al., 1986). Short exercise testing involved recording CMAPs over the abductor digiti minimi (ADM) of the right hand after a period of 10 minutes rest prior to starting the test. The hand was immobilized and supramaximal stimulation (20-30% above maximum) of the ulnar nerve performed every 60 seconds for two minutes to establish the baseline CMAP. The ADM was then maximally contracted for 10 seconds with CMAPs recorded immediately after exercise and then every 10 seconds for one minute. This was repeated three times. The long exercise

test involved recording CMAPs over the ADM with surface electrodes, similar to the short exercise test. The hand was rested for 10 minutes prior to starting the test. The hand was immobilized and supramaximal stimulation (20-30% above maximum) of the ulnar nerve performed every 60 seconds for two minutes to establish the baseline CMAP. The ADM was maximally contracted for five 72-second periods, each consisting of four 15-second activation intervals followed by a three-second rest period. After each 72-second interval, a supramaximal stimulus was delivered. After completion of exercise, the patient was asked to relax completely and CMAPs were recorded every two minutes for a minimum of 30 minutes and to a maximum of 50 minutes. Some patients also had EMG and short exercise testing following cooling and rewarming of the muscle (Fournier et al., 2006). All neurophysiology tests were performed by specialist neurophysiologists at the centre. 13 patients also had MRI of the lower limbs with both T1 and STIR sequences as previously described (Morrow et al., 2013).

4.3.3 Pregnancy and Anaesthetics Data

Data on pregnancy and anaesthetics was collected using a questionnaire given to patients who attended the muscle channelopathy patient day or who attended the specialist channelopathy clinic (Appendix C). Only patients who were genetically confirmed were included.

4.3.4 Statistical Analysis

Statistical analysis was performed on all data. All categorical data was analysed using Pearson's Chi-squared test to assess for differences between groups. When significant differences were found, adjusted residuals were used to determine the groups that contributed most strongly to the statistical result. Adjusted residuals of greater than ± 2.0 were considered significant. The Mann-Whitney U test was used to analyse continuous data between two groups that were not normally distributed and Kruskal-Wallis was used for analysing continuous data analysis of multiple groups. A small number of categories were normally distributed and they were analysed using a one way ANOVA for multiple group comparisons and independent samples t-test for two group comparison. Normality of continuous data was assessed using Shapiro-Wilk test for normality and confirmed with Normal Q-Q plots. The p-values were all two tailed and a p-value of <0.05 was considered significant. Correlations were performed using Spearman's rank order. A Bonferroni correction was calculated for all multiple comparisons by diving the significance level by the number of tests. Comparisons

that were significant after Bonferroni correction are indicated in the tables. All analysis was performed using SPSS version 21. Graphs were produced using GraphPad Prism 6.

4.4 Genetic Diagnosis study

4.4.1 Strategy for Genetic Diagnosis

To try and identify the underlying genetic diagnosis in this cohort of patients, I took the following approach. Patients were split into three main groups and analysed accordingly (Figure 4.1):

1. Cases with a single loss of function variation in CLCN1 from a recessive pedigree with no "second mutation" identified.
   - These cases underwent MLPA testing of CLCN1 to look for deletions and duplications at exon level.
2. Cases with an unusual phenotype who either did not have a pathogenic mutation identified or who had a variation that was inconsistent with the clinical features.
   - These cases had Sanger sequencing of other possible causative genes done in a systematic fashion based on their phenotype.
3. Cases with a phenotype consistent with a skeletal muscle channelopathy but with no pathogenic variations identified following first line genetic testing (all CLCN1 exons, KCNJ2 coding exon, SCN4A exons 13, 22 and 24 , CACNA1S exons 11 and 30).
   - These were split into those with a PP phenotype and those with a NDM phenotype. Those with a PP phenotype first had sequencing of all SCN4A exons and the exons that encode S4 segments of CACNA1S as well as MLPA of KCNJ2. Those that were still unexplained went on to have aCGH and whole exome sequencing. Those with a NDM phenotype had MLPA of CLCN1, sequencing of all SCN4A exons and DM1 and DM2 gene analysis. Those that remained unexplained went on to have aCGH and whole exome sequencing

4.4.1.1 Patient Selection for Genetic Analysis

Patients were selected from a database of 1464 patients referred to the National Channelopathy reference laboratory (as part of the National Commissioning Group channelopathy service) at NHNN. Approximately 20% of these patients (296) either did not have any pathogenic mutations identified following standard genetic screening, had an

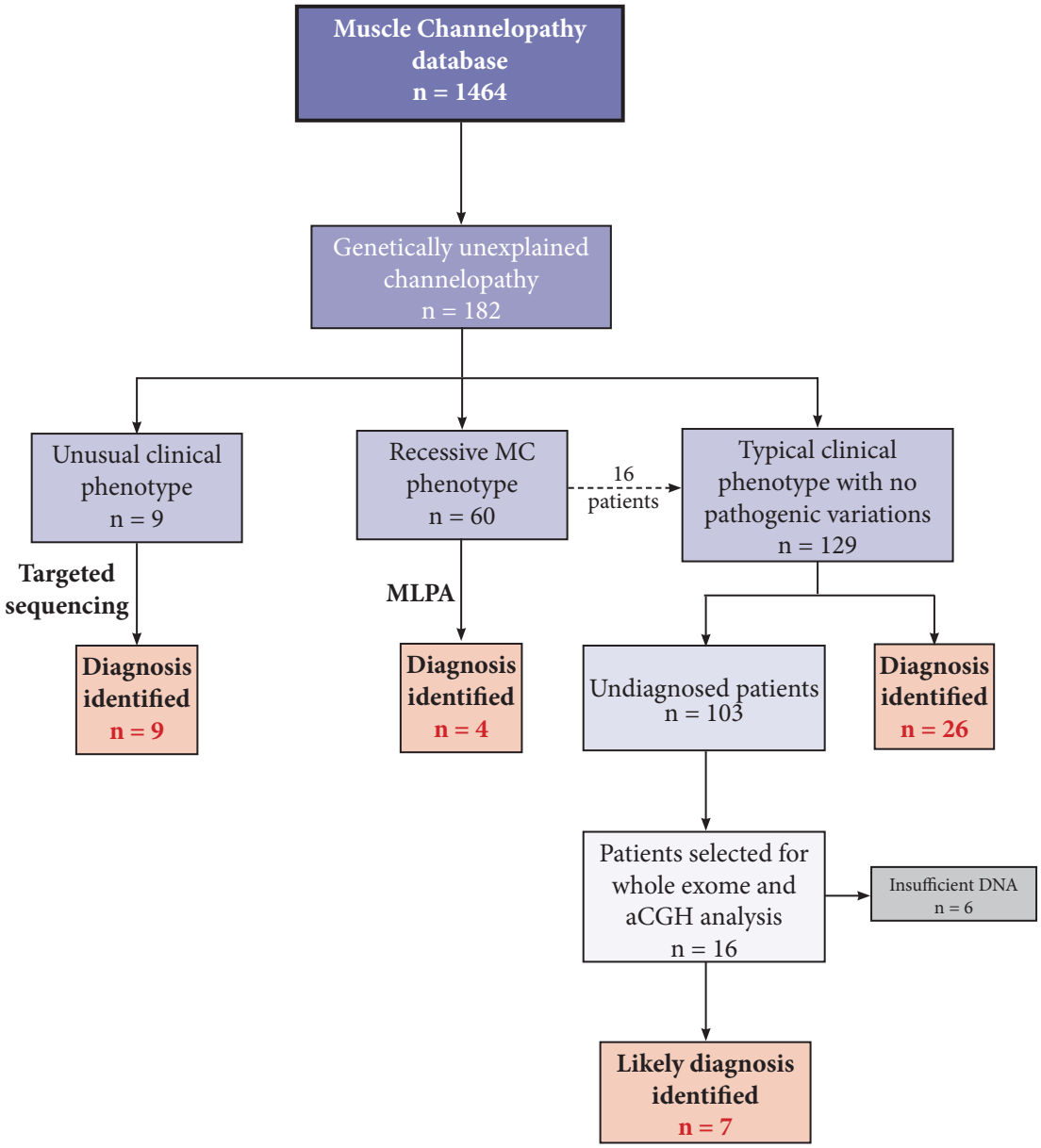


Figure 4.1: Flow chart of patient selection and overall results in genetic diagnostic study

unusual clinical phenotype in the context of their identified variation or had a single CLCN1 variation with a recessive pedigree. I carefully assessed these cases to identify only patients with convincing signs and symptoms consistent with a skeletal muscle channelopathy and accompanying objective neurophysiological evidence of a change in muscle membrane excitability. This identified 182 patients from 165 pedigrees for further analysis (Figure 4.1).

4.4.1.2 Further Analysis of Selected Patients

Of the 182 patients, 9 from 4 pedigrees were classified as having an unusual clinical phenotype and had strategic targeted genetic sequencing. 60 patients were found to have

a phenotype consistent with recessive MC and either no variations, a single loss of function CLCN1 variation or homozygous variations.

129 patients from 117 pedigrees (16 of which were also analysed in the CLCN1 group) had further genetic analysis. These patients all had full genetic sequencing of all known exons of CLCN1, SCN4A, KCNJ2 and the S4 segments of CACNA1S (exons 4,11,12,21 and 30) (either using Sanger sequencing by E.Matthews and S.Durran, or by A.Gardiner who analysed 69 patients using a muscle channel panel containing the same genes). Patients with myotonia also had testing for DM1 and DM2 at the appropriate UK service laboratory.

26 patients from 21 pedigrees (20%) had mutations identified in one of the four channelopathy genes following further sequencing. The remaining 103 patients were reassessed by myself to identify a smaller cohort of those with the highest likelihood of a channelopathy. 16 patients were identified as possible candidates for further testing using whole exome and aCGH techniques. Of these, 6 patients did not have sufficient DNA remaining, or DNA of insufficient quality to perform whole exome sequencing and aCGH (Figure 4.1).

4.4.2 Multiplex Ligation-dependent Probe Amplification

MLPA analysis of CLCN1 was used to investigate the possibility that patients with a phenotype of MC may have deletions or duplications in CLCN1 that underlie their disease.

4.4.2.1 Patient Selection

Samples were collected from patients referred to the National Channelopathy reference laboratory at the NHNN as described above, which included a small cohort of Sicilian patients (assessed by S.Portaro). All patients selected had a clear clinical diagnosis of recessive MC based on clinical assessment and electrophysiological studies with evidence of myotonia on EMG.

In total 350 MC cases, including patients from the cohort described in Fialho *et al* (Fialho et al., 2007), were assessed for suitability. These cases had previously had sequencing of all 23 coding exons of CLCN1 by the Neurogenetics laboratory at NHNN. From these a total of 50 UK patients and 10 Sicilian patients from 60 pedigrees were selected as candidates for MLPA testing (Figure 4.2). They were selected because they either had features of NDM and no pathogenic mutation, a single mutation despite a recessive pedigree or were apparent homozygotes for a novel or known pathogenic mutation (16 patients). Homozygous patients

were included because it is impossible to distinguish between a homozygous and hemizygous result by sequencing alone and parental samples were unobtainable in those cases to confirm the inheritance pattern.

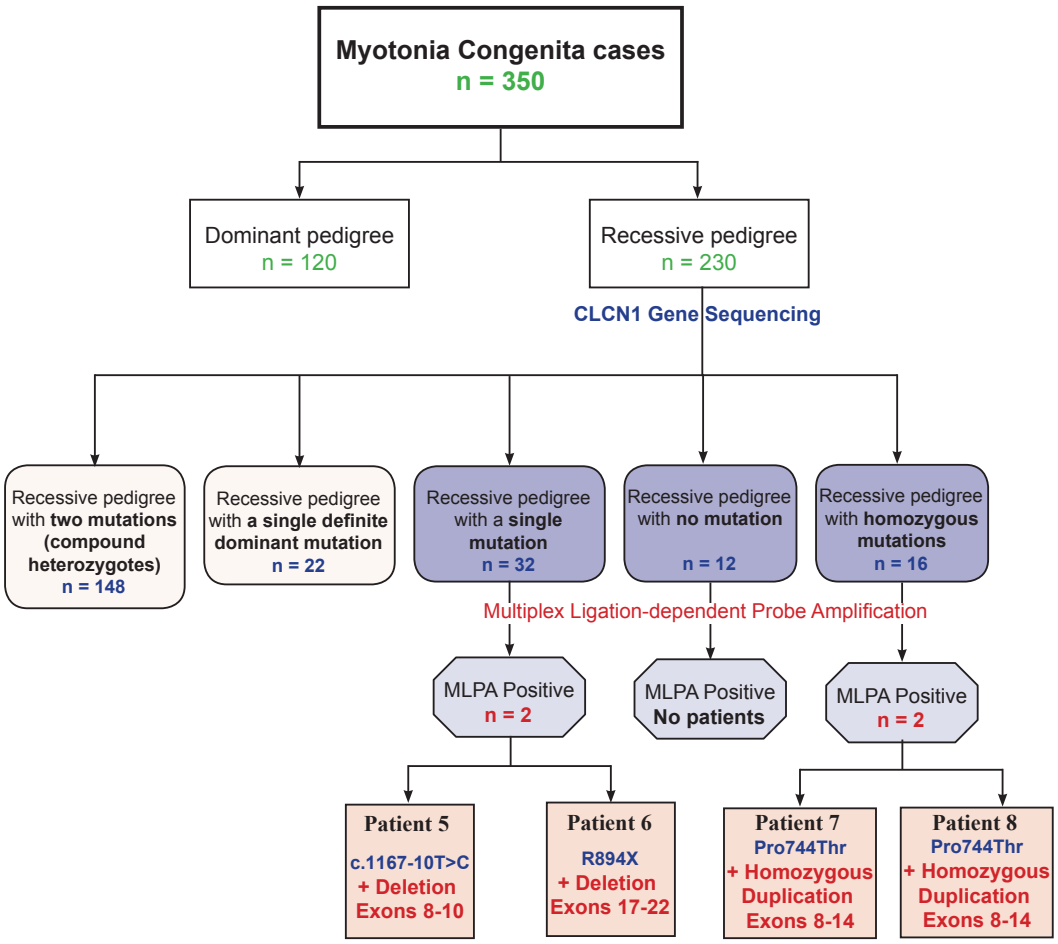


Figure 4.2: Flow chart of patient selection for MLPA and breakdown of results

Alongside this cohort, a group of patients who had a PP phenotype but no mutations identified were tested to look for CNVs in KCNJ2 as this was provided in the same MLPA kit (performed by V. Gibbons).

124 control chromosomes were also tested using the same technique from the European Collection of Cell Cultures Human Random Control DNA panels. This consisted of randomly selected UK Caucasian subjects.

4.4.2.2 MLPA Analysis

MLPA was performed using a commercially available kit (SALSA®MLPA kit P350-A1 CLCN1-KCNJ2), manufactured by MRC-Holland, Amsterdam. This kit contains probes for

all 23 exons of CLCN1, 4 probes for KCNJ2 and 16 reference probes detecting 16 different autosomal chromosomal locations. MLPA was performed as directed by the manufacturer's guidelines.

MLPA Technique

For denaturation, 1μl DNA mixed with 4μl Tris EDTA buffer was heated to 98°C for 5 minutes and then added to 1.5μl SALSA® Probemix and 1.5μl of SALSA® MLPA buffer. The DNA mix was heated to 95°C for 1 minute and kept at 60°C for 16 hours.

For ligation the DNA mix was added to a ligase mix of 3μl each of Ligase Buffer A & B, 1μl SALSA® Ligase-65 and 25μl water. The ligase and DNA mix was heated to 54°C for 15 minutes and then 98°C for 5 minutes and finally cooled to 4°C.

The polymerase chain reaction (PCR) was set up with 4μl SALSA® PCR buffer and 26μl Water added to 10μl of the DNA ligation mix. This was then combined at 60°C with a polymerase mix of 2μl SALSA® PCR primer mix, 2μl SALSA® Enzyme dilution, 0.5μl SALSA® polymerase and 5.5μl water. The PCR reaction was run at 35 cycles of 30s at 95°C, 30s at 60°C and 60s at 72°C with 20 minutes at 72°C at the end followed by cooling to 4°C.

Finally electrophoresis was done with 1μl of the MLPA PCR product mixed with 9μl Formamide and 0.3μl of GeneScan 500 LIZ® size standard. Amplified fragments were analysed using an ABI 3730xl capillary sequencer (Applied Biosystems, Foster City, California, USA).

A minimum of three reference samples were used per MLPA experiment and all positive samples were repeated. A peak ratio range of $>$1.25 and $<$0.75 was used to determine duplications and deletions.

MLPA Data Analysis

Results were quantitatively analysed using GeneMarker v1.85 software (SoftGenetics, State College, Pennsylvania, USA). Individual peaks corresponding to each probe were identified based on their difference in length. The peak heights were used, after standardisation, to determine the copy number status, using the MLPA ratio method: intra-sample normalisation by dividing the peak height of each probe's amplification product by the total height of the reference probes, followed by inter-sample normalisation by dividing the intra-normalised probe ratio in a test sample by the average intra-normalised probe ratio of all reference samples. Probe sequences were checked and it was confirmed that no point mutations or rare polymorphisms described in this cohort were located under the probe binding site. The

maximum size of the large scale alteration was calculated based on the maximum distance between the unaffected probe binding sites. The minimum size was calculated based on the minimum distance between the affected probe binding sites.

4.4.3 Array-based Comparative Genomic Hybridisation

aCGH was used to identify possible pathogenic CNVs throughout the genome in channelopathy patients without a genetic diagnosis.

4.4.3.1 Patient Selection

Of the 16 cases identified for testing, 7 were analysed with aCGH. This was because 9 patients had insufficient quantity or quality of DNA for testing and were not contactable to give further samples. 1 patient's sample failed analysis.

4.4.3.2 aCGH Technique

High-resolution aCGH was performed on genomic DNA and analysed against reference DNA (Kreatech, Amsterdam, The Nederlands). Two types of array were used for different purposes, the 720K probe exon-focused array and a 1.4 M probe whole-genome tiling array. The 1.4M array was used for patients with an ATS phenotype as, given their syndromic appearance, they were more likely to have large rearrangements located throughout the genome rather than small exonic deletions or duplications. The 720K exon-focussed arrays were used to test patients with a PP or NDM phenotype as they were more likely to have small exonic deletions or duplications and this array gave a better coverage of exons.

The standard NimbleGen 720K exon-focused CGH microarray had enhanced coverage at all known exons in the Human Genome 18 build. It had 701,702 probes with a probe spacing of 65 base pairs (bp) at the exons and 2,509bp for the rest of the gene. The array also included a genomic backbone probe set with an average probe spacing of 7,200bp. The standard NimbleGen 1.4M whole-genome tiling array had 1,400,139 probes with an even probe spacing of 916bp across the whole genome in the Human Genome 19 build. For both arrays, the DNA samples were labelled, the test sample with Cyanine(Cy)3 and reference with Cy5 and co-hybridised to the custom microarray in accordance with the manufacturer's instructions (NimbleGen Arrays User's Guide: CGH and CGH/LOH Arrays v9.1, Roche NimbleGen, Madison, WI USA). The microarray was washed and then scanned on an Axon GenePix 4400A Scanner using GenePix Pro 7 software (Molecular Devices, Sunnyvale, CA, USA). Raw data was normalized, LOESS correction applied and the data ratios calculated

using NimbleScan Software (Roche NimbleGen). The normalized data was processed using Infoquant Fusion v6.0 software (Infoquant, London, UK) with analysis call settings of 3 consecutive probes $\pm$ 0.4 Cy3/Cy5 ratio.

aCGH testing was performed by the North Thames Regional Cytogenetics Unit at Great Ormond Street Hospital, London, UK. Results were analysed by myself using oneClickCGH software from InfoQuant.

4.4.3.3 aCGH Analysis

All CNV calls with insufficient probe coverage (fewer than 4 probes) were eliminated as were those also identified in the Database of Genomic Variants (Iafrate et al., 2004). All remaining CNVs were cross-checked visually in a genome browser, checking firstly for any CNVs in the regions of the known channelopathy genes and then in the other ion channel genes. If negative the remaining CNVs were then checked.

4.4.4 Next-Generation Sequencing

4.4.4.1 Whole Exome Sequencing

Samples were processed by either Illumina or Axeq technologies and quality control checked and standard analysis performed by UCL genomics. Illumina TruSeq 62Mb exome enrichment kit was used for target enrichment with an average coverage of 50x for the targets. Illumina Hiseq DNA analyser was used to sequence the samples. Raw sequence data was aligned to the reference genome University of California, Santa Cruz (UCSC) hg19 (http://genome.ucsc.edu) using the Burrows-Wheeler Aligner (BWA) alignment algorithm version 0.5.9 (http://bio-bwa.sourceforge.net). All post alignment processing was done by Picard (http://picard.sourceforge.net) which includes sorting and marking PCR duplicates.

4.4.4.2 Variant Calling

Variant calling was done with the Genome Analysis ToolKit following best practice guidelines v3. This included quality score recalibration to normalise base quality score, indel realignment to improve indel detection and variable quality score recalibration which builds a model for accurate variant detection using publicly available data and ancillary datasets. Variant annotation was carried out using ANNOVAR (www.openbioinformatics.org/annovar) and SNPEFF (http://snpeff.sourceforge.net). Variants were filtered based on the assumption that the variant of interest is rare within the normal population, to this end dbSNP135,

1000genomes (2012 February release) and NHLBI ESP data were used. Furthermore, all variants with a minor allele frequency above 0.01 were not considered as likely candidates as the causative variant was likely to be a rare variant.

4.4.4.3 Identifying Causative Variants

Variants were narrowed down using the following techniques. All synonymous variants were excluded as it is highly likely that the causative variant would cause disease by altering an amino acid. Known causative genes were first cross-checked for possible variants to ensure none had been previously missed by Sanger sequencing. Samples were all checked for the presence of variants identified by Sanger sequencing previously, to check for accuracy of sequencing. Variants were then narrowed down to those located in genes known to cause muscle disease and those in ion channel genes. I specifically looked at ion channels that were expressed in skeletal muscle. All variants included in dbSNP were also excluded unless thought to be pathogenic. In patients with a KCNJ2 phenotype, genes known to cause long QT syndrome were also checked (Appendix B, Table B.3).

A list of all known muscle disease causing genes was identified using a combination of OMIM, DisGeNET and KEGG databases giving 130 possible genes (Appendix B, Table B.1). These were used for the first level of filtering of single nucleotide variants (SNVs) to identify patients who may have a known muscle disease presenting with an atypical channelopathy-like phenotype. A list of ion channels were then identified using the National Centre for Biotechnology Information (NCBI) Gene database (http://www.ncbi.nlm.nih.gov). I identified 374 voltage-gated ion channel genes and known associated genes in the genome by searching for human voltage-gated channel genes in NCBI Gene database (Appendix B, Table B.2). These genes were then checked for expression levels in skeletal muscle in the Database of mRNA gene expression profiles of multiple human organs (Son et al., 2005) and the UCSC genome bioinformatics database (Kent et al., 2002). Using this method I identified 88 genes that were expressed in low to high levels in normal skeletal muscle mRNA. I hypothesised that mutations in voltage-gated ion channels are most likely to be responsible for disease in this cohort, given the clinical and electrophysiological phenotypes. Since symptoms and signs were localised to the skeletal muscle it therefore follows that the critical genes should be expressed in normal skeletal muscle.

Once a list of potential variants for each patient was identified, they were then ranked according to whether they were indels, nonsense mutations or missense mutations. They

were analysed using SIFT, PolyPhen2 and Mutation taster to predict the likelihood of pathogenicity. Their protein expression and RNA expression in skeletal muscle were also checked using The Human Protein Atlas (www.proteinatlas.org).

4.4.5 Sanger Sequencing

All novel pathogenic variations identified by whole exome sequencing were validated by Sanger sequencing in the proband and any available relatives.

4.4.5.1 Primer Design

Possible pathogenic variations identified by whole exome sequencing in commonly tested genes were checked by the Neurogenetics department as part of standardised testing. The other variations were checked by Sanger sequencing by myself. Exon sequences were identified from the Ensembl database (www.ensembl.org/index.html) for each gene. These sequences were input into Primer 3, an online primer design tool (http://bioinfo.ut.ee/primer3), to identify potential primers. Primers were designed to include approximately 25 base pairs of intronic sequence upstream and downstream of the exon and produce a fragment size of approximately 400 base pairs. Potential forward and reverse primer sequences were then checked against the sequence in the *in silico* PCR software in the UCSC genome bioinformatics website (http://genome.ucsc.edu/cgi-bin/hgPcr), to cross-check its specificity to the particular gene and ensure it would not anneal to any other sequence in the genome.

Finally the potential primer sequence was input into a single nucleotide polymorphism (SNP) checker, SNP Check 3 (https://secure.ngrl.org.uk/SNPCheck/snpcheck.htm), to ensure it did not cover a region with any polymorphisms that would give a false positive result. Any primers that did not pass these checks were redesigned. Primers were ordered from SIGMA and tagged with a M13 universal primer sequence and had High Performance Liquid Chromatography purification (Table 4.1).

4.4.5.2 Sanger Sequencing Technique

Polymerase Chain Reaction

For the PCR, 200ng DNA was added to 12.5μl AmpliTaq Gold 360 PCR Master Mix (ABI), 10pmol/μl of forward and reverse primers and 9.5μl autoclaved nanopure water. This was added to a 96 well plate and covered and centrifuged for one minute at low speed. A negative and positive control were added for each set of primers. 2700 PCR machines were used for

Gene	Exon	Forward /Reverse	Sequence 5' to 3'
CRYAB	3	F	TGTAAAACGACGGCCAGTTCATCTCCAGGGAGTTCCAC
CRYAB	3	R	CAGGAAACAGCTATGACCTCATTCACTGGTGGGGAAAC
SCNN1B	9	F	TGTAAAACGACGGCCAGTacctcctcctgccacctaac
SCNN1B	9	R	CAGGAAACAGCTATGACCGGGTGTCActgaaagagaagg
RYR1	24	F	TGTAAAACGACGGCCAGTGATGAGCAATGGGTACAAGC
RYR1	24	R	CAGGAAACAGCTATGACCgggctgagtcaggtcagaga

Table 4.1: Primers designed for Sanger sequencing of novel variations(Red indicates M13 tag)

the reaction with the following cycling conditions: Denaturing at 95°C for 10min then 30 cycles of 95°C for 30s, 58°C for 30s, 72°C for 30s and the final extension step of 72°C for 7min.

Gel Electrophoresis

Gel electrophoresis was performed to analyse the quality and yield of the PCR. Gels were made with 1g agarose gel mixed with 50ml of 5ml 10% Tris Borate EDTA and 45ml water and microwaved for one to two minutes until the gel was fully dissolved. 1μl ethidium bromide was added to the gel and the gel was poured into a chamber and a gel comb inserted and left to set for approximately 15mins. Once set a further 50mls of 5ml 10% Tris Borate EDTA and 45ml water was added to the plate.

4μl of orange G dye was added to 4μl of the PCR reaction mix and 7μl of this mixture pipetted into the well. One well had 4μl of orange G added to 2μl of a 100 base pair DNA ladder to estimate the size of fragments. Electrodes were attached to the electrophoresis chamber and 65V was applied across it for 30min. The gel was then removed and placed under an ultraviolet illuminator to visualise the DNA fragments and estimate their size and confirm a successful PCR reaction.

PCR Purification

The PCR reaction mix was then purified by adding 79μl of nanopure water to the PCR product, centrifuging the mix and transferring to a clean-up plate. It was then placed on a vacuum manifold and a vacuum of 24mmHg applied for 10min until the wells had emptied. Samples were then reconstituted with 50μl of nanopure water and left to shake on a R100 rotatest shaker for 30min.

DNA Sequencing

All samples were sequenced bidirectionally using ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction kit version 1.1 with M13 universal primers. The sequencing was analysed on the ABI 3730 automated DNA sequencer.

For DNA sequencing, 3.5μl of purified PCR product was added to a mix of 1μl BigDye Premix, 2μl 5X BigDye sequencing buffer, 3.2pmol of either forward or reverse M13 primer (depending on which strand was being sequenced) and 2.5μl nanopure water. Samples were centrifuged and then placed into the ABI 9800 PCR machine. They were then run for 25 cycles at 96°C for 1min, 96°C for 10s, 50°C for 5s and then 60°C for 75s.

The extension product was then purified using the ABI gene Dye Terminator Removal Kit. This was first activated with centrifugation for 3 minutes at 2150rpm. 20μl of sterile deionized water was then added to each sequencing reaction and this mix was inserted into the removal plate. This was then centrifuged again for 3 minutes at 2150rpm and the collected sample was then sequenced using the ABI 3730 DNA sequencer. Data was analysed using 2.5 SeqScape Analysis software (ABI).

4.4.6 Expression Studies

Expression studies were performed to provide supporting evidence for pathogenicity for some novel variations. *Xenopus* oocyte studies were performed by R. Mannikko and ClC-1 expression in HEK293T cells by J. Burge.

4.4.6.1 Xenopus oocyte Studies

Molecular Biology and Oocyte Preparation

Human CLCN1 in pTLN vector (Lorenz et al., 1996) was used for this study. Site-directed mutagenesis was performed using the QuickChange®XL system (Stratagene, La Jolla, CA, USA). Successful mutagenesis was confirmed by sequencing. Capped mRNA was prepared using the mMESSAGE mMACHINE (Ambion, Austin, TX, USA). Integrity and concentration of the product was checked by ethidium bromide stained gel electrophoresis and spectrophotometry. Female *Xenopus laevis* frogs were sacrificed in accordance with the Animals (Scientific Procedures) Act 1986. Clusters of oocytes were incubated in 2mg/ml Collagenase A (Roche) in OR-2 (in mM: NaCl 82.5, KCl 2, $MgCl_2$ 1, Hepes 5, pH 7.5-7.6 with NaOH) for 2h, and stored in modified Barth's solution (in mM: NaCl 88, KCl 1, $MgSO_4$ 1.68, Hepes 10, $Ca(NO_3)_2$ 0.47, $NaHCO_3$ 2.4, $CaCl_2$ 0.41, pH 7.4 with NaOH) at 16°C. Stage

V and VI oocytes were injected with WT RNA, mutant RNA, or a 1:1 mixture of WT and mutant RNA to simulate the heterozygous state (2.5ng total RNA). Oocytes were incubated for 36-72 hours before the electrophysiological recordings.

Electrophysiology

Standard whole cell two-electrode voltage-clamp recordings were performed at room temperature (GeneClamp 500B and DigiData 1200 Interface, Axon Instruments) with ND96 (in mM: NaCl 96, KCl 2, $MgCl_2$ 1, $CaCl_2$ 1.8, Hepes 5, pH 7.4 with NaOH) as extracellular solution. Recording electrodes were filled with 3M KCL and had a tip resistance between 0.1-1MΩ. Data was filtered at 1kHz and sampled at 5kHz using Clampex (Axon Instruments) software. From holding potential of -80mV a 250ms pre-pulse to +60mV was applied, followed by 250ms step to test voltages between -150 and +150mV in 10 mV increments. Tail current amplitude was recorded at -100mV, 4ms after the end of the test voltage steps. Tail current amplitude was plotted against the test voltage and fitted with Boltzmann equation using Clampfit software (Axon Instruments):

$$I(V) = I_o + (I_{max} - I_o)/(1 + exp((V_{1/2} - V)/dV)$$

Where I_o is the minimum offset current at negative test voltages, I_{max} the maximal current amplitude, $V_{1/2}$ the voltage where half of the channels are activated and dV the slope factor. Value of I_o was fixed as the baseline current at most negative voltages. The data was acquired from >2 batches of oocytes, WT and mutant experiments were performed on the same day in time matched manner to avoid time dependent variation on expression levels and channel properties.

Data illustration and statistical analysis (Student's t-test) were performed using Excel 9 (Microsoft) and Origin 8.6 (Microcal) software. Data are given as mean ±S.E.M.

4.4.6.2 HEK293T Cell Studies

The Pro744Thr point mutation was introduced into the cDNA for WT CLCN1 in a mammalian cell expression vector (pCDH1, System Biosciences) using a Stratagene QuickChange Site-directed Mutagenesis Kit and overlapping primers encoding the mutation.

HEK293T cells were maintained in Dulbecco's modified Eagles Medium supplemented with L-glutamine and 10% fetal bovine serum. They were transfected in 1 well of a 6-well plate with 0.5μg of wild-type or mutant DNA using Fugene HD Transfection Reagent

(Roche) according to the user manual. Six hours after transfection the cells were plated onto glass coverslips coated with poly-D-lysine, and were studied by patch clamp 24-48 hours later.

Microelectrodes were pulled from borosilicate capillary glass on a Stutter 97 puller and backfilled with intracellular solution. Cells on a poly-D-lysine coated coverslip were placed in a 35mm dish containing extracellular solution to wash away culture medium before being transferred to the patch clamp set up. The intracellular solution contained in mM Cs Aspartate 110, CsCl 30, $MgCl_2$ 5, EGTA 10, HEPES 10. Extracellular solution contained in mM TEA-Cl 145, $CaCl_2$ 10, HEPES 10. Both solutions were pH 7.4.

Series resistance was 2-4mOhms, and was compensated 50-70%. From a holding potential of -40mV, channels were first activated by a 200ms step to +60mV. Then, to obtain the voltage dependence of activation, the instantaneous current (tail current) on stepping to -100 mV for 400ms was measured after 400ms prepulses to different voltages (-140mV to +120mV in 20mV increments).

4.5 Mexiletine in the Treatment of Non-dystrophic Myotonia

To investigate the efficacy of mexiletine in patients with NDM, a large multi-centred trial was set up. This enabled us to recruit sufficient patient numbers to adequately power the study. The study was designed by the CINCH consortium.

4.5.1 Trial Design

The study had a multi-centred randomised double-blind placebo-controlled crossover design. This was based over seven different centres in four different countries. I was responsible for recruiting, investigating and analysing the data from the UK cohort.

There were two treatment periods of four weeks, each with a one week wash out period in-between. Patients were randomised to either mexiletine 200mg three times a day or placebo three times a day for the first treatment period. Following the wash out they were then given the treatment that they had not previously received (Figure 4.3).

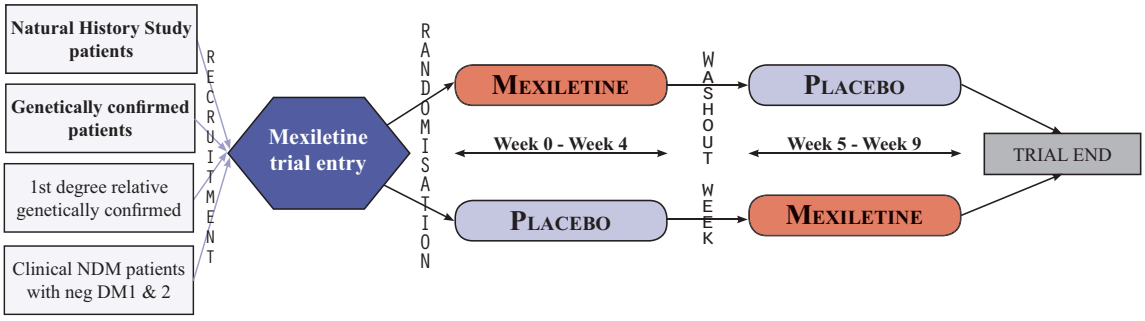


Figure 4.3: Flow chart of mexiletine study design

Mexiletine was purchased from TEVA Pharamaceutical. Placebo was Microcrystalline Cellulose (Avicel PH102). Both tablets were encapsulated to ensure blinding at the University of Iowa Research Pharmacy with Swedish Orange Capsule. A qualified person from Brecon Pharmaceuticals Limited inspected both sites to ensure compliance with EU regulations. Mexiletine drug level testing was conducted at Mayo Medical Laboratory.

The study was conducted between April 2008 and March 2011.

4.5.1.1 Sample Size

The power was set at a minimum of 90% with an alpha level of 0.05. The optimum sample size with these values was 60 patients. This allowed for a standard attrition rate of 10%. A

full data set (minimum of 5 data points in each collection period) for 54 patients allowed a 93% power to detect an effect-size of a quarter of a standard deviation within a subject for the primary outcome measure.

A linear mixed model was used for the power calculations. The primary model was:

$$Y_{ijk} \sim \mu_0 + \beta_M X_{ij} + \gamma_j + b_i + \varepsilon_{ijk}$$

Where Y_{ijk} is the severity score of stiffness occurring for the i^{th} patient, in the j^{th} treatment period, and the k^{th} daily episode (assumed that each patient called in 10 days of the possible 14 for the simulation). The symbol μ_0 represents the mean severity of stiffness score in the NDM population with no treatment (assumed to be 4 for the simulation - it has no substantive effect on the power), β_M is the fixed effect of mexiletine, X_{ij} is the design matrix (treatment assignment: placebo:0 and mexiletine:1), γ_j is the period effect (assumed to be zero for the simulation), b_i is the random effect of patient, and ε_{ijk} is the variation within patient. The two random terms are assumed to be normally distributed with a mean of zero and variance σ_b^2 and σ_ε^2 respectively. The effect size is expressed in standard deviation units (i.e. $\frac{\beta_M}{\sigma_\varepsilon}$) because the variance of the endpoint is unknown. The degree of correlation between two outcome measurements within the same participant is a product of the relative size of the variance of the two random components. The statistical power varies from 0.93 to 0.98 for an effect size of 0.25 ($\Delta = \frac{|\beta_M|}{\sigma_\varepsilon}$), a σ_b ranging from $0.5\sigma_\varepsilon$ to $2\sigma_\varepsilon$, and a two-sided Wald test with the α-level = 0.05. These results are based on 1000 Monte Carlo samples. The dependent variable (severity score) was generated by a random (normally distributed) number generator then rounding to the nearest whole number from 0 to 9. The random effect of subject has an inverse relationship with statistical power due to the truncated distribution of severity score at the extremes (i.e., 0 and 9).

The variation in power was due to the varying degree of between-participant standard deviation. Larger standard deviations lowered the power since the effect in the active treatment period for low-severity scores cannot be less than 0. These values were set before data was available but since the publication of the natural history study these values were shown to be reasonable. In that study the mean severity was 3.85 with a within-participant standard deviation (SD) of 1.5 and between participant standard deviation of 1.82 (Statland et al., 2011). The effect size of one-quarter of an SD was chosen to be conservative to

compensate for the unknown degree of participant adherence to treatment, and to have a sufficient sample size available for the secondary interactive voice response (IVR) diary end points for which some participants do not have the symptom.

4.5.1.2 Randomisation

Patients were randomised remotely using a computerised system that generated a randomisation list using fixed block sizes. The trial team had no knowledge of the randomisation codes. Sealed envelopes were held for safety but no envelopes were unsealed during the trial. All medication bottles were returned after each treatment period to check compliance.

4.5.2 Ethics and Safety

The UK part of the study had full ethical approval from the UCLH ethics committee and the Medicines and Healthcare products Regulatory Agency (MHRA). The US part of the study had Federal Drug Administration (FDA) and local US institutional review board approval. All patients gave full written informed consent.

The safety of the study was reviewed every six months by a Data Safety Monitoring Board established by the National Institute of Health in the US. In the UK the trial sponsor, UCL, also monitored the safety of UK patients and any serious adverse effects were reported to the MHRA.

4.5.3 Patient Selection

All UK patients were at least 18 years old. They had clinical, electrical and genetic confirmation of NDM. Patients from other centres without genetic confirmation either had clinical and electrical evidence of NDM and an affected first degree relative or were DM1 and DM2 negative. Patients were excluded if they were: unable or unwilling to provide informed consent; had other neurological, renal or hepatic disease, heart failure or seizure disorder; had genetically confirmed DM1 or DM2; had pre-existing cardiac conduction defects or a permanent pacemaker; were on an anti-arrhythmic, anti-myotonic or myotonia-inducing medications. Patients on fibrate acid derivatives or hydroxymethylglutaryl CoA reductase inhibitors were only included if they had been on a stable dose for at least 30 days. Pregnant or lactating women were excluded.

All patients were screened prior to starting the trial. This included confirming that patients were symptomatic, had a diagnosis of NDM and had no contraindications to taking

mexiletine. All patients had an electrocardiogram that was assessed by a cardiologist in the US. Patients also had baseline blood tests prior to randomisation.

4.5.4 Outcome Measures

The choice of outcome measures is central to the validity of a trial. Prior to this study the CINCH group performed a natural history study to understand the nature and progression of the disease and identify the optimum outcome measures. Some of these results have already been reported (Statland et al., 2011, 2012; Trivedi et al., 2013). The FDA have put recent emphasis on patient-reported outcome measures and ensuring that outcome measures are clinically meaningful and based on the patient's perspective (US Department of Health and Human Services Food and Drug Administration., 2009) therefore this was taken into account when identifying outcome measures.

4.5.4.1 Primary Outcome measures

The primary outcome measure was severity of patient-assessed stiffness in the third and fourth weeks of treatment. This was measured using an IVR diary which was an automated telephone service that patients called every day during the trial and ranked their stiffness severity from 1–9, one being minimal and nine being the worst ever experienced. The responses were automatically stored on a centralised database and patients did not have access to how they had previously ranked their stiffness, effectively blinding them to their previous responses. This system was previously validated in the NDM natural history study (Statland et al., 2011), which verified that patient-reported stiffness was the most severe and common symptom in NDM patients and therefore it was used as the primary outcome measure.

4.5.4.2 Secondary Outcome Measures

The secondary outcome measures included:

1. Daily patient-reported pain, weakness and fatigue, measured using the same IVR system over the last two weeks of treatment.

2. Clinical myotonia assessment: this included detecting percussion myotonia, triggered by a reflex hammer to the extensor digitorum communis and abductor pollicis brevis. It also included bedside measurement of eyelid myotonia and hand grip myotonia, when patients were asked to close their eyes or fist tightly for three seconds and then asked

to rapidly open it. The speed of opening was measured in seconds with a stopwatch. This was repeated five times to identify the presence of warm-up or paramyotonia.

3. Quantitative hand grip myotonia, measured using a strain gauge to measure the force of contraction on the handle of a grip ergometer, which was recorded on a computerised system. This was a standardised setup across all sites. Right hand grip relaxation times after maximal voluntary isometric contraction was recorded and relaxation time from 90% to 5% of maximal force measured as has been used by other groups (Logigian et al., 2005; Moxley R. T. et al., 2007). Patients did this for six trials, with maximal contraction for three seconds with a 10 second rest between each contraction. Each set of measurements was repeated two further times with a 10 minute rest period inbetween.

4. Measurement of CMAP amplitude during long and short exercise testing (Fournier et al., 2004, 2006).

5. Myotonia on needle EMG in the right ADM and tibialis anterior which was graded according to severity, 0 being no myotonia, 1+ being minimal myotonia, 2+ being myotonic discharges in more than half of needle locations and 3+ being myotonic discharges with each needle movement in all examined areas.

6. Quality of life measures as measured by the SF-36 and INQoL questionnaires for neuromuscular disorders (Trip et al., 2009a; Vincent et al., 2007).

4.5.5 Statistical Analysis

Statistical analysis and design for the entire cohort was performed by the trial statistician, Brian Bundy. All UK cohort data was collected and analysed by myself. An intention-to-treat principle was used but modified to remove missing values that were assumed to be missing at random. The primary endpoint and the secondary endpoints derived from the IVR patient-reported results were all ordinal data and therefore summary statistics used were medians. The majority of analysis was done using either the paired t-test or the linear mixed effects model. The mixed model adjusted for a number of variables including gender, age and difference in time period and took into account the linear structure of endpoints for the quantitative hand grip, short exercise test and long exercise test. It also included data from dropouts. The paired t-test was used for all the main treatment comparisons and

closely matched the results derived from the mixed model. It was however limited to subjects who provided data for both treatment periods. The mixed model was also used to perform homogeneity testing of the treatment effect in mutation subgroup analysis accounting for the variability in result depending on type of mutation.

For EMG myotonia, the score was converted to a numeric value as follows: no myotonia was set to 0, 1+ was set to 1, 2+ was set to 2, and 3+ was set to 3. The endpoint was the sum of the numerical scores of the two muscles. The paired Wilcoxon test was used to test the hypothesis that the medians of the two samples were equal.

Normality assumptions were made for all analyses. QQ plots were done for all the IVR severity scores and demonstrated deviation from normal. Therefore average severity score per week was used instead as these values were normally distributed. Modifications had to be made for some of the other outcomes to fulfil normality criteria. For clinical hand grip and eye closure times a $\log(t_i+0.1)$ transformation was used. For quantitative hand grip myometry a $\log(t_i)$ transformation was used. It was also assumed that there was no carry-over effect and that the treatment effect was the same across periods and the Wald test was used to check for carry-over effect. A significant carry-over effect was identified for cases where the Wald test gave a $p<0.10$ and the data for those categories were analysed for each period separately. Since this trial identified a primary endpoint, all other p-values presented were for secondary endpoints and are not adjusted for multiple testing. All p-values were two sided and 0.05 was considered statistically significant.

Chapter 5

RESULTS

5.1 Prevalence of Skeletal Muscle Channelopathies

5.1.1 Total Minimum Prevalence of Skeletal Muscle Channelopathies

665 patients from 453 pedigrees were selected according to the inclusion criteria. Of these, 593 (89%) were living in England, giving a minimum point prevalence of skeletal muscle channelopathies of 1.12/100,000 (95% CI: 1.03-1.21) (Figure 5.1). Overall 424 (63.8%) patients were male. The median age was 37.6 years (range:1.5-89.4 years) (Table 5.1).

5.1.2 Minimum Prevalence of Non-dystrophic Myotonia

Overall 449 patients had NDM, of which 395 patients were from England giving a minimum point prevalence of 0.75/100,000 (95% CI 0.67–0.82). The male to female ratio was 1.57 (Table 5.1, Figure 5.2).

5.1.2.1 Minimum Prevalence of MC

321 patients had MC with mutations in CLCN1, of which 277 were from England giving a minimum prevalence of MC in England of 0.52/100,000 (Table 5.1). 168 patients had a positive family history, of these, 99 patients (59%) from 50 pedigrees were classified as dominant MC and 69 patients (41%) from 49 pedigrees as recessive MC (Table 5.1). The type of MC was classified based on the patient's inheritance pattern and genotype. Of the remaining 153 patients, five were sporadic cases with de novo mutations and 148 had an uncertain family history. The male to female ratio was 1.97 for MC but varied significantly between dominant MC (1.30) and recessive MC (2.00) (Table 5.1, Figure 5.2).

Overall 104 different CLCN1 mutations were identified in this group, of which 65 could be classed as "private mutations". Of the remaining mutations, 15 particular mutations

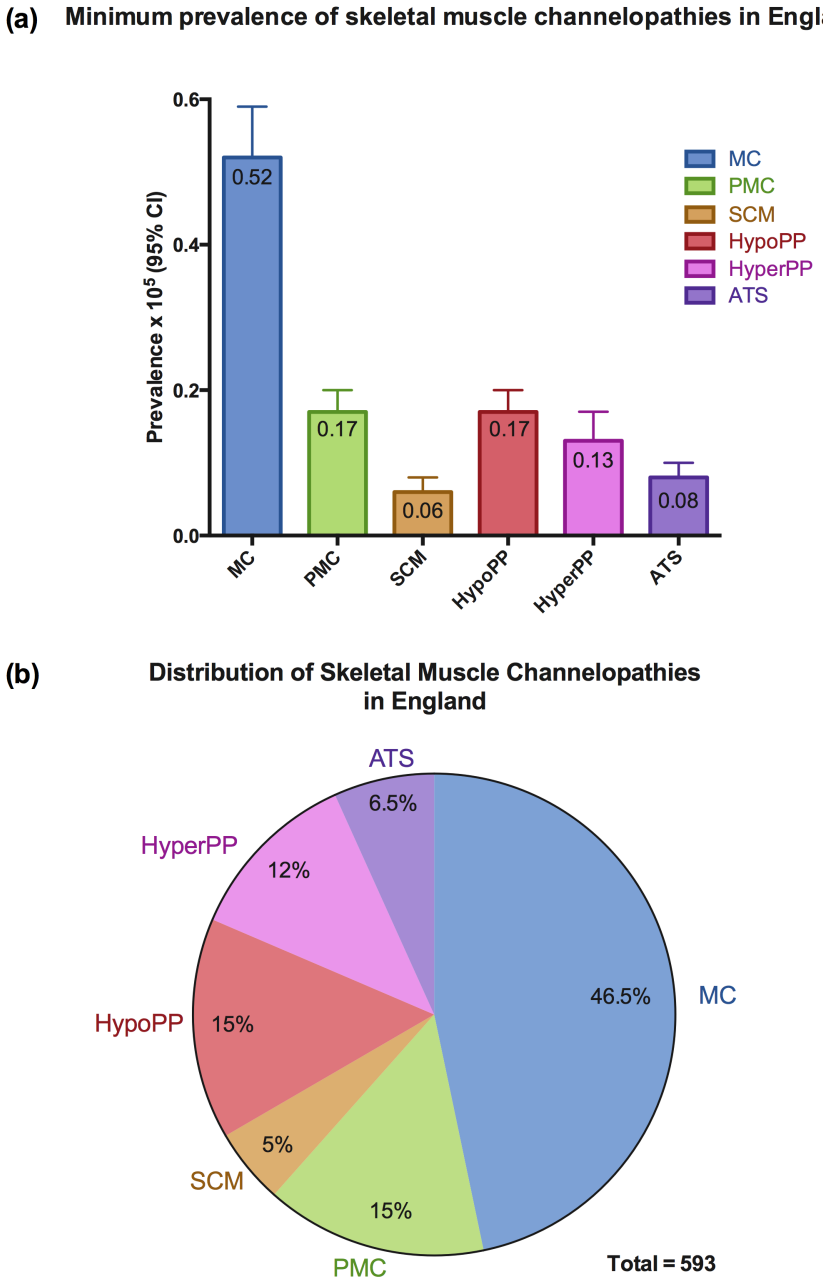


Figure 5.1: Graph of the minimum prevalence of the skeletal muscle channelopathies in England. (a) Minimum prevalence of individual skeletal muscle channelopathies in England (b) Distribution of skeletal muscle channelopathies in England

	All Patients (pedigrees)	Age (yr), mean ± SD[a]	Male/ female ratio	Patients from England, n (%)	Minimum Prevalence (x10^{-5}), rate in England (95% CI)
Skeletal Muscle Channelopathies	**665 (453)**	**38.3 ± 17.7**	**1.78**	**593 (89%)**	**1.12 (1.03–1.21)**
NDM	**449 (322)**	**38.0 ± 17.5**	**1.57**	**395 (88)**	**0.75 (0.67–0.82)**
MC	321 (252)	39.0 ± 16.3	1.97	277 (86)	0.52 (0.46–0.59)
Dominant MC	99 (50)	40.8 ± 17.3	1.30	73 (74)	0.14 (0.11–0.17)
Recessive MC	69 (49)	33.5 ± 17.7	2.00	63 (91)	0.12 (0.09–0.15)
Other[b]	153 (153)	40.2 ± 14.5	2.64	141 (92)	0.27 (0.22–0.31)
PMC[c, d]	96 (56)	35.1 ± 19.5	0.88	88 (92)	0.17 (0.13–0.20)
SCM	32 (14)	37.7 ± 21.3	1.00	30 (94)	0.06 (0.04–0.08)
PP	**216 (131)**	**39.1 ± 18.3**	**2.27**	**198 (92)**	**0.37 (0.32–0.43)**
HypoPP	95 (59)	36.6 ± 18.1	3.52	88 (93)	0.17 (0.13–0.20)
HyperPP	77 (48)	40.2 ± 19.3	2.08	70 (91)	0.13 (0.10–0.17)
ATS	44 (24)	42.7 ± 16.2	1.20	40 (91)	0.08 (0.05–0.10)

[a] At the time of the prevalence day. [b] Sporadic cases and patients with uncertain family history of MC.

[c] Three patients from three pedigrees displayed an overlapping PCM/SCM phenotype.

[d] Two patients with p.Arg1448Cys and one with p.Ile693Thr also had episodes of paralysis.

Table 5.1: Demographic characteristics of prevalence study data and minimum point prevalence rates

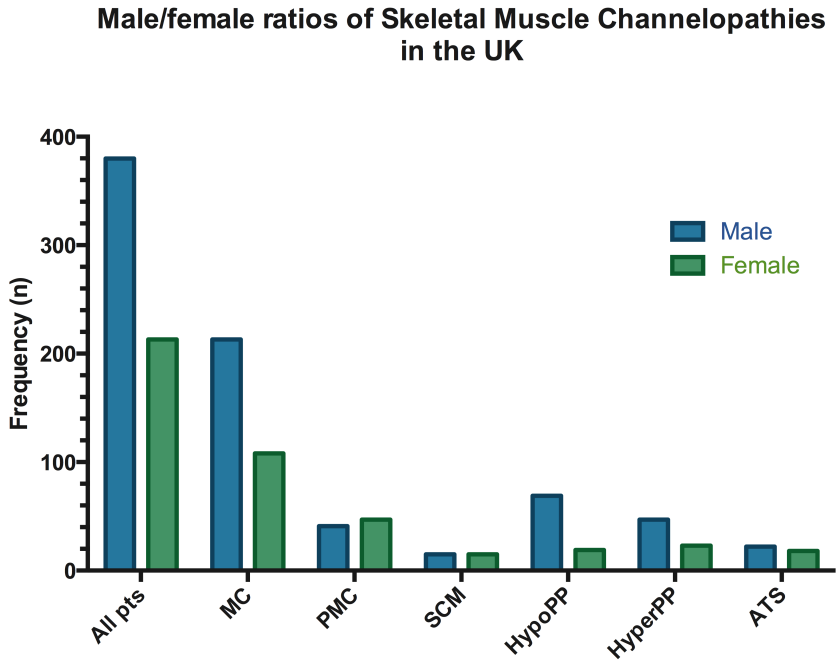


Figure 5.2: Graph of male to female ratios in the skeletal muscle channelopathies in the UK.

were responsible for disease in 83% of all MC patients (96% of dominant MC; 67% of recessive MC). The three commonest mutations were Gly230Glu(18%), Gly285Glu(10%) and Trp303Arg(9%) (Figure 5.3a, Figure 5.4a,Table 5.2).

186 heterozygous patients were identified, as well as 85 compound heterozygotes and 41 patients with homozygous mutations. 9 patients had a heterozygous mutation and a synonymous nucleotide substitution. Of the heterozygous patients, 95%(94) had dominant MC. Of the compound heterozygotes and homozygous cases, 95%(88) had recessive MC (Table 5.3, Figure 5.5). Seven mutations were identified that are commonly associated with dominant MC. 139 patients were identified with these mutations, of which 88%(123) were heterozygous (Table 5.3). The Phe307Ser mutation was the commonest dominant-acting mutation associated with a second mutation (36% of cases). Three mutations which are more commonly associated with recessive MC were found to be expressed heterozygously: Gly285Glu (35% of cases), Phe167Leu (38%) and Val327Ile (43%) (Figure 5.3a, Table 5.3).

5.1.2.2 Minimum Prevalence of PMC and SCM

93 patients had a diagnosis of PMC (88 from England), 32 had a diagnosis of SCM (30 from England) and three displayed an overlapping PMC/SCM phenotype based on clinical and electrophysiological features. This gives a minimum prevalence of 0.17/100,000 for PMC and 0.06/100,000 for SCM (Table 5.1, Figure 5.1). The male to female ratio was 0.88 for

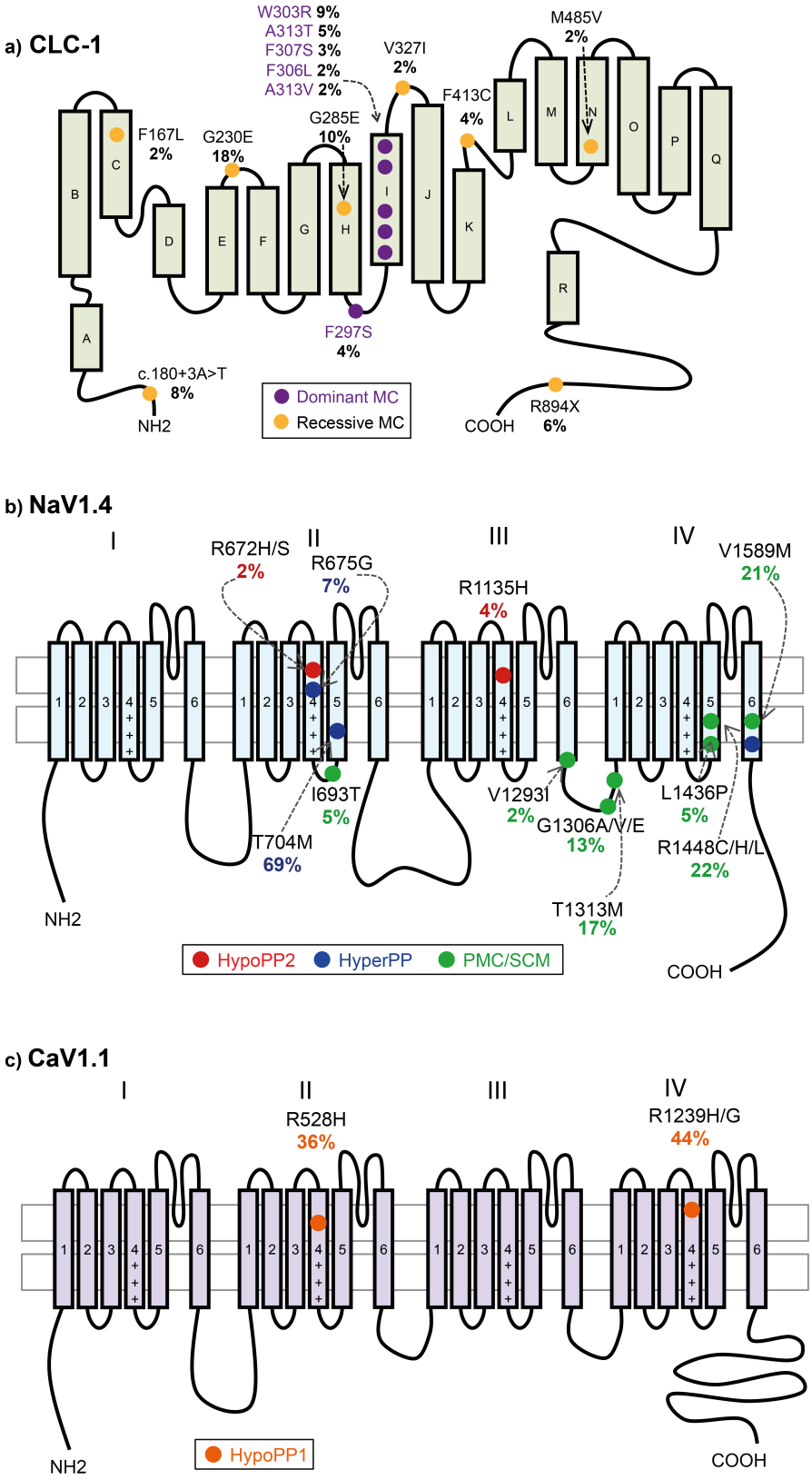


Figure 5.3: Diagrammatic representation of the skeletal muscle ion channels and the location of the common mutations identified in this prevalence study. (a) ClC-1 (b) NaV1.4 (c) CaV1.1. The location of the most common mutations and their percentage frequency in the study sample are shown in each figure.

Table 5.2: Frequency of common mutations in patients with chloride channel mutations in the prevalence study

CLCN1 mutations	All patients with MC, n (%) (n = 321)	Patients with positive family history	
		AD MC, n (%) (n = 99)	AR MC, n (%) (n = 69)
p.Gly230Glu	58 (18)	34 (34)	1 (1)
p.Gly285Glu	31 (10)	0	10 (14)
p.Trp303Arg	29 (9)	21 (21)	0
c.180+3A>T (splice site)	25 (8)	1 (1)[b]	16 (23)
p.Arg894X	18 (6)	2 (2)	5 (7)
p.Ala313Thr	15 (5)	8 (8)	0
c.1437_1450del	15 (5)	1 (1)[c]	2 (3)
p.Phe297Ser	13 (4)	9 (9)	0
p.Phe413Cys	13 (4)	0	4 (6)
p.Phe307Ser	11 (3)	7 (7)	1 (1)
p.Phe167Leu	8 (2)	1 (1)	2 (3)
p.Met485Val	8 (2)	1 (1)[d]	3 (4)
p.Ala313Val	7 (2)	5 (5)	0
p.Phe306Leu	7 (2)	5 (5)	0
p.Val327Ile	7 (2)	0	2 (3)

[a] Non–private mutations present in >2 pedigrees and ≥2% of all patients with MC.

[b–d] Compound heterozygous with a dominant mutation: [b] p.Arg894X; [c] p.Phe307Ser; [d] p.Gly230Glu.

Blue shading indicates common mutations associated with dominant MC.

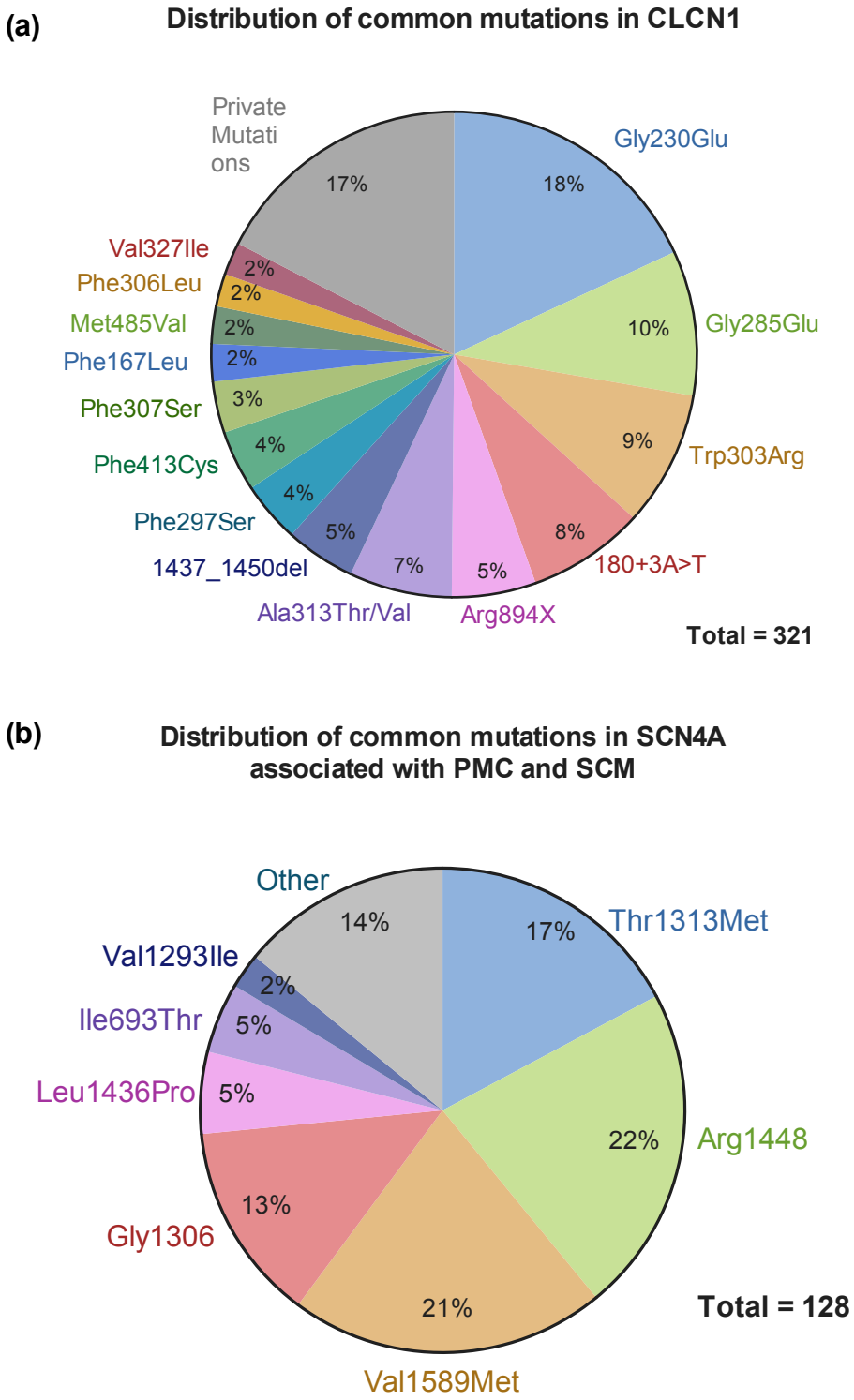


Figure 5.4: Pie charts of distribution of common mutations in Non-dystrophic myotonia (a) Mutations in CLCN1 associated with myotonia congenita (b) Mutations in SCN4A associated with PMC and SCM

Table 5.3: Breakdown of homozygous and heterozygous variations in MC patients in the prevalence study.(a) Overall breakdown of homozygous and heterozygous variations in MC patients, (b) Frequency of heterozygous, homozygous and compound heterozygous mutations amongst the common mutations in MC patients

a)

Myotonia congenita cases *(CLCN1* mutations)	Dominant MC n (%) (n = 99)	Recessive MC n (%) (n = 69)	Other n (%) (n = 153)	Total n (%) (n = 321)
Heterozygous	94 (95)	2 (3)	90 (59)	186 (58)
Compound heterozygous	3 (3)	39 (57)	43 (28)	85 (26)
Homozygous	2 (2)	22 (32)	17 (11)	41 (13)
Heterozygous + synonymous nucleotide substitution	0	6 (9)	3 (2)	9 (3)

b)

CLCN1 Mutations (n = 321)	Heterozygous n (%) (n = 186)	Heterozygous + p.(=) n (%) (n = 9)	Compound Heterozygous n (%) (n = 85)	Homozygous n (%) (n = 41)
p.Gly230Glu	54 (29)	1 (11)	3 (4)	0
p.Gly285Glu	11 (6)	0	20 (24)	0
p.Trp303Arg	28 (15)	0	1 (1)	0
c.180+3A>T	1 (1)	0	22 (26)	2 (5)
p.Arg894X	4 (2)	0	13 (15)	1 (2)
p.Ala313Thr	14 (8)	0	0	1 (2)
c.1437_1450del	3 (2)	1 (11)	9 (11)	2 (5)
p.Phe297Ser	13 (7)	0	0	0
p.Phe413Cys	3 (2)	0	9 (11)	1 (2)
p.Phe307Ser	7 (4)	0	3 (4)	1 (2)
p.Phe167Leu	3 (2)	0	5 (6)	0
p.Met485Val	1 (1)	0	7 (8)	0
p.Ala313Val	6 (3)	0	1 (1)	0
p.Phe306Leu	7 (4)	0	0	0
p.Val327Ile	3 (2)	0	4 (5)	0
N[a]	158 (85)	0	27 (32)	8 (20)

Table looking at non–private mutations present in >2 pedigrees and ≥2% of all patients with MC.

[a]Number of patients with myotonia congenita in which only common *CLCN1* mutations were detected.

Blue shading indicates common mutations associated with dominant MC.

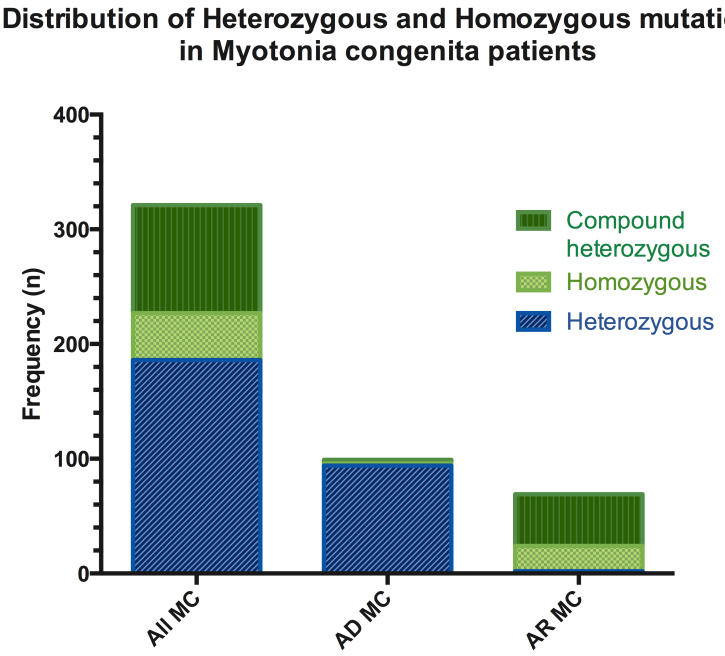


Figure 5.5: Graph of distribution of heterozygous and multiple mutations in myotonia congenita patients

PMC and 1.00 for SCM (Figure 5.2).

Overall, of the 22 different SCN4A mutations detected in patients with PMC and SCM, 11 were present in 86% of all pedigrees and 11 were private mutations (Figure 5.3b, Figure 5.4b). Of the common mutations, two were only seen in PMC patients, Thr1313Met and changes at the Arg1448 location. Two mutations were seen with equal frequency in both PMC and SCM patients, Val1589Met and changes at the Gly1306 location, although at this location, substitutions to a valine were more likely to be associated with PMC and those to alanine more likely to be associated with SCM (Table 5.4). Two patients with PMC from the same pedigree had one SCN4A mutation and a sequence variant of unknown clinical significance.

5.1.3 Minimum Prevalence of Periodic Paralysis

Overall, 216 patients from 131 pedigrees had PP, of which 198 were from England giving a minimum prevalence of 0.37/100,000 (Table 5.1).

5.1.3.1 Minimum Prevalence of HypoPP and HyperPP

95 patients (88 in England) had a diagnosis of HypoPP and 77 (70 in England) of HyperPP, giving a minimum prevalence of 0.17 and 0.13/100,000 respectively (Figure 5.1). The male to female ratio for HypoPP was 3.52 and for HyperPP 2.08 (Table 5.1, Figure 5.2). In patients

Table 5.4: Frequency of common mutations in patients with PMC and SCM a) Frequency of individual mutations across all patients, b) Frequency of mutations by clinical diagnosis (PMC versus SCM)

a)

***SCN4A* mutations**	**Patients, n (%) (n = 128)**	**Pedigrees, n (%) (n=70)**
p.Thr1313Met	22 (17)	15 (21)
p.Arg1448Cys/His/Leu	28 (22)	13 (19)
p.Val1589Met	27 (21)	11 (16)
p.Gly1306Ala[a]/Glu/Val	17 (13)	9 (13)
p.Leu1436Pro[a]	7 (5)	6 (9)
p.Ile693Thr	4 (3)	4 (6)
p.Val1293Ile	3 (2)	2 (3)

Non–private mutations present in all patients with PMC, SCM
[a] *SCN4A* mutations associated with an overlapping PMC/SCM phenotype

b)

***SCN4A* mutations**	**Clinical diagnosis**	
	PMC	**SCM**
p.Thr1313Met	22	0
p.Arg1448Cys/His/Leu	26	2
p.Val1589Met	14	13
p.Gly1306Ala[a]/Glu/Val	8 (+1)	8 (+1)
p.Leu1436Pro[a]	4 (+2)	1 (+2)
p.Ile693Thr	4	0
p.Val1293Ile	1	2

[a] 1 patient had an overlapping PMC/SCM phenotype

with HypoPP, a total of five different CACNA1S mutations causing HypoPP1 and nine SCN4A mutations causing HypoPP2 were detected. Of these mutations, six accounted for 86% of pedigrees (Figure 5.3b & c, Figure 5.6a, Table 5.5). Six different SCN4A mutations were found in patients with HyperPP, of which three accounted for 94% of pedigrees (Figure 5.3b, Figure 5.6b, Table 5.5).

	Patients, n (%)	**Pedigrees, n (%)**
HypoPP		
***CACNA1S* mutations**	78 (82)	47 (80)
p.Arg528His	34 (36)	24 (41)
p.Arg1239His	39 (41)	19 (32)
p.Arg1239Gly	3 (3)	2 (3)
***SCN4A* mutations**	17 (18)	12 (20)
p.Arg1135His	4 (4)	2 (3)
p.Arg672His/Ser	4 (2)	4 (7)
HyperPP		
***SCN4A* mutations**	77 (100)	48 (100)
p.Thr704Met	53 (69)	32 (67)
p.Met1592Val	14 (18)	11 (23)
p.Arg675Gly	5 (7)	2 (4)
Non–private mutations present in all patients with HypoPP and HyperPP		

Table 5.5: Frequency of common mutations in patients with HypoPP and HyperPP

5.1.3.2 Minimum Prevalence of ATS

44 patients (40 in England) in 24 pedigrees with ATS were identified with a minimum prevalence of 0.08/100,000 (Table 5.1, Figure 5.1). All had episodes of weakness with or without other features of the disorder (cardiac arrhythmias and distinctive physical features).

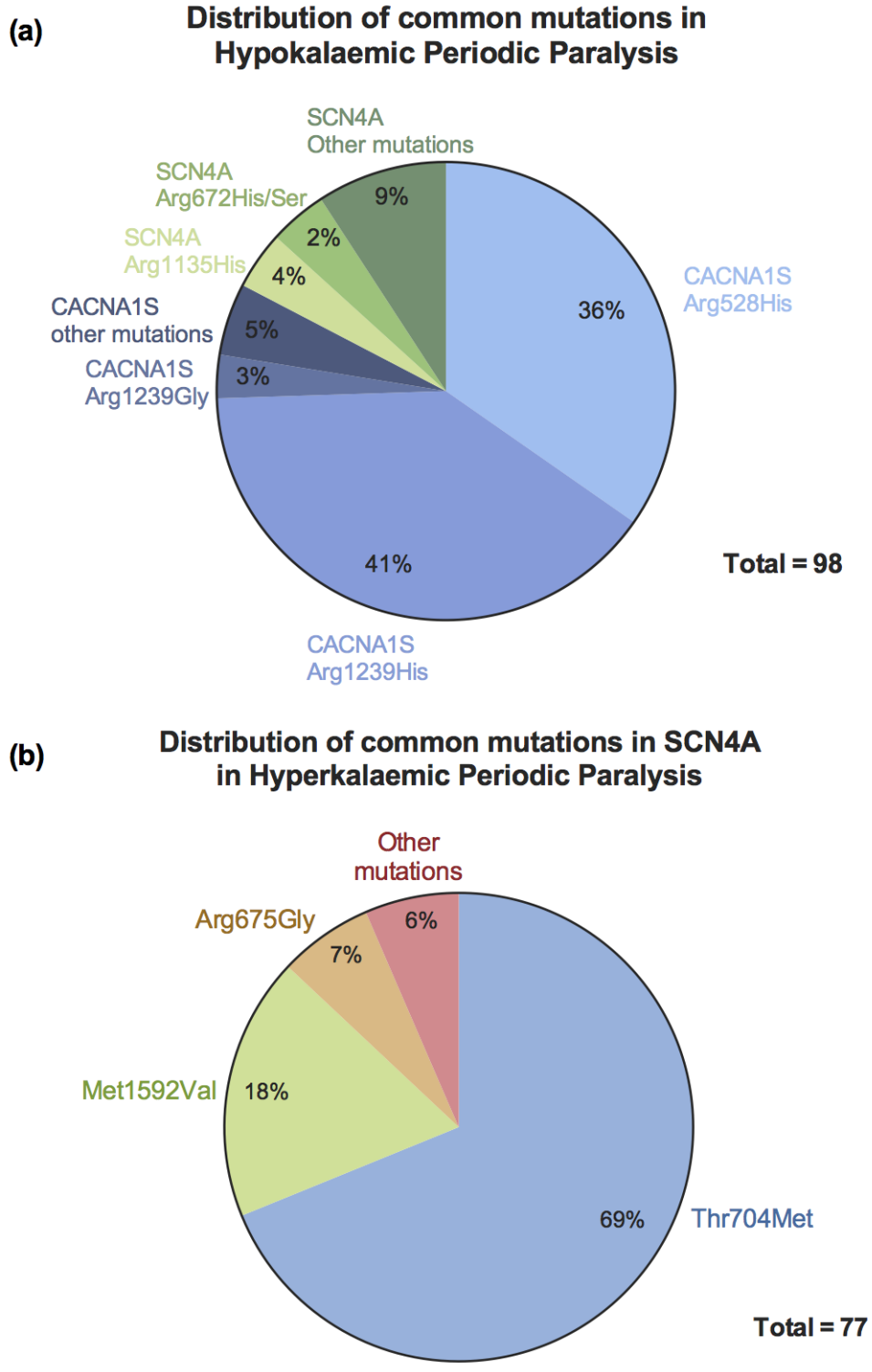


Figure 5.6: Pie charts of the distribution of common mutations in periodic paralysis (a) in Hypokalaemic Periodic Paralysis (b) in Hyperkalaemic Periodic Paralysis

The male to female ratio was 1.20. 17 different KCNJ2 mutations were detected: three were present in 42% of pedigrees and the remaining 14 were private mutations.

5.2 Clinical Phenotype Study of patients with Periodic Paralysis, PMC and SCM

Overall 220 patients with HypoPP, HyperPP, ATS, PMC and SCM were characterised (Figure 5.7). Comparative studies were then done to identify key differences between the groups.

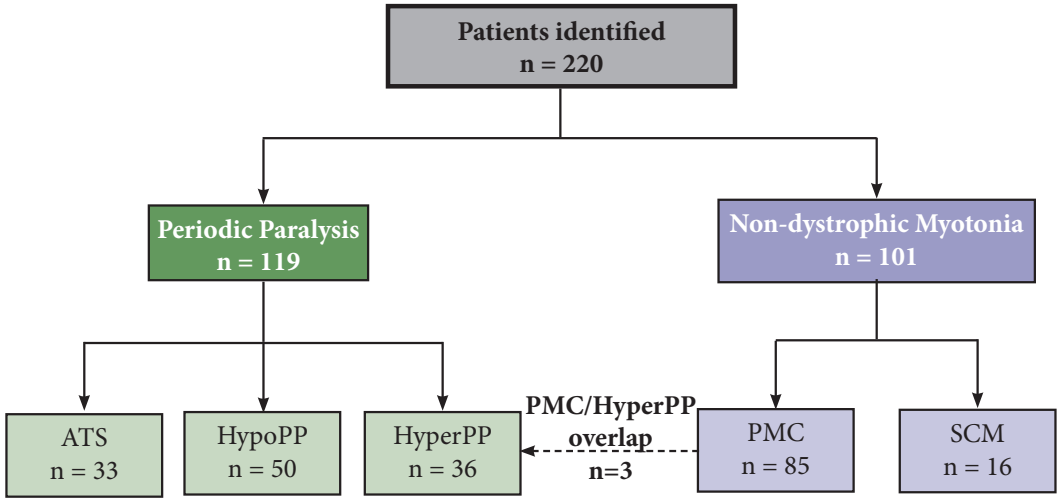


Figure 5.7: Flow chart of patients analysed in the clinical phenotypes study.

5.2.1 Distinguishing Clinical Phenotypes in PMC and SCM

5.2.1.1 Demographics

101 patients were identified as having myotonia and variations consistent with either PMC or SCM. Of these, 82 were classed by the reviewing clinician as having PMC, 3 had a diagnosis of PMC/HyperPP overlap and 16 as SCM (Figure 5.8). There was an equal distribution of males and females, with 70 males and 67 females. The median age was 45 years with a range of 7 to 86 years. All patients with either PMC or SCM had myotonia observed clinically and electrically.

5.2.1.2 Differences in Groups with and without Weakness

As the traditional differentiation between patients with PMC and SCM is based on the presence or absence of weakness, I assessed the number of patients classified as PMC or SCM that had either episodic or fixed weakness. I unexpectedly discovered that 3 (21%) patients with SCM had episodes of weakness and 27% of PMC patients (18/68) had no

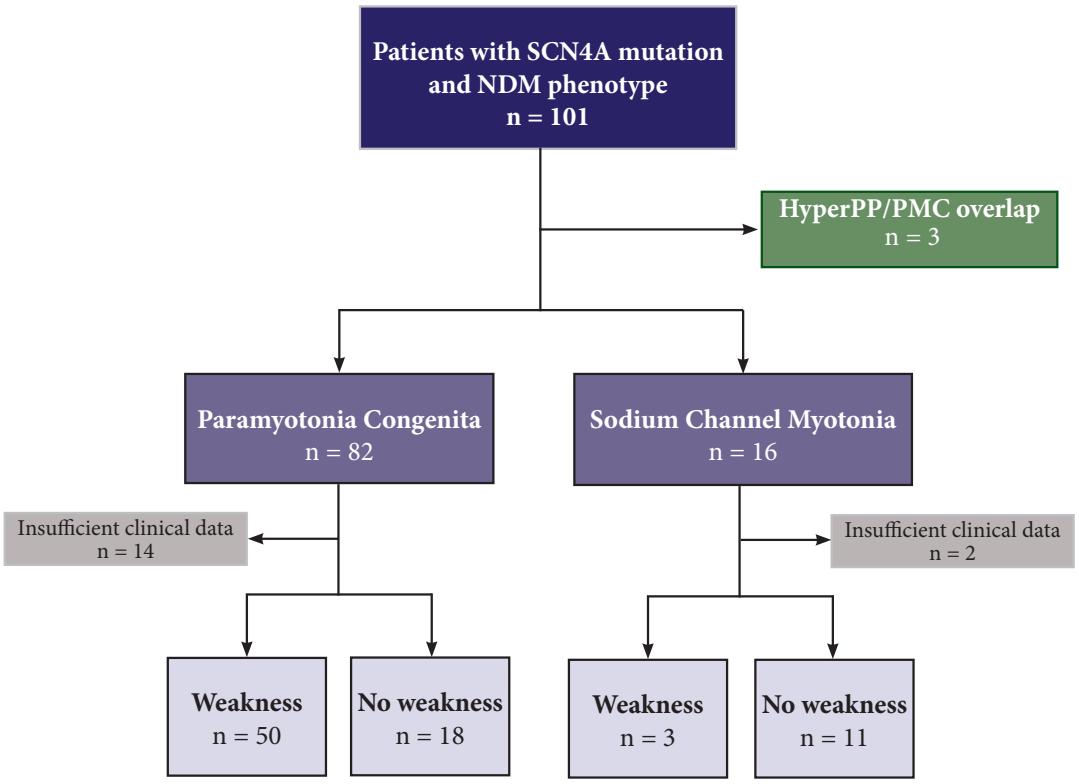


Figure 5.8: Flow chart of breakdown of patients with PMC and SCM and proportion of those with weakness.

episodes of weakness, despite traditionally being the key differentiating factor in diagnosing PMC and SCM (Figure 5.8). Due to these inconsistencies, I performed analysis of patients according to the presence or absence of weakness (rather than clinician diagnosis), with those with weakness being assigned a diagnosis of "PMC" and those without weakness assigned a diagnosis of "SCM". Data analysis using this breakdown demonstrated no significant differences between the two groups (Table 5.6).

The median age of onset for both groups was 5.0 years and frequency of attacks were very similar at 8.2 attacks per week for those with weakness, compared to 6.6 attacks per week for those without (p=0.621) (Figure 5.9a & b). Paramyotonia, which is thought to be more frequent in PMC, was very common in both groups, present in 77% (n=24) of patients with weakness versus 78% (n=14) of patients without weakness (p=0.561)(Figure 5.9e). Patients with PMC have also been reported to be more cold sensitive but in this group 89% (n=46) of patients with weakness were cold sensitive compared to 96% without weakness (p=0.214)(Figure 5.9e). Another key factor thought to distinguish these groups

	n	Weakness	No weakness	P-value
Number of cases	82	53	29	
Median age of onset of myotonia (range)	71	5.0 (0-58)	5.0 (0-50)	0.937
Frequency of attacks of myotonia per week (SD)	31	8.2 (5.7)	6.6 (3.7)	0.621
Severity of myotonia (1-4) (SD)	**59**	**2.7 (0.9)**	**2.1 (1.0)**	**0.037**
Distribution of myotonia				
- eyes	69	36 (78)	19 (83)	0.672
- hands	72	36 (80)	23 (85)	0.580
- limbs	59			0.999
- arms & legs		18 (47)	10 (47)	
- legs only		16 (42)	9 (43)	
- trunk	53	11 (33)	4 (20)	0.296
Precipitants of myotonia				
- cold	81	46 (89)	28 (96)	0.214
- exertion	64	36 (90)	20 (83)	0.435
- rest after exercise	54	15 (41)	7 (41)	0.965
- carbohydrates	33	3 (14)	0 (0)	0.199
- potassium	33	3 (13)	0 (0)	0.231
Relievers for myotonia				
- warmth	19	11 (79)	5 (100)	0.259
- exercise	11	5 (63)	1 (33)	0.545
Painful myotonia	50	28 (78)	10 (71)	0.637
Severity of pain (1-3) (SD)	34	2.2 (0.64)	2.0 (1.1)	0.686
Improvement with mexiletine	34	21 (96)	10 (83)	0.114
Examination				
Clinical myotonia	75	48 (100)	27 (100)	-
Eyelid myotonia	71	39 (87)	23 (89)	0.827
Warm-up myotonia	48	6 (19)	3 (18)	0.885
Paramyotonia	49	24 (77)	14 (78)	0.977
Limb hypertrophy	49	16 (49)	8 (50)	0.333
Investigations				
Creatine Kinase, IU/L (SD)	44	388 (597)	196 (105)	0.485
Interictal potassium, mmol/L (SD)	42	4.2 (0.4)	3.9 (0.5)	0.059
Neurophysiology Fournier pattern	51			
- I		19 (54)	8 (50)	
- III		15 (43)	8 (50)	
- IV		1 (100)	0 (0)	
EMG myotonia	62	42 (100)	20 (100)	-
Change in EMG with cooling	18			0.218
- increased myotonia		6 (46)	3 (60)	
- reduced myotonia		5 (39)	0 (0)	
Myopathic EMG	27	2 (12)	0 (0)	0.260
Short exercise test positive	49	7 (21)	6 (37.5)	0.226
Short exercise test positive following cooling	36	16 (70)	7 (54)	0.346
Long exercise test abnormal	44	10 (33)	5 (36)	0.877
MRI abnormal	10	8 (100)	2 (100)	-

Table 5.6: Characteristics of patients with PMC and SCM divided by the presence and absence of weakness. (Percentages are in brackets unless otherwise indicated. Continuous data have standard deviation in brackets as indicated)

was the presence of pain in patients with SCM but I did not see a difference between those without weakness (71% , n=10) versus those with weakness (78%, n=28) (p=0.637) (Figure 5.9e). The severity of pain was also comparable, with those without weakness having a mean score of 2.0/3 compared to those with weakness having a score of 2.2/3 (p=0.686). Interestingly there was also no significant difference in the neurophysiology pattern between patients in these two groups (p=0.734). There was also no difference in the number of patients with positive short exercise tests, with 70% (n=16) in those with weakness and 63% (n=10) in those without (p=0.226) (Figure 5.9e). The percentage decrement in the positive short exercise tests of both groups was also similar (p=0.337).

The only category in which I detected any difference was in the severity of myotonia. Severity of myotonia tended to be greater in patients with weakness than without (2.7/4 versus 2.1/4) with a p-value of 0.037, however this difference was not significant when multiple comparisons were taken into account (Figure 5.9c).

5.2.1.3 Differences in Groups Divided by Fournier Type I and Type III Classification

As there was no significant phenotypic difference between patients with and without weakness, I investigated whether splitting patients according to their neurophysiology patterns may be a better differentiator. All patients, apart from two with the Ile693Thr variation, had either Fournier type I or type III neurophysiology patterns. The two with Ile693Thr had type IV patterns, which are commonly seen in patients with a HyperPP/PMC overlap (Plassart et al., 1996). 29 patients had type I patterns suggestive of a PMC phenotype and 24 had type III patterns suggestive of a SCM phenotype. Again using this differentiation the phenotypes of the two groups were the same (Table 5.7). There was no significant difference in age of onset of myotonia (Figure 5.10a), frequency of myotonia (Figure 5.10b), sensitivity to cold or presence or severity of pain (Figure 5.10e). Attacks of weakness were common in both groups, 70% (n=19) of type I versus 65% (n=15) of type III (p=0.697) (Figure 5.10e). The frequency of paramyotonia on examination was greater in type 1 patients, 83% (19 patients), than in type III, 57% (9 patients), although this did not reach statistical significance due to the small numbers (0.072) (Figure 5.10e).

Figure 5.9: Graphs of comparison of phenotypes of myotonic patients with and without weakness. a) Age of onset of myotonia; b) Frequency of attacks of myotonia per week; c) Severity of myotonia (score 1-4); d) Creatine kinase levels; e) Key phenotypic comparisons in patients.

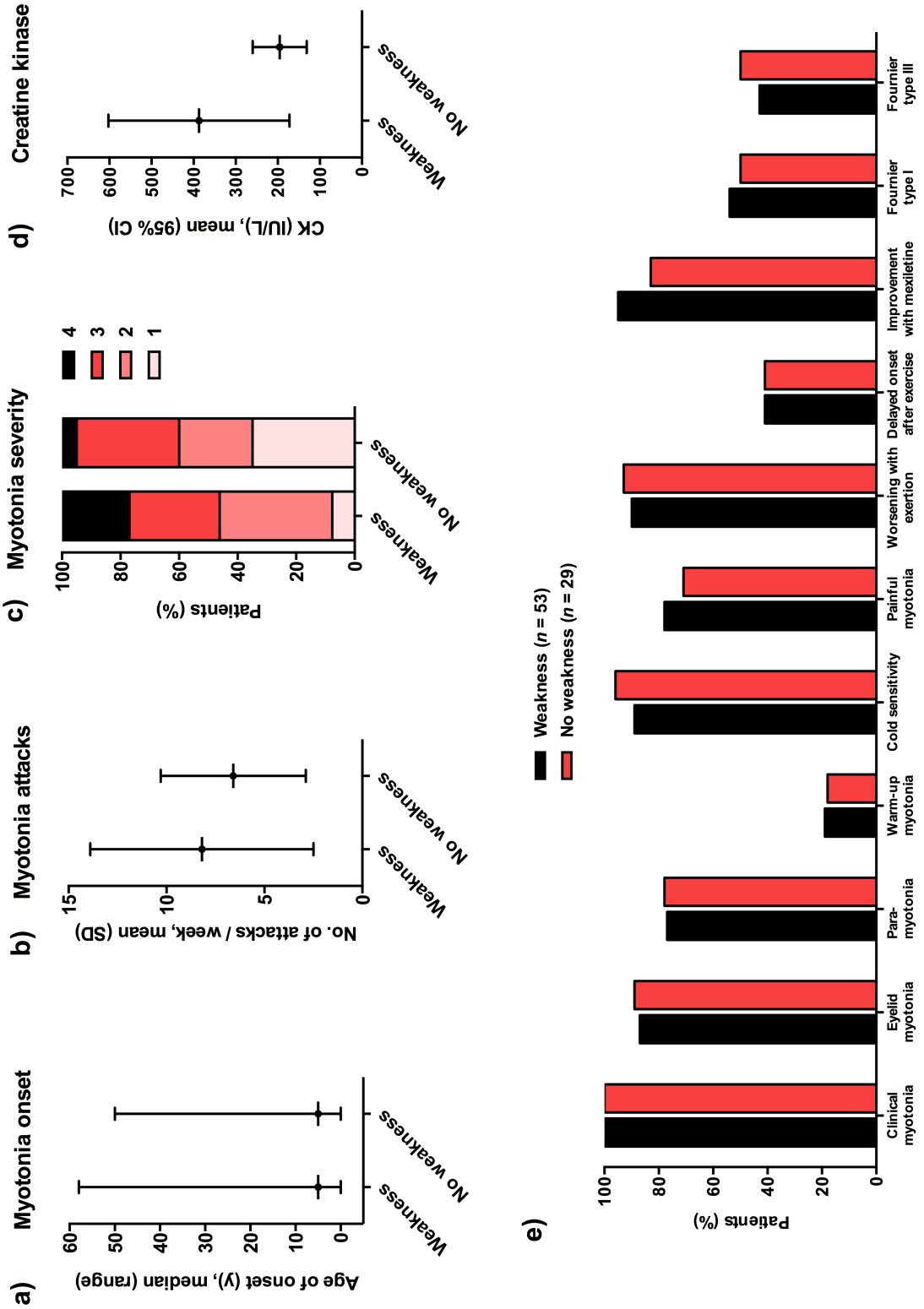


Figure 5.10: Graphs of comparison of phenotypes of myotonic patients divided by Fournier type I and type III neurophysiology patterns a) Age of onset of myotonia; b) Frequency of attacks of myotonia per week; c) Severity of myotonia (score 1-4); d) Interictal potassium levels; e) Key phenotypic comparisons in patients.

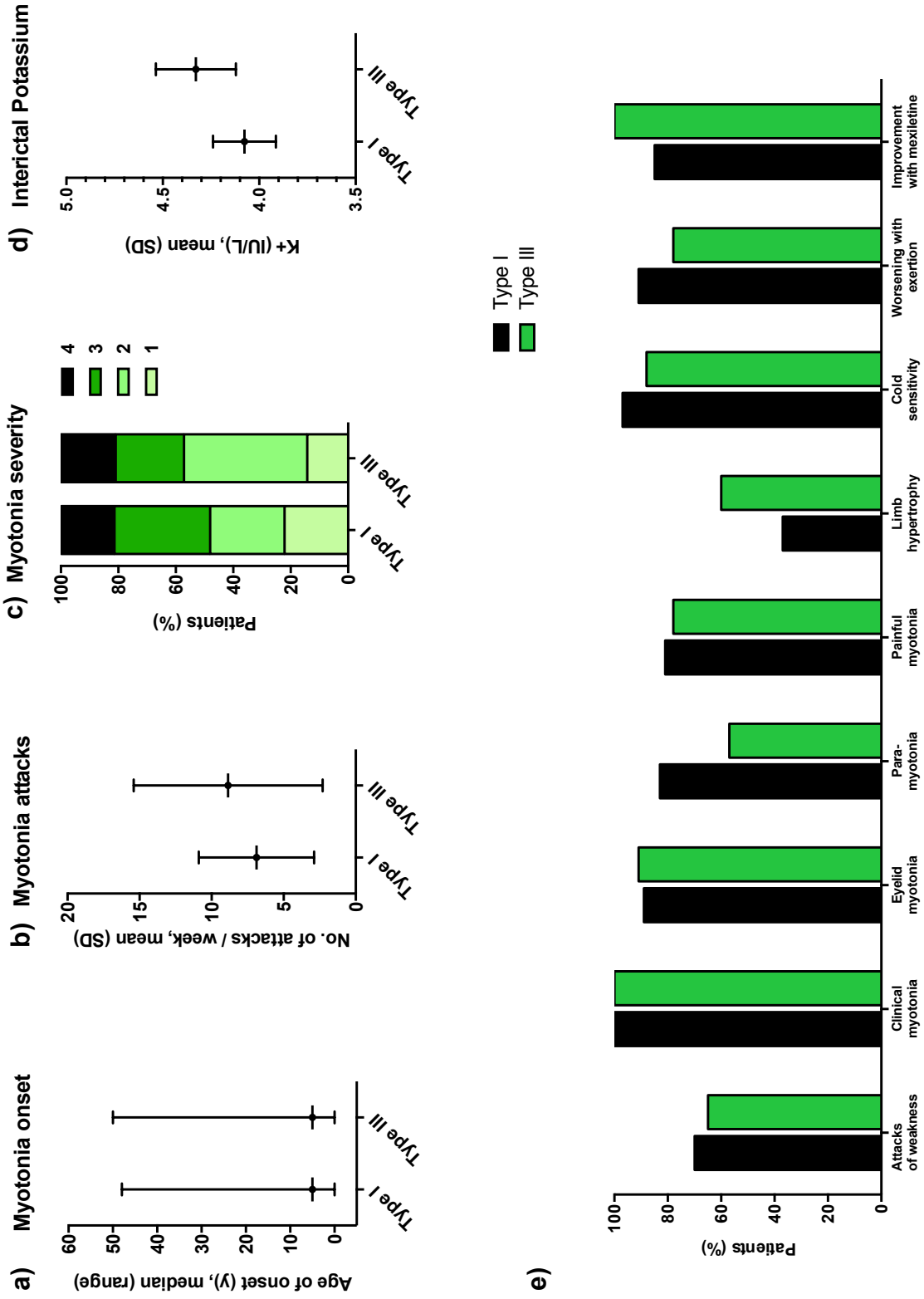


Table 5.7: Characteristics of patients divided by Fournier type I and type III neurophysiology patterns (Percentages are in brackets unless otherwise indicated. Continuous data have standard deviation in brackets as indicated)

	n	Type I	Type III	p-value
Number of cases	53	29 (53)	24 (44)	
Age of onset of myotonia (median & range)	50	5 (0-48)	5 (0-50)	0.280
Characteristics of myotonia				
Frequency of attacks of myotonia per week (SD)	30	6.9 (4.0)	8.9 (6.6)	0.472
Severity of myotonia (1-4) (SD)	48	2.5 (1.0)	2.5 (1.0)	0.940
Distribution of myotonia				
- eyes	45	18 (72)	15 (75)	0.821
- hands	48	19 (73)	18 (82)	0.473
- limbs	43			0.139
- arms & legs		7 (32)	12 (57)	
- legs only		11 (50)	9 (43)	
Precipitants of myotonia				
- cold	53	28 (97)	21 (88)	0.214
- exertion	46	21 (91)	18 (78)	0.218
- rest after exercise	39	10 (50)	11 (58)	0.621
- carbohydrates	25	1 (7)	1 (10)	0.763
- potassium	24	1 (7)	1 (10)	0.803
Relievers for myotonia				
- warmth	18	11 (92)	4 (67)	0.180
- exercise	10	3 (60)	3 (60)	1.000
Painful myotonia	39	17 (81)	14 (78)	0.807
Severity of pain (1-3) (SD)	29	2.0 (0.8)	2.4 (0.7)	0.195
Characteristics of attacks of weakness				
Attacks of weakness	50	19 (70)	15 (65)	0.697
Distribution of weakness in limbs	21			0.812
- arms & legs		6 (50)	4 (44)	
- legs only		3 (25)	4 (44)	
Precipitants of attacks of weakness				
- strenuous exercise	26	10 (67)	10 (91)	0.147
- rest after exercise	21	4 (29)	4 (57)	0.204
- cold	23	14 (93)	6 (75)	0.214
- potassium	16	1 (11)	0 (0)	0.362
Fixed progressive weakness	29	3 (21)	6 (40)	0.280
Improvement of myotonia with mexiletine	28	17 (85)	8 (100)	0.511
Examination				
Clinical myotonia	51	27 (100)	24 (100)	-
Eyelid myotonia	48	23 (89)	20 (91)	0.782
Warm-up myotonia	39	3 (13)	6 (38)	0.075
Paramyotonia	39	19 (83)	9 (57)	0.072
Proximal limb weakness	27	1 (10)	2 (12)	0.888
Limb hypertrophy	39	7 (37)	12 (60)	0.251
Investigations				
Creatine Kinase, IU/L (SD)	38	303 (463)	287 (221)	0.231
Interictal potassium, mmol/L **(SD)**	**39**	**4.1 (0.4)**	**4.3 (0.4)**	**0.049**
MRI abnormal	10	5 (100)	5 (100)	-

5.2.1.4 Phenotype of Common Variations in SCN4A

To investigate whether there were any distinctive characteristics for specific genotypes, I analysed phenotypes of the four main variations present in the cohort of PMC and SCM patients. I investigated phenotypes for variations at positions Gly1306, Arg1448 and mutations Thr1313Met and Val1589Met. For variations at position Gly1306, phenotypes were first analysed individually for the individual mutations Gly1306Ala and Gly1036Val and as there was no significant difference the data was combined for this analysis. This was the same for Arg1448Cys and Arg1448His variations. Unlike when comparing PMC and SCM, there were significant differences seen between the four genotypes as shown in Table 5.8 and distinctive characteristics associated with particular variations.

Firstly, all patients with Arg1448 position variations or Thr1313Met variations were diagnosed with PMC by their clinician whereas there was a mixture of PMC and SCM diagnoses for those with Val1589Met and Gly1306 variations. The age of onset of myotonia in patients with Gly1306 and Val1589Met variations tended to be older (6 years and 5 years respectively) than Arg1448 and Thr1313Met variations (1 year and 3 years respectively) although there was not sufficient data to demonstrate a significant difference (p=0.084) (Figure 5.11a). Warm-up was more common in Gly1306 and Arg1448 variations (67% in both cases) but again the difference fell short of significance (p=0.051) (Figure 5.12). Although the majority of patients were sensitive to cold, unusually Gly1306 variations were also more likely to be sensitive to warmth as well (33%, p=0.042). There were differences in the factors that relieved myotonia although once corrected for multiple comparisons they may not be significant (Figure 5.12). No patients reported relief with warmth or exercise with Val1589Met variations whereas the majority of patients reported this with Gly1306 and Arg1448 variations. All patients with Thr1313Met improved with warmth but none noticed an improvement with exercise. There was no significant difference in potassium sensitivity or in delayed myotonia after exercise. However, those with Gly1306 variations were more likely to have delayed myotonia than the other genotypes (67% Gly1306 versus 40% Arg1448, 25% Thr1313Met, 21% Val1589Met) (Figure 5.12, Table 5.8).

The phenotypic analysis showed no significant difference in paramyotonia, pain or severity of pain between any of the genotypes, although all patients with Gly1306 mutations had pain and higher pain scores (2.6/3 versus 2.0, 1.8 and 2.1 in Arg1448, Thr1313Met and Val1589Met) (Figure 5.12, Figure 5.11e). Myotonia was very common in the face in

Table 5.8: Characteristics of patients with common SCN4A variations. Percentages are in brackets unless otherwise indicated.(* Indicates significant p-values after Bonferroni correction. p-values <0.05 in bold. Continuous data have standard deviation in brackets as indicated)

	G1306A/V	R1448C/H	T1313M	V1589M	P value
Number of Cases	9	20	27	18	
Clinician Diagnosis - PMC - SCM	 7 2	 20 0	 27 0	 10 8	
Characteristics of myotonia					
Age of onset of myotonia [median] (range)	6 (0-50)	1 (0-10)	3 (0-9)	5 (0.33 - 14)	0.084
Severity of myotonia (1-4) (SD)	2.0 (1)	2.3 (0.9)	2.4 (1.2)	2.3 (0.7)	0.762
Painful myotonia	7 (100)	5 (71)	10 (77)	7 (64)	0.355
Severity of pain (1-3) (SD)	2.6 (0.5)	2.0 (0.8)	1.8 (0.6)	2.1 (0.9)	0.195
Warm-up	4 (67)	4 (67)	3 (21)	2 (18)	0.051
Location of myotonia					
- Eyes	5 (63)	11 (92)	16 (73)	14 (82)	0.397
- Hands	7 (88)	14 (82)	18 (78)	14 (88)	0.871
- limbs - arms & legs - legs only - arms only	 4 (50) 4 (50) 0 (0)	 1 (10) 5 (50) 3 (30)	 10 (46) 8 (37) 2 (9)	 10 (67) 4 (27) 1 (7)	0.203
- trunk	**4 (67)**	**0 (0)**	**3 (18)**	**4 (33)**	**0.023**
Precipitants of myotonia					
- cold	9 (100)	20 (100)	25 (96)	16 (84)	0.122
- hot	**3 (33)**	**0 (0)**	**0 (0)**	**3 (18)**	**0.042**
- exertion	7 (78)	5(71)	18 (100)	14 (82)	0.177
Delayed myotonia after exercise	6 (67)	2 (40)	4 (25)	3 (21)	0.116
Relievers of myotonia					
- warmth	**2 (100)**	**3 (75)**	**8 (100)**	**0 (0)**	**0.037**
- exercise	**2 (100)**	**3 (100)**	**0 (0)**	**0 (0)**	**0.029**
Characteristics of attacks of weakness					
Attacks of weakness	4 (50)	9 (69)	15 (68)	8 (47)	0.458
Frequency of attacks of paralysis per week (SD)	2.4 (4.0)	3.3 (3.4)	5.9 (6.5)	-	0.353
Duration of attacks of weakness in hours (SD)	0.8 (1.0)	4.9 (8.5)	9.3 (9.7)	-	0.107
Precipitants of weakness					
- strenuous exercise	3 (100)	3 (43)	8 (73)	4 (100)	0.129
- rest after exercise	1 (33)	3 (50)	3 (33)	-	0.792
- immobility	**0 (0)**	**3 (75)**	**1 (14)**	**-**	**0.047**
- cold	2 (67)	7 (100)	9 (100)	-	0.060
Progressive weakness	2 (33)	2 (33)	5 (50)	0 (0)	0.253
Improved with mexiletine	5 (100)	4 (100)	9 (90)	8 (100)	0.622
Examination					
Clinical myotonia	9 (100)	14 (100)	23 (100)	18 (100)	-
Eyelid myotonia	6 (75)	10 (77)	21 (88)	17 (100)	0.190
Handgrip myotonia	7 (88)	9 (69)	18 (82)	16 (94)	0.331
Paramyotonia	4 (67)	6 (86)	17 (94)	9 (75)	0.322

Table 5.8: Characteristics of patients with common SCN4A variations (cont.)

	G1306A/V	R1448C/H	T1313M	V1589M	P value
Limb weakness	0 (0)	0 (0)	2 (20)	1 (6)	0.435
Limb hypertrophy	**3 (43)**	**0 (0)**	**12 (75)**	**8 (89)**	**0.001***
Investigations					
CK, IU/L **(SD)**	**213 (95)**	**134 (50)**	**374 (534)**	**771 (1044)**	**0.033**
Interictal potassium, mmol/L (SD)	4.2 (0.3)	3.8 (0.5)	4.3 (0.4)	4.2 (0.3)	0.315
Neurophysiology pattern **Type I** **Type III**	 **2 (29)** **5 (71)**	 **5 (83)** **1 (17)**	 **12 (86)** **2 (14)**	 **4 (33)** **8 (67)**	**0.009**
EMG myotonia	7 (100)	9 (100)	17 (100)	17 (100)	-
Change in myotonia in cold - **Increased** - **Reduced**	 **1 (33)** **0 (0)**	 **5 (100)** **0 (0)**	 **2 (29)** **5 (71)**	 **-**	**0.002***
Positive short exercise test at room temperature	**2 (29)**	**0 (0)**	**8 (57)**	**1 (9)**	**0.019**
Positive short exercise test with cooling	**2 (50)**	**5 (100)**	**11 (92)**	**3 (43)**	**0.035**
Abnormal long exercise test	**1 (17)**	**1 (17)**	**10 (71)**	**1 (13)**	**0.011**
Abnormal MRI	3 (100)	-	2 (100)	2 (100)	-

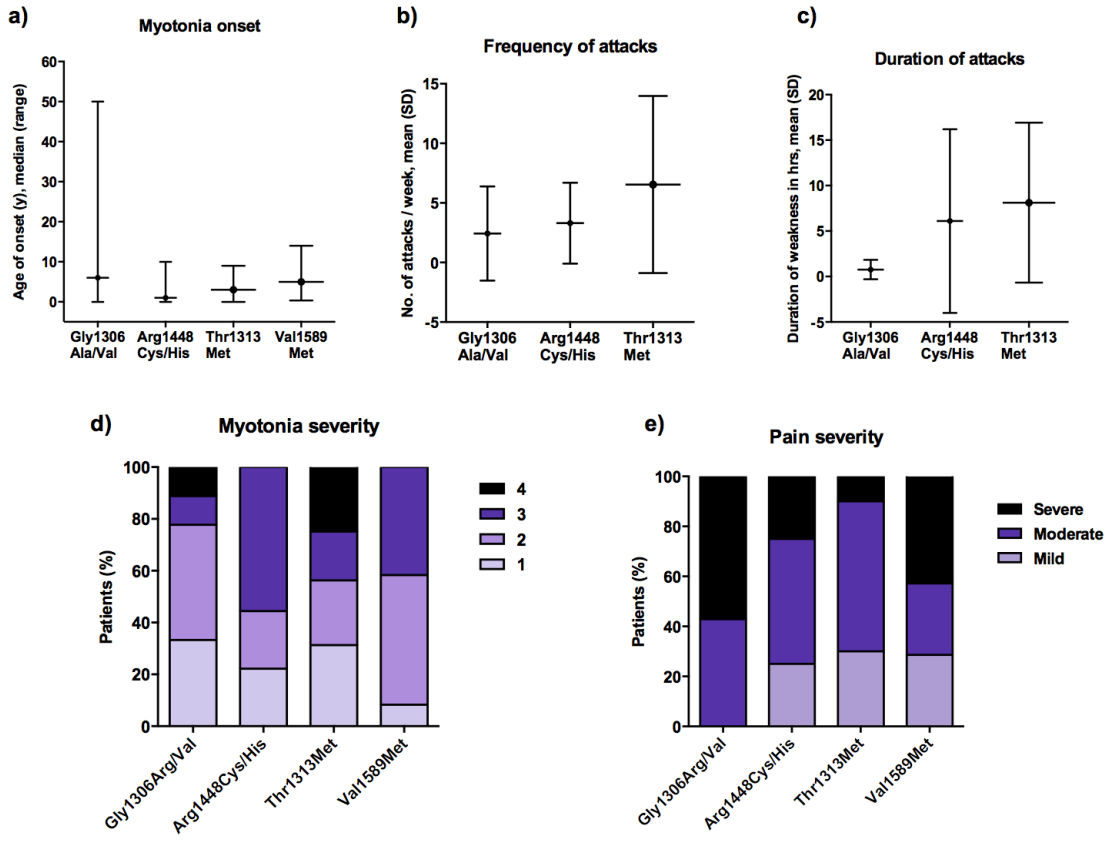
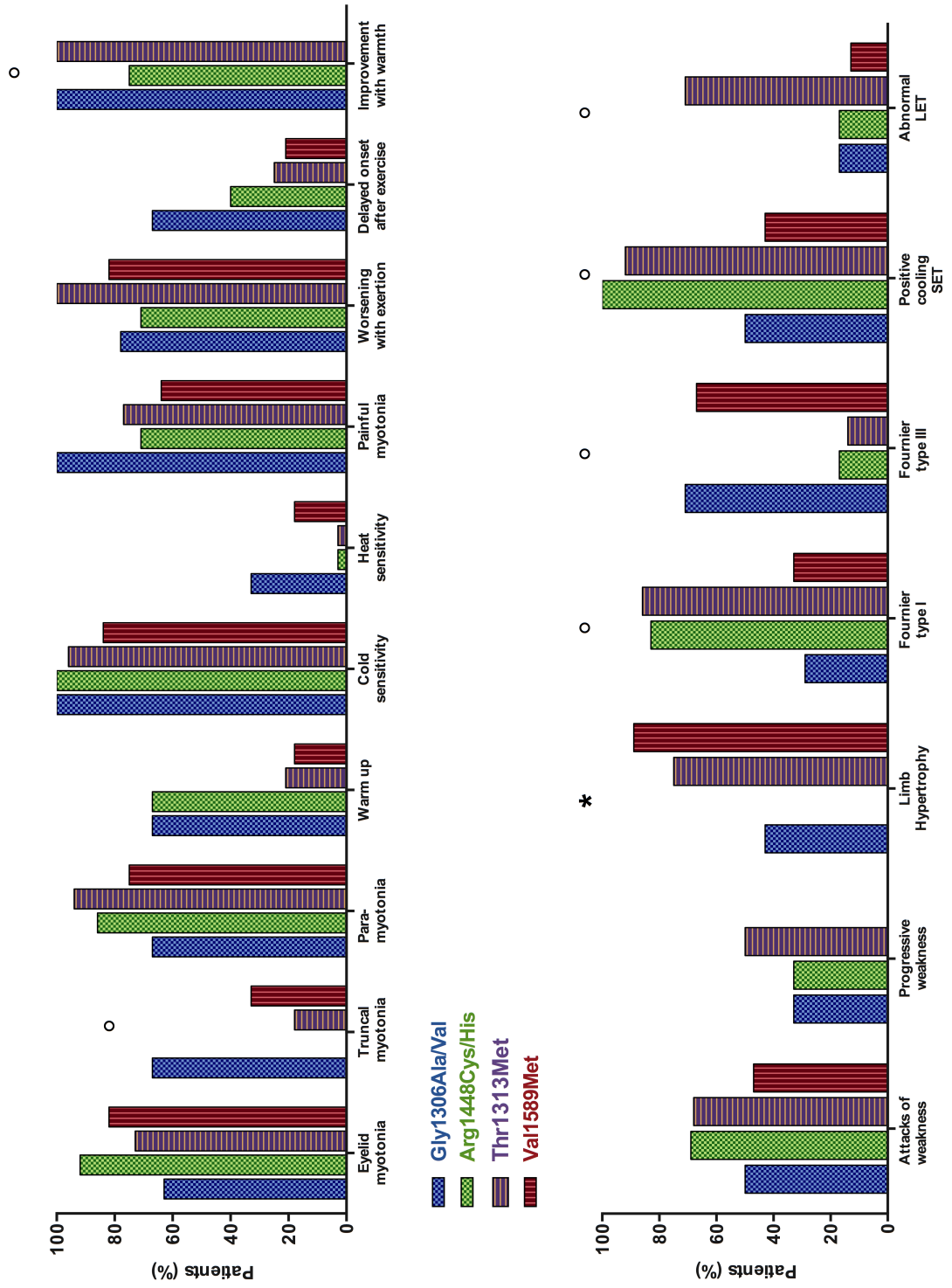


Figure 5.11: Graphs comparing attacks of weakness and myotonia in the common SCN4A genotypes. a) Age of onset of myotonia b) Frequency of attacks of weakness c) Duration of attacks of weakness d) Severity of myotonia (score 1-4) e) Severity of pain (score 1-3)

Figure 5.12: Graph of key phenotypic comparisons between common SCN4A genotypes (* indicates significant difference after Bonferroni correction, ° indicates p<0.05 before correction)



all groups but also frequently seen in the hands. Truncal myotonia was more common in Gly1306 variation carriers compared with the other variations (p=0.023)(Figure 5.12).

Weakness was reported by patients with all types of variation with a frequency from 47% to 69% and therefore did not help differentiate between the variations (Figure 5.12). Attacks of weakness tended to be more frequent and longer in Thr1313Met patients compared with Arg1448 and Gly1306 variations (Figure 5.11b & c). Strenuous exercise was a common precipitant in all groups but immobility triggering attacks was most commonly reported in Arg1448 patients (p=0.047) (Table 5.8).

On examination, limb hypertrophy was more common in Val1589Met and Thr1313Met patients (p=0.001), which was significant after Bonferroni correction (Figure 5.12). There were differences in neurophysiology patterns between the mutations. Arg1448 and Thr1313Met variations had primarily Fournier type I patterns (83% and 86% respectively) and Gly1306 and Val1589Met variations had primarily type III patterns (71% and 67% respectively, p=0.009) (Figure 5.12). All patients with Arg1448 variations had increased electrical myotonia in the cold whereas 71% of Thr1313Met had reduced myotonia in the cold (p=0.002). Almost all patients with Arg1448 and Thr1313Met had positive short exercise tests after cooling compared to only half of patients with Gly1306 and Val1589Met (Figure 5.12). The long exercise test was commonly positive for Thr1313Met patients (71%) compared with 13-17% of other variations (p=0.011) (Figure 5.12). All MRI scans were abnormal (Table 5.8).

5.2.1.5 Comparing HyperPP and PMC/SCM patients

Finally to confirm the finding in other studies that there is a significant difference between HyperPP patients and those with SCM or PMC, I directly compared these two groups. PMC and SCM groups were combined for this analysis as there was no significant difference between them. This revealed marked differences between the two groups that were significant after correction for multiple comparisons (Table 5.9). All patients with PMC/SCM had myotonia compared with 72% of HyperPP ($p<0.0001$) (Figure 5.13a). Age of onset was younger in PMC/SCM patients (5 years versus 8 years) (Figure 5.13b). Severity of myotonia, presence of paramyotonia and warm-up were all the same in both groups (Figure 5.13c & d). Patients with PMC/SCM had myotonia sensitive to exertion (88% versus 56%, p=0.004) and were more likely to have painful myotonia than those with HyperPP (78% versus 30%, $p<0.0001$) (Figure 5.13d).

Table 5.9: Characteristics of patients with PMC/SCM and HyperPP. Percentages are in brackets unless otherwise indicated. (* Indicates significant p-values after Bonferroni correction. Continuous data have standard deviation in brackets as indicated)

	n	PMC/SCM	HyperPP	p-value
Number of cases	137	98	39	
Characteristics of myotonia				
Symptomatic myotonia	**130**	**98 (100)**	**23 (72)**	**<0.0001***
Age of onset of myotonia (median & range)	83	5 (0-58)	8 (3-18)	0.087
Severity of myotonia (1-4) (SD)	76	2.4 (1.0)	2.4 (1.1)	0.796
Distribution of myotonia				
- eyes	93	63 (82)	11 (69)	0.238
- hands	98	68 (83)	11 (69)	0.189
- limbs	78			0.024
- arms & legs		32 (47)	6 (60)	
- legs only		26 (38)	1 (10)	
- arms only		7 (10)	0 (0)	
- none		3 (5)	3 (30)	
Precipitants of myotonia				
- cold	111	88 (93)	15 (94)	0.873
- exertion	81	57 (88)	9 (56)	0.004
- rest after exercise	71	21 (38)	3 (20)	0.189
Painful myotonia	**70**	**39 (78)**	**6 (30)**	**<0.0001***
Characteristics of attacks of weakness				
Attacks of weakness	**116**	**49 (66)**	**39 (100)**	**<0.0001***
Frequency of attacks per week (SD)	**41**	**3.9 (4.8)**	**1.0 (2.1)**	**0.001***
Duration of attacks of weakness in hours (SD)	**49**	**8.3 (13)**	**63.4 (89)**	**<0.0001***
Severity of attacks of weakness (1-4) (SD)	60	1.8 (1.0)	2.4 (0.9)	0.015
Time of attacks	34			0.030
- on waking		2 (9)	3 (25)	
- morning		3 (13)	5 (42)	
- night		4 (18)	3 (25)	
- anytime		13 (59)	1 (8)	
Distribution of weakness in limbs	61			0.030
- arms & legs		12 (50)	30 (81)	
- legs only		7 (29)	7 (19)	
- arms only		4 (17)	0 (0)	
Precipitants				
- strenuous exercise	64	23 (77)	16 (47)	0.015
- rest after exercise	56	8 (37)	22 (65)	0.038
- cold	**56**	**22 (92)**	**14 (44)**	**<0.0001***
- potassium	49	0 (0)	3 (9)	0.193
Progressive fixed weakness	62	11 (31)	14 (54)	0.065
Improvement with mexiletine	44	34 (92)	6 (86)	0.639
Improvement with acetazolamide	28	3 (43)	16 (77)	0.145
Examination				
Clinical myotonia	**109**	**82 (100)**	**21 (78)**	**<0.0001***
Eyelid myotonia	97	68 (90)	18 (86)	0.631
Handgrip myotonia	**95**	**63 (85)**	**6 (29)**	**<0.0001***
Fixed Proximal Limb Weakness	**77**	**6 (13)**	**18 (56)**	**<0.0001***

Table 5.9: Characteristics of patients with PMC/SCM and HyperPP (cont.)

	n	PMC/SCM	HyperPP	p-value
Limb appearance **- hypertrophy** **- atrophy**	**67**	 **27 (52)** **1 (2)**	 **4 (27)** **7 (47)**	**<0.0001***
Investigations				
CK IU/L (SD)	64	370 (572)	735 (998)	0.026
Interictal potassium mmol/L (SD)	65	4.1 (0.4)	4.0 (0.5)	0.068
EMG myotonia	**86**	**67 (100)**	**16 (84)**	**0.001***
Myopathic EMG	46	3 (10)	7 (41)	0.014
Short exercise test positive	61	13 (27)	1 (8)	0.061
Short exercise test positive following cooling	47	25 (68)	3 (30)	0.065
Long exercise test abnormal	59	15 (33)	11 (79)	0.003
MRI abnormal	13	10 (100)	3 (100)	-

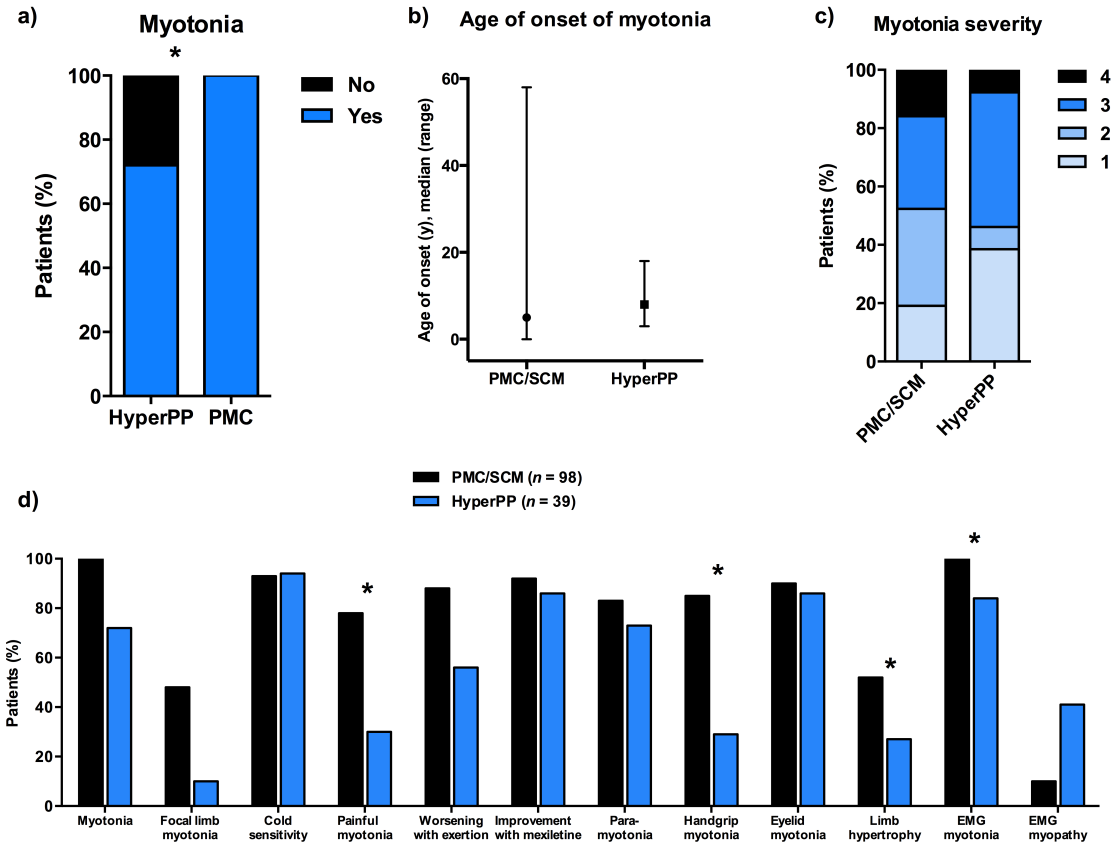


Figure 5.13: Graph of comparisons of characteristics of myotonia in HyperPP versus PMC and SCM a) Presence of myotonia b) Age of onset of myotonia c) Severity of myotonia (score 1-4) d) Key phenotypic comparisons of characteristics of myotonia (* indicates significant difference after Bonferroni correction)

As expected, attacks of weakness were present in all HyperPP patients compared with only 62% PMC/SCM patients ($p<0.0001$)(Figure 5.14e). The nature of attacks between the two groups was markedly different, with PMC/SCM patients having more frequent attacks

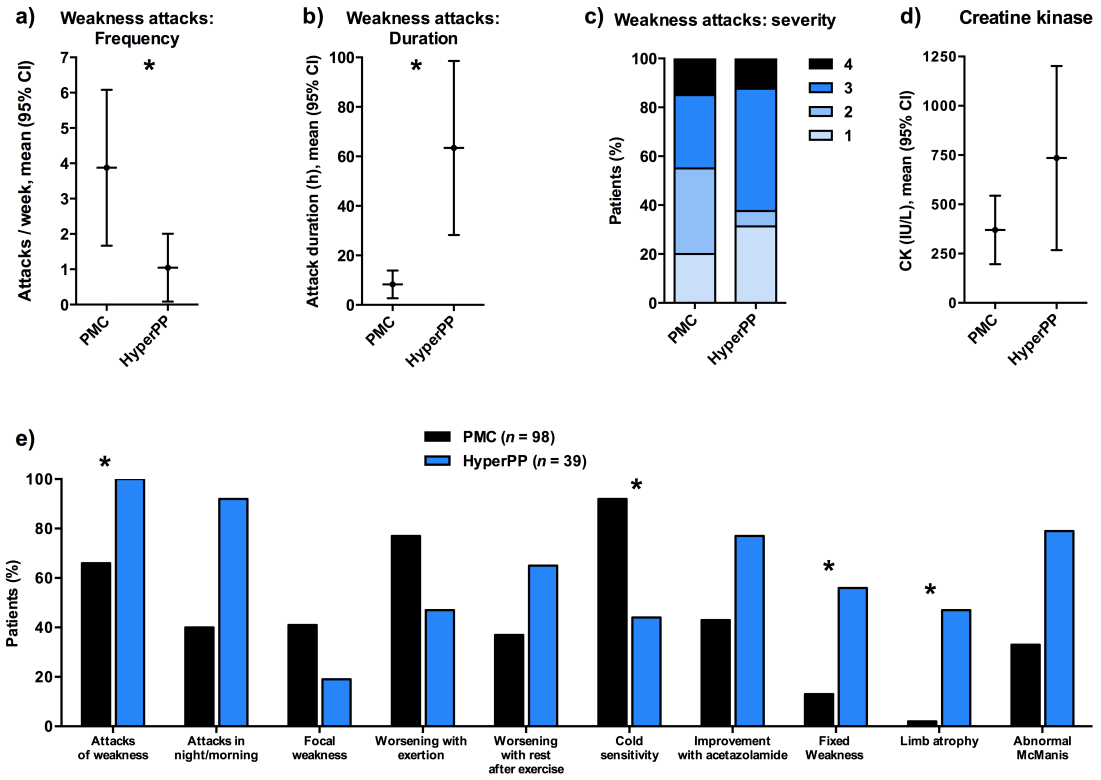


Figure 5.14: Graph of comparisons of characteristics of attacks of weakness in HyperPP versus PMC/SCM a) Frequency of attacks of weakness b) Duration of attacks of weakness c) Severity of attacks of weakness (score 1-4) d) Creatine kinase levels e) Key phenotypic comparisons of characteristics of attacks of weakness (* indicates significant difference after Bonferroni correction)

of weakness (3.9 per week versus 1.0 per week, p=0.001) and much shorter attacks (8.3 hours versus 63.4 hours, p<0.0001) (Figure 5.14a & b). The severity of attacks in HyperPP was also worse (2.4/3 versus 1.8/3, p=0.015) although not significant after correction (Figure 5.14c). Attacks of weakness in HyperPP tended to occur primarily at night or early morning whereas with PMC/SCM they could occur at anytime of day (p=0.030). Patients with PMC/SCM also had focal weakness more commonly than complete weakness of arms and legs. Interestingly, patients with PMC/SCM were more sensitive to strenuous exercise (77% versus 47%, p=0.015) whereas patients with HyperPP were more sensitive to rest after exercise (65% versus 37%, p=0.038) (Figure 5.14e). Although both groups had a high frequency of sensitivity of myotonia to cold (93% and 94%), patients with PMC/SCM had more cold-sensitive attacks of weakness compared with HyperPP (92% versus 44%, p<0.0001) (Figure 5.14e).

On examination hand grip myotonia was much more frequent in PMC/SCM than

HyperPP (85% versus 29%, p<0.0001) (Figure 5.13d). Fixed proximal limb weakness was more common in HyperPP patients compared with PMC/SCM (56% versus 13%, p<0.0001)(Figure 5.14e). Hypertrophy was more common in PMC/SCM whereas atrophy was more common in HyperPP (Figure 5.14e, Figure 5.13d). On neurophysiology, electrical myotonia was present in all patients with PMC/SCM but in only 84% of HyperPP (p=0.001). 41% of patients with HyperPP had a myopathic EMG compared with only 10% of PMC/SCM (Figure 5.13d). The long exercise test was more commonly abnormal in HyperPP (79%) than in PMC/SCM (33%) but the percentage decrement was not significantly different between the two groups (Figure 5.14e). All MRIs were abnormal.

5.2.2 Distinguishing Clinical Phenotypes in Periodic Paralysis

5.2.2.1 Demographics

119 patients were identified with PP of which 50 had HypoPP, 36 HyperPP and 33 ATS (Figure 5.7, Table 5.10). There was a gender bias with approximately two thirds males to females (81:38). The median age of patients at the time of the study was 43 years with a range of 8-64 years.

As with other studies, there were significant differences identified between HypoPP, HyperPP and ATS. Age of onset in HyperPP was noticeably lower than HypoPP (5 years versus 11.5 years) (p=0.005) but ATS was more intermediate at 8 years (Figure 5.15b). In all groups there was a male predominance and this was greater in HypoPP with a 3:1 ratio of males to females (Figure 5.15a). Family history was positive in 90-94% of patients suggesting an incidence of less than 10% of sporadic cases amongst genetically confirmed individuals.

5.2.2.2 Comparing HypoPP, HyperPP and ATS

There was a difference in the number of patients with HypoPP (98%) compared to HyperPP (76%) that had documented attacks of complete paralysis from the neck down (p=0.004) (Figure 5.16). The frequency of all types of attacks were similar between the conditions. It was greatest for HypoPP (9.9 per month), intermediate for HyperPP (8.8 per month) and least frequent for ATS (6.9 per month) (Figure 5.15d). There was a significant difference between maximum attack duration in HypoPP and HyperPP (109 hours versus 88 hours, p<0.001) and an even greater difference between HyperPP and ATS (163 hours) (Figure 5.15e). During attacks there was a significant difference in ictal serum potassium, with HyperPP associated with abnormally high potassium (5.16mmol/L), HypoPP associated

Table 5.10: Characteristics of patients with HypoPP, HyperPP and ATS. Percentages are in brackets unless otherwise indicated. (* Indicates significant p values after Bonferroni correction. P-values <0.05 in bold. Continuous data have standard deviation in brackets as indicated)

	HypoPP	**HyperPP**	**ATS**	**p- value**
Cases	50	36	33	
Gender: Male Female	38 (76) 12 (24)	23 (64) 11 (36)	20 (61) 13 (39)	
Age of onset (median,yrs)	11.5	5	8	**0.005**
Attacks of complete paralysis	48 (98)	25 (76)	-	**0.004**
Time of attacks (main attacks at night)	23 (72)	6 (55)	9 (56)	0.425
Generalised episodes of weakness	46 (94)	28 (82)	26 (93)	0.191
Ictal serum potassium, mmol/L **(SD)**	**2.39 (0.79)**	**5.16 (1.20)**	**3.63 (0.95)**	**<0.001***
Precipitants				
Strenuous exercise	19 (54)	14 (45)	21 (75)	0.061
Rest after exercise	16 (46)	21 (68)	15 (54)	0.194
Immobility	6 (18)	15 (52)	12 (43)	**0.013**
Cold	10 (29)	12 (41)	9 (33)	0.558
Carbohydrate	**29 (81)**	**5 (17)**	**12 (43)**	**<0.001***
High potassium	0 (0)	2 (7)	2 (7)	0.279
Relievers				
Mild exercise	**1 (6)**	**7 (70)**	**2 (40)**	**0.002***
Potassium supplement	**24 (89)**	**0 (0)**	**2 (67)**	**<0.001***
Glucose	0 (0)	3 (30)	0 (0)	**0.049**
Hospitalisation for attacks	18 (82)	4 (33)	-	**0.037**
Pain in attacks	2 (9)	5 (28)	2 (15)	0.289
Co-existing conditions				
Cardiac	**6 (18)**	**3 (14)**	**19 (66)**	**<0.001***
Respiratory	2 (6)	3 (14)	3 (19)	0.384
Diabetes	2 (6)	2 (9)	2 (13)	0.743
Musculoskeletal	4 (12)	9 (41)	1 (7)	**0.011**
Depression	**0 (0)**	**3 (16)**	**8 (45)**	**0.002***
Treatment				
Acetazolamide: - improved - worsened	 21 (72) 0 (0)	 16 (84) 0 (0)	 20 (91) 0 (0)	 0.227
Dichlorphenamide: - improved - worsened	 8 (80) 2 (20)	 4 (67) 1 (17)	 4 (67) 0 (0)	 0.355
SandoK: - improved	 19 (95)	 -	 3 (100)	 0.901
Potassium-sparing agent: - improved - worsened	 5 (83) 1 (17)	 - -	 3 (100) 0 (0)	 0.690
Thiazide diuretic: - improved	 -	 6 (86)	 1 (100)	 1.00

Table 5.10: Characteristics of patients with HypoPP, HyperPP and ATS (cont.)

	HypoPP	**HyperPP**	**ATS**	**p-value**
Salbutamol - improved	-	4 (80)	-	-
Mexiletine - improved	-	4 (80)	-	-
Examination				
Fixed Proximal weakness				
- Upper limb	12 (28)	10 (36)	13 (46)	0.280
- Lower limb	25 (58)	17 (59)	19 (68)	0.680
Limb appearance				
- Atrophy	5 (29)	7 (47)	3 (14)	**0.025**
- Hypertrophy	0 (0)	4 (27)	6 (27)	
Clinical myotonia	**0 (0)**	**17 (71)**	**0 (0)**	**<0.001***
Investigations				
EMG				
- myopathic	9 (43)	6 (40)	7 (32)	0.743
- **myotonia**	**0 (0)**	**13 (81)**	**0 (0)**	**<0.001***
Short exercise test				
- abnormal at room temperature	0 (0)	0(0)	0(0)	1.00
- abnormal with cooling	0 (0)	3 (33)	0 (0)	0.068
Long exercise test - abnormal	10 (67)	10 (77)	18 (90)	0.236
Biopsy				
- abnormal	9 (100)	4 (80)	6 (75)	0.291
- vacuoles present	8 (89)	3 (60)	1 (17)	**0.020**
- tubular aggregates present	1 (13)	0 (0)	2 (33)	0.302
- “myopathic”	6 (67)	3 (60)	1 (20)	0.228
MRI legs abnormal	12 (86)	3 (100)	3 (100)	0.621

Figure 5.15: Graphs of key aspects of attacks of weakness in patients with HypoPP, HyperPP and ATS. a) Male/female ratios b) Age of onset of attacks of weakness c) Functional status of patients (mRS) d) Frequency of attacks per month e) Maximum attack duration in hours f) Severity of attacks (1-4) (* indicates significant difference)

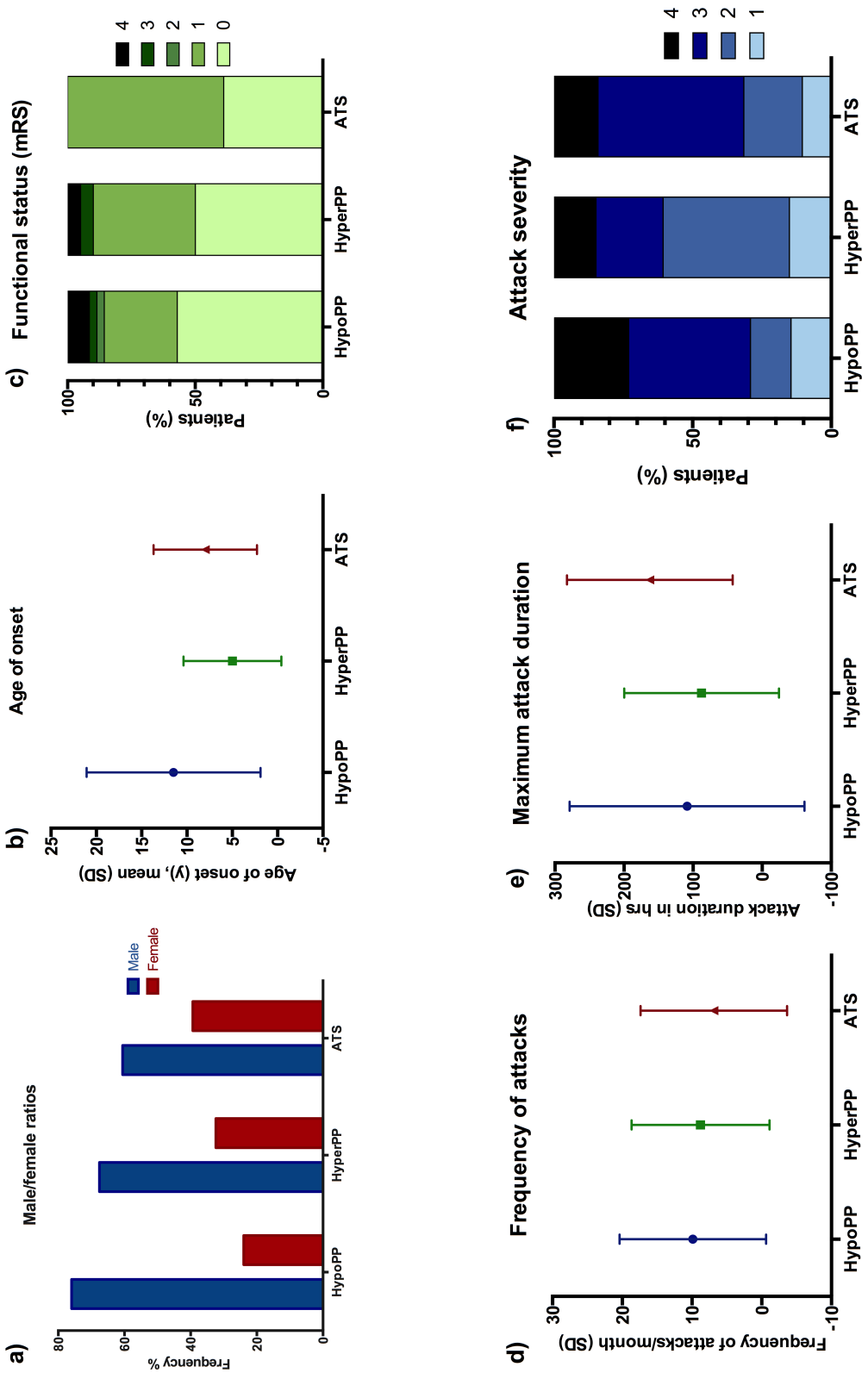
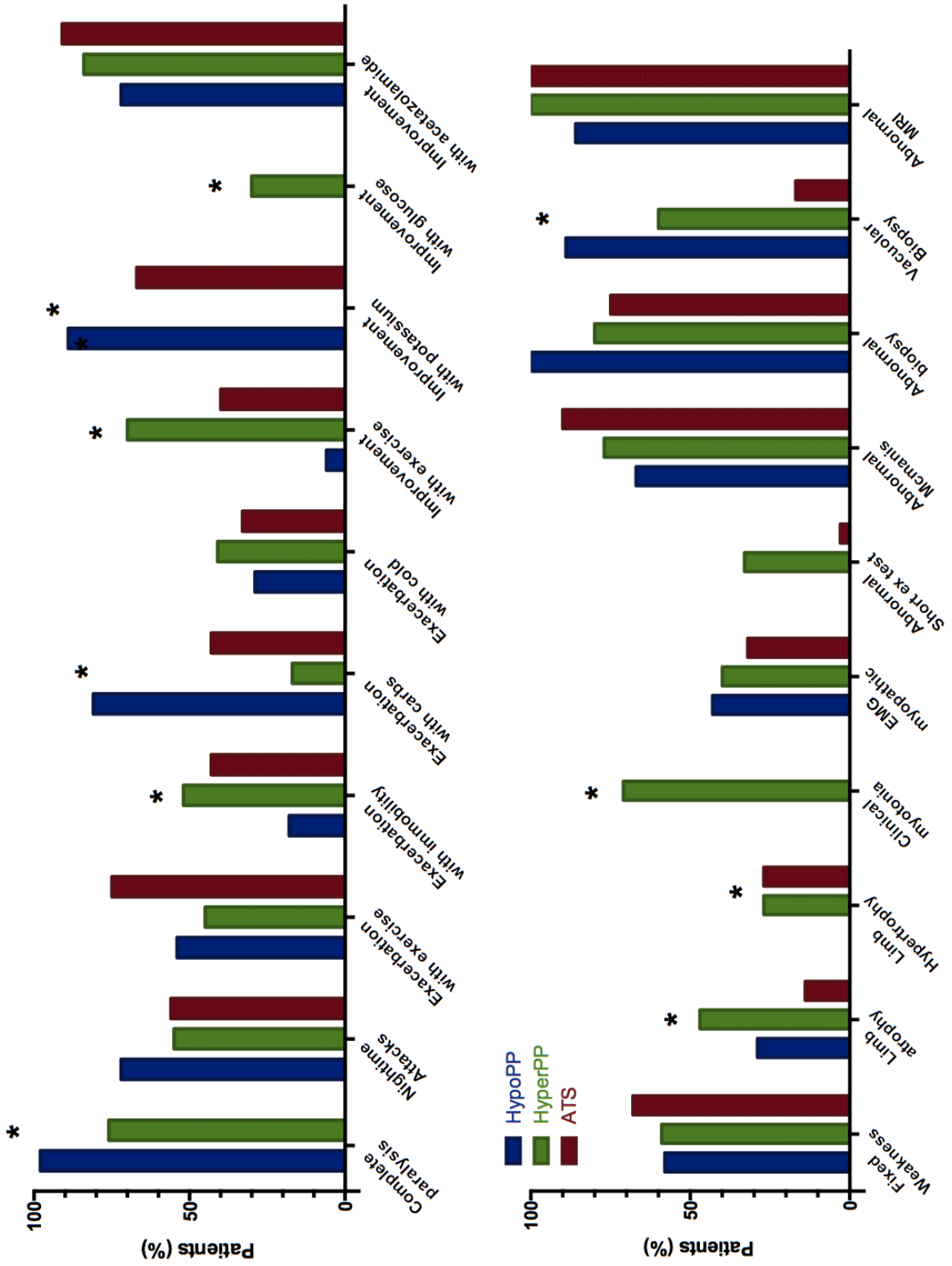


Figure 5.16: Graph of key phenotypic comparisons between types of periodic paralysis (* indicates significant difference after Bonferroni correction)



with abnormally low potassium (2.39mmol/L) and ATS associated with normal potassium (3.63mmol/L) ($p<0.001$). There was no difference in the number of patients with generalised versus focal attacks of weakness between the diseases. Severity of attacks were also similar (Figure 5.15e)f, Table 5.10).

A number of attack precipitants were investigated. The most significant difference was seen with carbohydrate loading. Carbohydrate was more likely to precipitate an attack in HypoPP than in HyperPP or ATS (81% versus 17% versus 43%, $p<0.001$). Strenuous exercise was more likely to precipitate attacks in ATS than in HypoPP or HyperPP but this was not significant (75% versus 54% and 45%, p=0.061) and immobility was more likely to trigger an attack in HyperPP than HypoPP (52% versus 18%, p=0.013) (Figure 5.16, Table 5.10).

There were significant differences between groups in what factors relieved each disease. Mild exercise much more frequently improved attacks in HyperPP compared with HypoPP and ATS (70% versus 40% and 6%, p=0.002). Potassium was more likely to improve attacks in HypoPP compared to ATS and never improved attacks in HyperPP (89% versus 67% versus 0%, $p<0.001$). Glucose only improved attacks in HyperPP but the numbers were small (p=0.049) (Figure 5.16).

As would fit with the triad of disease in ATS, patients were significantly more likely to have cardiac disease ($p<0.001$). They were also more likely to have depression with 45% of patients affected ($p<0.002$). HyperPP patients, however, were more likely to have musculoskeletal disease (41%, $p<0.011$) (Table 5.10).

When response to treatment was analysed, a large proportion of patients reported improvement with acetazolamide, especially amongst those with ATS (91%) (Figure 5.16). Fewer patients had tried dichlorphenamide but the numbers that noted improvement were similar. Oral potassium improved 95-100% of patients with HypoPP or ATS. A potassium-sparing diuretic also improved symptoms in 5 out of the 6 patients with HypoPP who tried it (Table 5.10).

Examination findings only revealed a small number of differences. Functional status, measured by mRS score, was similar through the groups with most scoring close to 1 (no significant disability, able to carry out all usual activities despite some symptoms) (Figure 5.15c). Presence of fixed proximal weakness was similar in all groups although marginally more common in ATS. MRC scoring of power was also very similar. The biggest difference

was in clinical myotonia which was only seen in HyperPP ($p<0.001$). There was also a difference in numbers with atrophy and hypertrophy of muscles. Surprisingly, not only patients with HyperPP but also ATS had hypertrophy. As expected patients with HypoPP had atrophy but more patients with HyperPP had atrophy (29% versus 47%) and few patients with ATS had atrophy (14%) (p=0.025) (Figure 5.16, Table 5.10).

On neurophysiology there was, as expected, a marked difference in myotonia with it present in 81% of patients with HyperPP and no patients with HypoPP or ATS (Figure 5.16). There was no significant difference between the number of patients with myopathy on EMG and no difference in short or long exercise testing results although they were more likely to be positive in ATS (90%) than in HypoPP (67%) and HyperPP (77%) (Figure 5.16). There was no significant difference in the percentage decrement either although again in ATS there was more likely to be a greater decrement. Biopsy results were abnormal in the majority of patients (100% HypoPP, 80% HyperPP and 75% ATS). The only notable difference was in the presence of vacuoles which were commoner in HypoPP and ATS than HyperPP (89% & 60% versus 17%, p=0.02) (Figure 5.16). MRI scans were also abnormal in the majority of patients (86% HypoPP and 100% HyperPP and ATS) although numbers were very small (Table 5.10).

5.2.2.3 HypoPP1 versus HypoPP2

I compared the phenotypes of HypoPP1 and HypoPP2 but there were only 9 cases of HypoPP2 allowing for only a trend analysis rather than true statistical comparison (Table 5.11). Age of onset for HypoPP2 was younger (8.6 versus 12.4 years) (Figure 5.17a). Frequency of attacks was similar but maximum attack length was more variable and tended to be longer in HypoPP2 (Figure 5.17b & c). However the severity of actual attacks were similar (ictal potassium was similarly low in both groups) (Figure 5.17f). Common precipitants were similar between the groups but potassium was more likely to improve episodes of weakness in HypoPP1 than HypoPP2 (96% versus 60%, p=0.023) (Figure 5.18). Proximal weakness was equally common in the two diseases but age of onset of proximal weakness was earlier in HypoPP2 than HypoPP1 (26 years versus 41 years) (Figure 5.17d, Figure 5.18).

There was a notable difference in response to treatment. Acetazolamide was much less likely to improve attacks in HypoPP2 than HypoPP1 (33% versus 83%, p=0.016) (Figure 5.18). The response to dichlorphenamide was better although two patients did worsen with treatment (Table 5.11). There was also a difference in functional status with, on average,

Table 5.11: Characteristics of patients with HypoPP1 and HypoPP2. (Percentages are in brackets unless otherwise indicated. Continuous data have standard deviation in brackets as indicated)

	HypoPP1	HypoPP2	p-value
Number	41	9	
Characteristics of Attacks			
Age of onset in yrs (SD)	12.4 (9.8)	8.6 (8.8)	0.189
Frequency of major attacks per year (SD)	12.3 (25)	10.5 (20)	0.910
Maximum attack length in hours (SD)	28.7 (24)	153 (218)	0.455
Generalised (vs focal) weakness during attacks	37 (93)	9 (100)	0.396
Ictal potassium level, mmol/L (SD)	2.37 (0.67)	2.50 (1.25)	0.810
Precipitants:			
Cold	8 (29)	2 (29)	1.00
Immobility	6 (21)	0 (0)	0.211
Rest after exercise	14 (50)	2 (29)	0.309
Strenuous exercise	15 (54)	4 (57)	0.865
Relievers:			
Potassium	21 (96)	3 (60)	0.023
Hospitalisations with attacks	15 (68)	3 (75)	0.786
Pain during attacks	2 (12)	0 (0)	0.421
Severity of attacks (1-4) (SD)	2.7 (1.1)	3.2 (0.7)	0.229
Proximal weakness	15 (47)	5 (63)	0.429
Age of onset of proximal weakness in yrs (SD)	41 (17)	26 (12)	0.165
Treatment			
Improvement with acetazolamide	19 (83)	2 (33)	0.016
Dichlorphenamide - improved - worsened	 5 (100) 0 (0)	 3 (60) 2 (40)	 0.114
Examination			
Functional status mRS (0-6)	0.5 (1.0)	1.7 (1.7)	0.037
Muscle atrophy	2 (17)	3 (60)	0.074
Proximal weakness			
- upper limb	8 (24%)	4 (44%)	0.214
- lower limb	19 (56%)	6 (67%)	0.560
Investigations			
Bloods			
CK, IU/L (SD)	200 (105)	420 (293)	0.126
Interictal potassium, mmol/L (SD)	4.32 (0.41)	4.20 (0.37)	0.531
Neurophysiology			
Myopathic EMG	5 (31)	4 (80)	0.055
Long exercise test abnormal	7 (70)	3 (60)	0.699
Long exercise test percentage decrement (SD)	48 (20)	40 (16)	0.441
Abnormal biopsy	6 (100)	3 (100)	-
Abnormal MRI	8 (80)	4 (100)	0.334

patients with HypoPP2 having some disability and those with HypoPP1 being normal or having no significant disability (Figure 5.17e). This was not however reflected in MRC scores.

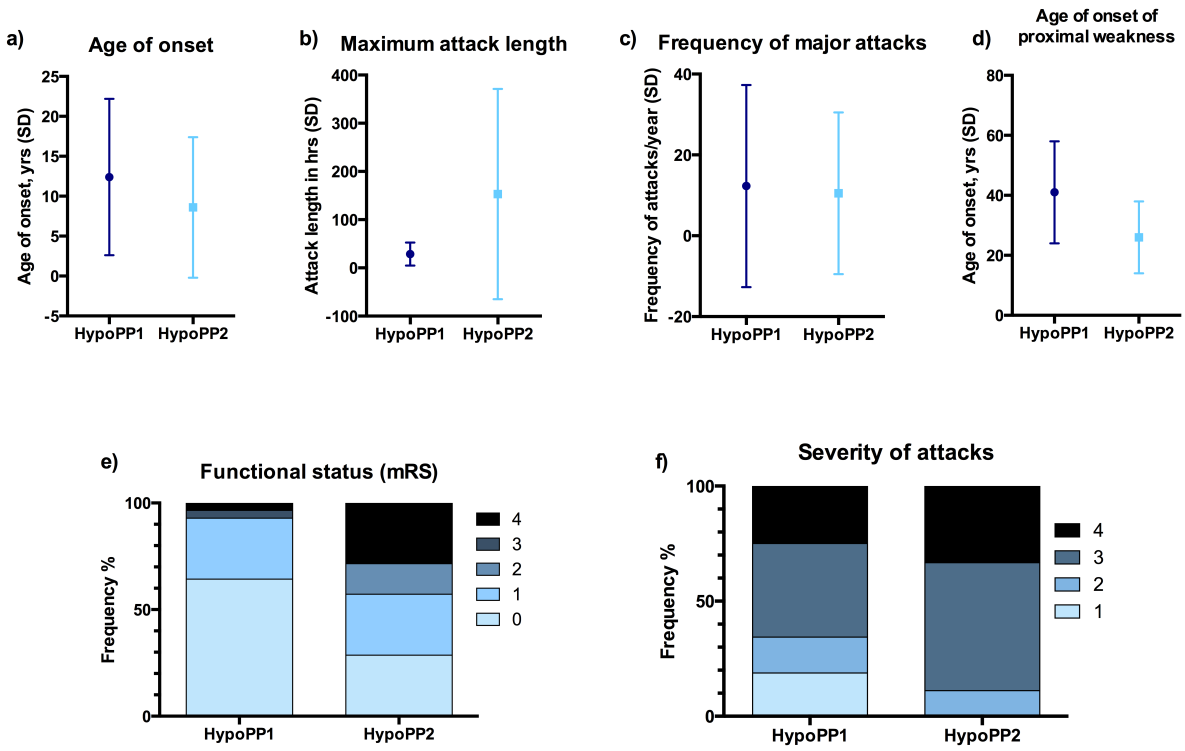


Figure 5.17: Graph of comparisons of attacks of weakness in HypoPP1 versus HypoPP2 a) Age of onset b) Maximum attack length in hours c) Frequency of major attacks per year d) Age of onset of proximal weakness e) Functional status, mRS (0-6) f) Severity of attacks (0-4)

HypoPP2 patients were more likely to have myopathic EMGs (80% versus 31%). Long exercise tests and the percentage decrement were very similar and all patients that had biopsies had abnormalities noted (Figure 5.18).

5.2.2.4 Genotype-Phenotype Correlations in HypoPP

The commonest variants identified in patients with HypoPP were Arg1239His and Arg528His. I therefore compared the data for these two variations to identify any distinguishing features. Overall, the variations had very similar phenotypes (Table 5.12). The key difference was in age of onset, with Arg1239His begin associated with a younger age of onset (11.4 years) when compared with Arg528His (13.4 years) patients (p=0.006) (Figure 5.19a). Although the frequency of severe attacks was greater in Arg1239His the duration and frequency of minor attacks were similar (Figure 5.19b & c). Similar to the variation in age of onset between the two groups, the age of onset of proximal weakness

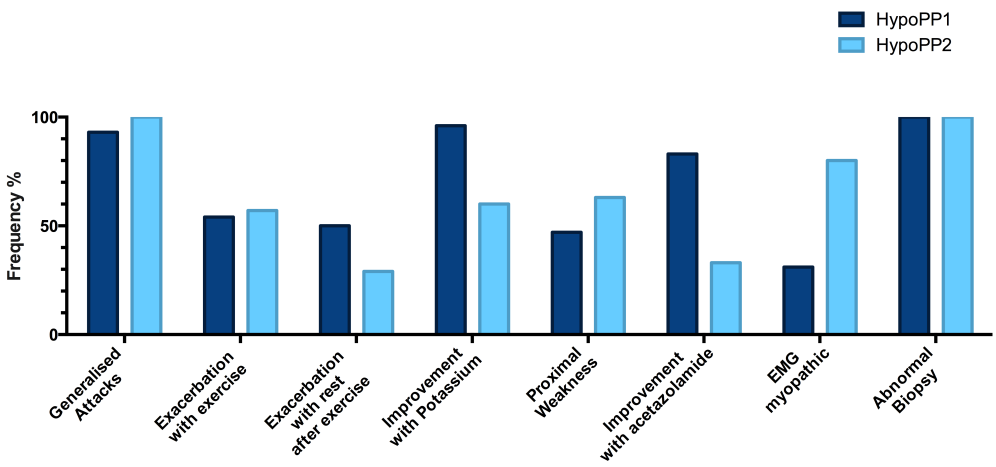


Figure 5.18: Graph of key phenotypic comparisons between HypoPP1 and HypoPP2

was earlier in Arg1239His (28.3 years versus 44.7 years, p=0.05) (Figure 5.19e). Severity of attacks was similar between the two groups (Figure 5.19f).

Sensitivity to different precipitants was similar in the two groups. The only differences were in sensitivity to carbohydrates and stress (Figure 5.20). All patients with Arg1239His were sensitive to carbohydrates, compared with 69% of patients with Arg528His (p=0.036). 64% of Arg1239His patients were sensitive to stress compared with 8% of Arg528His. Arg1239His patients were also more sensitive to rest after exercise (73% versus 33%) (Figure 5.20). Investigation results between the two groups were very similar, however those with Arg1239His were more likely to have a myopathy on EMG (80% versus 14%, p=0.023) (Figure 5.20).

5.2.2.5 Genotype-Phenotype Correlations in HyperPP

The main variants identified in patients with HyperPP were Thr704Met and Met1592Val (Table 5.13). The age of onset of attacks was younger for Thr704Met (4.2 years) than Met1592Val (9.5 years) (p=0.027) (Figure 5.21a). Although severity of attacks was similar, the duration of attacks in Met1592Val was notably longer (147.4 hours versus 38.7 hours, p=0.032) (Figure5.21c). The most marked difference in precipitants was in strenuous exercise, which was more commonly a precipitant in Met1592Val (86% versus 28%, p=0.009); and illness which was also more likely to precipitate attacks in this group of patients (Figure 5.21d). Progressive weakness was more common in Thr704Met patients although not significantly different (Figure 5.21d, Table 5.13).

Stiffness was more common in patients with Met1592Val mutations and tended to be

Table 5.12: Characteristics of patients with HypoPP variations Arg528His and Arg1239His (Percentages are in brackets unless otherwise indicated. Continuous data have standard deviation in brackets as indicated. p-values$<$0.05 in bold, * indicates significant difference after Bonferroni correction)

	Arg528His	Arg1239His	p-value
Number	19	17	
Age of onset, yrs (SD)	**13.4 (4.7)**	**11.4 (14.7)**	**0.006**
Frequency of severe attacks per year (SD)	2.6 (3.6)	22.4 (34.0)	0.203
Frequency of minor attacks per month (SD)	8.4 (9.7)	10.4 (11.5)	1.000
Maximum attack length in hours (SD)	28.8 (24.8)	32.5 (25.2)	0.718
Generalised (vs focal) weakness during attacks	19 (100%)	14 (93%)	0.253
Ictal potassium level, mmol/L (SD)	2.50 (0.63)	2.17 (0.86)	0.331
Precipitants:			
Cold	3 (25%)	5 (46%)	0.304
Immobility	2 (17%)	3 (27%)	0.538
Rest after exercise	4 (33%)	8 (73%)	0.059
Strenuous exercise	7 (58%)	6 (55%)	0.855
Carbohydrates	**9 (69%)**	**12 (100%)**	**0.036**
Stress	**1 (8%)**	**7 (64%)**	**0.005**
Relievers:			
Potassium	11 (100%)	9 (100%)	-
Hospitalisations with attacks	8 (89%)	7 (64%)	0.194
Pain during attacks	1 (14%)	1 (14%)	1.000
Severity of attacks (1-4) (SD)	2.8 (0.9)	2.7 (1.2)	0.693
Proximal weakness	4 (31%)	9 (60%)	0.122
Age of onset of proximal weakness, yrs (SD)	**44.7 (5.7)**	**28.3 (12.7)**	**0.050**
Treatment			
Improvement with acetazolamide	11 (92%)	7 (78%)	0.368
Improved with Daranide	1 (100%)	4 (100%)	-
Functional status (0-4)	0.4 (1.1)	0.7 (0.9)	0.135
Muscle atrophy	2 (40%)	0 (0%)	0.067
Proximal weakness			
- upper limb	3 (20%)	3 (20%)	1.000
- lower limb	7 (47%)	9 (60%)	0.464
Bloods			
CK, IU/L (SD)	208 (123)	234 (64)	0.573
Interictal potassium, mmol/L (SD)	4.30 (0.44)	4.25 (0.36)	0.640
Neurophysiology			
Myopathic EMG	**1 (14%)**	**4 (80%)**	**0.023**
Long exercise test abnormal	2 (67%)	4 (80%)	0.673
Long exercise test percentage decrement (SD)	56% (22%)	48% (21%)	0.655
Abnormal MRI	2 (67%)	3 (75%)	0.809

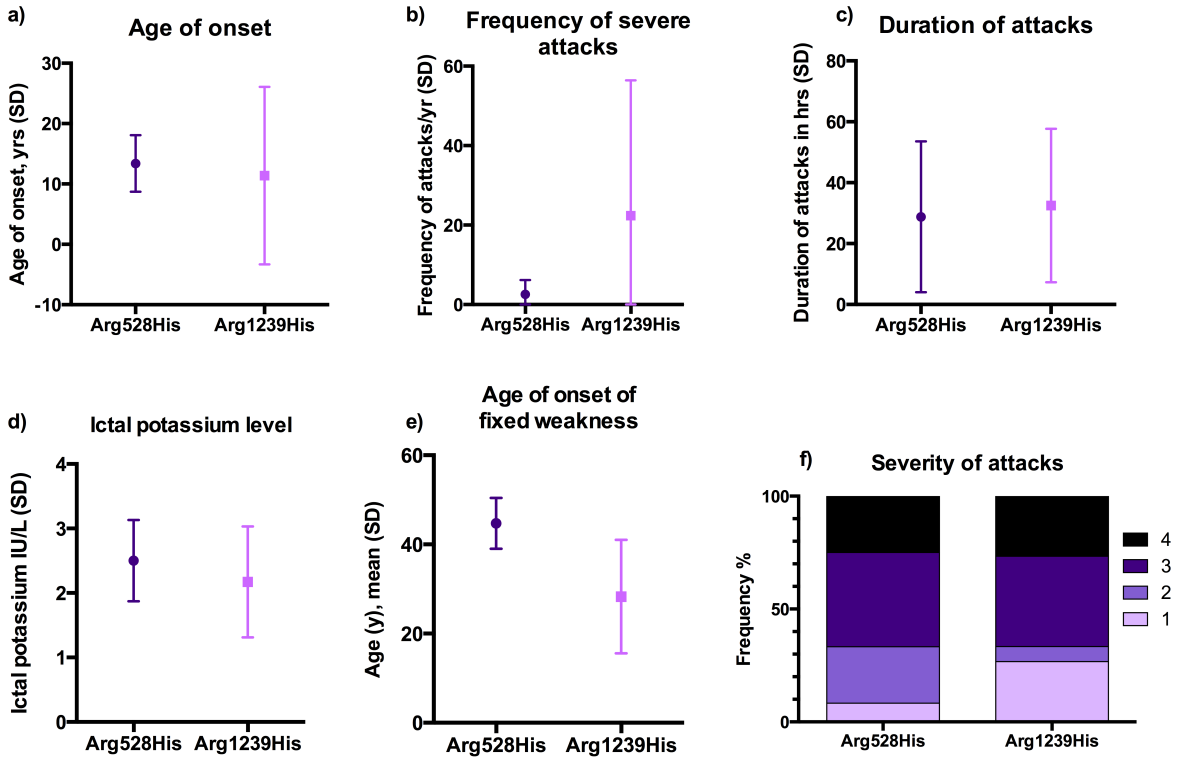


Figure 5.19: Graph of comparisons of attacks of weakness in patients with HypoPP with mutations Arg528His and Arg1239His a) Age of onset b) Frequency of severe attacks per year c) Maximum attack length in hours d) Ictal serum potassium level e) Age of onset of proximal weakness f) Severity of attacks (0-4)

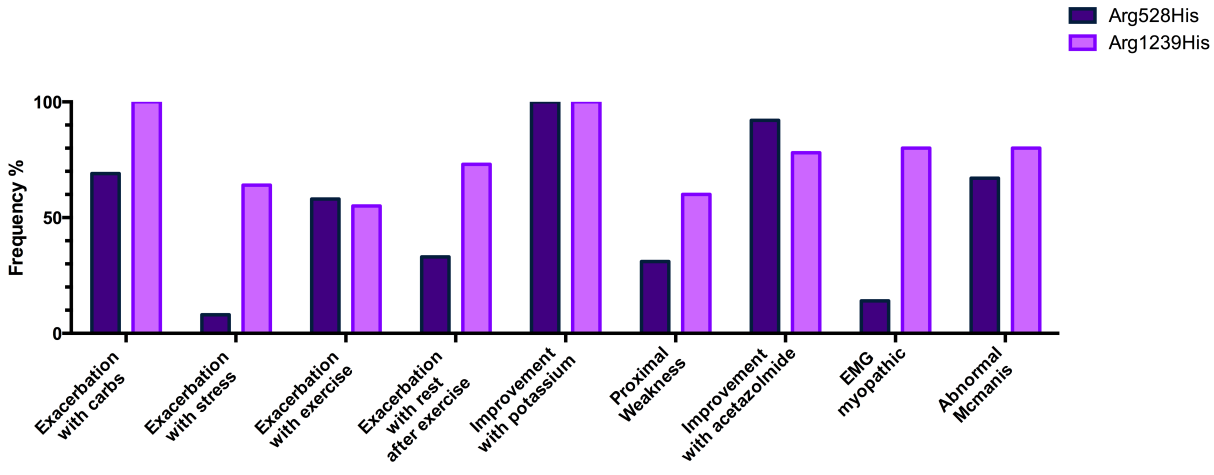


Figure 5.20: Graph of key phenotypic comparisons between Arg528His and Arg1239His

Table 5.13: Characteristics of patients with HyperPP variations Thr704Met and Met1592Val (Percentages are in brackets unless otherwise indicated. Continuous data have standard deviation in brackets as indicated. P-values$<$0.05 in bold, * indicates significant difference after Bonferroni correction)

	Thr704Met	**Met1592Val**	**p-value**
Number of cases	22	8	
Age of onset of attacks, yrs	4.2 (4.2)	9.5 (5.6)	**0.027**
Attacks of complete paralysis	16 (76%)	8 (100%)	0.129
Frequency of major attacks per year (SD)	48.5 (103)	27.2 (32)	0.864
Maximum duration of attacks in hours (SD)	38.7 (40)	147.4 (137)	**0.032**
Severity of attacks (1-4) (SD)	2.4 (0.8)	2.3 (1.1)	0.511
Focal attacks of weakness in limbs	4 (20%)	1 (12.5%)	0.707
Precipitants			
- strenuous exercise	5 (28%)	6 (86%)	**0.009**
- rest after exercise	14 (78%)	4 (57%)	0.302
- cold	4 (25%)	4 (57%)	0.136
- potassium	2 (12.5%)	0 (0%)	0.328
Progressive weakness	9 (64%)	2 (29%)	0.122
Hospitalisations for attacks	2 (40%)	1 (33%)	0.850
Myotonia	8 (50%)	7 (88%)	0.074
Severity of myotonia (1-4) (SD)	1.6 (0.9)	2.2 (1.1)	0.356
Distribution of myotonia			
- eyes	5 (100%)	1 (20%)	**0.010**
- hands	3 (60%)	4 (80%)	0.490
Precipitants of myotonia			
- cold	4 (100%)	5 (83%)	0.389
- hot	0 (0%)	1 (20%)	0.343
- exertion	2 (50%)	4 (67%)	0.598
- rest after exercise	0 (0%)	2 (33%)	0.197
Abnormal early milestones	1 (33%)	0 (0%)	0.273
Improvement with acetazolamide	10 (77%)	4 (100%)	0.290
Examination			
Clinical myotonia	9 (64%)	4 (67%)	0.919
Eyelid myotonia	8 (89%)	4 (80%)	0.649
Handgrip myotonia	1 (11%)	2 (40%)	0.207
Functional status (0-4) (SD)	1.0 (1.3)	0.3 (0.6)	0.386
Limb Weakness	13 (72%)	2 (33%)	0.088
Limb appearance:			
- **hypertrophy**	**4 (44%)**	**0 (0%)**	**0.002***
- **atrophy**	**5 (56%)**	**0 (0%)**	
Reduced or absent reflexes	5 (38.5%)	0 (0%)	0.264
Investigations			
CK, IU/L (SD)	597 (492)	1307 (1778)	0.739
Interictal potassium, mmol/L (SD)	4.01 (0.57)	3.95 (0.41)	0.826
EMG myotonia	4 (57%)	4 (100%)	0.125
Myopathic EMG	4 (57%)	1 (33%)	0.490
Short exercise test positive	0	0	-
Short exercise test positive with cooling	1 (33%)	2 (100%)	0.136
Long exercise test abnormal - % decrement (SD)	4 (80%) 61% (23%)	2 (50%) 48% (11%)	0.343 0.439

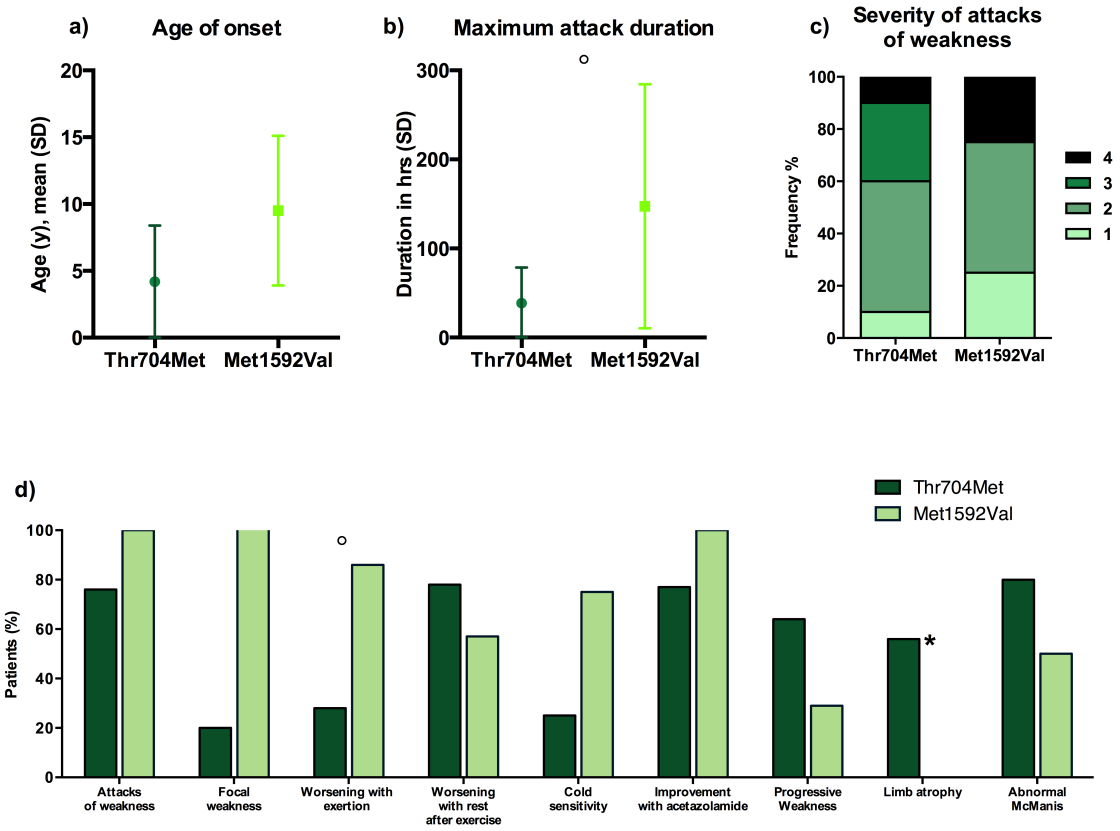


Figure 5.21: Graph of comparisons of attacks of weakness in patients with HyperPP with mutations in Thr704Met and Met1592Val a) Age of onset b) Maximum attack length in hours c) Severity of attacks (0-4) d) Key phenotypic comparisons of characteristics of attacks of weakness (* indicates significant difference after Bonferroni correction, ° indicates $p<0.05$)

more symptomatic in the hands than the eyes compared with Thr704Met (p=0.010) (Figure 5.22b). However, on examination, eyelid myotonia was equally present in both groups and an equal proportion of patients had myotonia on examination. Warm-up was noted in some patients with Met1592Val but not in those with Thr704Met where paramyotonia predominated. Precipitants of myotonia were similar between the two groups (Figure 5.22b). Limb weakness was more common in Thr704Met and as a result these patients tended to have a poorer functional status. They had a significant number of patients with hypertrophy and atrophy (p=0.002), compared with none of the patients with Met1592Val (Figure 5.22b, Table 5.13).

5.2.2.6 ATS Phenotypes

Data from 33 ATS patients was analysed. All patients had episodes of paralysis, 61% had cardiac symptoms related to ATS and all patients had skeletal abnormalities. Amongst this group, the commonest presenting system was paralysis (88%). Age of onset of paralysis

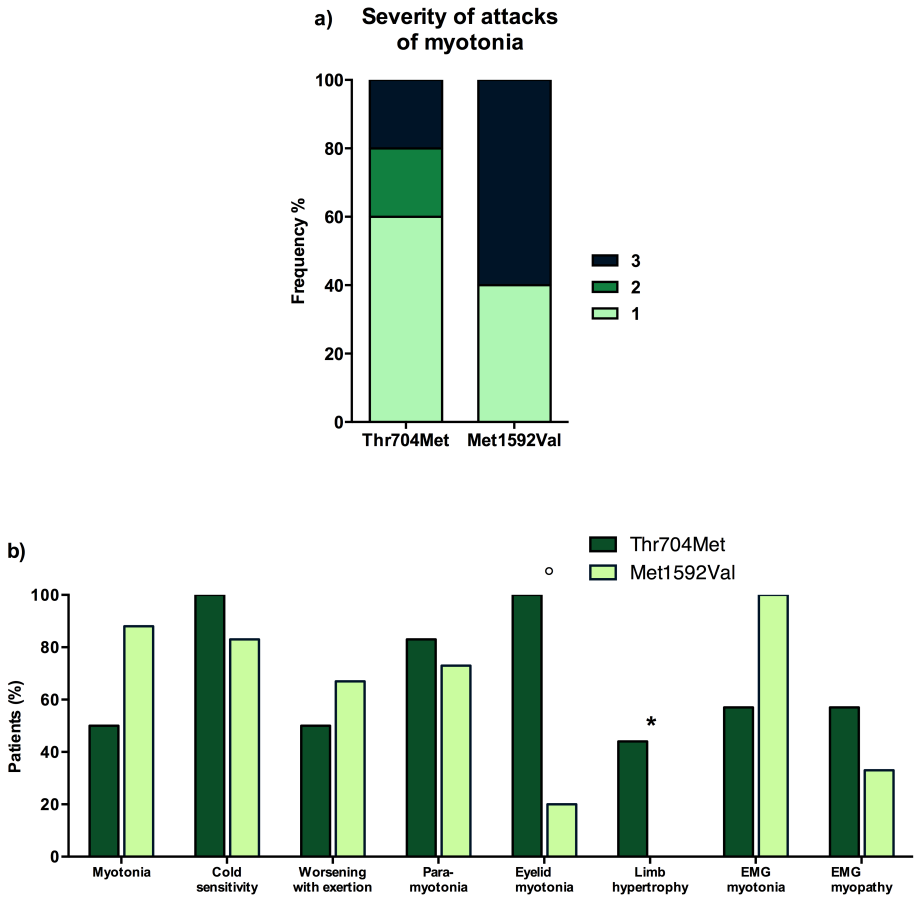


Figure 5.22: Graph of comparisons of characteristics of myotonia in patients with HyperPP with mutations in Thr704Met and Met1592Val a) Severity of myotonia (0-3) b) Key phenotypic comparisons of characteristics of myotonia (* indicates significant difference after Bonferroni correction, ° indicates $p<0.05$)

was younger than onset of cardiac symptoms (9.9 years versus 12.9 years) (Table 5.14). The phenotype of attacks of paralysis are described in Section 5.2.2.2.

The commonest cardiac symptoms were palpitations (94%), with almost half having syncopal events (43%) but only one reporting a cardiac arrest. Two patients had severe enough arrhythmias to require a pacemaker and implantable cardioverter defibrillator. 50% of patients were on at least one cardiac treatment, of which a quarter were on more than one treatment. The commonest treatment was beta blockers, which was prescribed in 83% of those on medication. Despite only 61% of patients having cardiac symptoms, 85% of patients had an abnormal electrocardiogram (ECG). The commonest abnormality was an abnormal U wave although a third of patients also had frequent ventricular ectopics. The average QTc interval was at the upper limit of normal for both men and women. A third of patients had abnormal 24 hour ECGs and 23% had abnormal echocardiograms (Table 5.14).

Table 5.14: Characteristics of patients with ATS. (Percentages are in brackets unless otherwise indicated. Continuous data have standard deviation in brackets as indicated)

	N (%)
Number	33
Episodes of paralysis	33 (100)
Cardiac symptoms	17 (61)
Dysmorphism	27 (100)
Presenting Symptom	
- Paralysis	29 (88)
- Cardiac	4 (12)
Age of onset of paralysis, yrs (SD)	9.9 (5.7)
Age of onset of cardiac symptoms, yrs (SD)	12.9 (8.9)
Palpitations	16 (94)
Syncope	6 (43)
Cardiac arrest	1 (7)
PPM/ICD	2 (14)
Severity of cardiac symptoms	
- mild	4
- mild-moderate	4
- moderate	2
- severe	2
Learning difficulties	4 (27)
Memory impairment	5 (36)
Depression	8 (45)
Dysmorphisms	
- low set ears	17 (71)
- hypertelorism	13 (57)
- micrognathia	20 (83)
- cleft palate	2 (8)
- high arched palate	3 (13)
- micromelia	15 (63)
- clinodactyly	18 (75)
- syndactyly	5 (22)
- short stature	9 (36)
Number on cardiac treatment	12 (50)
Abnormal ECG	23 (85)
ECG abnormalities - U waves - Premature ventricular contractions - Abnormal QTc - Borderline QTc - QTc interval, ms (SD) - male - female	 17 (81) 7 (32) 1 (5) 8 (38) 425 (20) 447 (6)
Abnormal 24hr ECG	7 (32)
Abnormal Echocardiogram	3 (23)

The incidence of cognitive and psychiatric problems in this group was high. 27% had learning difficulties of varying severity, 36% reported memory impairment and almost half had a history of depression. The pattern of skeletal abnormalities were variable but the majority had micrognathia, low set ears, clinodactyly, micromelia and hypertelorism. All patients who had abnormal features had a minimum of two features identified although on average four different features were noted (Table 5.14).

5.2.3 Anaesthetics & Pregnancy in Channelopathy Patients

109 patients with genetically confirmed channelopathies responded to the survey. 51% were female and 48% were male. 25 patients had MC, 37 had PMC, 13 HypoPP, 23 HyperPP and 12 ATS (Figure 5.23a). The median age of respondents was 47.5 years (Table 5.15).

5.2.3.1 Anaesthetics in Channelopathy Patients

Overall, 70 patients had been given local anaesthetic, either via direct injection or through a spinal or epidural injection. Of the 70 who had received local anaesthetic, 15 (21%) expressed having a problem with either or both direct injection or spinal/epidural injection. The most common problem being that the local anaesthetic was ineffective (10 patients). Four patients described the effect of the local anaesthetic as being prolonged. Two suggested that it worsened their symptoms, one of whom felt it triggered a prolonged attack of weakness and the other felt it worsened their myotonia (Figure 5.23b). Of those patients who had problems with local anaesthetic, two patients who had not had a problem with direct injection, experienced a problem with spinal/epidural injection, one reporting a prolonged effect of spinal anaesthetic after back surgery and the other reporting ineffective epidural during childbirth. One patient who had received both epidural and local anaesthetic injection reported a prolonged effect of the anaesthetic after both procedures (Table 5.15). When dividing patients up according to disease type there was a very similar percentage of those with NDM and those with PP who found local anaesthetic was ineffective. All patients who found the effect of anaesthetic was prolonged had NDM (Figure 5.23d, Table 5.16).

71 patients received general anaesthetic at some point in their life. Of these, 22 (31%) reported a problem with general anaesthetic, either with prolonged recovery (41%) or worsening symptoms (45%). None reported MH-like reactions. The remaining three patients had respiratory arrest, cardiac complications and a reaction to propofol (Figure 5.23c, Table 5.15). When dividing up the data by disease group, problems with general anaesthetic

Table 5.15: Table of problems encountered by patients following local and general anaesthetics

	Number (%)
Total respondents	109
Diagnosis	
- Myotonia congenita	25
- PMC/SCM	37
- HyperPP	13
- HypoPP	23
- ATS	12
Gender	
- Male	43 (48%)
- Female	57 (51%)
Median age (range)	47.5 (18-80)
Local Anaesthetic	
Exposed to local anaesthetic (via direct injection or spinal/epidural)	70
Problems with injection of local anaesthetic	13 (19%)
Local anaesthetic ineffective	9 (69%)
Local anaesthetic prolonged	3 (23%)
Worsened symptoms	2 (15%)
Number had spinal/epidural	16
Problems with spinal/epidural	3 (19%)
Spinal ineffective	1
Spinal prolonged	2
General Anaesthetic	
Exposed to General Anaesthetic	71
Problems with General Anaesthetic	22 (31)
GA ineffective	0
GA prolonged	9
GA worsened symptoms	10
Malignant Hyperthermia-like reaction	0

Table 5.16: Table of problems reported by muscle channelopathies patients following local and general anaesthetics in individual channelopathy disease groups

	MC	PMC/SCM	HyperPP	HypoPP	ATS
Local Anaesthetic					
Problems with LA	4 (19%)	6 (35%)	1 (14%)	2 (13%)	0 (0%)
Ineffective LA	3	4	1	1	
Prolonged LA	1	2	0	0	
General Anaesthetic					
Problems with GA	5 (31%)	6 (30%)	3 (38%)	5 (31%)	3 (27%)
Prolonged GA	3 (19%)	4 (21%)	0 (0%)	1 (7%)	1 (9%)
GA worsened symptoms	3 (19%)	1 (6%)	2 (29%)	3 (20%)	1 (9%)

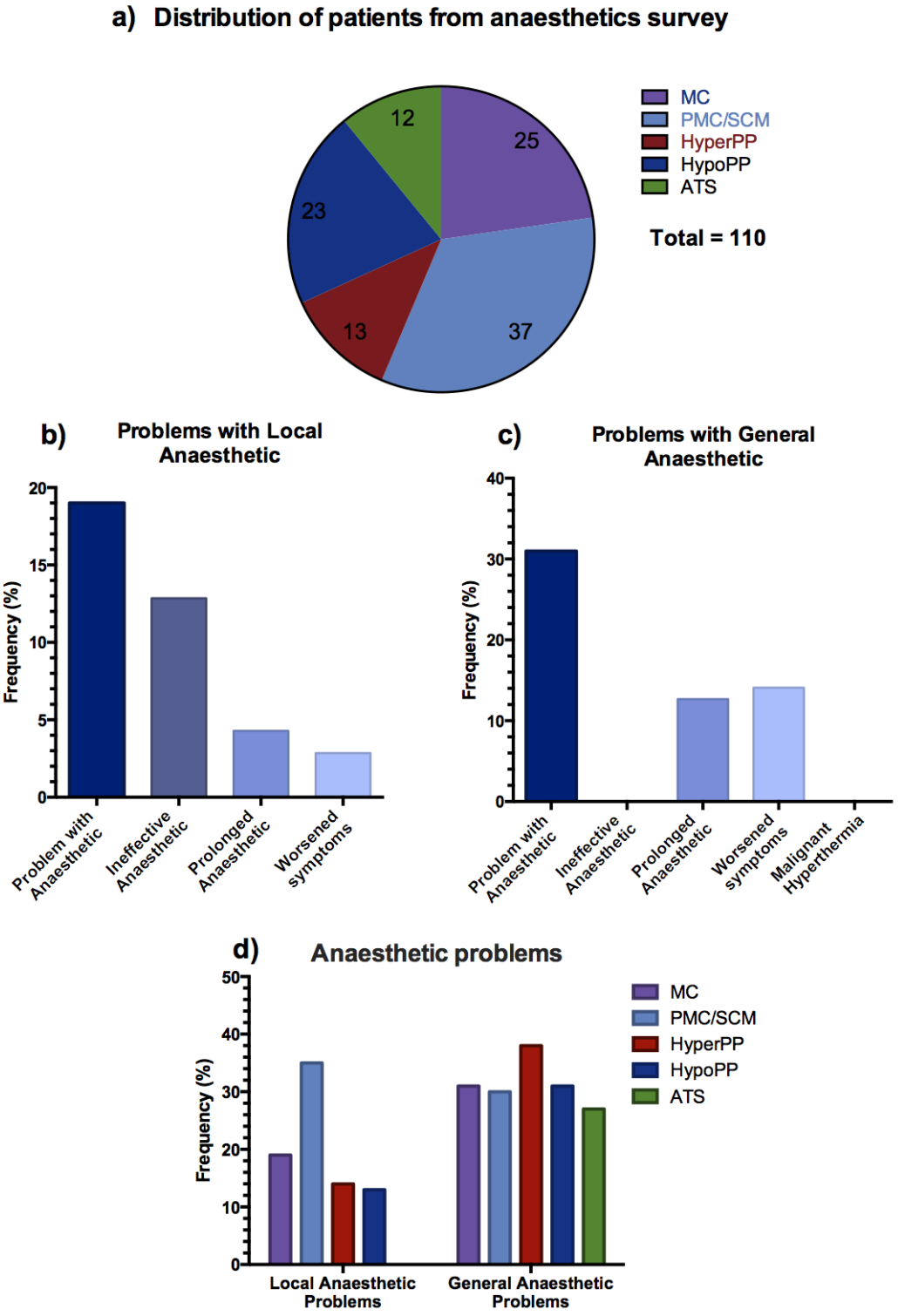


Figure 5.23: Graphs of problems reported by patients following anaesthetics. a) Distribution of channelopathy patients within the survey b) Problems reported with local anaesthetics c) Problems reported with general anaesthetics d) Problems with local and general anaesthetic split according to disease group

were more common in those who had NDM. Results for worsening symptoms were similar between the two groups (Figure 5.23d, Table 5.16).

5.2.3.2 Pregnancy in Channelopathy Patients

Overall 35 women responded to questions about pregnancy, of which 25 had at least 1 pregnancy. 29 men also supplied information about conception and numbers of children conceived. 30 patients also gave information regarding the effect of menstruation on their channelopathy (Table 5.17).

In total 42 patients had tried to conceive, of which 25 were women and 17 were men. Of these, 8 (22%) patients had problems trying to conceive. A similar proportion of men and women had difficulty conceiving, 3/15 men and 5/21 women. The average time to conceive for those with difficulties was 28 months compared to 3 months for those with no difficulties. Patients did not qualify why they had difficulties conceiving (Table 5.17). There was no significant difference in difficulty conceiving between patients with NDM and PP (Figure 5.24a).

In total, 87 children were born to patients with channelopathies of which 57 were born to female patients with channelopathies. There were 114 pregnancies overall of which 84 were in females with channelopathies. Of the 27 pregnancies that were not viable, three were elective terminations of pregnancy and 24 were miscarriages from nine women. Of the miscarriages that patients gave information about, only three were after the first trimester. No information was given about the possible causes of miscarriages. There was no significant difference between miscarriage rates in any of the diseases (Figure 5.24b, Table 5.18).

Overall 12 patients reported problems in pregnancy and 12 reported problems during delivery. The commonest complication in pregnancy was urinary tract infection (four patients). Two patients had gestational hypertension, one pre-eclampsia and one gestational diabetes (Figure 5.25a). One patient had a stillbirth. Problems during delivery included six emergency caesarean-sections, six deliveries requiring forceps or ventouse, and seven premature births. There were three postpartum haemorrhages and one placental abruption (Figure 5.25c). Four babies were reported to have neonatal problems, three of which had PMC or SCM and were reported to have either been floppy or have breathing problems (Figure 5.25b, Table 5.18).

Pregnancy worsened channelopathy symptoms in a significant number of patients with MC, PMC and HyperPP (Figure 5.24d). It affected 50% of patients with HypoPP and

Table 5.17: Table of pregnancy and births in channelopathy patients

	Number (%)
Number of pregnancies overall	110
Number of children overall	87
Average number of children	2.0
Average number of pregnancies	2.5
Problems trying to conceive	8 (22%)
Time taken to conceive (SD)	7.7 months (13.4)
Number of patients with miscarriages	9 (35%)
Total number of miscarriages	24
Stage of miscarriage	
- 1st trimester	10
- 2nd trimester	3
- 3rd trimester	0
Problems in pregnancy	12
Pre-eclampsia	1
Hypertension	2
Gestational diabetes	1
UTI	4
Problems in delivery	12
Emergency C-section	6
Forceps	5
Ventouse	1
Post-partum Haemorrhage	3
Placental abruption	1
Stillbirth	1
Uterine rupture	1
Premature births (<38wks)	7
Post-natal problems	4
Floppy baby	1
Breathing difficulties	3
Change in channelopathy	
- better	3 (8%)
- worse	29 (76%)
- unchanged	6 (16%)
When symptoms changed	
-1st trimester	1
-2nd trimester	1
-3rd trimester	3
Symptoms changed in pregnancy	
- myotonia	15 (79%)
- weakness	7 (39%)
- pain	6 (38%)
Symptoms settled after birth	17 (90%)
Time to settle symptoms	
- immediately	7 (54%)
- <1 month	4 (31%)
- 1-3 months	1
- >3 months	1
Worsening in symptoms with menses	15 (50%)

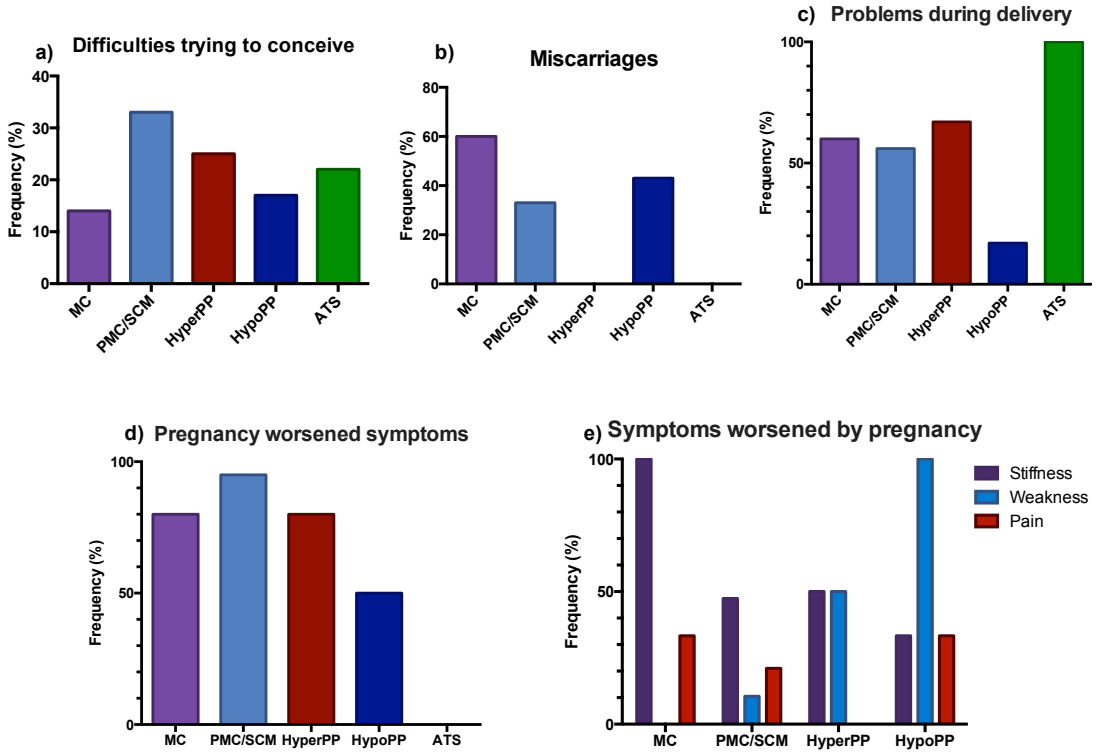


Figure 5.24: Graphs of problems in pregnancy and birth in individual channelopathies a) Difficulties with conception b)Miscarriages c)Problems during delivery d)Proportion of patients in whom pregnancy worsened symptoms e) Specific symptoms that worsened in pregnancy

improved symptoms in three patients with PP. It did not improve symptoms in any patients with NDM (Figure 5.25d). Only one patient with PMC did not improve symptoms after delivery. 54% of patients improved immediately after delivery (Figure 5.25e). In MC and PMC, pregnancy primarily worsened stiffness (Figure 5.24e). Menstruation worsened the channelopathy symptoms in over half of MC, PMC and ATS patients. It was less likely to affect HyperPP and HypoPP patients. None of the patients felt that it improved their symptoms (Table 5.18).

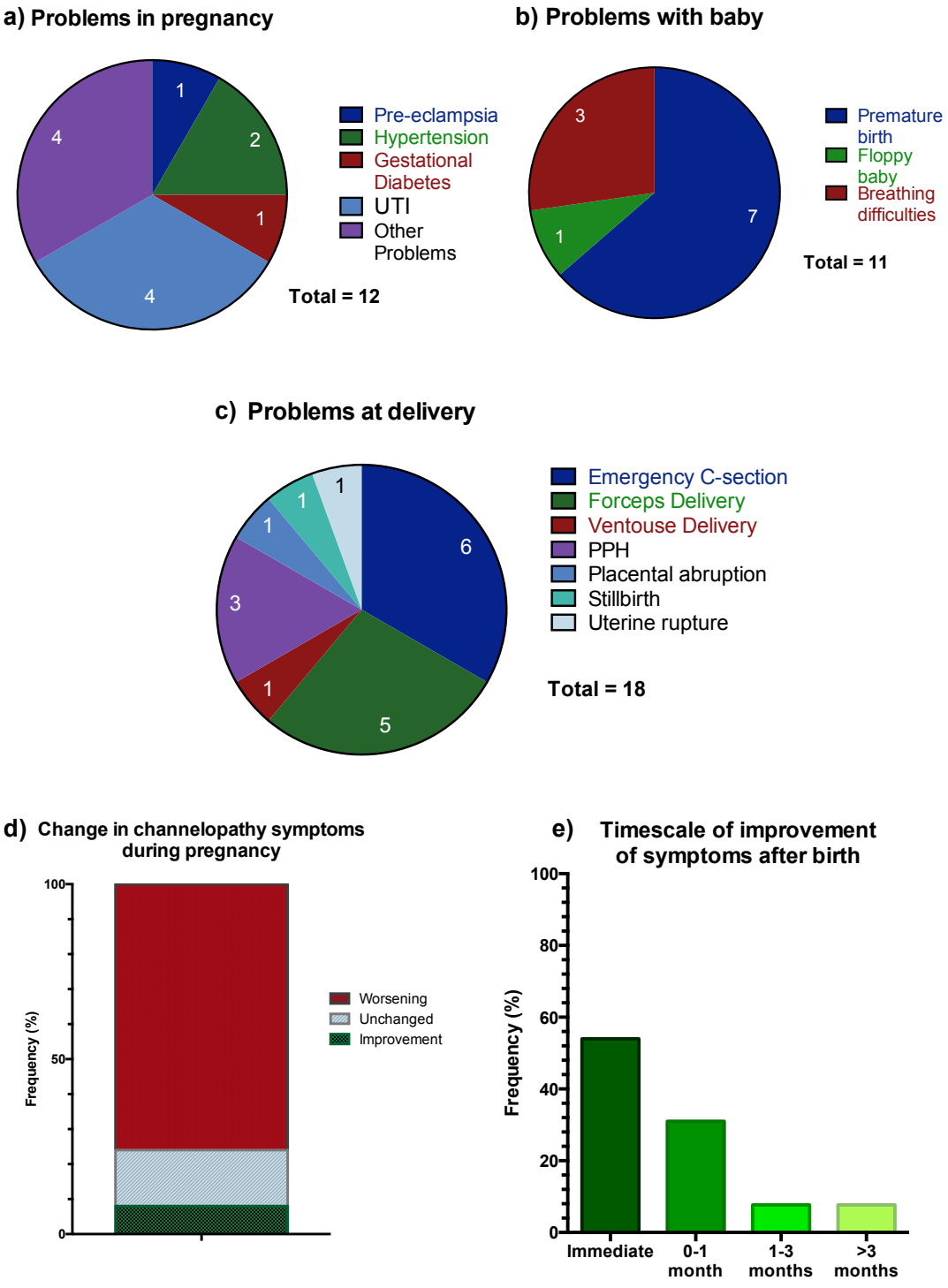


Figure 5.25: Graphs of problems in pregnancy and birth in all channelopathy patients a) Problems encountered during pregnancy b) Problems with babies at birth c) Problems encountered at delivery d) Change in channelopathy symptoms during pregnancy e) Timescale for improvement of worsening symptoms after childbirth.

Table 5.18: Table of pregnancy and births in individual channelopathy disease groups

	MC	PMC/SCM	HyperPP	HypoPP	ATS
Number of respondants	15	12	4	10	4
Number of female respondants who have been pregnant	5	9	4	6	1
Number of pregnancies	42	28	6	42	3
Number of children	33	23	6	19	3
Problems trying to conceive	2 (14%)	3 (33%)	1 (25%)	1 (17%)	1 (22%)
> 1 year to conceive	1	2	0	0	1
Number of patients with miscarriages	3 (60%)	3 (33%)	0 (0%)	3 (43%)	0 (0%)
Miscarriages after 1st trimester	1	1	0	1	0
Problems in pregnancy	2 (40%)	4 (44%)	2 (67%)	3 (50%)	1 (100%)
Problems during delivery	3 (60%)	5 (56%)	2 (67%)	1 (17%)	1 (100%)
Assisted delivery	1	4	0	2	-
Premature birth	0	4	1	1	1
Postnatal problems	1	3	0	0	0
Floppy baby	0	1	0	0	0
Neonatal Breathing difficulties	0	3	0	0	0
Pregnancy worsen channelopathy (p=0.022)	**3 (80%)**	**19 (95%)**	**4 (80%)**	**3 (50%)**	**0 (0%)**
Stiffness worsened	3	9	2	1	-
Weakness worsened	0	2	2	3	-
Pain worsened	1	4	0	1	-
Menses worsened channelopathy	3 (50%)	8 (67%)	2 (40%)	1 (20%)	1 (50%)

5.3 Genetic Diagnosis in Skeletal Muscle Channelopathy Patients

As described in the methods section, three distinct groups of patients were analysed (Figure 4.1, p 77). The targeted sequencing group consisted of nine patients from four pedigrees seen in the channelopathy service who had an unusual phenotype. These patients had strategic sequencing of relevant genes.

The second group, the MLPA group, consisted of 60 patients with either a single loss of function mutation or no mutations with a recessive MC phenotype. They had MLPA analysis to look for large scale deletions and duplications.

The final next generation sequencing group, consisted of 10 patients with no mutations but clinical and electrical evidence of a skeletal muscle channelopathy. They went on to have whole exome sequencing, and if sufficient DNA remained, aCGH.

5.3.1 Targeted Sequencing in Patients with Unusual Phenotypes

Nine patients from four pedigrees were identified as having unusual phenotypes. Two of these families had a pathogenic variation already identified but when assessed clinically had a severe atypical phenotype. Following further strategic gene analysis further variations were found in all patients.

5.3.1.1 Case 1

Case 1 was a family with marked variation in phenotypes between two branches of the same family. One branch having a particularly severe presentation and unusual neurophysiology findings.

Phenotype of the Family

The proband was female and initially presented with marked stiffness from the age of 4 years, primarily of the hands and eyes. It later progressed to affect the throat, arms and legs. It was markedly exacerbated by cold but there was never any accompanying weakness. It partially responded to treatment with mexiletine and was severely exacerbated during the second trimester of pregnancy. There was a strong dominant family history as shown in the pedigree (Figure 5.27). On examination she had generalised muscle hypertrophy and grip, eyelid and percussion myotonia.

Her investigations demonstrated a mildly elevated CK of 391IU/L. Her neurophysiology

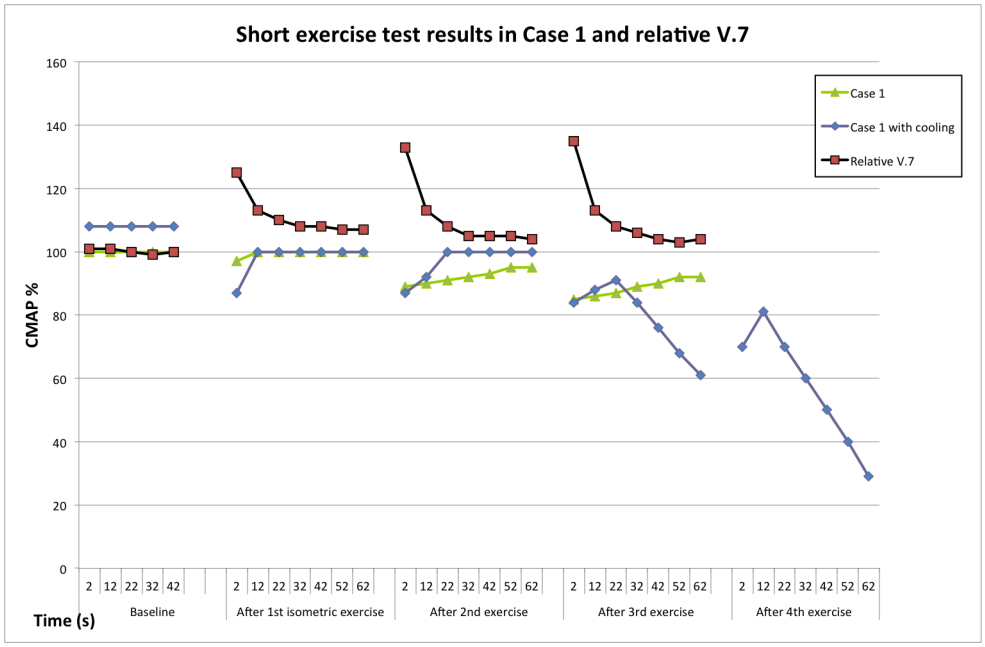


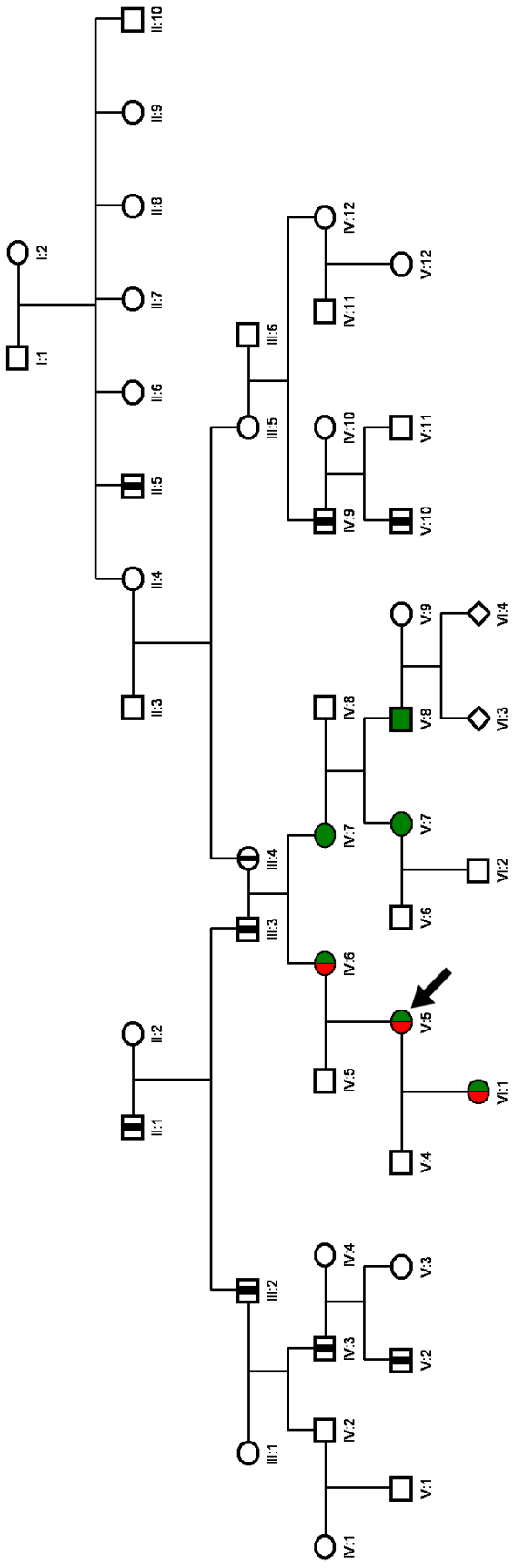
Figure 5.26: Graph of short exercise test results of Case 1 and relative V.7

testing demonstrated profuse myotonia but had an atypical pattern on short exercise testing. At room temperature, she had very little decrement with initial exercise but with repeated exercise in the third and fourth trials demonstrated mild worsening. However in the fifth and sixth trials of exercise she then showed warm-up (not shown in the figure). However, after cooling, there was a marked decrement, especially after fourth and fifth trials of exercise (Figure 5.26). Paradoxically this improved with repeated exercise after rewarming although, despite rewarming, the limb did not recover back to normal. MRI of the calves and thighs demonstrated moderate fatty infiltration in the tibialis anterior and the gastrocnemius bilaterally. Short tau inversion recovery (STIR) sequences demonstrated oedema of the medial head of the gastrocnemius (Figure 5.28).

The proband's mother (IV.6) was affected in early childhood. She also had primarily leg stiffness, worse in the cold. She, like her daughter, had a severe exacerbation in pregnancy and again later in life when she took hormone replacement therapy. On examination she had generalised muscle hypertrophy and hand grip myotonia. She was noted to also have moderate myotonia on EMG when assessed previously (Table 5.19).

The proband's daughter (VI.1) was severely affected from 6 months old. She had eye and hand stiffness which was improved with mexiletine treatment. On examination she had generalised muscle hypertrophy, a stiff gait and eyelid and hand grip myotonia. Her CK was slightly elevated at 376IU/L and had profuse myotonia on EMG (Table 5.19).

Figure 5.27: Pedigree of Case 1. (Black arrow indicates the proband. Green indicates those carrying the Gly1306Val variation in SCN4A. Red indicates those carrying the Ala313Val variation in CLCN1. Black band indicates patients thought to be affected but not genetically analysed)



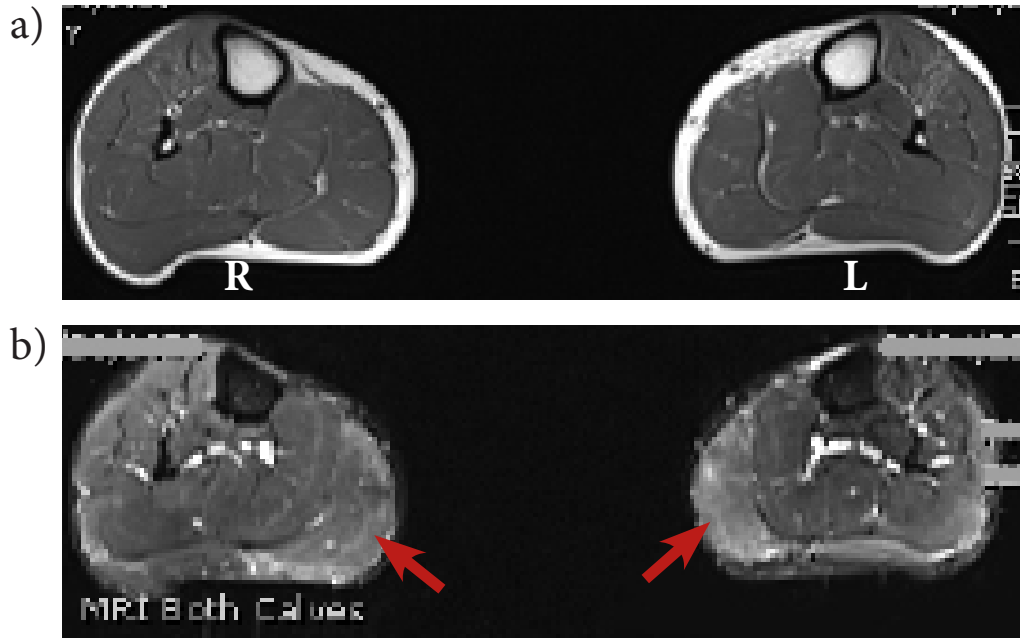


Figure 5.28: MRI of calves of proband in Case 1 a) T1 MRI of calves demonstrating muscle hypertrophy b) STIR MRI of calves demonstrating oedema primarily in the medial gastrocnemius (indicated by red arrows)

The proband's cousin (V.7) was also found to be affected but with much milder symptoms. We were able to assess her after she had marked worsening in the third trimester of pregnancy. She had onset in late childhood with mild cramping and stiffness primarily after running. She would most frequently develop stiffness after exercise, affecting her eyes, hands, legs and face. They were significantly worsened by cold. Her mother (IV.7) and brother (V.8) were also mildly affected. When examined during pregnancy she had moderate grip and eyelid myotonia with no proximal weakness or hypertrophy. Her EMG demonstrated profuse myotonia in all tested muscles. Her short exercise test demonstrated an increment with subsequent trials of exercise rather than a decrement with less of an increment following cooling (Figure 5.26). Her mother (IV.7) also had a history of stiffness in late childhood with mild eye stiffness which was worsened by cold (Table 5.19).

Genotype of Case 1

Initial genetic analysis of the proband revealed a known pathogenic variation in CLCN1, c.938C>T; p.Ala313Val in exon 8 which is commonly associated with dominant MC. However, given the marked severity of symptoms, which is unusual in dominant MC, and the predominance of eyelid myotonia and cold-sensitivity, we also sequenced SCN4A.

	Gender	Age	Onset of symptoms	Symptoms	Signs
Family branch 1					
V.5 Proband (Case 1)	♀	52yrs	4yrs old, Hand stiffness	Stiffness worst in cold. No weakness Severe exacerbation in pregnancy (2nd trimester) Now unable to work.	Generalised muscle hypertrophy Hand grip, eyelid & percussion myotonia
VI.1 Daughter of proband	♀	27yrs	6 months old, Eye & hand stiffness	Severe myotonia, improved with mexiletine	Generalised muscle hypertrophy, Stiff gait Eyelid & hand grip myotonia
IV.6 Mother of proband	♀	73yrs	Early childhood, Leg stiffness	Moderate myotonia, Severe exacerbation in pregnancy & after HRT	Proximal muscle hypertrophy Hand grip myotonia
Family branch 2					
V.7 Cousin of proband	♀	40yrs	Late childhood, Eye and hand stiffness	Mild myotonia Exacerbated by cold Worsened in pregnancy	Hand grip & eyelid myotonia
IV.7 Aunt of proband	♀	67yrs	Late childhood, Eye stiffness	Mild myotonia Exacerbated by cold	Eyelid myotonia
Founders					
III.3 Grandfather of proband	♂	Died		Severe myotonia	Grip myotonia EMG myotonia
III.4 Grandmother of proband	♀	Died		Mild myotonia	Eyelid myotonia EMG myotonia

Table 5.19: Clinical details of the family members in Case 1

We discovered a second known pathogenic variation, c.3917G>T; p.Gly1306Val which is associated with PMC, suggesting that the combination of variations are responsible for the unusual phenotype. When the proband's mildly affected cousin and aunt were tested they were found to only carry the c.3917G>T; p.Gly1306Val variation. It was later established that both the proband's grandparents were thought to be affected, not just the grandfather (III.3), although the grandmother (III.4) was only mildly affected (Figure 5.27).

5.3.1.2 Case 2

Case 2 was a consanguineous family with evidence of myotonia but also unusual features of proximal weakness and arrhythmias in some family members.

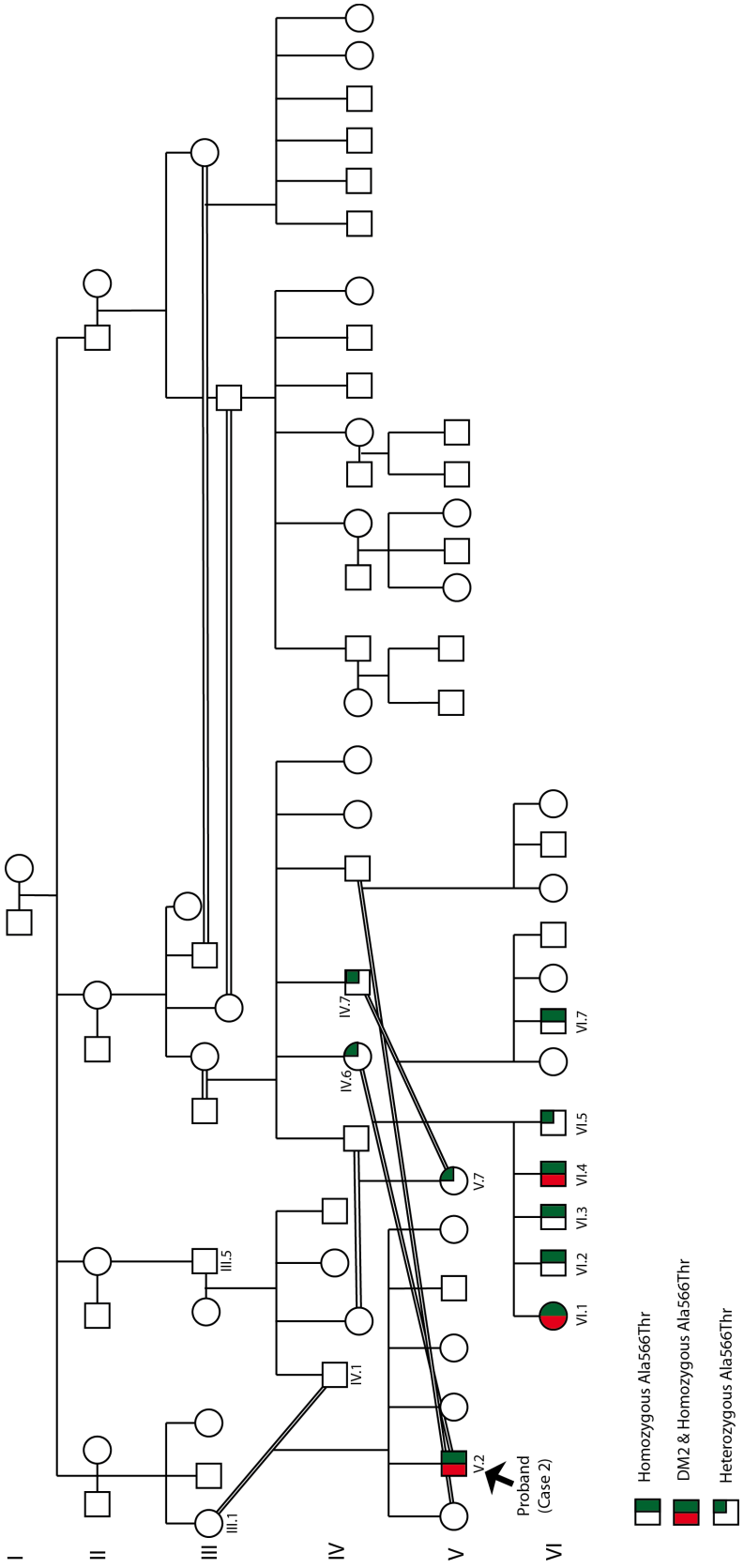
Phenotype of the Family

Proband: was a 36 year old Pakistani male, who first noticed symptoms at the age of 15 when he had an episode of muscle stiffness after a prolonged fever. His stiffness was present in the hands, limbs and axial muscles and warmed-up with repetitive movement. Prior to this he reached normal developmental milestones and was able to function normally but had always noticed that his muscles were bulky. At the age of 19 years he started to notice difficulty climbing stairs which gradually progressed. Over time he developed difficulty lifting heavy objects. He did not have a history of palpitations but did develop excessive daytime somnolence and memory loss in the 5 years prior to presentation. He came from a large consanguineous family with first cousin marriages over many generations (Figure 5.29). He and his wife (IV.6) were second cousins both through his paternal and maternal lines. Four of his five children were symptomatic with muscle stiffness and weakness as described below. His youngest child (VI.5) was unaffected. His father (IV.1) had a history of palpitations and type 2 diabetes mellitus and his mother (III.1) had cataracts and daytime somnolence. His paternal grandfather (III.5) had increased muscle bulk but no history of stiffness.

On examination he had generalised muscle hypertrophy and significant grip and limb myotonia with warm-up. He had mild neck flexion weakness and shoulder abduction weakness (grade 4 MRC). He had severe proximal leg weakness (hip extension grade 3 MRC, hip flexion and knee flexion grade 4- MRC). Deep tendon reflexes were normal. He did not have cataracts or frontal balding and his ECG was normal. EMG showed profuse myotonic discharges with a normal long exercise test and cold immersion exercise testing.

Proband's daughter (VI.1): The proband's 16 year old daughter (VI.1) had a normal neonatal period but was a late walker, walking at two years. At four years she had difficulty climbing stairs. At five years she noticed mild stiffness, especially in the legs and hands. In the few years prior to presentation, she developed progressive leg weakness and difficulty running. At the age of 14 she began to have episodes of palpitations with dizziness, nausea and chest pain. She was subsequently diagnosed with a supraventricular tachycardia on Holter 24 hour ECG monitoring. She had also noted increased daytime somnolence. As described above she came from a large consanguineous family (Figure 5.29). Her parents were second cousins but her mother (IV.6) was unaffected. All her mother's siblings had a history of early cataracts in their 40s. Her mother's brother (IV.7) was married to her father's paternal first cousin (V.7) and one of their four children (VI.7) also had stiffness

Figure 5.29: Pedigree of Case 2 Green represents Ala566Thr variation in CLCN1 (quarter-filled = heterozygous, half-filled = homozygous). Red represents pathological expansion in ZNF9 consistent with DM2.



with no weakness. On examination she had increased muscle bulk with both eyelid and grip myotonia with warm-up. She had mild proximal muscle weakness with grade 4 MRC shoulder abduction weakness and grade 4- MRC hip flexion weakness. She had profuse myotonic discharges on EMG. Her standard 12 lead ECG was normal.

Proband's eldest son (VI.2): The proband's 14 year old son (VI.2) had a normal neonatal period but had congenital dislocation of the left hip at 18 months which was surgically reduced at two years. He walked late, after 2 years, as a result of the hip problems but was always able to run and jump. He had difficulty with handwriting due to stiffness but never had weakness. On examination he had grip myotonia with warm-up. He had normal tone, power and deep tendon reflexes. He had a normal ECG and echocardiogram. EMG demonstrated occasional myotonic discharges.

Proband's second son (VI.3): The proband's 10 year old son (VI.3) was also a late walker at two years. He noticed stiffness and falls at seven years. He later developed pain on chewing. He was always able to run and jump. On examination he had calf hypertrophy and vitiligo. He had very mild grip and eyelid myotonia with warm-up and was the least severely affected of the proband's affected children. EMG demonstrated occasional myotonic discharges.

Proband's third son (VI.4): The proband's eight year old son (VI.4) walked at one year but had a mild speech delay. He noted stiffness of grip and limbs in early childhood with frequent falls and clumsiness. He always had problems at school with poor attention and disruptive behaviour. He had one episode of palpitations at five years old but had a normal ECG and echocardiogram. On examination he had significantly increased muscle bulk and grip and eyelid myotonia. Tone, power and reflexes were normal. EMG showed occasional myotonic discharges.

Genotype of case 2

Given the phenotypes suggestive of recessive MC the service laboratory sequenced all exons of CLCN1 revealing a homozygous mutation in exon 15, c.1696G>A;p.Ala566Thr in the proband. This homozygous mutation was also found in the proband's children (VI.1, VI.2, VI.3 and VI.4). The reportedly affected wife's nephew (VI.7) also carried this homozygous mutation. The unaffected wife (IV.6) and unaffected youngest child (VI.5) were heterozygous for this mutation (Figure 5.29).

Given the unusual phenotype with proximal weakness and cardiac arrhythmias, and

the variability in phenotype between the children, DNA was sent for sequencing of DM1 and DM2. The proband and two of his children, VI.1 and VI.4 (the more severely affected children) were all found to have relatively small DM2 expansions of approximately 81 CCTG repeats. Children VI.2, VI.3 and unaffected child VI.5 did not have abnormal expansions, consistent with their milder MC phenotypes.

Expression of the Variation

As Ala566Thr has not been previously described as causing MC it was expressed in *Xenopus* oocytes by R.Mannikko to assess its pathogenicity. In contrast to WT CLC-1 channels, homomeric Ala566Thr channels (homA566T) displayed no or very small voltage dependent currents when expressed in *Xenopus* oocytes (Figure 5.30). Only 2 of 12 cells displayed voltage dependent currents. Mean maximal amplitude of these currents was -1.3$\pm$0.1A compared to mean maximal amplitude of WT currents -8.7$\pm$0.6A (n=13). When WT and Ala566Thr RNA was injected in 1:1 ratio to simulate the heterozygous state found in the unaffected family members, the resulting currents were significantly smaller than the WT currents with a mean maximal amplitude of -5.9 $\pm$0.6A, n=10 ($p<0.005$). The voltage dependence of the WT and heterozygous Ala566Thr were similar (WT: -33.2 $\pm$3.0mV, heterozygous Ala566Thr: -28.2$\pm$2.6mV, p=0.23). The findings that homomeric Ala566Thr channels display no or tiny measurable currents, the fact that current amplitude of heterozygous Ala566Thr is greater than half of the WT currents, and that coexpression of A566T with WT had no effect on voltage dependence of the channel activation are all typical for a recessive ClC-1 mutation (Wollnik et al., 1997). These results fit with the clinical picture seen in this family of unaffected heterozygous carriers and affected homozygous carriers.

5.3.1.3 Case 3

Case 3 was of two brothers with symptoms suggestive of a sodium channelopathy with an alternative final diagnosis.

Phenotype of Case 3

The proband was a 25 year old male who was the product of a normal pregnancy and delivery. He was not floppy at birth. He walked late at 3 years, and had multiple falls, difficulty rising from seated and was slower at running than peers at school. At age 3.5 years he had a muscle biopsy which was reported to have non-specific findings. He then improved and developed normally until 10 years when he began to have progressive gait difficulties, and

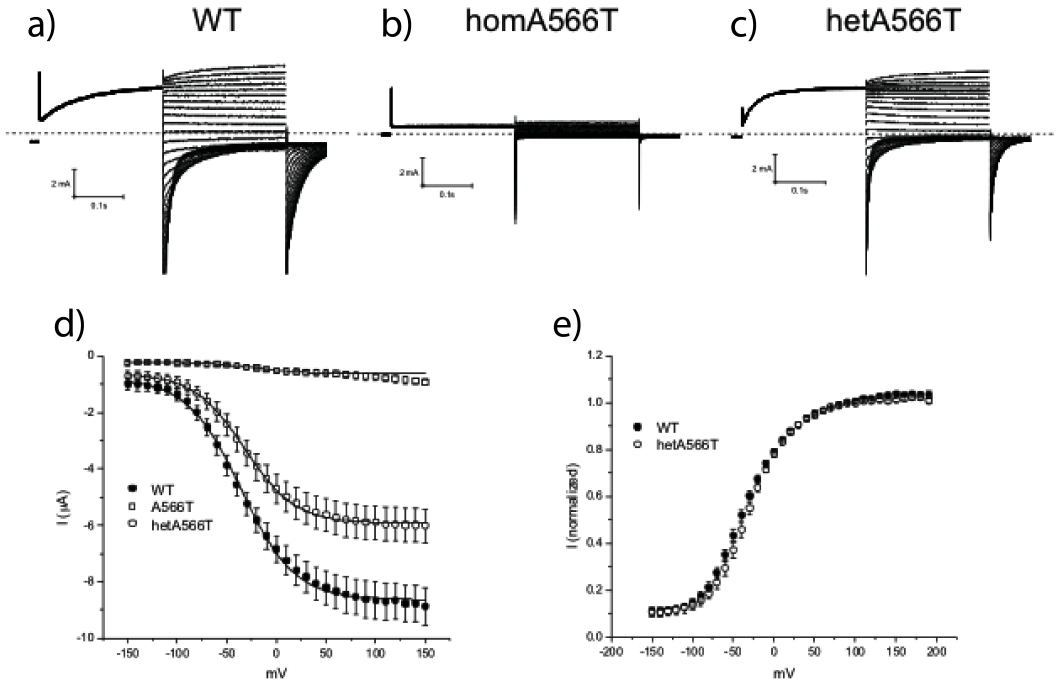


Figure 5.30: Functional expression of wild-type ClC-1 and Ala566Thr mutation by whole-cell patch clamp of *Xenopus* Oocytes a-c) Representative whole-cell two-electrode currents recorded from intact *Xenopus* oocytes heterologously expressing wild-type, homA566T or hetA566T channels. Voltage steps from -150 to + 90 mV are shown. The first 2ms of the capacitative current peaks of each voltage step are not shown. d) Current-voltage relationship of WT (solid circles, n=13), hetA566T (open circles, n=10) and homA566T channels (open squares, n=12). The data were fitted to Boltzmann equation with values, WT: I_0= -0.8μA, I_{max}= -8.6μA, $V_{\frac{1}{2}}$= -35.8mV, dV=27.1mV, hetA566T I_0=-0.6μA, I_{max}=-5.9μA, $V_{\frac{1}{2}}$=-32.2mV, dV=25.5 mV, homA566T curve was drawn by eye. e) Same as in (d) but the data was normalized to I_{max} for WT and hetA566T channels to illustrate similar voltage dependence and similar minimal offset current. (figure courtesy of R.Manniko)

could only walk short distances with a stick and needed a wheelchair for long distances. His repeat muscle biopsy, however, was unchanged. Again at 18 years his symptoms settled and he began to steadily improve, he no longer needed a stick to mobilise. At presentation his primary complaints were of stiffness and pain especially with prolonged sitting or cold, and improved with walking. He had occasional episodes of legs giving way and suddenly becoming weak in the cold. He had tiredness & lethargy with exertion but no association with carbohydrate meals or bananas. He had no diplopia or dysarthria. His was one of four siblings and his younger brother was also severely affected (Figure 5.31).

His examination was normal with no proximal weakness or myotonia. His investigations demonstrated a raised CK at 647IU/L. Electrophysiology demonstrated a positive McManis

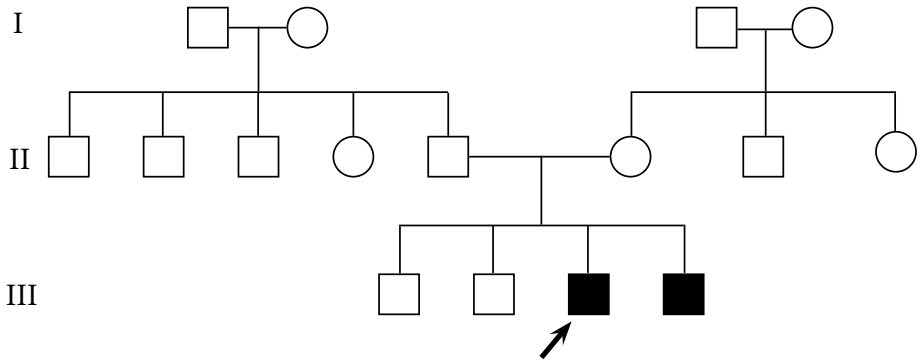


Figure 5.31: Pedigree for Case 3 Black squares indicate affected individuals, arrow indicates proband.

with 44% decrement from the peak amplitude (although there was only a 12% decrement from the baseline) (Figure 5.32a). On short exercise testing there was a drop in CMAPs with exercise, however there was an increase in area suggesting an alteration in the morphology of the curves with cooling rather than a true decrement. There was no myotonia or myopathy on EMG. MRI of the lower limbs demonstrated mild fatty infiltration in the thighs and calf. There was high signal on STIR suggesting oedema present mainly in the soleus in the calves, subtly in the anterior compartment of the thighs and more significantly in the posterior compartment of the thighs (Figure 5.33).

The proband's brother was a 16 year old boy who was also the product of a normal pregnancy but with a delivery complicated by meconium aspiration with foetal distress. He was in special care intensive baby unit for 8 days requiring nasogastric feeding but no ventilation. He also had significantly delayed milestones with delayed standing at 1 year and walking at 20 months with a waddling gait. At 2 years he started to have multiple falls and difficulty rising from a seated position and was unable to climb stairs. He had a biopsy at 3 years which demonstrated non-specific abnormalities with type 1 fibre predominance and normal immunohistochemistry. Initially he was less affected than the proband. As he grew up he was noted to have mild learning and communication difficulties and then slowly became more progressively weak and by age 10 he was predominantly using a wheelchair but with some variability from day to day. He was always able to swim unaided. He would have attacks of weakness precipitated by cold and these would last for minutes. He had no cardiac abnormalities but 78% predicted FVC on spirometry. On examination he had a thoracolumbar scoliosis and proximal limb weakness to MRC grade 3 in the upper and lower limbs.

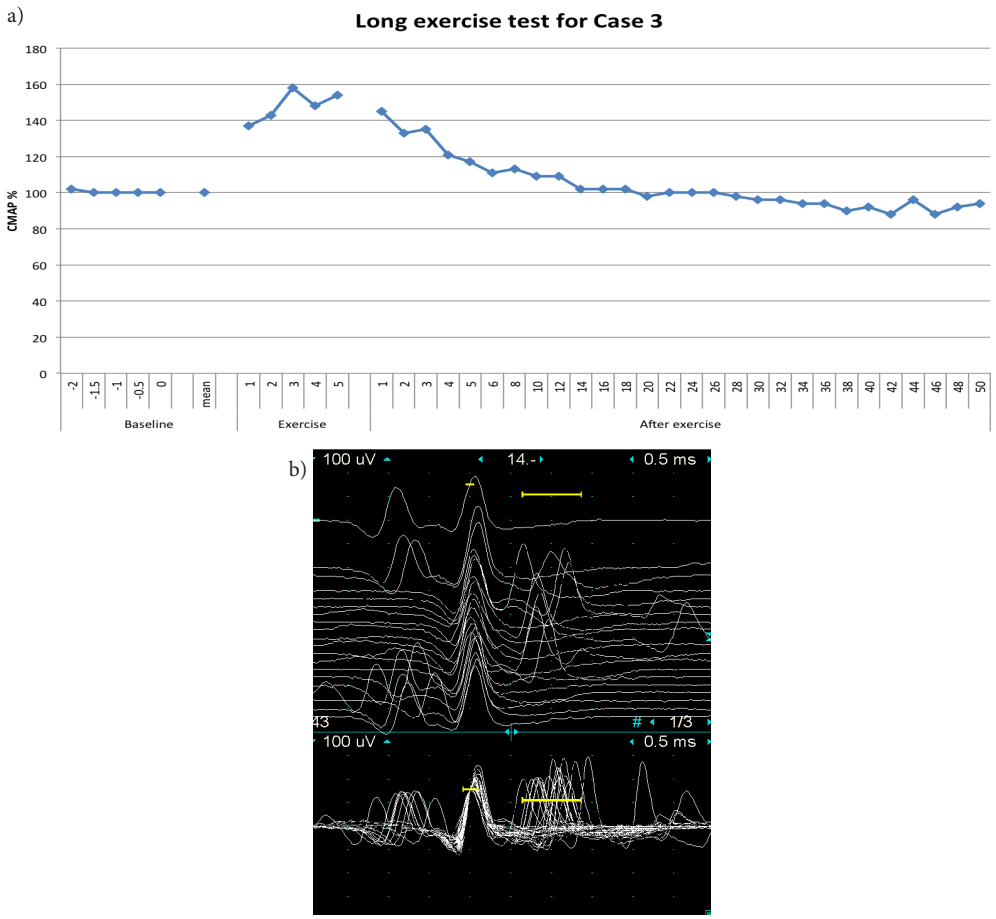


Figure 5.32: Case 3 Neurophysiology a) Long exercise test demonstrating significant decrement. b) Single fibre EMG of trapezius demonstrating jitter

Genotype of Case 3

Given the electrophysiology findings and the periodic nature of the symptoms, the proband had sequencing of SCN4A, CLCN1, DM1 and 2 and RYR1. No pathogenic mutations were identified. Given the unusual phenotype he had further EMG with repetitive stimulation and single fibre studies which demonstrated an abnormal decrement and jitter in the trapezius muscle (Figure 5.32b). Both subjects went on to have screening of the congenital myasthenia genes which revealed compound heterozygous variations in DOK7. The c.1124_1127dupTGCC;p.Leu375fs, which is a known pathogenic mutation and c.533-37_c.533-11del27 which encompasses a consensus branch point and therefore may lead to aberrant splicing (Alamut v1.5). This was carried by both the proband and his brother and given their phenotypes, is likely to account for their disease. Both individuals were therefore started on salbutamol treatment, which significantly improved their symptoms, adding weight to the theory that the DOK7 variations were causative.

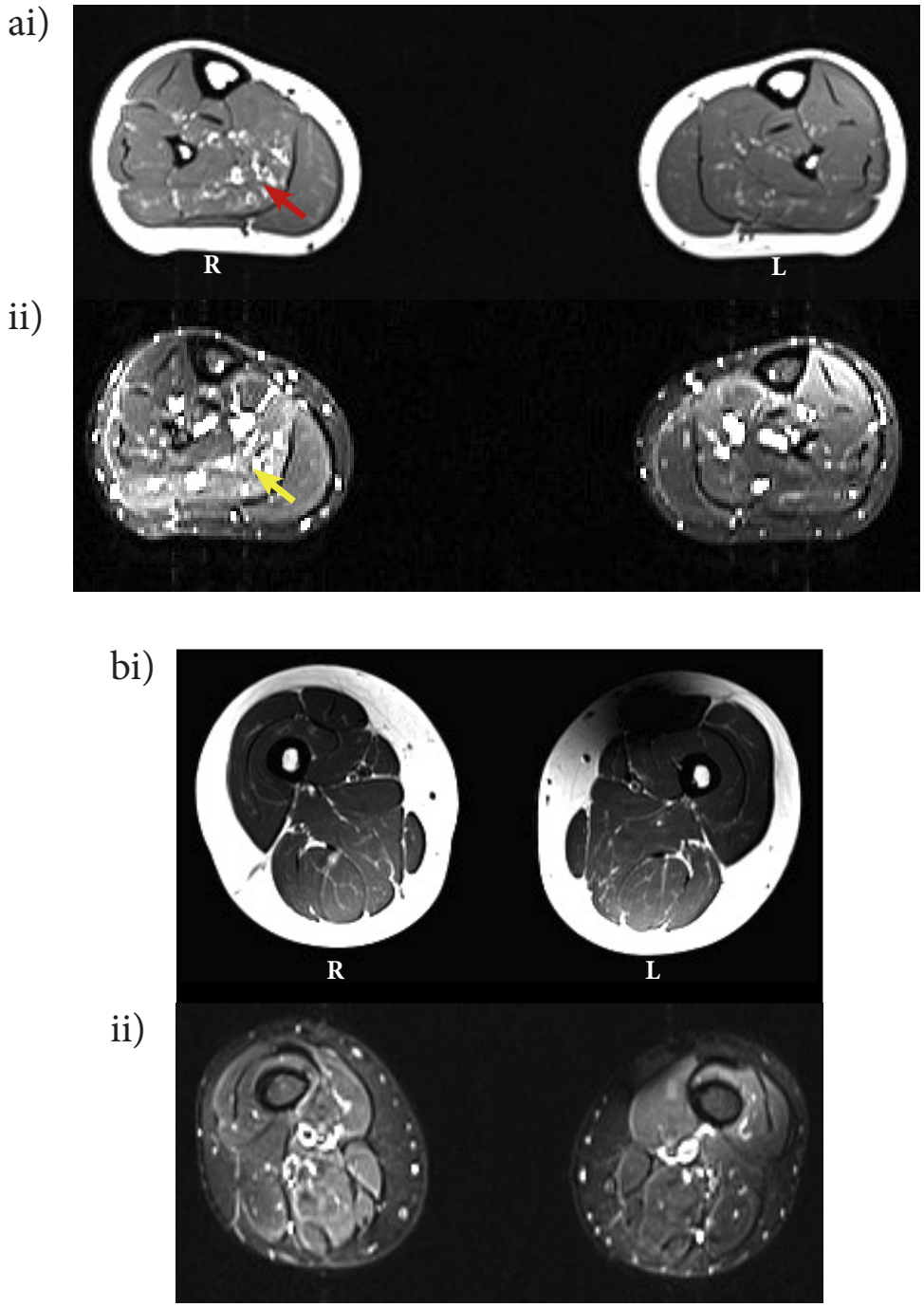


Figure 5.33: MRI of proband in Case 3 ai) T1 MRI of calves demonstrated mild fatty infiltration primarily in soleus (indicated by red arrow) aii) STIR MRI of calves demonstrating high signal suggestive of oedema primarily in soleus (indicated by yellow arrow), bi) T1 MRI of thighs demonstrating very subtle fatty infiltration of the posterior compartment, bii) STIR MRI of thighs with oedema in the posterior compartment and subtle oedema in the anterior compartment

5.3.1.4 Case 4

Case 4 was a patient with a complex mixed phenotype with features of periodic paralysis and congenital myasthenia.

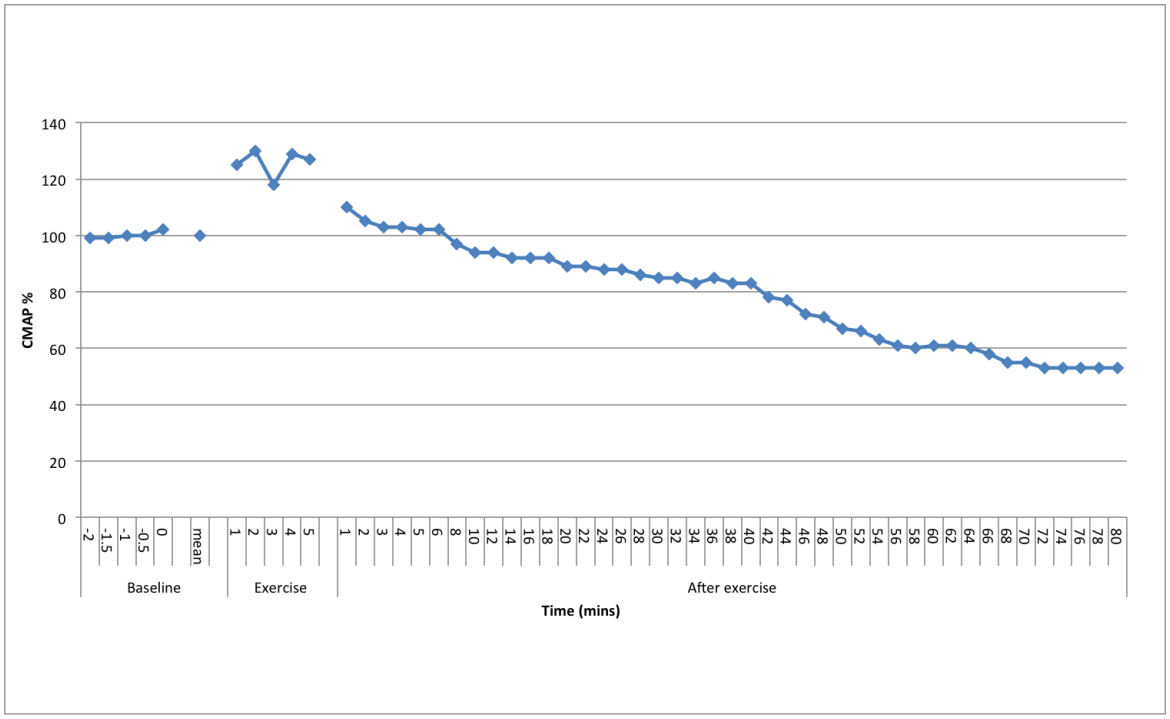
Phenotype of Case 4

The 55 year old proband was born 7 weeks premature and required special schooling. In childhood he had difficulty rising from the floor and climbing up stairs. He also had difficulty with running and was poor at sports at school and had always noted fatiguability. In his second decade he began to have episodes of paralysis, usually with no precipitant. These would consist of myalgia and then marked leg weakness and respiratory compromise lasting usually two to seven days. He had a number of occasions when he was so severely affected that he required intubation and ventilation. He often lost his ability to swallow during these attacks. He also had more frequent minor episodes of weakness of his neck or arm. He would have episodes of pharyngeal weakness that required use of a gastrostomy tube when prolonged. He had a history of hyperthermia reactions to anaesthetic in the past often accompanied by an episode of paralysis. His other medical history included an atrial septal defect that was closed, mild mitral valve prolapse, type II diabetes mellitus and atrial flutter which resolved following DC cardioversion. His parents were unaffected, he had three siblings, his older sister developed scoliosis at 5 years requiring surgery and had been diagnosed with fibromyalgia but she had otherwise normal development and her examination was normal. His sister's two children were unaffected.

On examination he was mildly dysmorphic and had pes cavus. He had a convergent strabismus and complex ophthalmoplegia on examination. He had poor eye abduction bilaterally, limited upgaze and partially limited downgaze. He had mild facial weakness and neck flexion and extension weakness. He had proximal muscle atrophy and weakness of shoulder abduction with MRC grade 4 and hip flexors of MRC grade 3 but had calf hypertrophy. He was areflexic and sensation was preserved. His FVC was 1.85L (approximately 50% predicted).

His investigations revealed normal routine bloods with a CK just above normal at 247IU/L. His ECG demonstrated first degree heart block. His 24 hour ECG revealed intermittent Wenckebach and a broad complex tachycardia. An echocardiogram showed mild bi-atrial dilatation with normal left ventricular diastolic and systolic function, mild bi-leaflet mitral valve prolapse with mild to moderate mitral regurgitation.

Figure 5.34: Case 4 Long exercise test



His electrophysiology was very interesting. The EMG was myopathic but with no myotonia. He had a markedly abnormal long exercise test with a decrement of 59% (Figure 5.34) but normal short exercise tests both at room temperature and cooling. Repetitive stimulation was normal and single fibre EMG revealed some mild abnormalities in orbicularis oculi which was in keeping with the severity of the ophthalmoplegia making it less likely to be a primary neuromuscular junction disorder. However, as a result he had antibody testing for anti-acetylcholine receptor antibodies, anti-MuSK antibodies and anti-voltage-gated calcium channel antibodies, all of which were negative. The muscle biopsy showed a myopathy with core-like structures and Type I fibre predominance (Figure 5.35). MRI of the calves demonstrated moderate fatty infiltration of tibialis anterior and soleus bilaterally and hypertrophy of the lateral head of the gastrocnemius (Figure 5.36). He had trialled a number of treatments including pyridostigmine and mexiletine, neither of which improved symptoms and acetazolamide which had improved his attacks of weakness.

Genotype of Case 4

The patient therefore had testing of all exons in SCN4A, S4 segments of CACNA1S and all exons of KCNJ2 which were all normal. Given the decrement seen on single fibre he was also screened for CHRNE, DOK7, RAPSN, CHRNA1, CHRNB1, CHRND, COLQ and CHAT none of which revealed pathogenic variations. Given his malignant hyperthermia

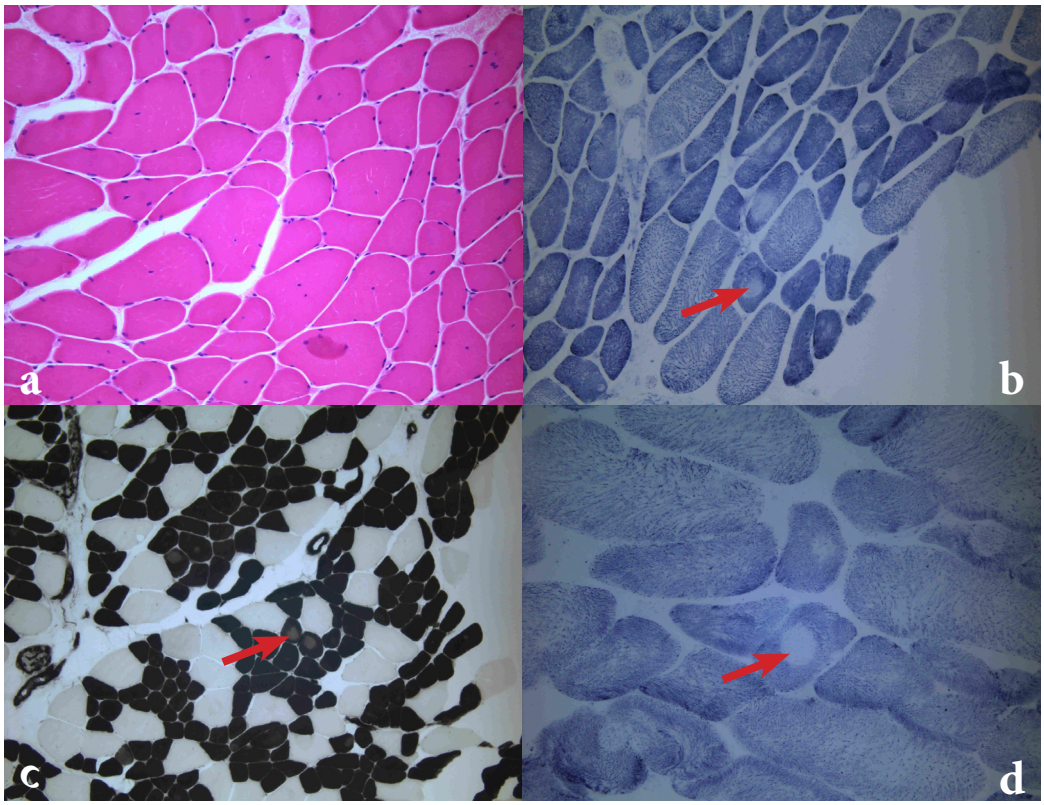


Figure 5.35: Case 4 Biopsy a) Haematoxylin and eosin stain demonstrating variation in fibre size and shape and internalised central nuclei b) NADH stain demonstrating type 1 fibre predominance and presence of core-like structures (indicated by red arrow) c) SDH stain demonstrating the core-like structures (indicated by red arrow) d) ATPase stain demonstrating core-like structures visible in the type 1 fibres corresponding to areas of myofibrillar disruption within the type 1 fibres (indicated by red arrow)(images supplied by F.Jaffer)

reaction he was then screened for RYR1 variations and found to have three variations: a heterozygous variation in exon 3, c.208C>T;p.Gln70X, the c.325C>T;Arg109Trp in exon 4 and the c.1453A>G;p.Met485Val variation. The Gln70X variation results in a premature stop codon and therefore is likely to be pathogenic. The Arg109Trp variation has been previously reported in a patient with a core myopathy and has been shown to affect the function of the RYR1 protein making it also highly likely to be pathogenic (Zhou et al., 2006). The Met485Val variation, has been reported as being *in cis* with the Arg109Trp mutation with unknown significance. Given the patient's phenotype these variations are highly likely to represent the underlying cause of his symptoms.

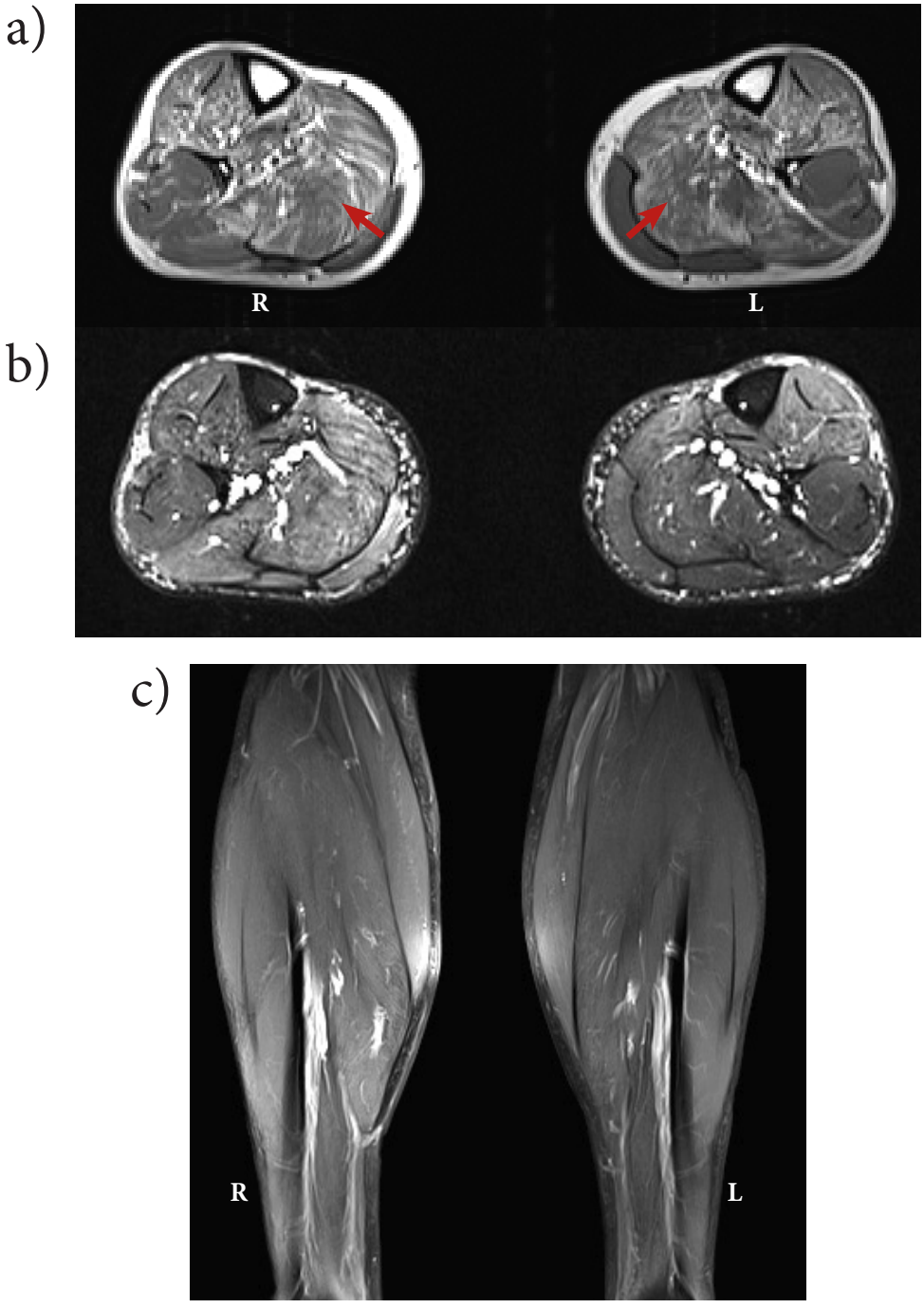


Figure 5.36: Case 4 MRI of calves a) T1 MRI of calves showing fatty infiltration primarily of the soleus (indicated by red arrows), b) STIR MRI of calves, c) T1 MRI coronal section of calves demonstrating hypertrophy of the calves.

5.3.2 Diagnosing Patients with Single Mutations in CLCN1 using MLPA

Overall MLPA was performed on 60 patients from the UK and Sicilian cohorts. 4 out of the 60 (6.7%) displayed altered peak ratios consistent with whole or partial exon deletion or duplication (Figure 5.37). The clinical details of each of these patients are described in Table 5.20.

Overall in the group of recessive patients with a single mutation, I identified exon deletions in two patients (6%). No large scale rearrangements were detected in the 12 mutation negative patients. In the group of patients with apparently homozygous mutations, two out of 16 had abnormal peak ratios on MLPA. I also identified one false positive result in a patient who was apparently homozygous for a previously described 14 base pair deletion in exon 13, c.1437_ 1450del;p.[Pro480fs] (Meyer-Kleine et al., 1994). MLPA identified a relative peak ratio of 0 for exon 13, indicating a homozygous deletion of this exon. However, as the 14 base pair deletion mutation encompasses part of the MLPA exon 13 probe recognition site this most likely results in the loss of probe binding, rather than representing a true exon deletion.

No rearrangements were detected in CLCN1 by MLPA in the 124 control chromosomes analysed suggesting that exon deletions and duplications are not common in the normal population.

5.3.2.1 Patient 5

Phenotype of Patient 5

Patient 5 was an Italian male with muscle stiffness and difficulty walking from age 12 that progressed to marked disability by late teenage years. He experienced the warm-up phenomenon and had a significant improvement in stiffness with mexiletine. The pedigree was consistent with recessive inheritance, with his parents and sibling unaffected. On examination there was marked calf hypertrophy and generalised limb percussion myotonia but no weakness.

His investigations demonstrated a CK of 576IU/L. EMG showed frequent myotonic potentials and short exercise testing was consistent with a Fournier type II pattern (Figure 5.38a). MRI of the patient's calf showed high signal on the STIR images bilaterally in the lower part of the medial head of the gastrocnemius with normal signal on T1-weighted images

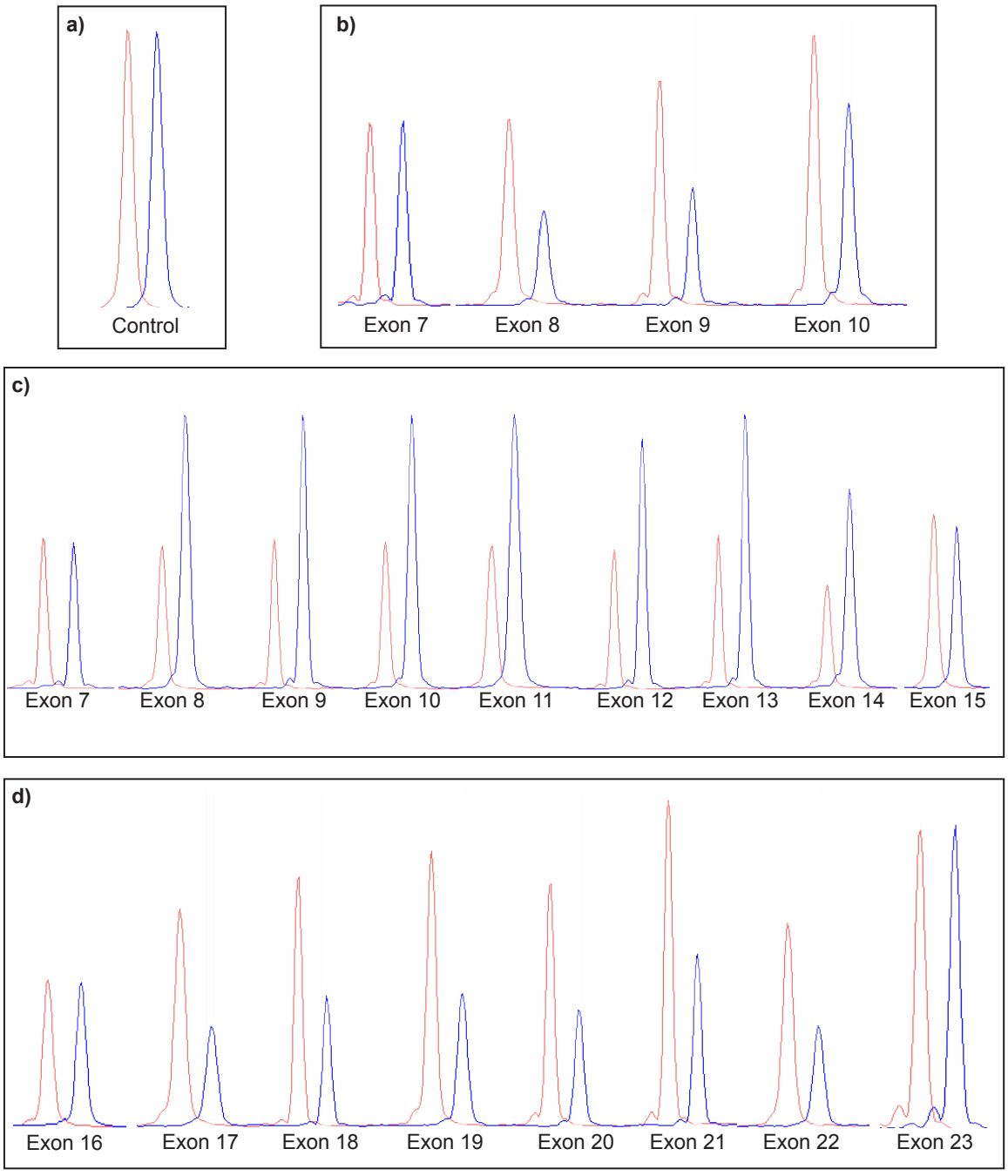


Figure 5.37: Relative MLPA probe heights in patients with large scale alterations (Red peaks represent average peak height of target probes from reference samples; blue peaks represent peak height of target probes in patient samples) (a) Control sample MLPA: both peaks are of equal height, representing normal copy number for that exon. (b) Patient 5: exons 8-10 have probe ratios of approximately 0.5 representing a heterozygous deletion. (c) Patients 7 & 8: exons 8-14 have probe ratios of >1.8, representing a homozygous duplication (4 copies of each exon). (d) Patient 6: exons 17-22 have probe ratios of approximately 0.5 representing a heterozygous deletion.

Table 5.20: Clinical details and genetic results of patients with confirmed exon deletions or duplications in the MLPA study (* sequence variant - non-pathogenic mutation based on expression studies)

	Age/ Gender	Clinical Details	Family History	Investigations	CLCN1 sequence analysis	CLCN1 MLPA
Patient 5	40yr/ Male	Italian. Onset at 12yrs. Stiffness of legs with warm up. Calf hypertrophy and myotonic grip	No family history	EMG: Myotonia, SET: Fournier type II CK 576 IU	c.1167-10T>C; p.? (intron 10)	Deletion of exons 8-10
Patient 6	15yr/ Male	Polish. Onset in childhood. Generalised limb and tongue myotonia with warm-up. No weakness	No definite family history	EMG: Myotonia CK: Normal	c.2680C>T; p.Arg894X (exon 23)	Deletion of exons 17-22
Patient 7	31yr/ Female	Iraqi. Onset from infancy. Leg stiffness with warm-up. Myotonic grip & mild proximal weakness	Consanguinity, father and 3 siblings affected.	EMG: Myotonia	c.2230C>A;p.Pro744Thr homozygous (exon 18) (*sequence variant)	Homozygous duplication of exons 8-14
Patient 8	30yr/ Female	Iraqi. Onset at 1yr. Arm and leg stiffness with warm-up. Calf hypertrophy, grip and percussion myotonia. Mild weakness.	Recessive pedigree. No consanguinity.	EMG: Myotonia SET: Fournier type II CK: normal	c.2230C>A;p.Pro744Thr homozygous (exon 18) (*sequence variant)	Homozygous duplication of exons 8-14

indicating the presence of oedema in that muscle (Figure 5.39).

Genotype of Patient 5

Altogether the clinical picture and pedigree were consistent with recessive MC, DNA sequencing of CLCN1 identified a heterozygous intronic point mutation, c.1167-10T>C; p.?, which has previously been described in recessive pedigrees (Trip et al., 2008). This mutation did not lie within the MLPA probe binding region for exon 11. MLPA identified peak ratios of approximately 0.5 in exons 8, 9 and 10 (Figure 5.37b). Exons 7 and 11 had normal peak ratios. This data is consistent with a contiguous deletion of exons 8-10. Break point analysis performed by the lab confirmed the deletion as c.854-363_1077del;p.?

5.3.2.2 Patient 6

Phenotype of Patient 6

Patient 6 was a 13 year old Polish boy with generalised stiffness from childhood and warm-up phenomenon. His paternal grandfather was reported to have grip stiffness, but a clinical examination report from Poland of the asymptomatic father was negative for both clinical and electrical myotonia. The mother was also asymptomatic by her own history and on clinical examination. A half-sister and half-brother on the maternal side were clinically unaffected. On examination there was calf hypertrophy and generalised percussion myotonia but no weakness. Investigations demonstrated a normal CK and the EMG demonstrated myotonia.

Genotype of Patient 6

The patient's pedigree and clinical phenotype were consistent with recessive MC. CLCN1 sequencing of the patient's DNA identified the previously published mutation c.2680C>T;p.Arg894X (George et al., 1994). The unaffected mother carried the Arg894X mutation only. The father was not resident in the UK and therefore it was not possible to test him. MLPA in the patient identified a contiguous deletion of exons 17-22 with a minimum size of 4901-9268 base pairs (Figure 5.37d).

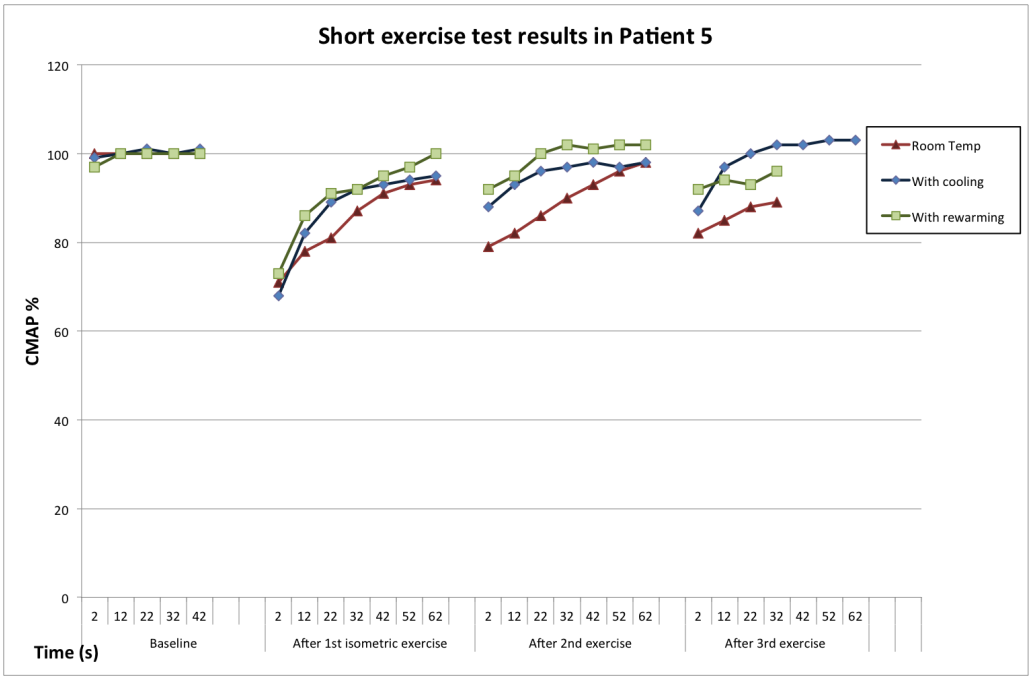
5.3.2.3 Patient 7

Phenotype of Patient 7

Patient 7 was a female patient from Northern Iraq with muscle stiffness and warm-up from infancy. She had also developed mild proximal weakness in childhood. Her three siblings and father were similarly affected. In addition, four out of seven of her father's siblings had similar symptoms. She was from a consanguineous pedigree, her parents were second

Figure 5.38: Short exercise test results of patients 5 & 8 from MLPA study a) Patient 5 b) Patient 8. (Both graphs illustrate significant transient decrement (>20%) post-exercise which rapidly normalises and shows a less significant decrement with subsequent trials of isometric exercise)

a)



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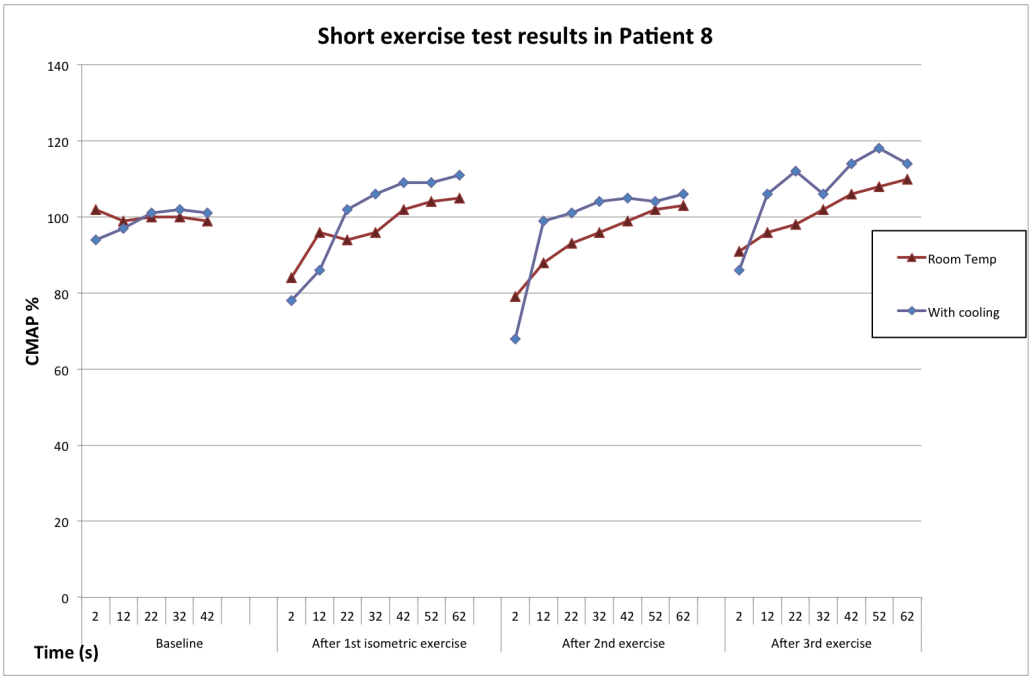


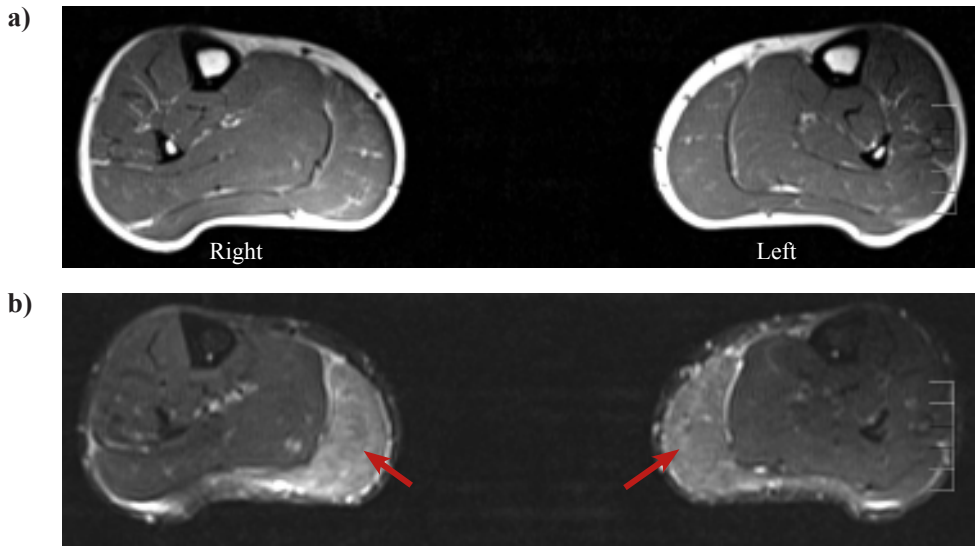


Figure 5.39: MRI scan of calves of Patient 5 (a) T1 weighted image of calves (b) STIR sequence, shows high signal in the medial head of the gastrocnemius bilaterally suggesting the presence of oedema (indidcated by red arrows)

cousins and paternal grandparents were first cousins consistent with a recessive pedigree. On examination she had severe generalised myotonia demonstrable on percussion and grip. There was mild proximal muscle weakness (MRC score 4/5). EMG showed myotonia and the short exercise test revealed a Fournier type II pattern.

Genotype of Patient 7

CLCN1 exon sequencing identified the homozygous sequence variant, c.2230C>A; p.Pro744Thr, not previously described in the literature. This variation was not found in the UK control samples but was predicted by PolyPhen2 and SIFT to be non-pathogenic. Expression studies were therefore performed by J.Burge in HEK293T cells to assess pathogenicity. Both WT and mutant Pro744Thr yielded fast deactivating inward currents in HEK cells (Figure 5.40a & b). Current magnitude varied depending on the amount of vector taken up by the cell being studied (as indicated by brightness of fluorescence). Brightly fluorescing cells produced currents in the 1020nA range. In contrast to published studies of dominant mutations, which strongly reduce or eradicate channel activity, there was no systematic difference when comparing Pro744Thr with WT CLCN1 (Figure 5.40c), suggesting that this variation was non-pathogenic and did not cause the patient's phenotype.

MLPA identified relative peak ratios of approximately 1.8 in exons 8 to 14 consistent

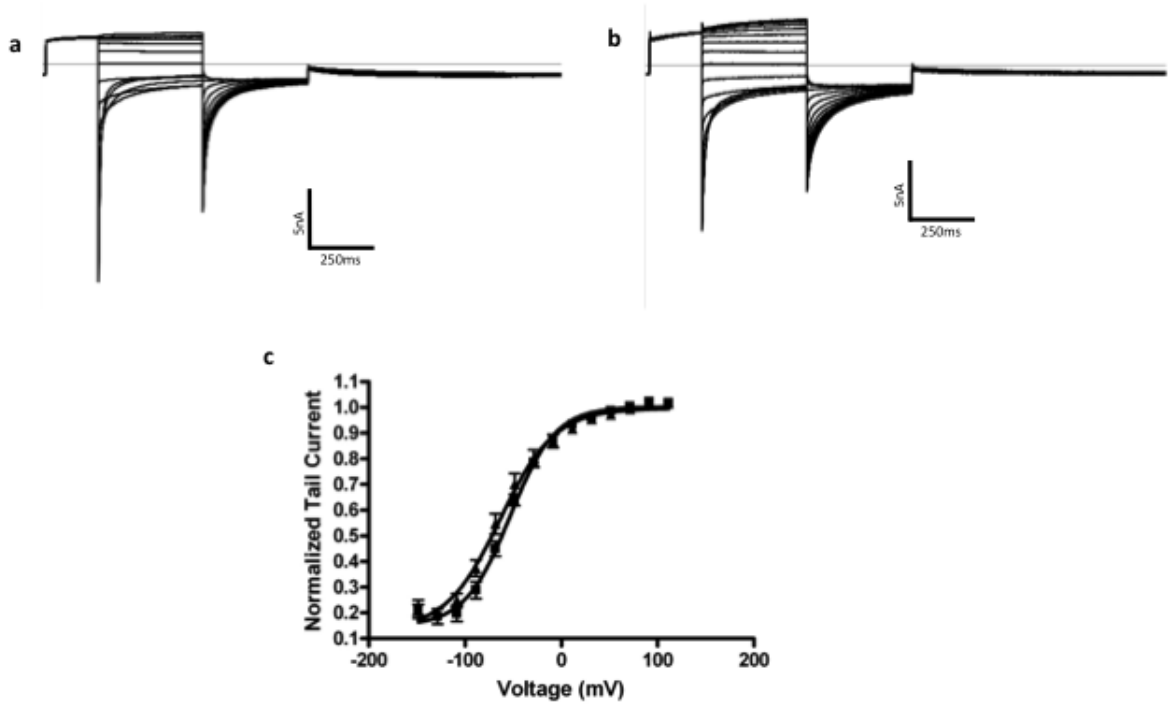


Figure 5.40: Functional expression of wild-type ClC-1 and Pro744Thr mutation by whole-cell patch clamp of transfected HEK293T cells (a) Representative wild-type recording (2mOhm series resistance, 70% series resistance compensation). (b) Representative mutant recording (3mOhm series resistance, 70% series compensation) (c) Boltzmann fits of the normalized tail currents from 6 wildtype (squares) and 4 mutant (triangles) recordings to show the similar voltage dependence of activation (figure supplied by J.Burge)

with either a homozygous duplication or a heterozygous triplication (Figure 5.37c).

5.3.2.4 Patient 8

Phenotype of Patient 8

Patient 8 was an Iraqi female with stiffness affecting the arms and legs at one years old. She had warm-up phenomenon and was resistant to anti-myotonic treatment including mexiletine. She also developed mild proximal muscle weakness. There was a strong family history with two out of six siblings affected. Her parents were distant cousins. Neither of the patient's two children were affected. A nephew from a consanguineous kindred was also reported to be affected suggesting a recessive pedigree. On examination she had generalised muscle hypertrophy and grip and percussion myotonia. There was mild proximal muscle weakness (MRC score 4/5). Her investigations included a normal CK. EMG studies demonstrated myotonic discharges and the short exercise test demonstrated a Fournier type II pattern (Figure 5.38b).

Genotype of Patient 8

CLCN1 sequencing identified the same homozygous sequence variant c.2230C>A; p.Pro744Thr as identified in patient 7. The patients were not known to be related but both originated from Iraq. The six intragenic SNPs in both patients 7 and 8 were consistent with the mutation being on a shared haplotype but heterozygosity of the SNPs studied was high. MLPA analysis of patient 8 also demonstrated the same homozygous duplication/heterozygous triplication of exons 8-14 as detected in patient 7 with relative peak ratios of approximately 1.9 (Figure 5.37c).

5.3.3 Diagnosing Genetically Unconfirmed Cases using aCGH and Whole Exome Sequencing

Overall DNA from 10 patients were analysed using whole exome sequencing. All patients had no mutations identified but objective evidence of a skeletal muscle channelopathy.

Of the 10 patients, seven (70%) had likely causative pathogenic variations identified. Two had variations identified in the known channelopathy genes and the others had variations in unusual genes which were likely to underlie their phenotype.

5.3.3.1 Case 9

Phenotype of Case 9

The first case I analysed was a 65 year old man with stiffness primarily of the hands, arms and legs but also in the face since he was 5 years old. The stiffness was severe with only mild pain. He had warm-up phenomenon but no sensitivity to cold or particular foods. He had a good response to mexiletine. He also had hypertension, hypercholesterolaemia and type II diabetes mellitus. His family history included one affected older brother and an unaffected sister and brother. His parents and grandparents were unaffected. His daughter and her four sons were unaffected as were their children. His parents were not known to be consanguineous although they were from the same community. On examination he had generalised calf hypertrophy and hand grip myotonia. He did not have any eyelid myotonia and had normal strength.

He had a normal ECG and his CK was elevated at 485IU/L. His electrophysiology demonstrated prominent myotonic discharges in all limb muscles. The short exercise test showed a marked decrement on initial exercise which improved over time and with subsequent exercise. There was less decrement seen on rewarming than cooling (Figure 5.41). The long exercise test was normal. This pattern is most suggestive of MC. His MRI

Table 5.21: Table of total number of variants identified in whole exome sequencing cases

	Variants identified in genome	Novel variants identified in genome	Variants identified in exome	Novel variants in exome	Novel Indels in exome	Novel stop gain/loss variants in exome	Novel nonsynonymous variants in exome
Case 9	203331	13628	18573	863	173	12	392
Case 10	207646	12763	18312	671	173	8	278
Case 11	205731	12341	18192	616	187	9	246
Case 12	190372	10491	18126	593	153	5	245
Case 13	188725	10679	18139	594	144	7	263
Case 14	199977	11793	18338	663	183	3	272
Case 15	416148	10376	22539	348	123	3	114
Case 16	194199	11653	18038	692	165	6	283
Case 17	250363	6211	21939	352	90	6	153
Case 18	344866	7829	22198	313	94	4	129

scan demonstrated a mild degree of diffuse symmetrical fatty infiltration of the thigh and calf muscles. The distal part of the medial head of the gastrocnemius muscle was completely replaced by fatty tissue. The was no evidence of oedema (Figure 5.42).

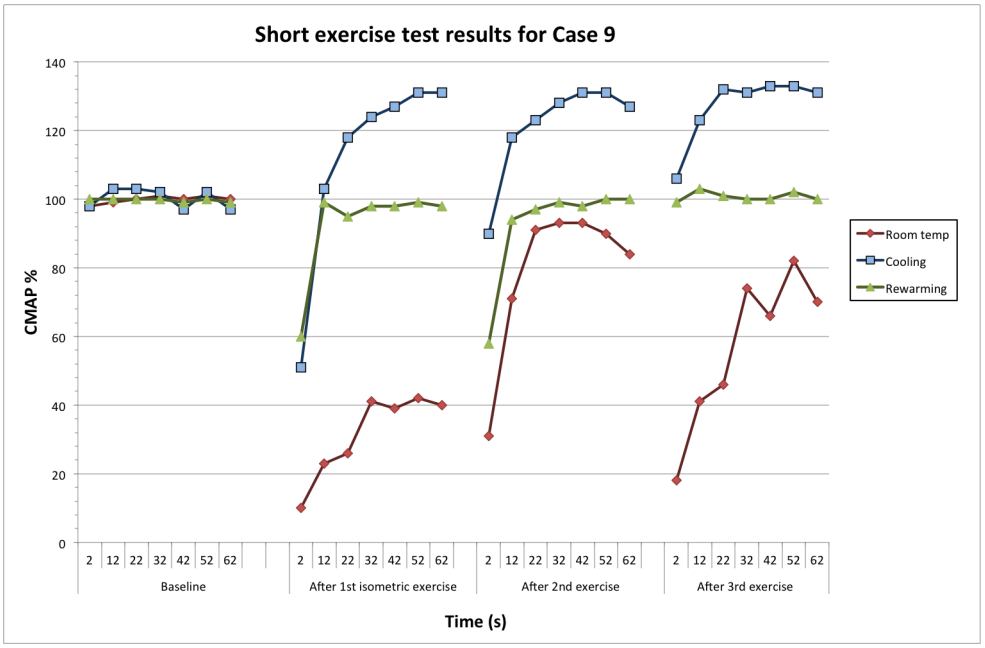


Figure 5.41: Graph of short exercise test for Case 9 Red diamonds represent CMAP percentage at room temperature, blue squares represent CMAP percentage after cooling and green triangles represent CMAP percentage after rewarming

Given the pattern of stiffness, neurophysiology and MRI it was consistent with recessive MC. However, full sequencing of CLCN1, SCN4A and MLPA of CLCN1 did not reveal any pathogenic variants. Testing for DM1 and DM2 expansions was also normal. This patient was therefore selected for next generation sequencing.

aCGH for Case 9

This patient had aCGH using the 720K array. 112 anomalies were detected of which six were novel and located within the exome (Table 5.22). None of these six anomalies were in ion channel genes expressed in skeletal muscle. The patient therefore did not have any relevant CNVs that could explain his phenotype.

Whole Exome Results for Case 9

The whole exome sequencing identified 203,331 SNVs in the exome and surrounding parts of the genome. Of these, 863 were found in the exome and not identified in dbSNP or 1000 Genomes (Table 5.21). These variations were then filtered for those located in the known channelopathy genes. One homozygous variation was identified in the 5'UTR region

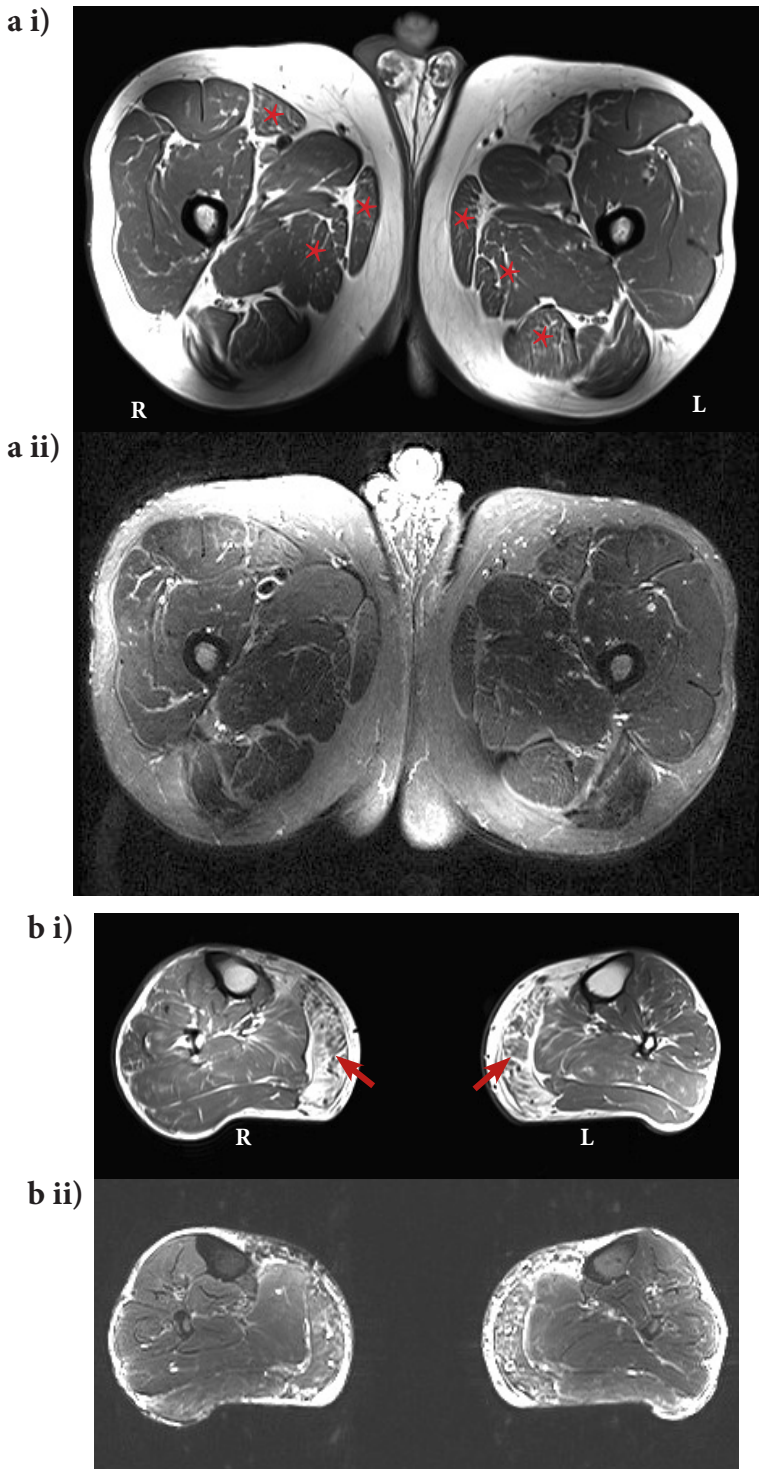


Figure 5.42: MRI scan of thighs and calves of Case 9 ai) T1 weighted image of thighs with mild fatty infiltration primarily in posterior compartment (most affected muscles indicated by red $\star$) aii) STIR sequence of thighs, bi) T1 weighted image of calves with complete fatty replacement of medial gastrocnemius (indicated by red arrows) bii) STIR sequence of calves.

Chr	Size of copy number variation	Loss/ Gain	Average ratio	Genes	Gene function
1	1q41(218,155,426-218,179,977)	Gain	1.42	RNU5F-1, SLC30A10	manganese transporter
5	5q31.1(131,567,703-131,572,738)	Loss	0.74	P4HA2	catalyses posttranslational formation of 4-hydroxyproline in collagens
8	8q12.1(56,177,665-56,178,359)	Loss	0.75	XKR4	unknown
10	10q25.2(114,700,599-114,701,271)	Loss	0.56	TCF7L2	diabetes mellitus susceptibility
17	17q12(34,352,565-34,364,646)	Gain	1.40	FBXO47	associated with papillary cell carcinoma
18	18q21.2(50,004,390-50,004,848)	Loss	0.65	MBD2	T helper cells and IL4 induction

Table 5.22: Table of novel copy number variations identified by aCGH for Case 9

of CLCN1 that has not been previously reported, c.1-59C>A (g.143013247C>A) (Table 5.23). Its presence was confirmed by Sanger sequencing of exon 1 by the genetics unit at NHNN. It was not identified in the most current versions of exome variation server, dbSNP or 1000 Genomes. It was also absent in 504 control chromosomes. It was not identified in the original sequencing of CLCN1 for this patient as it was located under the forward primer for exon 1. Although this region is conserved in primates it is not highly conserved in other groups.

Gene	Type of variation	Variation	Hetero/ homozygous	Associated disease	Expression in skeletal muscle	*in silico* prediction
Muscle Disease Genes						
CLCN1	**Nonsyn**	**c.1-59C>A (g.143013247C>A)**	**Homo**	**Myotonia congenita**	**High**	
DYSF	NFmS insertion	c.3139_3140insAGGCGG: p.Q1047delinsQAE	Hetero	LGMD2B, AR	High	
BAG3	Nonsyn	c.418G>A:p.G140S	Hetero	Myofibrillar Myopathy, AD	High	Tolerated
TTN	Nonsyn	c.16707T>A:p.H5569Q	Hetero	LGMD2J, AR	High	Tolerated
TTN	Nonsyn	c.12997A>G:p.K4333E	Hetero	LGMD2J, AR	High	Tolerated
Ion channel genes						
GRIN2A	Nonsyn	c.3482G>C:p.G1161A	Hetero	Epilepsy	None	
ALG10	Nonsyn	c.887C>T:p.S296L	Hetero	Reduced susceptibility to LQTS	Medium	Tolerated
ANO10	Nonsyn	c.647A>G:p.Y216C	Hetero	SCA10, AR	Medium	Tolerated
ANO7	Nonsyn	c.2234G>T:p.R745L	Hetero		Low	Tolerated
SCN9A	Nonsyn	c.1741A>G:p.N581D	Hetero		None	Damaging
KCNH6	Stop gain	c.2176C>T:p.R726X	Hetero		None	

Table 5.23: Table of novel variations identified by whole exome sequencing in Case 9

The remainder of the exome data was analysed to ensure no other likely variations were identified. When data was filtered for muscle disease causing genes, four variations in three different genes were identified (Table 5.23). Variations in DYSF are associated

with LGMD type 2B but both alleles need to be affected to cause disease and only one was identified in this case. Two variations were identified in TTN which is associated with LGMD type 2J. Both of these are predicted to be benign or tolerated by PolyPhen2 and SIFT. The phenotype of LGMD2J is also very different from this patient, as it is normally associated with cardiomyopthy and a tibial muscular dystrophy. I did however, identify a variation in BAG3, c.418G>A;p.Gly140Ser (Table 5.23). There have been reports of patients with autosomal dominant myofibrillar myopathy type 6 with variations in BAG3 having electrical myotonia but this variation is predicted by PolyPhen2, SIFT and mutation taster as having a very high probability of being a polymorphism. This makes it likely that the causative variation is the 5'UTR homozygous variation in CLCN1.

5.3.3.2 Case 10

Phenotype of Case 10

The second case was a 66 year old man who was well until he was 17 years old when he began to notice occasional episodes of shoulder abduction weakness. His legs were unaffected at that stage. 14 years later, at 31 years old, he had his first significant episode of weakness when his legs gave way after getting up from a chair. This was associated with significant pain and lasted four days. From this point on he continued to have attacks approximately once a month. Attacks primarily occurred in the evenings or night-time. Severe attacks would involve arms, legs and neck usually lasting approximately eight hours. His speech, swallow, breathing and eye movements were all normal during attacks. There were no obvious precipitants. Unfortunately a potassium level was never recorded during an attack. The attacks were at their worst in his mid to late 40s but tailed off in the last 10 years and reverted to progressive weakness with a significant painful component. He did not find any improvement with treatment with acetazolamide. At the point of review he could only mobilise to 50 yards without stopping.

His past medical history includes three myocardial infarctions, the last eight years ago. He had hypertension diagnosed 20 years ago and type 2 diabetes mellitus diagnosed around the same time. He also had a history of migraine. He was on metformin, insulin, carvedilol, felodipine, prednisolone, omeprazole, aspirin and ezetamibe. His parents were unaffected, his sister had hypertension and was diagnosed with fibromyalgia, his brother was unaffected. He had two daughters, the older daughter was diagnosed with fibromyalgia. His younger

daughter had hypertension and in her 30s had episodes of weakness after the delivery of each of her children but has refused all medical investigations.

On examination he had marked proximal wasting and weakness of arms and legs with shoulder abduction MRC grade 4+ and hip flexion MRC grade 4. He was unable to rise from a chair unaided and needed a stick to mobilise. His reflexes were absent at the knees but were otherwise normal. Investigations demonstrated a mildly elevated CK of 270IU/L. His mitochondrial respiratory chain enzymes were also normal. Electrophysiology done elsewhere demonstrated a normal short exercise test but an abnormal long exercise test with a 45% decrement . There was no myotonia on EMG but some myopathic changes. Single fibre EMG was normal. MRI demonstrated complete fatty replacement of circumscribed areas of the vastus muscles, many of which had a ring of oedema around them. There was also some fatty infiltration of the tibialis anterior and soleus bilaterally and mild oedema of the gastrocnemius bilaterally (Figure 5.43).

His biopsy demonstrated variation of fibre size and and scattered atrophic fibres (Figure 5.45a). There was a mild focal increase in endomysial connective tissue. A high proportion of fibres had multiple basophilic inclusions of varying size (Figure 5.45b). These inclusions stained strongly for NADH (Figure 5.45c) and electron microscopy confirmed these were tubular aggregates (Figure 5.45i & j). Two rimmed vacuoles of unclear significance were also identified but did not contain any protein aggregation (Figure 5.45e, f, g & h).

I was also able to fully assess the patient's eldest daughter. She was 35 years old with a history of two episodes at night in her early twenties of waking unable to move her right arm. She had weakness of the arm for two days with full recovery of strength afterwards. Following that she had a number of mild episodes of weakness in the arms and legs but was still able to mobilise independently and these recovered fully. She also had longstanding problems with lower back and neck pain. She was not known to have hypertension and was on gabapentin, meloxicam and citalopram. She had one unaffected child. On examination she had very subtle proximal weakness in the upper and lower limbs. Her CK was normal at 54IU/L. Her EMG and long exercise test were normal with a decrement of 24% from peak. Her MRI demonstrated mild fatty infiltration of the adductors of the thigh and atrophy of the lateral head of gastrocnemius in the calf. There was no evidence of oedema (Figure 5.44).

Given the episodic weakness and positive long exercise test, S4 segments of CACNA1S, all exons of SCN4A and KCNJ2 were tested but no pathogenic variations were found.

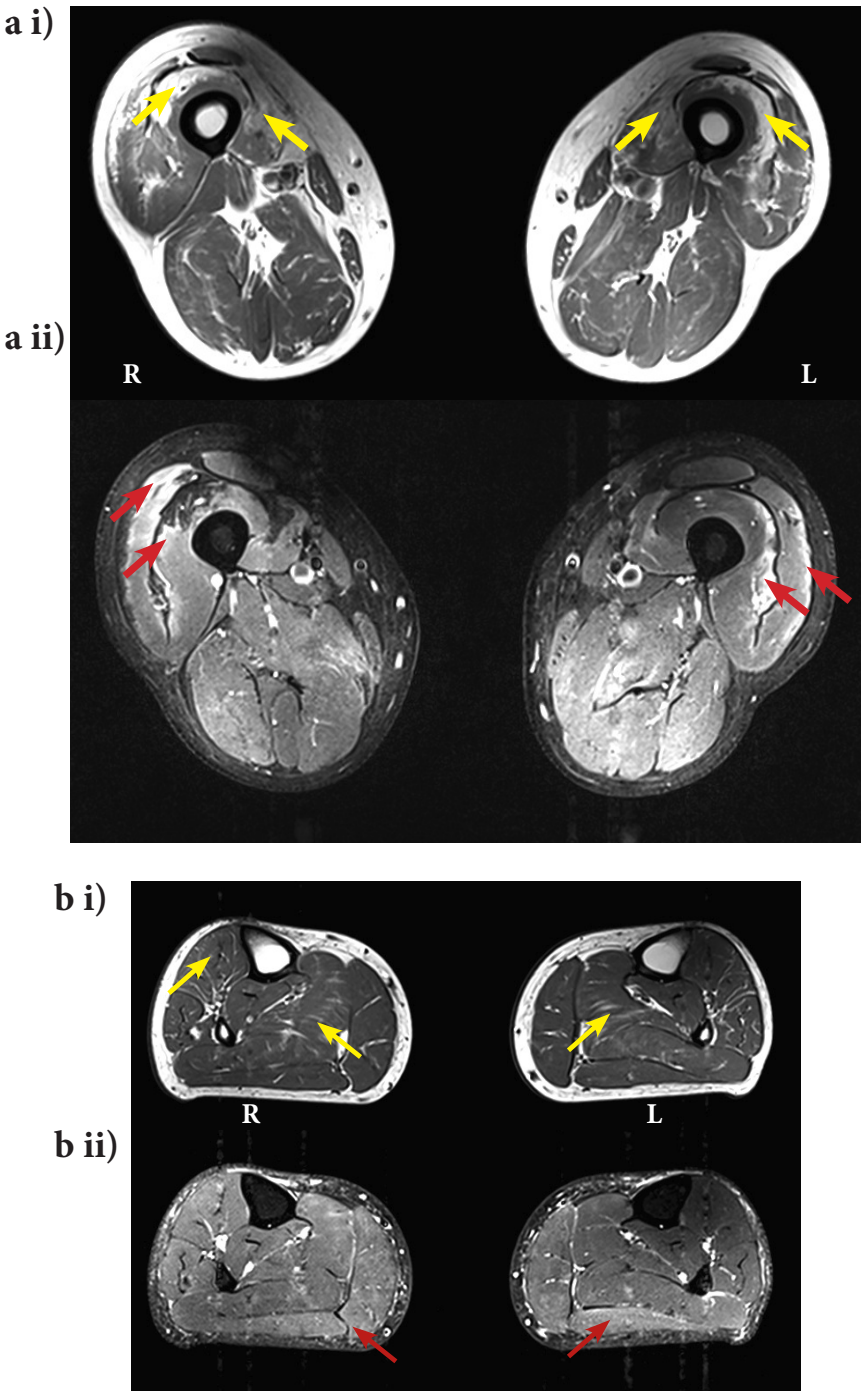


Figure 5.43: MRI scan of thighs and calves of Case 10 ai) T1 weighted image of thighs with fatty infiltration in areas of vastus muscle (indicated by yellow arrows) aii) STIR sequence of thighs with ring of oedema around vastus muscle (indicated by red arrows), bi) T1 weighted image of calves with mild fatty infiltration of tibialis anterior and soleus (indicated by yellow arrows) bii) STIR sequence of calves with mild oedema of the gastrocnemeii (indicated by red arrows).

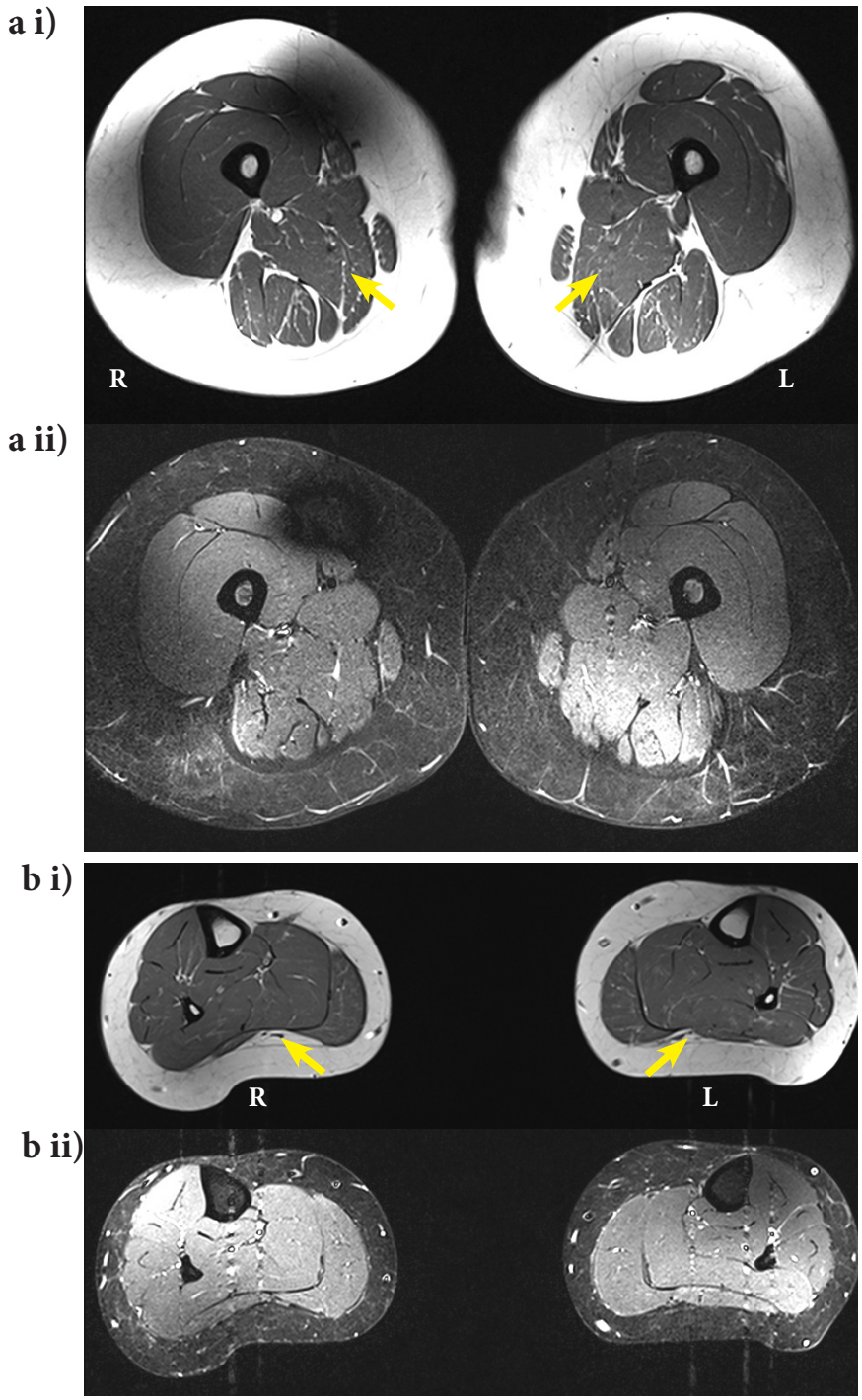


Figure 5.44: MRI scan of thighs and calves of daughter of Case 10 ai) T1 weighted image of thighs showing mild fatty infiltration of adductors (indicated by yellow arrows) aii) STIR sequence of thighs, bi) T1 weighted image of calves with atrophy of lateral head of gastrocnemius (indicated by yellow arrows) bii) STIR sequence of calves

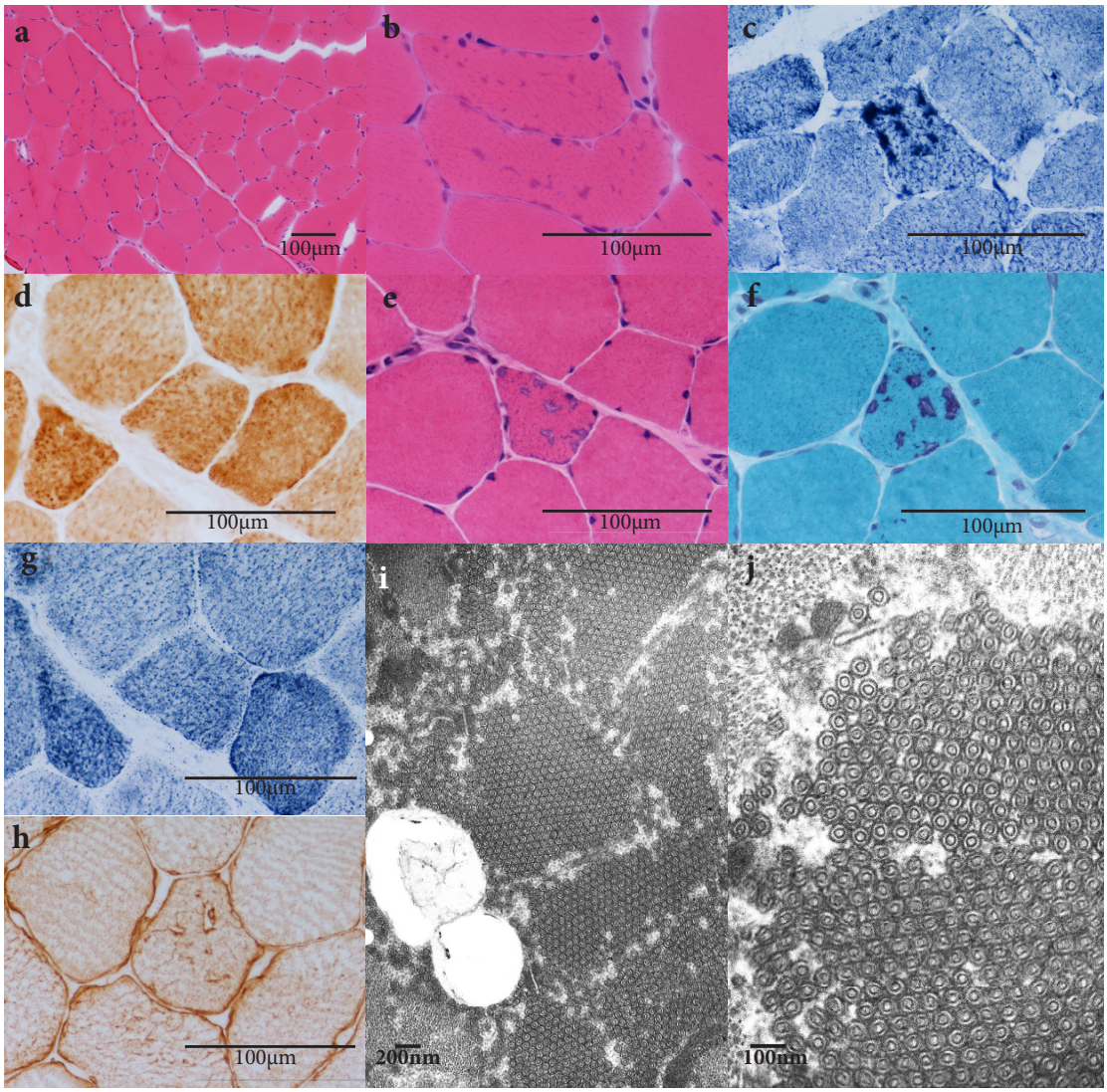


Figure 5.45: Muscle biopsy of vastus lateralis of Case 10 a) Haematoxylin and eosin stain showing variation in fibre size b) Haematoxylin and eosin stain at higher power demonstrating tubular aggregates c) NADH staining of tubular aggregates d) COX stain showing no staining of tubular aggregates e) Haematoxylin and eosin stain at higher power demonstrating rimmed vacuoles f) Gomori trichrome stain demonstrating rimmed vacuoles g) SDH stain showing no staining of rimmed vacuoles, h) Desmin stain showing no staining of rimmed vacuoles, i) Electron microscopy of tubular aggregates j) High power electron microscopy of tubular aggregates

Mitochondrial DNA analysis was also normal. He was therefore selected for next generation sequencing. Although aCGH was performed the quality of DNA was too poor to use.

Whole Exome Results for Case 10

Overall 207,646 SNVs were identified in the exome and surrounding parts of the genome. Of these, 671 were novel and located within the exome (Table 5.21). No novel variations were identified in the known channelopathy genes. I then looked at variations in known muscle channel genes and identified 2 novel variations (Table 5.24). The first variation was located in CRYAB, the gene for αB-crystallin, at location c.460G>A;p.Gly154Ser. Pathogenic variations in this gene have been associated with myofibrillar myopathy. This particular variation was previously reported in the literature in a patient with a late-onset distal myopathy (Reilich et al., 2010). The reported case had no history of episodes of weakness and had a much later onset of a primarily distal myopathy, different from Case 10. However, this variation has also been reported in dbSNP as having uncertain significance (rs150516929). SIFT and PolyPhen2 both predict that the variation is benign but Mutation taster suggests it is pathogenic, possibly because it has been published as pathogenic. The second variation identified was in TTN which is associated with LGMD type 2J causing an autosomal dominant late-onset distal myopathy. Again the phenotype of Case 10 was different from the cardiomyopathy and tibial muscular dystrophy that is seen in TTN mutations. This variation was predicted *in silico* to be a polymorphism.

Gene	Type of variation	Variation	Hetero/ homozygous	Associated disease	Expression in skeletal muscle	*in silico* prediction
Muscle Disease Genes						
CRYAB	**Nonsyn**	**c.460G>A:p.G154S**	**Hetero**	**Myofibrillar Myopathy**	**High**	**?tolerated (published as pathogenic)**
TTN	Nonsyn	c.13948C>T:p.P4650S	Hetero	LGMD2J, AR Late onset distal myopathy, AD	High	Tolerated
Ion channel genes						
SCNN1B	**Deletion**	**c.1307_1310del: p.436_437del**	**Hetero**	**Liddles Disease**	**None**	**Pathogenic**

Table 5.24: Table of novel variations identified by whole exome sequencing in Case 10

As there was some question as to the pathogenicity of the variation in CRYAB, I also looked at the variations located in ion channel genes. Only one variation was identified that was not seen in other patients from my exome panel or in the updated

version of dbSNP (Table 5.24). This variation was a frameshift deletion in SCNN1B, c.1307_1310delAGAG;p.Arg438PfsX17 which would prematurely terminate the transcript. SCNN1B encodes an amiloride-sensitive sodium channel β subunit that is specifically expressed in the kidney, acting as a distal renal sodium channel. The β subunit augments the channel activity of the α subunit which is responsible for conductance of sodium ions (Canessa et al., 1994). Variations in this gene are associated with Liddle's syndrome (Shimkets et al., 1994). This syndrome is characterised by hypertension, metabolic alkalosis and hypokalaemia due to excessive sodium reabsorption in the distal nephron and therefore could potentially cause secondary HypoPP (Liddle et al., 1963).

I confirmed the presence of both the CRYAB and SCNN1B variations with Sanger sequencing in both the patient and his eldest daughter (Figure 5.46). His youngest daughter declined genetic testing. Given the possible diagnosis of Liddle's syndrome I also tested plasma aldosterone which was low at 122pmol/L (600-1200) and renin which was normal 2.67nmol/h/l (1.2-4.4). Bicarbonate was normal 22mmol/L. Urine aldosterone was also normal at 19 nmol/24hr (10-50). Plasma aldosterone and renin levels in the proband's daughter however, were normal at 954pmol/L and 2.23nmol/h/l respectively. There are therefore two possible pathogenic variations that may be responsible for the patient's disease.

5.3.3.3 Case 11

Phenotype of Case 11

Case 11 was a 27 year old right handed female. She developed recurrent attacks of weakness from 24 years old. Her first episode was of bilateral leg weakness one evening after a 45 minute period of sitting whilst at a bar. Her weakness recovered the next day although she continued to have mild heaviness of her left leg. Her next episode was a year later when, after an asthma attack and using her salbutamol inhaler several times, she developed marked bilateral weakness which resolved after two hours. Her potassium was recorded as normal during this attack. Following that episode she continued to have minor attacks every couple of months which were always during the day and usually after a period of exertion. She had never awoken with weakness or had night-time attacks. Her attacks improved with acetazolamide. She also has a history of palpitations from 24 years old but no associated syncope or chest pain. She was the product of a normal pregnancy and delivery and reached all her developmental milestones at the appropriate age. She was, however, very clumsy at

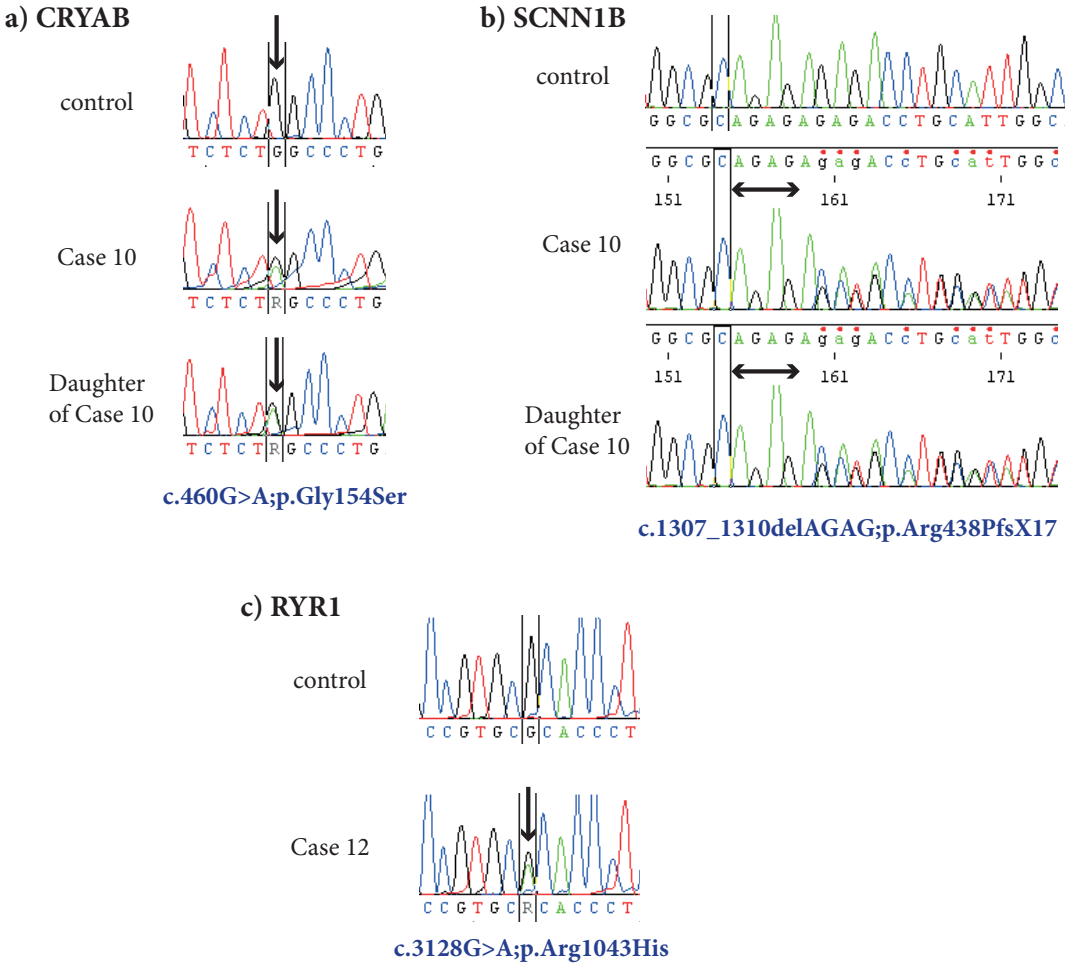


Figure 5.46: Electropherogram for variants that were Sanger sequenced to confirm the findings from whole exome sequencing a) CRYAB sequencing demonstrating variant c.460G>A;p.Gly154Ser present in case 10 and his daughter, b) SCNN1B sequencing demonstrating frameshift deletion c.1307_1310delAGAG;p.Arg438PfsX17 in case 10 and his daughter, c) RYR1 sequencing demonstrating variant c.3128G>A;p.Arg1043His identified in case 12.

school and poor at sports. Her past medical history was of asthma and migraine.

Her family history included a 32 year old sister who also had palpitations and was found to have a prolonged QT interval of 580ms. She never had any attacks of weakness, no skeletal abnormalities and had a normal long exercise test. The proband's mother and two of her mother's 11 siblings all had epilepsy. Her father and her grandparents were unaffected.

On examination the proband was of small stature and had low set ears. She had subtle clinodactyly and syndactyly of the second and third toes and looked very different from her sister. She had mild proximal weakness, although there was some functional overlay to her weakness. Her reflexes were normal. Her blood tests were normal including potassium during attacks. Her CK was 58IU/L. Her ECG demonstrated U waves and a QTc of 460ms, the upper limit of normal for a woman, although in the past it had been recorded as 500ms. Her 24 hour tape was normal, as was her echocardiogram. Electrophysiology testing was initially abnormal with a CMAP decrement of 51% of the amplitude and a corresponding decrement of area of 59%, however repeated testing at our centre was normal. MRI of thighs and calves were normal (Figure 5.47).

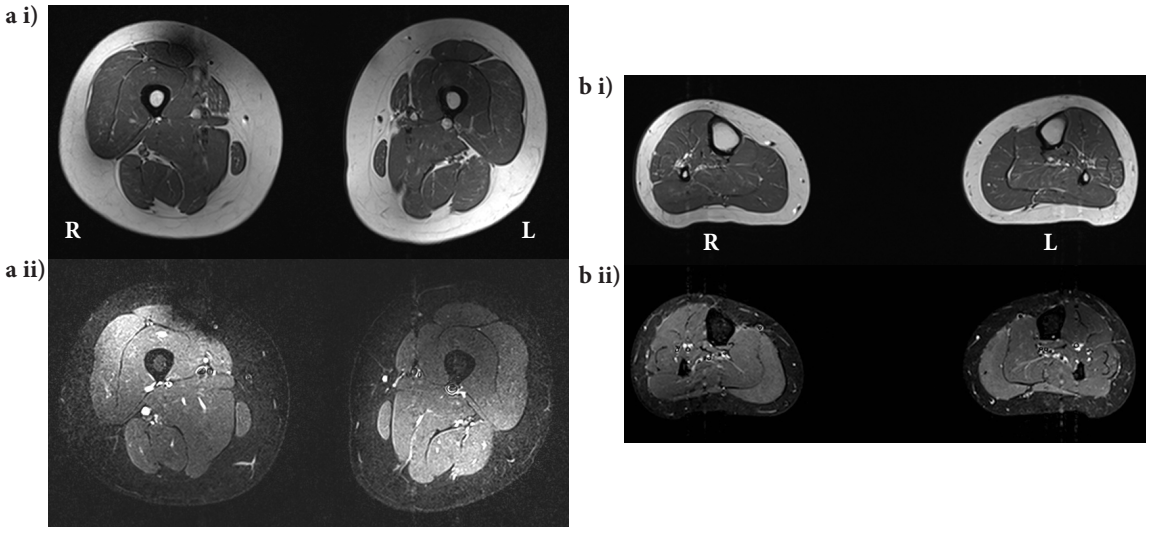


Figure 5.47: MRI scan of thighs and calves of Case 11 ai) T1 weighted image of thighs aii) STIR sequence of thighs, bi) T1 weighted image of calves bii) STIR sequence of calves.

The positive long exercise test, episodes of weakness, subtle dysmorphic features and borderline QT interval suggested a diagnosis of ATS. However, sequencing and MLPA of KCNJ2 was negative as was sequencing of SCN4A exons and S4 segments of CACNA1S.

She was therefore selected for next generation sequencing.

aCGH in Case 11

Array CGH was performed using the 1.4M array. 106 anomalies were identified of which 28 were novel anomalies. Of these, seven were located within the exome and not detected in other samples from the same analysis set (Table 5.25). The only CNV located in a relevant gene was a gain of copy number of the MRPL44 gene. This was a single gain of approximately 16,052 base pairs long and included the entire MRPL44 gene (Figure 5.48). This gene is a large subunit of the mitochondria ribosome. Homozygous variations and compound heterozygous variations in the gene have been documented as causing childhood hypertrophic cardiomyopathy (Carroll et al., 2013). However, as this is a heterozygous CNV it would only cause disruption to expression of the MRPL44 gene from one allele and therefore would be unlikely to be responsible for the patient's phenotype.

Chr	Position of copy number variation	Width	Loss/ Gain	Average ratio	Genes	Gene function
2	2q36.1(224,818,710-224,834,762)	16,052	Gain	1.31	MRPL44, SERPINE2, WDFY1	large subunit of the mitochondrial ribosome
7	7p14.3(29,594,671-29,605,644)	10,972	Loss	0.74	PRR15	
8	8q13.2(68,132,759-68,143,371)	10,612	Gain	1.30	ARFGEF1	regulate vesicular traffic
11	11p15.1(17,896,787-17,917,301)	20,513	Loss	0.75	SERGEF	
18	18q12.1(28,883,480-28,916,303)	32,823	Gain	1.33	DSG1	
19	19q12(31,791,099-31,807,952)	16,853	Loss	0.80	TSHZ3	neuronal and smooth muscle differentiation
X	Xq13.1(71,349,754-71,357,559)	7,804	Loss	0.69	FLJ44635, NHSL2, RGAG4	

Table 5.25: Table of novel copy number variations identified by aCGH for Case 11

Whole Exome Results in Case 11

Overall 205,731 SNVs were identified. Of these 616 were not in dbSNP (Table 5.21). No novel variations were identified in the known channelopathy genes. I then looked at the variations identified in known muscle disease causing genes and genes associated with long QT syndrome, given the family history of long QT and the patient's borderline prolonged QT. Only two novel variations were identified in muscle disease genes, one in PLEC and one

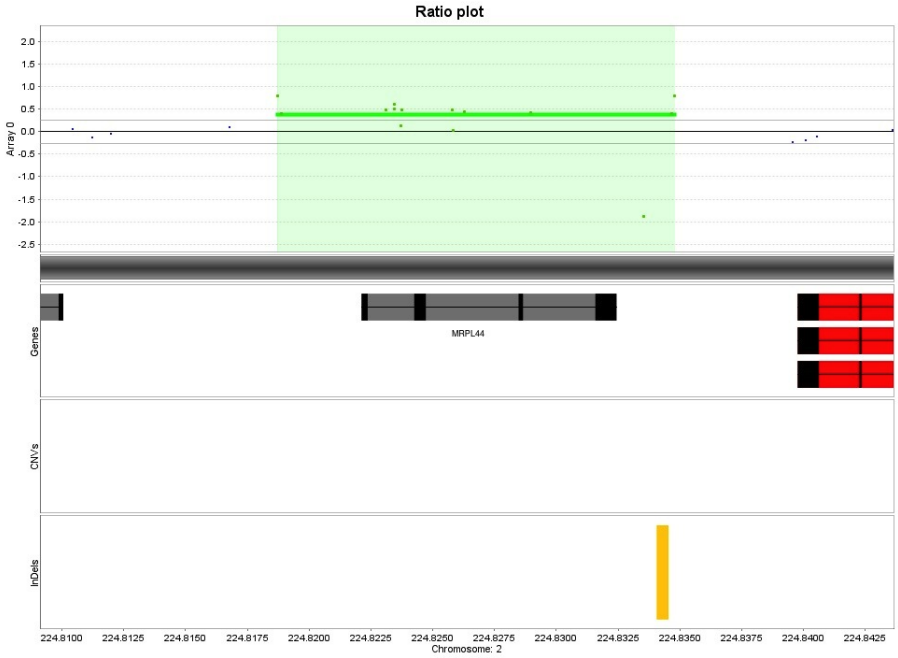


Figure 5.48: ArrayCGH ratio plot with gain in copy number of gene MRPL44 and surrounding region.

in CHRNE (Table 5.26). Both genes are only associated with autosomal recessive diseases, LGMD2Q and congenital myasthenia respectively, and were therefore unlikely to be relevant. However, looking at the long QT genes, two variations were identified including one that has been reported in the literature as pathogenic (Table 5.26). I identified the variation c.805G>A;p.Gly269Ser in KCNQ1. Variations in this gene are associated with LQT1 and this variation has been reported in a number of families with syncope and sudden cardiac death both heterozygously and homozygously expressed (Donger et al., 1997; Ackerman et al., 1999; Murray et al., 2002). The presence of this variation was confirmed by the service laboratory in the patient, her sister and her father by Sanger sequencing. Her father remained unaffected with a normal QTc and cardiac investigations.

Gene	Type of variation	Variation	Hetero/ homozygous	Associated disease	Expression in skeletal muscle	*in silico* prediction
Muscle Disease Genes						
PLEC	Nonsyn	c.7550G>A:p.R2517H	Hetero	LGMD2Q, AR	High	Tolerated
CHRNE	Nonsyn	c.1036C>G:p.L346V	Hetero	Congenital Myasthenia, AR	High	Possibly damaging
Long QT genes						
KCNQ1	**Nonsyn**	**c.805G>A:p.G269S**	**Hetero**	**LQTS**	**Low**	**Known Pathogenic**

Table 5.26: Table of novel variations identified by whole exome sequencing in Case 11

This variation, however does not explain the patient's episodes of weakness as none of the reported cases and the proband's sister and father who carry the variation did not have symptoms of recurrent weakness. I therefore looked at the variations in other ion channels but did not find any other plausible variations.

5.3.3.4 Case 12

Phenotype of Case 12

Case 12 was a 45 year old right handed man who was fit and well until the age of 14. At that point he started having episodes of weakness of the hands, arms and legs following activity, which would last a couple of hours. It would frequently occur after weight training, although he did have a few spontaneous attacks. These episodes were never accompanied by a change in urine colour. He had a number of episodes of waking in the middle of the night feeling that his arms and legs were weak but would recover by the morning. At this point he was having episodes a couple of times per year. In his early twenties however, these became more frequent, with clusters a couple of times a week but with long periods attack free. His first major attack was at 29 years when he awoke at 7am unable to move from the neck down. His potassium level was checked and was 2.31mmol/L and he was given potassium replacement. He returned to normal strength in 10 hours. After this he began to have very frequent attacks of at least one a week where he would wake unable to lift his arms and legs. In the last few years his attacks began to last longer, sometimes taking up to 48 hours to return back to normal strength. His attacks were precipitated by heat, strenuous exercise, eating after 8pm, fasting and alcohol. He was started on regular Sando-K but felt it worsened attacks and on stopping his attack frequency did improve. At 42 years he was started on dichlorphenamide which improved his attack frequency and exercise tolerance considerably. He had been given two general anaesthetics, one at 9 years old when he had no problems and the second at 18 years which did not bring on an attack but he was told he had problems due to volatile anaesthetics which may have been MH. No further details of this were available.

His only relevant family history was of hypertension and vitamin D deficiency. He had a positive family history, with his father also affected although not as severe. He would get weakness after strenuous exercise but never had attacks of complete paralysis. It was not known if his father had problems with general anaesthetic as his father died in his 60s with a heart condition. The proband had one older brother who was unaffected. His

father's siblings and parents were thought to be unaffected. His mother was adopted and therefore had no knowledge of her family history. He has one daughter who was 20 years and currently unaffected. His examination was completely normal with no fixed weakness, facial dysmorphisms or ECG abnormalities.

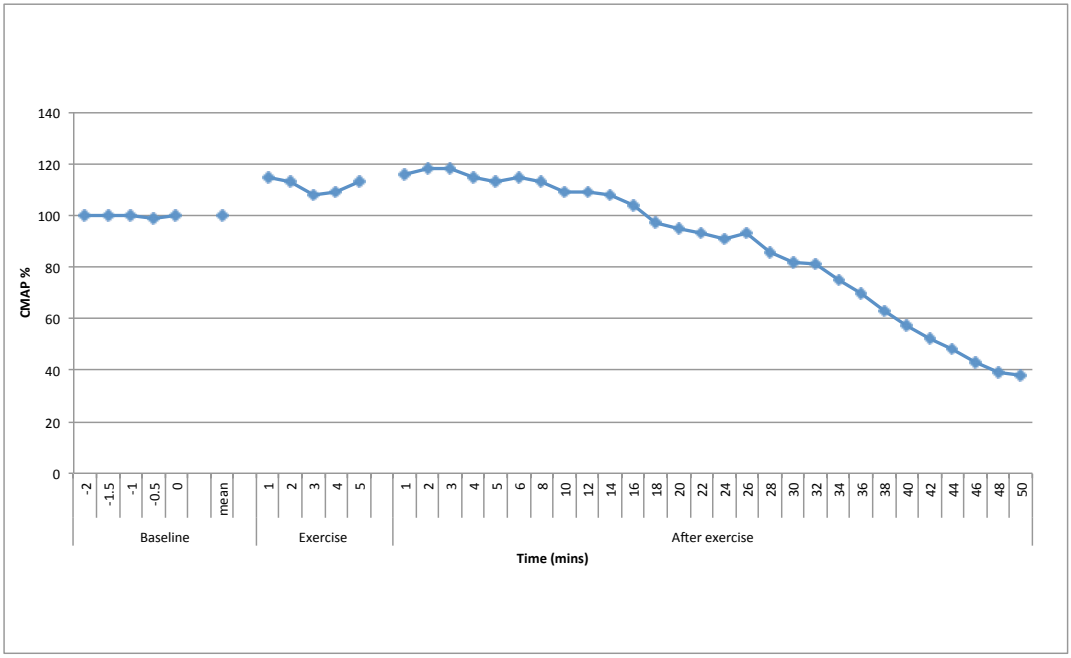


Figure 5.49: Case 12 long exercise test (Positive with 68% decrement from peak)

His blood results between episodes were normal with a CK of 92IU/L and normal electrolytes including interictal potassium level. He had endocrine investigations at his local hospital to try and identify a secondary cause of his episodes of hypokalaemia but they were all negative. His EMG was normal but his long exercise test was markedly abnormal with a decrement of 68% from peak and 62% from baseline (Figure 5.49). His MRI showed mild fatty infiltration in the posterior compartment of the thighs and the calves, more evident in the soleus (Figure 5.50). His needle muscle biopsy demonstrated increased fibre size variation, occasional ring fibres and regenerating fibres and a single cytochrome oxidase (COX)-deficient ragged red fibre (Figure 5.51).

Given the typical pattern of episodes of weakness and positive long exercise test, full exon sequencing of KCNJ2 and SCN4A and sequencing of S4 segments of CACNA1S was done, all of which were negative. He was therefore selected for next-generation sequencing. There was insufficient DNA for aCGH as well.

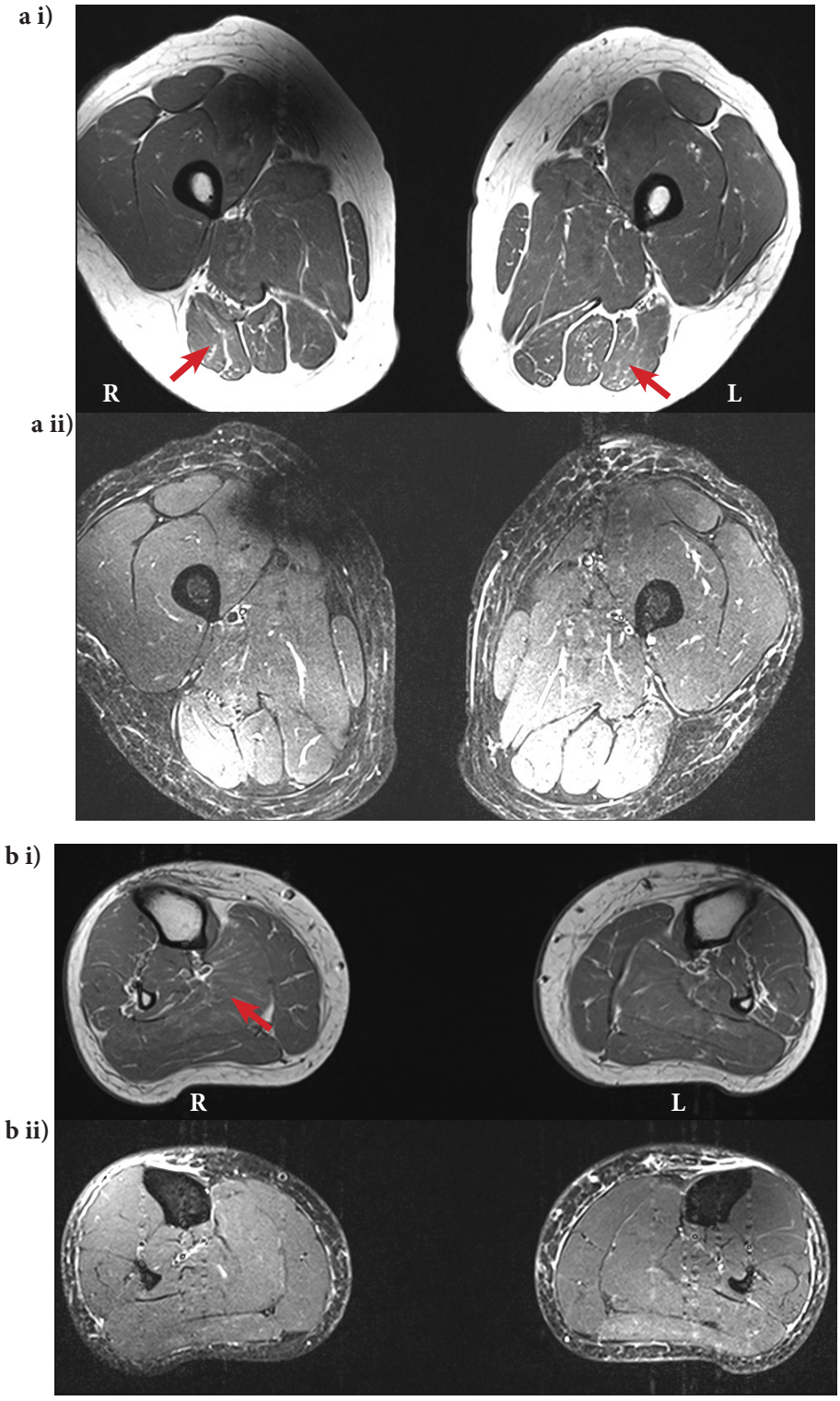


Figure 5.50: MRI scan of thighs and calves of Case 12 ai) T1 weighted image of thighs showing mild fatty infiltration in the posterior compartment (indicated by red arrows) aii) STIR sequence of thighs, bi) T1 weighted image of calves with mild fatty infiltration in the soleus (indicated by red arrows) bii) STIR sequence of calves.

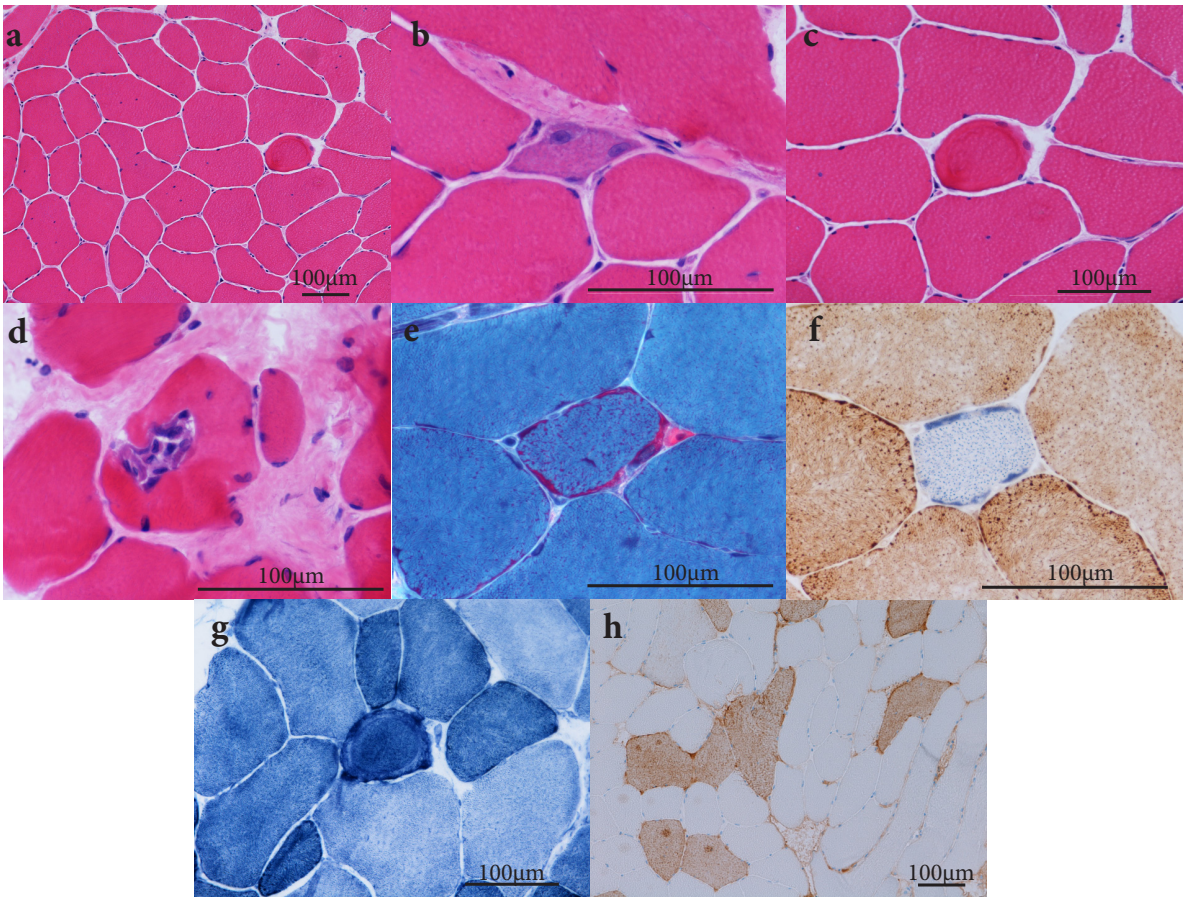


Figure 5.51: Muscle biopsy of vastus lateralis of Case 12 a) Haematoxylin and eosin stain showing variation in fibre size, internal nuclei and fibre hypertrophy b) Haematoxylin and eosin stain demonstrating a regenerating fibre c) Haematoxylin and eosin stain demonstrating a ring fibre d) Haematoxylin and eosin stain demonstrating a necrotic fibre e) Gomori trichrome stain demonstrating a ragged red fibre f) COX-SDH stain with a ragged red fibre stained blue g) NADH staining of a ring fibre h) NADH stain showing type 1 fibre predominance

Whole exome results of Case 12

Overall 190,372 SNVs were identified, 10,491 of which were novel (Table 5.21). No novel variations were identified in the channelopathy genes. I looked at variations in the known muscle disease genes and identified four novel variations (Table 5.27). The first was in RYR1 c.3128G>A;p.Arg1043His. This gene is known to be associated with a case of atypical PP and multi-minicore disease and so could be relevant (Zhou et al., 2010). This particular variation lies in a highly conserved region and was predicted to be pathogenic by SIFT (0.01), PolyPhen2 (1.000) and Mutation Taster (0.99989) as it lies at a splice site as well as altering the protein. This would be expected to considerably disrupt the protein downstream.

Gene	Type of variation	Variation	Hetero/ homozygous	Associated disease	Expression in skeletal muscle	*in silico* prediction
Muscle Disease Genes						
RYR1	**Nonsyn**	**c.3128G>A:p.R1043H**	**Hetero**	**Multi-minicore disease Central core disease Malignnat hyperthermia**	**High**	**Pathogenic**
AR	Non FM ins	c.170_171insGCA: p.L57delinsLQ	Hetero	Spinal muscular atrophy (with CAG expansion)	Low	
ATXN1	Non FM del	c.624_626del: p.208_209del	Hetero	Spinocerebellar ataxia 1 (with CAG expansion)	High	
SNRPN	Splicing	g. 25131738T>C	Hetero	Prader-Willi (with deletion)	Low	
Ion channel genes						
BEST3	FM del	c.177delA:p.K59fs	Hetero	Anion channel	Medium	Pathogenic
KCNMA1	Nonsyn	c.1499C>T:p.A500V	Hetero	Paroxysmal dyskinesia	None	Pathogenic
LYN	Nonsyn	c.170A>C:p.H57P	Hetero	Regulation of mast cell degranulation	None	Pathogenic

Table 5.27: Table of novel variations identified by whole exome sequencing in Case 12

Two of the other three variations identified were in genes that are only associated with disease in the presence of CAG expansions (AR and ATXN1) (Table 5.27) and the final variation was in a splice site for SNRPN which is associated with Prader-Willi syndrome but only in the presence of deletions. These are therefore unlikely to be relevant.

Given the presence of the single ragged red fibre on biopsy, I checked for possible variations in known nuclear mitochondrial disease genes but no novel variations were identified.

Three variations were identified in ion channel genes, two of which were in genes not expressed or expressed in very low levels in skeletal muscle (Table 5.27). The other variation was a frameshift deletion identified in BEST3, c.177delA;p.K59fs. This gene encodes an anion channel and is expressed in skeletal muscle. Its role in skeletal muscle is unknown but as it is a frameshift mutation it would be expected to produce a non-functioning protein.

Given the patient's phenotype, however, the likeliest relevant variation is the one identified in RYR1.

5.3.3.5 Case 13

Phenotype of Case 13

Case 13 was of a 16 year old female with problems since birth. She was the product of a normal pregnancy with normal foetal movements. She was born at 38 weeks and was admitted to the Specialist Care Baby Unit for feeding difficulties and temperature

instability and was noted to have a cleft palate which was repaired at one year. Her initial developmental milestones were normal, sitting without support at 6 months, standing at 10 months, walking independently at one year and running at 15 months. However, she was always slower than her peers and had speech delay requiring speech therapy for phonation and articulation difficulties. At the age of 5 she had difficulty rising and walking after a long period of sitting which would improve over a couple of hours. Over the next few years this progressed to distinct episodes of weakness after illness or after she had done exercise. Her episodes of weakness primarily occurred on waking and were associated with arm weakness and then leg weakness, resulting in difficulties walking. She had no abnormalities of swallowing, breathing or ocular muscles. At the time of assessment her episodes were occurring approximately once a month, lasting for 48 to 72 hours.

Her other problems included bilateral conductive hearing loss, short stature (for which she was on growth hormone), special educational needs, recurrent chest infections, a small right kidney and scoliosis. At the age of nine she had an episode of cardiac syncope which was found to be secondary to ventricular tachycardias. She had another episode two years later and, as a result, had an implantable cardioverter defibrillator inserted. She had a negative family history with an unaffected sister and parents, although her father had similar facial features.

On examination she was 30.4 kg weight and 137.7cm in height, two blocks below the 0.4th centile. Her VC was 1.27L. She had microcephaly, micrognathia, broad nasal bridge, low hanging columella, low set ears, syndactyly, clinodactyly, short toes and dysplastic nails (Figure 5.52). She had asymmetrical scoliosis with a thoracic convexity to the right and a waddling gait. She had a positive Gowers' manoeuvre on rising from the floor. She had neck flexion weakness, MRC score 3-/5, trunk flexion weakness (4-/5) and extensor weakness (4+/5). Proximal upper arm strength was 3-/5 but distally she had full power. Her proximal lower limb strength was only mildly impaired at 4+/5 with normal distal strength. She had a mildly impaired vibration sense in the lower limbs but otherwise sensory examination was normal.

Her blood tests were normal with a CK of 99 IU/L. Her echocardiogram demonstrated normal biventricular size and function with mild mitral regurgitation and tricuspid regurgitation. Due to her defibrillator she was only able to have needle EMG which demonstrated myopathic changes. Her sleep study demonstrated occasional desaturations

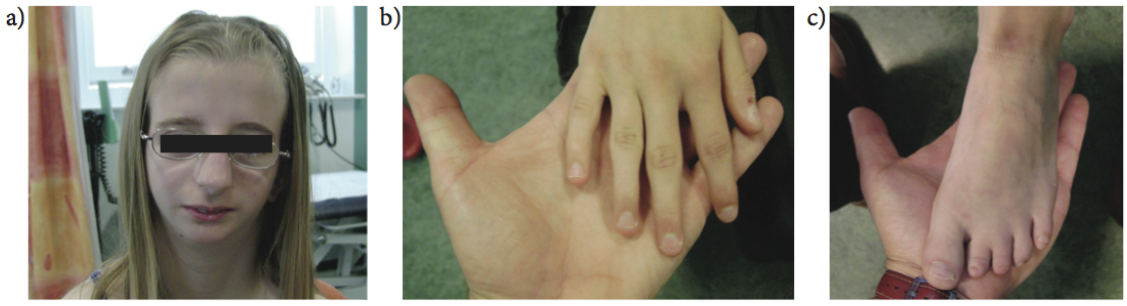


Figure 5.52: Photos of distinctive facial features and skeletal abnormalities in Case 13 a) Face demonstrating microcephaly, micrognathia, broad nasal bridge and low hanging columella, b) Hand demonstrating syndactyly and clinodatyly, c) Foot demonstrating short toes, syndactyly and dysplastic nails. (Images reproduced with permission from the patient)

suggestive of mild obstructive sleep apnoea. She was unable to have an MRI of the lower limbs because of her defibrillator. She had a muscle biopsy which demonstrated variation in fibre size, primarily of type I fibres, with occasional round or angular atrophic fibres and rare nuclear bag fibres (Figure 5.53a). Occasional fibres contained internal nuclei. There were at least six COX deficient fibres seen and several fibres with intermediate staining (Figure 5.53c). Given the presence of COX negative fibres she had mitochondrial respiratory chain enzyme analysis which were all normal, although complex IV activity was at the lower limit of normal.

Given the combination of arrhythmias, skeletal abnormalities and episodes of weakness, DNA was sequenced for variations in KCNJ2. This was negative as was MLPA of KCNJ2, excluding deletions or duplications within the gene. Analysis of S4 segments of SCN4A and CACNA1S were also negative. Given the presence of COX negative fibres she had full sequencing of the mitochondrial genome and analysis for rearrangements which were all negative. She therefore went on to have aCGH and whole exome sequencing.

aCGH in Case 13

Array CGH was performed using the 1.4M array. 119 CNVs were detected of which 27 were novel. Of these 18 were identified within the exome (Table 5.28). One CNV with possible relevance was identified in this group, a gain of one copy of 9,826 base pairs in SYNE1 based on the reading from 3 probes (Figure 5.54). The extra copy was located from exons 112 to 117. Missense mutations in this gene are associated with Emery-Dreifuss muscular dystrophy type 4 and autosomal recessive spinocerebellar ataxia (SCA) type 1 (Gros-Louis et al., 2007).

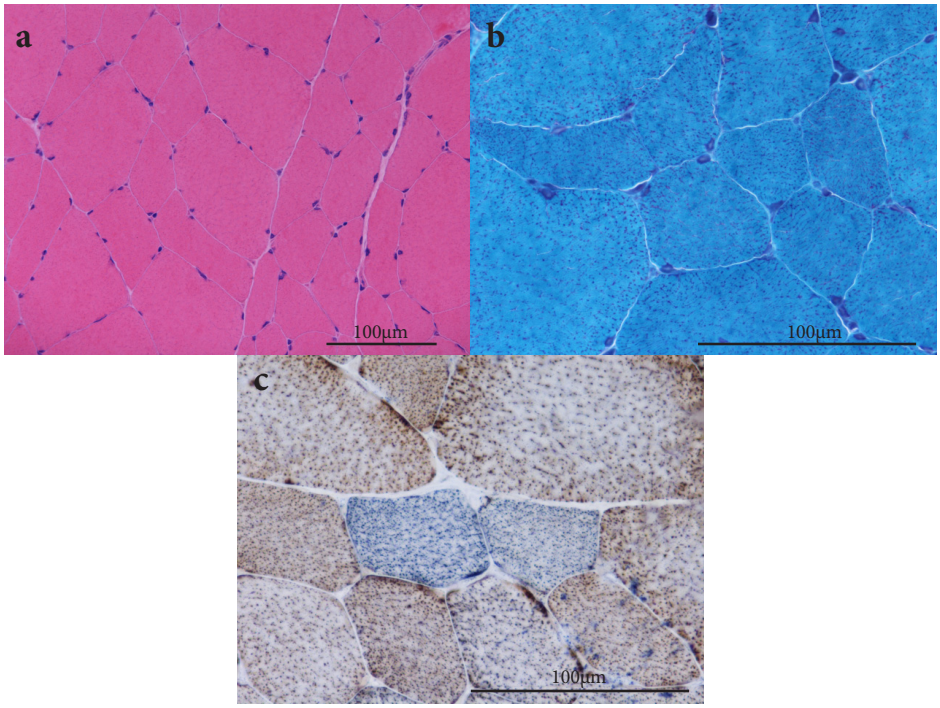


Figure 5.53: Muscle biopsy of vastus lateralis of Case 13 a) Haematoxylin and eosin stain showing variation in fibre size b) Gomori trichrome stain c) COX-SDH stain with COX negative fibre stained blue

The phenotype in SCA1 is associated with adult-onset ataxia, dysarthria and oculomotor abnormalities. This is distinct from the phenotype in this case. Emery-Dreifuss muscular dystrophy caused by variations in SYNE1 is an autosomal dominant condition associated with contractures, slowly progressive skeletal muscle wasting and weakness and cardiomyopathy (Zhang et al., 2007). This phenotype was similar to this patient's presentation, however the call was based on the presence of only 3 probes questioning the validity of the result.

Whole Exome Results in Case 13

Overall 188,725 SNVs were identified, 10,679 of which were not in dbSNP (Table 5.21). No novel variations were identified in the channelopathy genes. There were only two variations identified in known muscle disease genes (Table 5.29). Both variations were in the nebulin gene, c.5567G>A;p.Arg1856Glu and c.10383G>C;p.Met3461Ile. However both variations have now been reported in single cases in the most recent release of dbSNP making them less likely to be pathogenic. The Arg1856Glu variation is predicted to be pathogenic but the Met3461Ile variation is predicted by SIFT and PolyPhen2 to be tolerated making it less

Chr	Position of copy number variation	Width	Loss/ Gain	Average ratio	Genes	Gene function
1	1q24.2(170,125,366-170,127,166)	1,800	Loss	0.55	METTL11B, MIR3119-1, MIR3119-2	
1	1q25.3(185,099,060-185,130,966)	31,906	Gain	1.46	SWT1, TRMT1L	
1	1q42.13(229,760,620-229,762,527)	1,907	Loss	0.54	TAF5L, URB2	Type 1 Diabetes
3	3p26.1(4,622,159-4,637,074)	14,914	Loss	0.77	ITPR1	deep in intron
3	3q21.2(124,909,065-124,921,424)	12,359	Loss	0.76	SLC12A8	Psoriasis vulgaris
6	6q22.31(125,384,574-125,394,752)	10,177	Loss	0.75	RNF217	
6	**6q25.2(152,544,036-152,553,863)**	**9,826**	**Gain**	**1.76**	**SYNE1**	**ED type 4, SCA8**
7	7p14.3(32,930,248-32,933,067)	2,819	Loss	0.71	KBTBD2	
8	8q23.1(108,325,294-108,334,609)	9,315	Gain	1.45	ANGPT1	microvascular leakage
9	9q21.11(71,937,583-71,942,207)	4,623	Loss	0.66	FAM189A2	
14	14q13.2(35,653,392-35,689,422)	36,029	Gain	1.33	KIAA0391	sbunit of mitochondrial RNAse P
15	15q25.2(84,576,950-84,585,731)	8,781	Gain	1.46	ADAMTSL3	
17	17p12(11,143,638-11,145,026)	1,387	Loss	0.46	SHISA6	
17	17q24.2(64,801,841-64,806,678)	4,837	Loss	0.62	PRKCA	processing self motion signals
20	20p13(4,980,741-5,001,791)	21,049	Loss	0.75	SLC23A2	sodium coupled vit c transporter
22	22q13.1(40,439,815-40,445,411)	5,596	Loss	0.71	TNRC6B	
X	Xp22.31(6,983,859-6,996,188)	12,329	Gain	1.44	HDHD1	
X	Xp22.12(21,751,932-21,754,047)	2,115	Gain	1.60	SMPX	Deafness x-linked

Table 5.28: Table of novel copy number variations identified on aCGH for Case 13

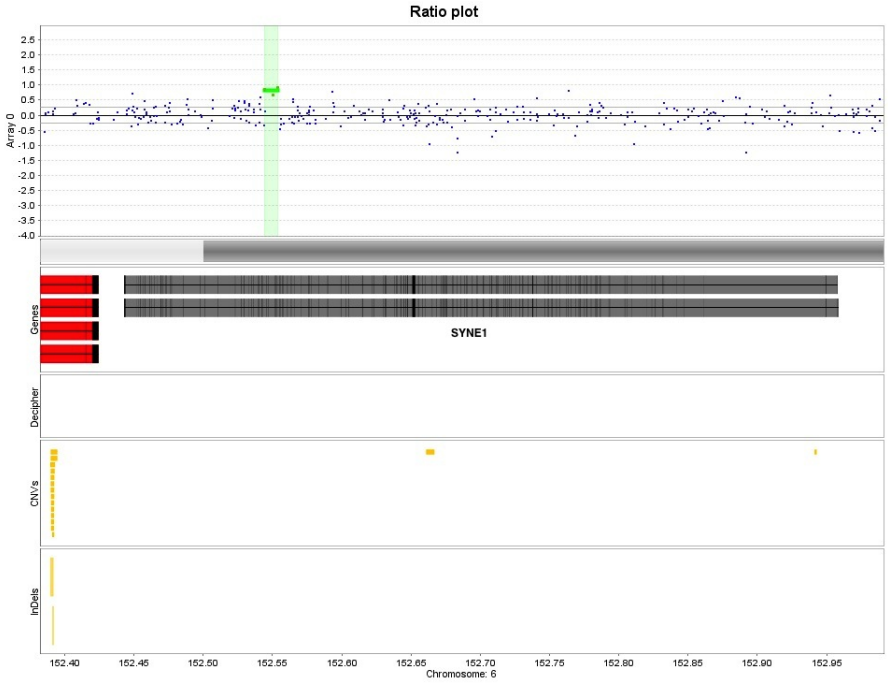


Figure 5.54: ArrayCGH ratio plot showing gain in copy number of part of the SYNE1 gene

likely to be the responsible pathogenic variations. Variations in nebulin are associated with autosomal recessive nemaline myopathy 2.

Gene	Type of variation	Variation	Hetero/ homozygous	Associated disease	Expression in skeletal muscle	*in silico* prediction
Muscle Disease Genes						
NEB	Nonsyn	c.5567G>A:p.R1856Q	Hetero	Nemaline myopathy	High	Pathogenic In dbSNP 144
NEB	Nonsyn	c.10383G>C:p.M3461I	Hetero	Nemaline myopathy	High	Tolerated In dbSNP 144
Mitochondrial disease genes						
NUBPL	Nonsyn	c.125G>A:p.G42D	Hetero	Complex I deficiency , AR	Low	Pathogenic
Ion channel genes						
PKD1L2	Stop loss	c.2974T>A:p.X992R	Hetero		Unknown	Tolerated

Table 5.29: Table of novel variations identified with whole exome sequencing in Case 13

Given the COX negative fibres, I looked at variations in known nuclear mitochondrial genes. I first looked at genes associated with complex IV deficiency and no novel variations were found. I then expanded to all other nuclear mitochondrial genes. This only revealed one novel variation (Table 5.29), a heterozygous variation in NUBPL which is associated with complex 1 deficiency with autosomal recessive inheritance. As there was only a heterozygous variation in this patient, it is unlikely that it is responsible for the patient's phenotype. I also checked for variations in genes associated with arrhythmias but no novel variations were identified. Finally I looked at the variations in ion channels. Four novel variations were

identified. Only one of the genes was expressed in skeletal muscle, PKD1L2. This was a stop loss variation, c.2974T>A;p.X992Arg which results in an extra 25 amino acids added on to the end of the transcript. It is however predicted to be a polymorphism by Mutation taster (>0.999).

Given the presence of facial dysmorphism, I input her distinctive facial features and symptom cluster into Face2Gene, a facial dysmorphology package that analyses facial features and compares them to a database of other patients with dysmoprhic features and confirmed diagnoses (www.fdna.com/face2gene). The patient did not have any novel variations in genes associated with the diagnoses suggested based on her profile. It therefore seems likely that the only variations that may have relevance are those in the nebulin gene or, less likely, the extra copy of exons 112-117 of SYNE1.

5.3.3.6 Case 14

Phenotype of Case 14

Case 14 was a 23 year old man with a history of episodes of severe calf pain accompanied by weakness from the age of 7. The episodes lasted five days to a few weeks and were always associated with significant pain. Episodes primarily occurred in the evening or at night. As he grew older the episodes of weakness improved but he continued to have painful episodes. These episodes were also associated with blurring of vision. His past medical history was of migraine but also of postural syncope and palpitations. There was no family history. On examination he had mild proximal weakness of MRC score 4+/5 but the rest of the examination was normal.

His investigations demonstrated an abnormal long exercise test with a 52% decrement but normal short exercise test and EMG (Figure 5.55). His nerve conduction studies did demonstrate a reduced left sural sensory nerve action potential (SNAP) of 7μV but the rest of the study was otherwise normal. His CK during one attack in childhood was 10,000IU/L but in recent attacks was normal. The MRI demonstrated a mild degree of fatty infiltration in the semimembranosus and the long head of the biceps bilaterally. There was also a mild increase of STIR signal in the gastrocnemius bilaterally (Figure 5.56).

aCGH in Case 14

Array CGH was performed using the 720K array. 109 anomalies were identified of which 28 were novel. 12 of these were not seen in other samples and were located within the exome

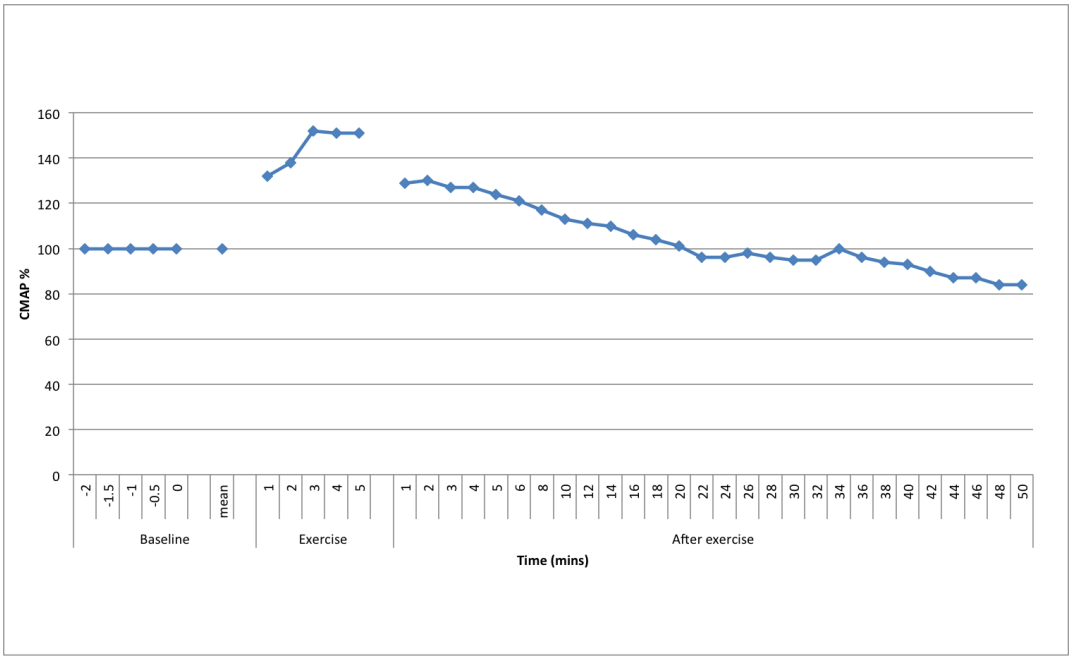


Figure 5.55: Case 14 long exercise test (Positive with 52% decrement from peak)

(Table 5.30). None of the CNVs were in ion channel genes expressed in skeletal muscle.

Whole Exome Results in Case 14

Case 14 had 199,977 variations identified of which 663 were novel exome variations (Table 5.21). There were no novel variations in muscle disease genes but one variation was found in the ion channel gene SCN11A, c.2776G>A;p.Val926Ile (Table 5.31). This is predicted by Mutation taster, PolyPhen2 and SIFT to be a polymorphism ($p>0.999$). This channel is primarily expressed in the dorsal root ganglion and variations are associated with hereditary sensory and autonomic neuropathy type VII (Leipold et al., 2013) and more recently in painful peripheral neuropathies (Huang et al., 2014). Given that this patient has a pain syndrome with autonomic features and an abnormal sural SNAP it is possible that this mutation may account for some of his symptoms, although it does not explain his episodes of weakness.

5.3.3.7 Cases 15-18

The remaining cases did not reveal any plausible explanations on whole exome sequencing. A brief summary of the cases is therefore given.

Case 15 was a 50 year old man with episodes of weakness of the arms and legs with an associated potassium of 1.9mmol/L. His power returned to normal after his potassium level returned to normal. His long exercise test, done at another centre, was positive.

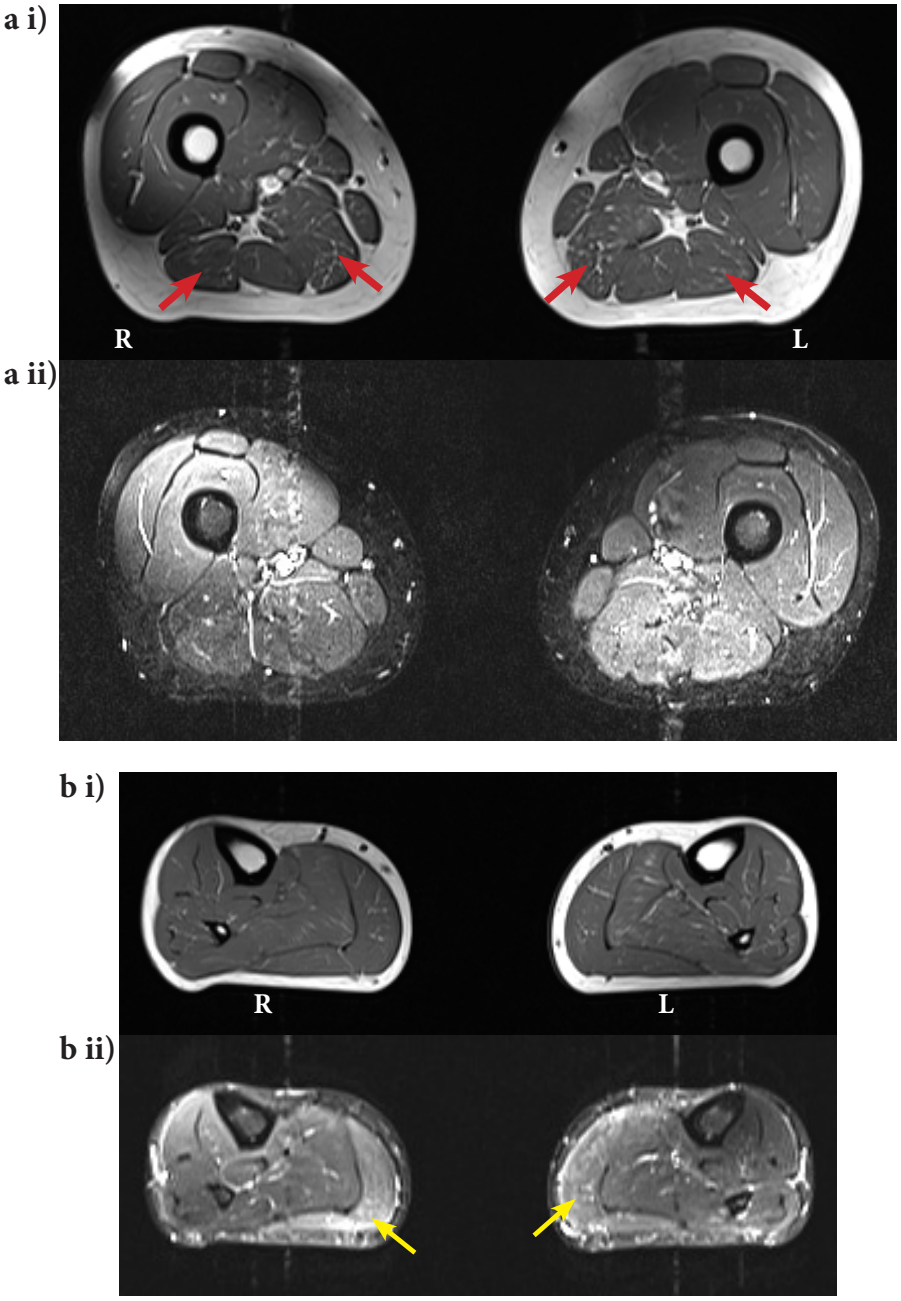


Figure 5.56: MRI scan of thighs and calves of Case 14 ai) T1 weighted image of thighs showing mild fatty infiltration of semimembranosus and the long head of the biceps (indicated by red arrows) aii) STIR sequence of thighs, bi) T1 weighted image of calves bii) STIR sequence of calves demonstrating subtle oedema in gastrocnemeii (indicated by yellow arrows)

Chr	Position of copy number variation	Width	Loss/ Gain	Average ratio	Genes	Gene function
1	1p36.32(4,671,850-4,671,870)	20	Loss	0.72	AJAP1	
1	1p34.2(41,022,552-41,022,567)	15	Loss	0.72	KCNQ4	AD hearing loss
2	2p11.2(85,214,454-85,215,011)	556	Loss	0.66	TCF7L1	transcription factor, skin and hair formation
5	5q35.3(179,430,648-179,431,182)	534	Loss	0.66	RNF130	
7	7q32.1(127,457,366-127,457,901)	534	Loss	0.74	LRRC4, SND1	
11	11p15.4(7,066,989-7,067,876)	886	Loss	0.72	RBMXL2	spermatogenesis
11	11q13.1(65,567,077-65,568,214)	1136	Loss	0.67	GAL3ST3	
13	13q12.13(26,231,483-26,231,889)	406	Loss	0.76	GPR12	
13	13q13.1(32,489,212-32,489,768)	556	Loss	0.76	KL	assoc tumour calcinosis and coronary artery disease
16	16q13(56,120,006-56,120,494)	488	Loss	0.73	CCDC102A	
17	17q11.2(27,838,830-27,839,332)	501	Loss	0.69	CDK5R1	pain hypersensitivity
X	Xp21.3(26,122,068-26,122,278)	209	Loss	0.72	MAGEB6	

Table 5.30: Table of novel copy number variations identified by aCGH for Case 14

Gene	Type of variation	Variation	Hetero/ homozygous	Associated disease	Expression in skeletal muscle	*in silico* prediction
Ion channel genes						
SCN11A	**Nonsyn**	**c.2776G>A:p.V926I**	**Hetero**	**Neuropathy, hereditary sensory and autonomic, type VII**	**Low** (mainly in DRG)	**Tolerated**

Table 5.31: Table of novel variations identified with whole exome sequencing in Case 14

Case 16 was a 68 year old female with a history of episodes of weakness in the context of low potassium. She had a positive glucose provocation test, with a low potassium and weakness after glucose loading and a family history of episodes of weakness.

Case 17 was a 19 year old man with onset of symptoms at 8 years old. His primary problem was his legs locking up whilst playing football with stiffness and transient weakness. In his mid teens this progressed to stiffness of the upper arms. The stiffness would improve with warm-up and there were no discrete episodes of weakness. His symptoms were improved by mexiletine. There was no family history of stiffness and his parents were thought to be unrelated. On examination he had generalised muscle hypertrophy and grip and percussion myotonia. The blood tests demonstrated a CK 537IU/L. His EMG demonstrated profuse myotonia. The short exercise test demonstrated immediate decrement of the CMAP after exercise which improved with subsequent trials. There was no significant difference following cooling or rewarming (Figure 5.57). The long exercise test was normal. These findings and the clinical phenotype were suggestive of recessive MC.

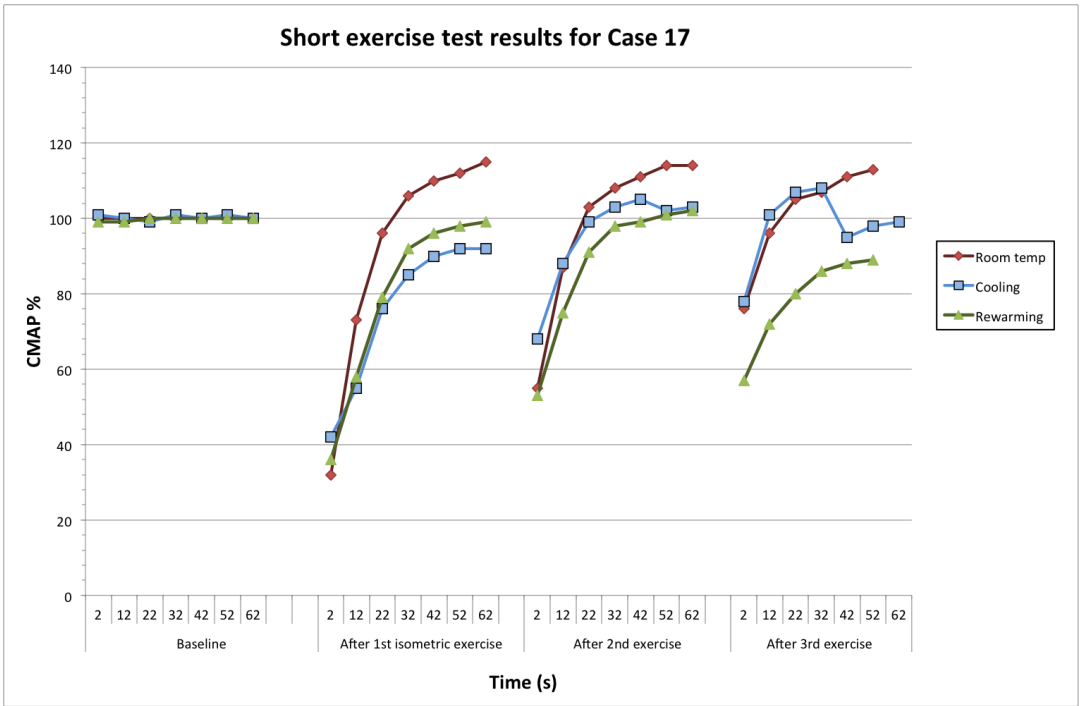


Figure 5.57: Graph of short exercise test for Case 17 (Red diamonds represent CMAP percentage at room temperature, blue squares represent CMAP percentage after cooling and green triangles represent CMAP percentage after rewarming)

Case 18 was a 41 year old with onset of attacks of weakness from his early twenties. Initially these episodes only affected his legs but over time progressed to involve the arms and legs. At the start of the attacks he developed redness of the eyes progressing to slurring

of speech. Following this his limbs would become weak and he would be unable to move for several hours. It would often take up to a week before his function completely returned to normal. Attacks were worsened by activity and were often precipitated by changes in temperature or heavy carbohydrate meals. He had no cardiac or respiratory problems. There was no family history and his parents were unrelated, his mother being from the Western Isles and his father from Southern Ireland. His examination was normal.

His CK was normal at 93IU/L, as was his interictal potassium. His electrophysiology demonstrated a positive long exercise test with a decrement of 76% (Figure 5.58). There was no decrement on short exercise testing and no myotonia on EMG. MRI demonstrated mild fatty infiltration of the medial and posterior compartment of the thighs and the superficial posterior compartment muscles in the calves. There was no evidence of water deposition (Figure 5.59).

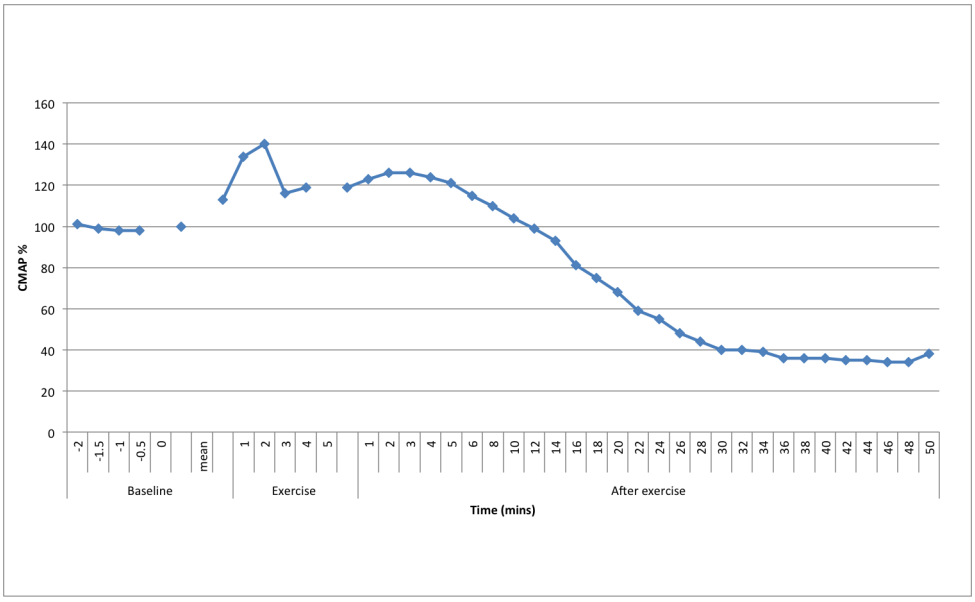


Figure 5.58: Case 18 long exercise test (Positive with 76% decrement from peak.)

Whole Exome Results for Cases 15-18

The overall numbers of SNVs for each case are described in the Table 5.21.

Case 15 had 416,148 SNVs, 348 of which were in the exome and not in dbSNP (Table 5.21). There were no novel variations in known muscle disease genes and only one variation in an ion channel gene expressed in skeletal muscle (Table 5.32a). This was in CACNA2D3, c.1156C>G;p.Pro386Ala. It was predicted by Mutation taster and PolyPhen2 to be pathogenic (>0.999) although predicted to be tolerated by SIFT (0.27). The gene is highly expressed in skeletal muscle. It is associated with problems with impaired pain

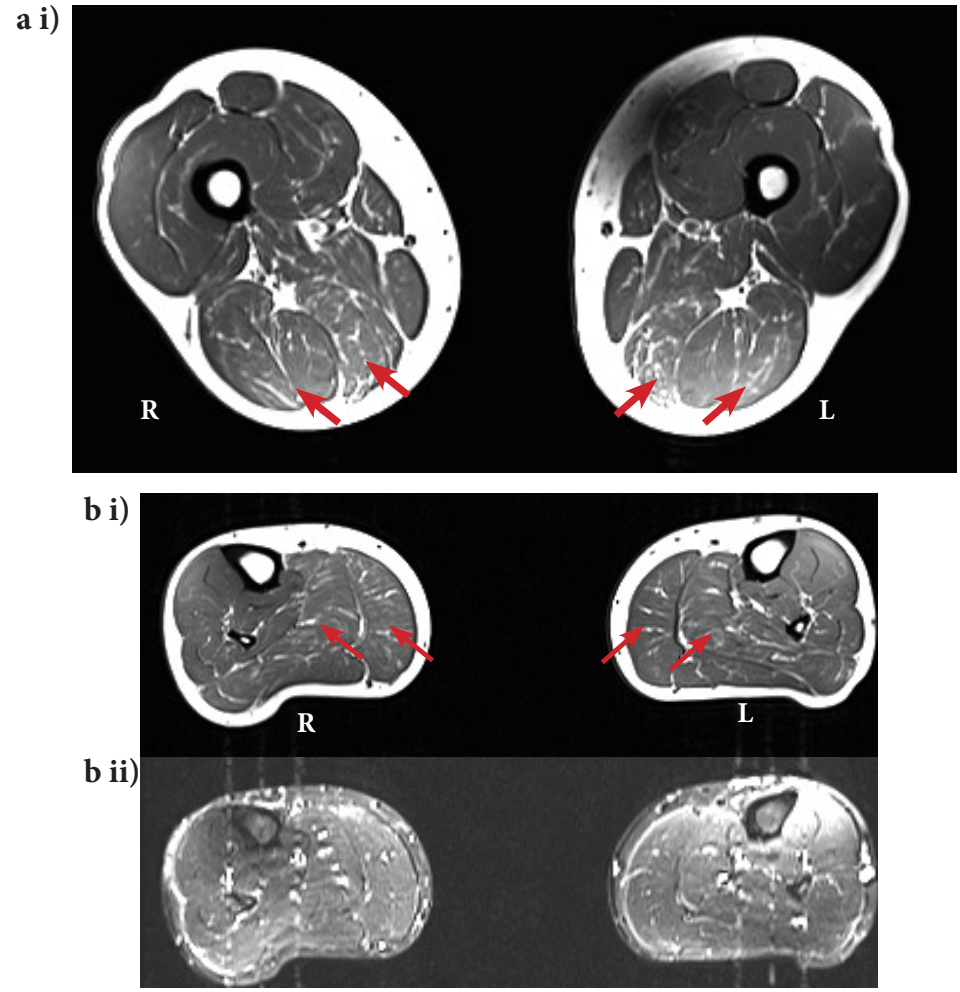


Figure 5.59: MRI scan of thighs and calves of Case 18 ai) T1 weighted image of thighs showing mild fatty infiltration in the medial and posterior compartment (indicated by red arrows), bi) T1 weighted image of calves with mild fatty infiltration in the superficial posterior compartment (indicated by red arrows) bii) STIR sequence of calves.

perception but has not been associated with weakness. It is therefore unlikely to be causative variation. Array CGH of this case was performed using the 720K array. 101 anomalies were identified of which 21 were novel. 4 of these were not seen in other samples and were located within the exome (Table 5.32b). None of the CNVs were in ion channel genes expressed in skeletal muscle.

Case 16 had 194,199 SNVs, of which 692 were exonic and not in dbSNP (Table 5.21). Only one novel variant was identified in muscle channel genes in CLCN1, c.412G>A;p.Val138Ile (Table 5.33). However, as there was no of myotonia, the phenotype in this patient was not consistent with this being a pathogenic variation. All the novel variations in ion channels were in genes that did not have significant expression in skeletal muscle and

a) Whole exome sequencing

Gene	Type of variation	Variation	Hetero/ homozygous	Associated disease	Expression in skeletal muscle	*in silico* prediction
Ion channel genes						
CACNA2D3	Nonsyn	c.1156C>G:p.P386A	Hetero	von Hippel Lindau (deletions) Knockout mice have impaired pain	High	Pathogenic (PolyPhen) Tolerated (SIFT)

b) aCGH

Chr	Position of copy number variation	Width	Loss/ Gain	Average ratio	Genes	Gene function
2	2p25.1(9,263,829-9,264,756)	926	Loss	0.53	ASAP2	
2	2p11.2(85,214,454-85,215,011)	556	Loss	0.69	TCF7L1	difficulty wound healing
4	4q24(106,683,227-106,704,025)	20797	Loss	0.51	ARHGEF38	
5	5q31.3(140,031,158-140,032,577)	1419	Loss	1.46	DND1	congenital teratoma risk in mice

Table 5.32: Table of novel variations identified in Case 15 a) Novel variations idenitifed in whole exome sequencing, b) Novel copy number variations identified in aCGH.

therefore were not likely to be causative. There was insufficient DNA for aCGH analysis.

Gene	Type of variation	Variation	Hetero/ homozygous	Associated disease	Expression in skeletal muscle	*in silico* prediction
Muscle Disease Genes						
CLCN1	Nonsyn	c.412G>A:p.V138I	Hetero	Myotonia congenita	High	Tolerated
Ion channel genes						
GABRA4	Nonsyn	c.1102C>T:p.R368C	Hetero		Very low	Tolerated
GRID2	Nonsyn	c.2110A>C:p.M704L	Hetero		Very low	Pathogenic
GLRA1	Nonsyn	c.74A>C:p.E25A	Hetero	Hyperekplexia, hereditary 1, AD	Very Low	Tolerated

Table 5.33: Table of novel variations identified with whole exome sequencing in Case 16

Case 17 had 250,363 variations identified of which 352 were novel exome variations (Table 5.21). There were no novel variations identified in muscle disease genes or ion channel genes. DM1 and DM2 testing were both normal. He was, however homozygous for the intronic variant c.1930+6T>G in CLCN1 and given that the remainder of his sequencing was normal it seems likely that this variation is responsible for his phenotype. There was insufficient DNA for aCGH analysis.

Case 18 had 344,866 variations of which 313 were novel exome variations (Table 5.21). The only variation identified in muscle or ion channel genes was in TTN, c.76381G>C;p.Glu25461Gln (Table 5.34a). This was, however, predicted by Mutation

Taster and PolyPhen2 to be a polymorphism (p>0.999). Mutations in TTN are not known to cause the phenotype seen in this patient and it is therefore unlikely to be the causative variation. Array CGH was performed using the 1.4M array. 137 CNVs were detected of which 15 were novel exome CNVs (Table 5.34b). None of the CNVs were in ion channel genes expressed in skeletal muscle.

a) Whole exome sequencing

Gene	Type of variation	Variation	Hetero/ homozygous	Associated disease	Expression in skeletal muscle	*in silico* prediction
Muscle disease genes						
TTN	Nonsyn	c.76381G>C:p.E25461Q	Hetero	LGMD2J, AR	High	Polymorphism

b) aCGH

Chr	Position of copy number variation	Width	Loss/ Gain	Average ratio	Genes	Gene function
2	2p23.3(24,172,948-24,226,142)	53,194	Loss	0.61	MFSD2B, UBXN2A	
2	2q24.1(158,973,419-158,977,935)	4,516	Loss	0.60	UPP2	
3	3p13(72,936,126-72,942,214)	6,088	Loss	0.73	GXYLT2	
3	3q26.33(179,673,767-179,676,437)	2,670	Gain	1.45	PEX5L	
6	6q22.1(117,738,906-117,741,851)	2,944	Loss	0.66	ROS1	tyrosine kinase
7	7p22.2(2,881,402-2,884,784)	3,381	Loss	0.57	GNA12	autophagy + cilia
7	7q36.1(151,121,398-151,132,934)	11,535	Loss	0.70	CRYGN, MIR3907	
9	9q22.33(101,868,408-101,870,269)	1,861	Loss	0.60	TGFBR1	Loeys-Dietz Syndrome
12	12p13.1(14,104,394-14,109,484)	5,089	Loss	0.68	GRIN2B	NMDA receptor subunit 2b, epileptic enecephalopathy
12	12q13.11(46,653,133-46,657,444)	4,310	Loss	0.61	SLC38A1	glutamate transporter
13	13q12.13(27,740,968-27,744,739)	3,770	Loss	0.58	USP12	deubiquinating of androgen receptor
17	17p12(11,137,471-11,145,786)	8,315	Loss	0.67	SHISA6	
19	19p13.11(16,471,925-16,473,249)	1,323	Loss	0.44	EPS15L1	
19	19p13.11(17,749,656-17,769,491)	19,834	Loss	0.70	UNC13A	APP processing
21	21q22.3(43,932,365-43,933,471)	1,106	Loss	0.54	SLC37A1	sugar phosphate transporter

Table 5.34: Table of novel variations identified in Case 18 a) Novel variations idenitifed in whole exome sequencing, b) Novel copy number variations identified in aCGH.

5.4 Efficacy of Mexiletine in Treating Non-dystrophic Myotonia

5.4.1 Study Population

Overall, 62 patients were recruited across all sites between December 2008 and January 2011. Three patients were ineligible at screening due to prolonged QTc interval, elevated alanine transaminase or no myotonia seen on clinical examination. 59 patients were randomised, 29 patients received mexiletine and then placebo and 30 patients received placebo then mexiletine. Two patients data were not usable as they did not call into the IVR system enough times during weeks three and four of either period. There were three dropouts, one for gastric discomfort, one with exacerbation of migraine and one with failure to attend study visits (Figure 5.60). 57 patients were included in the modified intention to treat analysis (two were excluded as they did not call the IVR during either period of treatment). In the UK group, 14 patients were recruited and randomised, seven to mexiletine and then placebo and seven to placebo and then mexiletine. One of the drop outs was from the UK cohort due to exacerbation of migraines.

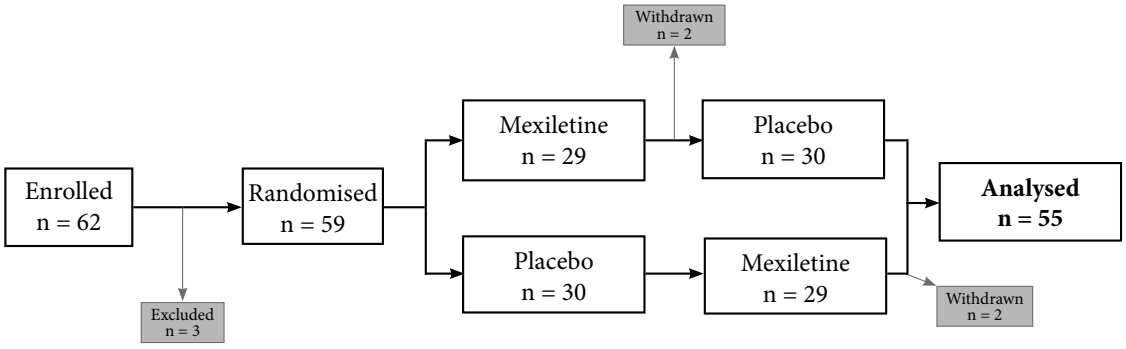


Figure 5.60: CONSORT flow diagram of patients assessed in the study of the efficacy of mexiletine in NDM

5.4.1.1 Demographics

In the whole cohort there were 33 men (56%) and 26 women (44%). The median age was 42 years and mean age 43 years. Patients were predominantly Caucasian (57/59). 34 patients had chloride channel mutations of which 20 had dominant MC and 14 had recessive MC. 21 patients had a sodium channel mutation, 12 of which had PMC and 9 of which had SCM

(Figure 5.61). 4 patients had no mutations identified. 17 patients were on drug treatment for myotonia prior to starting the study of which 13 (76%) were taking mexiletine. All patients had the appropriate washout period (equivalent of 5 times the half life of drug) prior to starting the study. The groups in the different treatment sequences (those who received mexiletine then placebo versus placebo then mexiletine) were well balanced (Table 5.35).

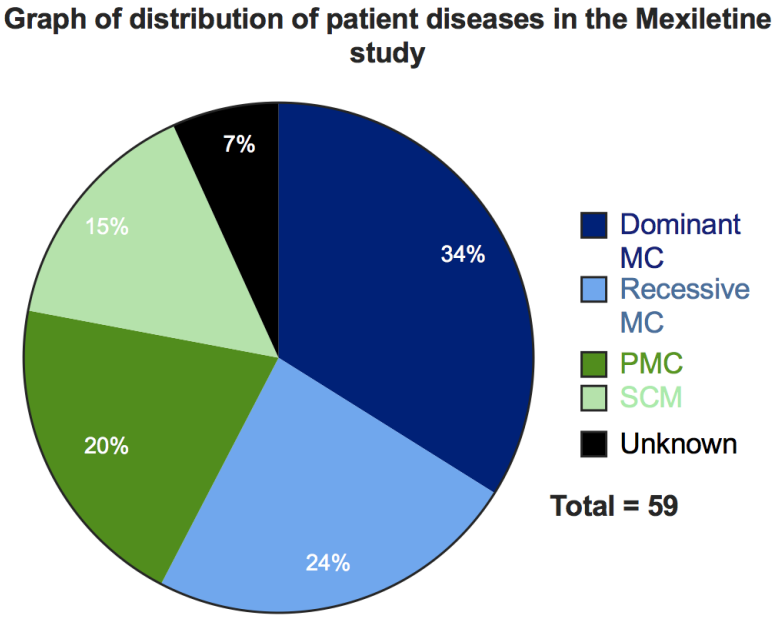


Figure 5.61: Distribution of patient diagnoses in the mexiletine study.

5.4.2 Primary Outcome Measure

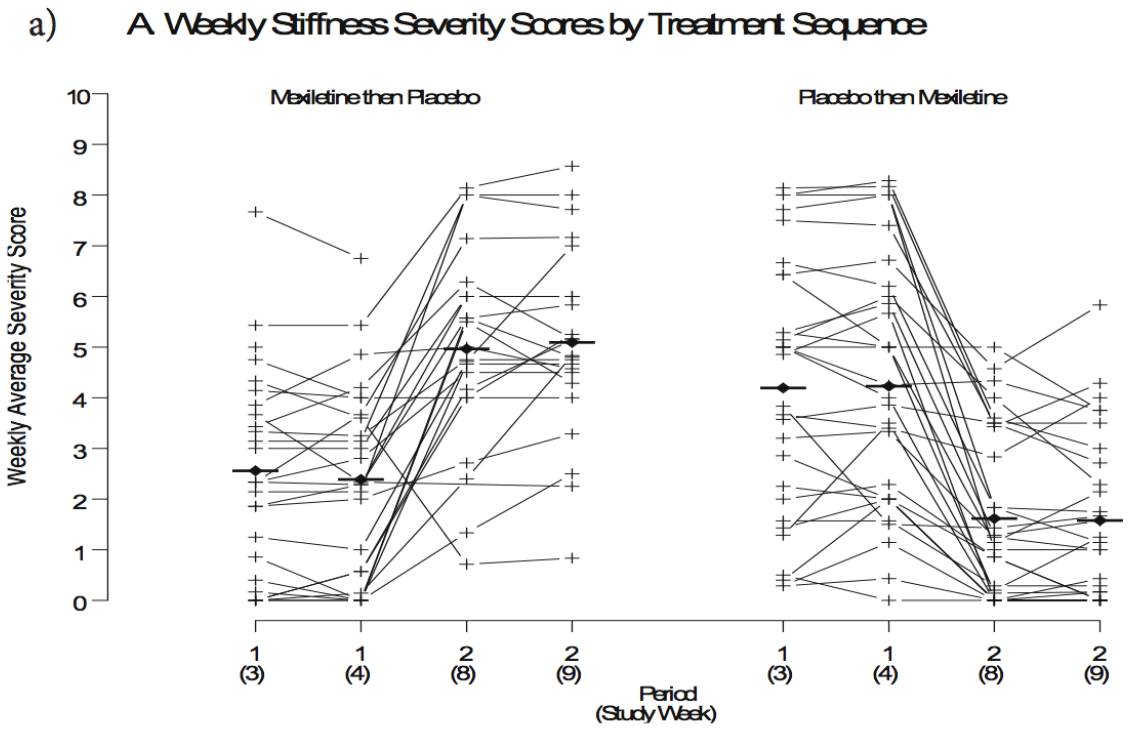
There was a significant improvement in stiffness as recorded by IVR when patients were on mexiletine (Figure 5.62). In period 1 the mean stiffness score was 2.53 on mexiletine and 4.21 on placebo and for period 2 it was 1.60 for mexiletine and 5.27 for placebo. When group scores were compared in treatment period 1 this gave a difference of 1.68 (95% CI 2.66 to 0.706; $p<0.001$) and in period 2 a difference of 3.68 (95% CI 3.85 to 0.139 $p=0.04$). The Wald test comparing the difference in scores from period 1 to period 2 was significant (estimate 0.997, $p=0.04$) and therefore the p-value for the analysis of period 2 was corrected based on the Wald estimate to account for the carry-over effect.

5.4.3 Secondary Outcome Measures

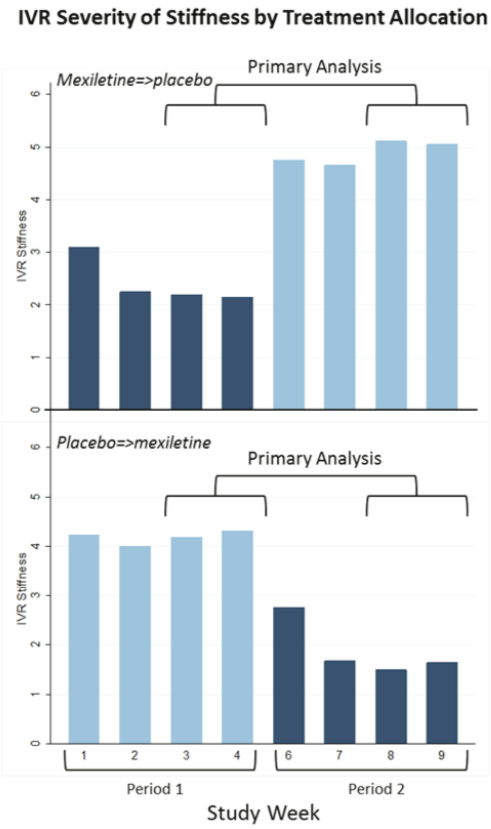
Mexiletine improved the majority of secondary outcome measures (Table 5.37). There was a significant improvement in patient-reported pain, weakness and tiredness ($p<0.001$). There were also significant improvements in both eye closure myotonia (mexiletine 0.161s vs

Figure 5.62: Stiffness severity by treatment group in the mexiletine study a) Individual treatment severity scores by treatment group in second two weeks of treatment, b) Average stiffness severity by treatment group in all four weeks of treatment (supplied by J. Statland)

a)



b)



	Mexiletine then Placebo group (n=29)	**Placebo then mexiletine group (n=30)**
Age, mean (range), yrs	41.1 (16-66)	44.7 (22-68)
Male sex	13 (44.8)	20 (66.7)
Caucasian	28 (96.6)	29 (100.0)
Medication prior to study		
Mexiletine	7 (24.1)	6 (20.0)
Other	3 (10.3)	1 (3.3)
IVR diary–stiffness, mean (SD)	3.89 (2.39)	4.63 (2.99)
SF-36, mean (SD)		
Physical, norm-based	38.7 (9.65)	40.8 (11.0)
Mental component	44.5 (13.3)	47.6 (9.8)
INQOL–QOL score, mean (SD)	14.0 (9.03)	15.9 (12.5)
Geometric-like mean (pseudo SD), secs		
Clinical hand-opening time	1.11 (0.898-3.48)	0.605 (0.510-1.84)
Clinical eye-opening time	0.507 (0.486-2.42)	0.466 (0.455-2.31)
Quantitative handgrip myotonia	0.651 (0.288-0.518)	0.507 (0.211-0.361)
Electromyographic grade = 3 Abductor digiti minimi	18 (62.1)	18 (62.1)
Short exercise test (% of baseline), mean (SD)	78.7 (24.5)	80.8 (28.7)
Mutation		
Chloride	17 (58.6)	17 (56.7)
Sodium	10 (34.5)	11 (36.7)
None identified	2 (6.9)	2 (6.7)

Table 5.35: Screening baseline characteristics of the 2 treatment sequence groups

placebo 0.474s; $p<0.001$) and hand grip myotonia (mexiletine 0.164s vs placebo 0.494s; $p<0.001$). Quantitative hand grip assessment, measuring the relaxation time to drop from 90% to 5% of isometric contraction also showed a significant improvement (mexiletine, 0.321s versus placebo, 0.429s; $p<0.001$). There was no difference between results in the long ($p=0.50$) or short exercise tests ($p=0.09$). However, there was a marked improvement in severity of EMG myotonia when on mexiletine compared to placebo ($p<0.001$) (Figure 5.63d, Table 5.37).

Quality of life measures showed significant improvements in the majority of physical aspects with mexiletine treatment (Table 5.37). INQoL had a significant improvement in the summary quality of life (QOL) score (mexiletine 14.0 vs placebo 16.7; $p<0.001$) with improvement in all aspects apart from weakness (Figure 5.63b). Of note muscle locking, pain and fatigue were all improved. SF-36 showed improvement of overall physical function

(mexiletine 42.8 vs placebo 37.8; p<0.001) and bodily pain (mexiletine 49.8 vs placebo 42.0; p<0.001) (Figure 5.63a).

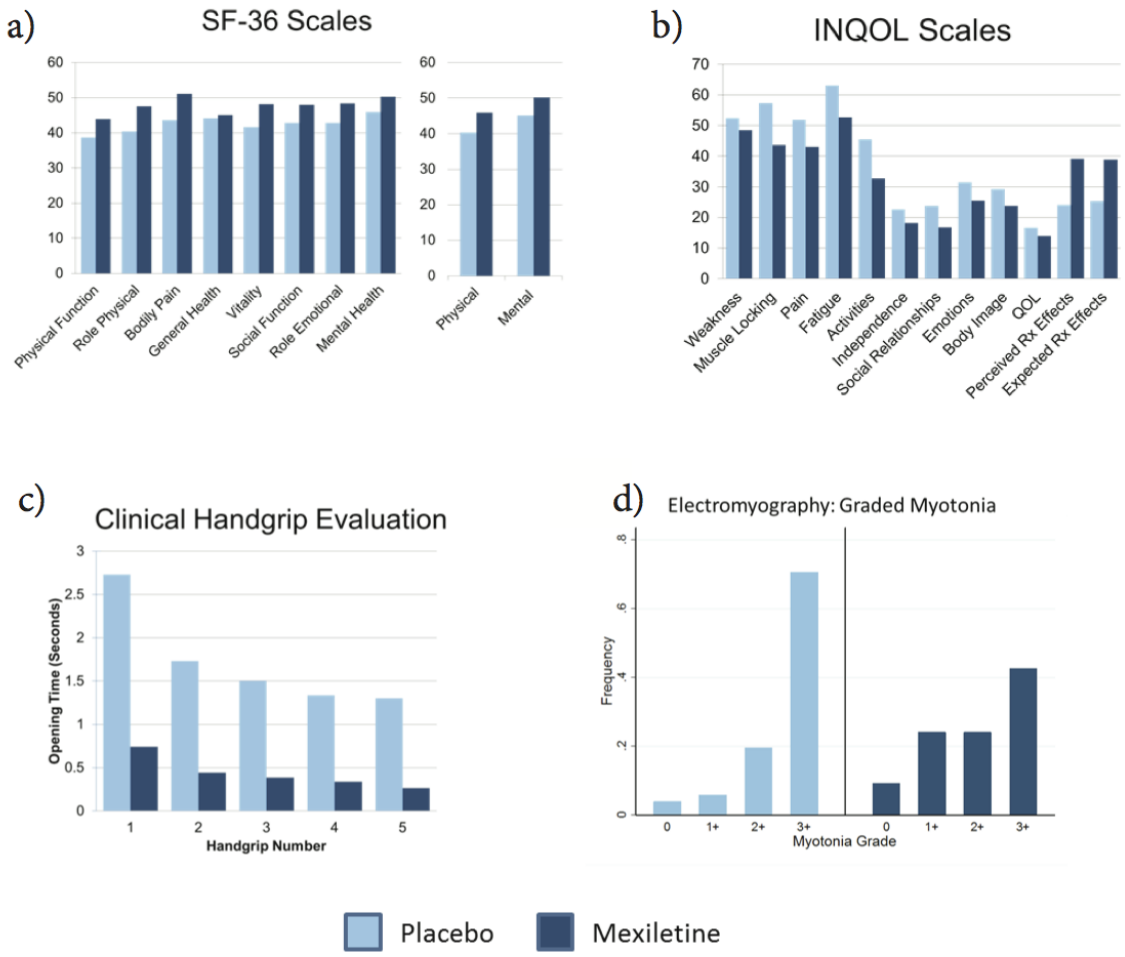


Figure 5.63: Graphs of change in secondary outcome measures in the mexiletine study a) SF-36 scales in mexiletine versus placebo groups, b) INQoL scales in mexiletine versus placebo groups, c) Hand grip opening time in mexiletine versus placebo groups, d) EMG myotonia grade in placebo versus mexiletine groups (Graphs supplied by J.Statland)

5.4.4 Subgroup Analysis

Subgroup analysis of the effect of mexiletine in chloride channel patients versus sodium channel patients was performed. It demonstrated a more marked decrease in hand grip myotonia in chloride channel patients compared with sodium channel patients (chloride channel, -1.24s versus sodium channel, -0.355s; p=0.04). The reduction in stiffness severity was greater for patients with chloride channel mutations in treatment period 2, however, patients with sodium channel mutations showed a greater reduction in stiffness severity during treatment period 1. There was no significant difference between chloride and sodium

channel patients in the reduction in eye closure myotonia or patient-reported weakness. This was despite there being a significant difference between the proportion of patients who had eye closure myotonia with sodium channel mutations compared to chloride channel mutations (91% vs 46%, p=0.002) and a significant difference in the maximum duration of stiffness between the two groups (8.5s vs 0.89, p=0.008). In comparison, although there was a significant difference between those who had hand grip myotonia with chloride channel mutations versus those with sodium channel mutations (97% vs 68%, p=0.012), there was no significant difference in the maximum duration of stiffness between the two groups (3.3s vs 3.4s, p=0.94). Subgroup analysis of neurophysiology testing was not practical giving the large variation in response between subgroups.

5.4.5 Drug Safety and Tolerability

Mexiletine was well tolerated and safe. There was one serious adverse event that was deemed to be unrelated to the study (due to narcotics withdrawal) (Table 5.36). The commonest side effect was gastrointestinal, affecting nine subjects in the mexiletine group and one subject in the placebo group. The majority of symptoms were relieved with over the counter medication or a reduction of mexiletine to twice daily dosing. One patient did not continue with the second phase of the study due to gastric discomfort. There were two reported cardiac events, both incidentally identified on ECG at the end of the treatment phase. One patient had bradycardia whilst on mexiletine, which resolved on follow-up and the other had premature ventricular complexes whilst on placebo. Both patients were asymptomatic and did not require termination of study participation.

5.4.6 Compliance and Blinding

Compliance with treatment was 90.2% for mexiletine and 92.7% for placebo for period 1 and 93% for mexiletine and 92.7% for placebo in period 2. Mexiletine levels in all patients at baseline and following washout periods were undetectable. The mean (SD) mexiletine level at the end of the treatment period was 0.54(0.35)μg/ml (therapeutic anti-arrhythmic level, 0.5-2.0µg/ml).

Compliance with calling into the IVR diary during the primary outcome time period (weeks 3 to 4) was 74.3% (78.6% in period 1 and 70.0% in period 2).

Patients were surveyed to determine if they were fully blinded to treatment allocations given the side effect profile of mexiletine. In treatment period 1, 64% (18/28) correctly

Category	Mexiletine	Placebo
Cardiac	1	1
Constitutional	3	0
Dermatology/Skin	1	2
Gastrointestinal	9	1
Infection	1	3
Lymphatics	0	1
Musculoskeletal/Soft Tissue	0	2
Neurologic	5	1
Pain	4	0
Total	24	11

Table 5.36: Adverse events in the mexiletine study

identified that they were on mexiletine and 69% (20/29) correctly identified that they were on placebo. In period 2, 79% (23/29) correctly identified being on mexiletine and 80% (20/28) on placebo.

Table 5.37: Table of secondary outcome measures for the mexiletine study

Endpoint – Period (No. of Participants)	Mean Mexiletine Treatment (95% CI)	Mean Placebo Treatment (95% CI)	Treatment Effect Estimate (95% CI)	Effect Size	p-value
IVR: Stiffness – First (57)	2.53 (1.80, 3.17)	4.21 (3.40, 5.20)	-1.68 (-2.66, -0.706)	-1.36	<0.001
IVR: Stiffness - Second (57)	1.60 (1.04, 2.20)	5.27 (4.44, 6.27)	-3.68 (-3.85, -0.139)	-2.97	0.04
IVR: Pain – Overall (48)	1.54 (0.924, 2.13)	3.17 (2.43, 3.93)	-1.63 (-2.00, -1.26)	-1.36	<0.001
IVR: Weakness – Overall (44)	1.96 (1.42, 2.63)	3.22 (2.52, 3.98)	-1.26 (-1.67, -0.861)	-0.994	<0.001
IVR: Tiredness – Overall (49)	2.9 (2.12, 3.68)	3.82 (3.03, 4.53)	-0.918 (-1.30, -0.532)	-0.709	<0.001
Short Exercise – Overall (% baseline; 56)	83.1 (77.5, 88.4)	78.6 (71.9, 84.7)	4.54 (-0.680, 9.75)	0.347	0.09
Prolonged Exercise – Overall (% baseline; 56)	81.8 (76.8, 87.0)	80.1 (74.7, 86.4)	1.69 (-3.34, 6.73)	0.134	0.50
Needle EMG: ADM – Overall (56)	2.05 (1.75, 2.33)	2.62 (2.39, 2.86)	-0.568 (-0.812, -0.325)	-0.947	<0.001
Needle EMG: TA – Overall (56)	2.07 (1.73, 2.37)	2.54 (2.28, 2.76)	-0.464 (-0.675, -0.254)	-0.900	<0.001
Clinical Assessment: Eye Closure – Overall (seconds; 57)	0.161 (0.0704, 0.314)	0.474 (0.261, 0.871)	-0.313 (-0.602, -0.149)	-0.888	<0.001
Clinical Assessment: Hand Grip – Overall (seconds; 57)	0.164 (0.0858, 0.294)	0.494 (0.281, 0.872)	-0.330 -0.633, -0.142)	-0.748	<0.001
QMA Hand Grip – Overall (seconds; 54)	0.321 (0.274, 0.370)	0.429 (0.365, 0.517)	-0.109 (-0.177, -0.0560)	-0.518	<0.001

Table 5.37: Table of secondary outcome measures for the mexiletine study (cont.)

Endpoint – Period (No. of Participants)	Mean Mexiletine Treatment (95% CI)	Mean Placebo Treatment (95% CI)	Treatment Effect Estimate (95% CI)	Effect Size	p-value
SF36: Physical Function – Overall (57)	42.8 (40.1, 46.1)	37.8 (34.9, 41.3)	5.00 (2.81, 7.20)	.904	<0.001
SF36: Role Physical – Overall (57)	46.5 (43.6, 49.2)	39.2 (35.7, 42.6)	7.23 (4.55, 9.92)	1.07	<0.001
SF36: Bodily Pain – Overall (57)	49.8 (46.4, 52.6)	42.0 (38.6, 45.5)	7.78 (5.08, 10.5)	1.14	<0.001
SF36: General Health – Overall (57)	45.5 (41.9, 48.7)	44.5 (41, 47.7)	0.977 (-0.659, 2.61)	0.240	0.24
SF36: Vitality - First (57)	45.5 (41.1, 49.6)	43.7 (39.7,48.1)	1.76 (-4.34, 7.85)	0.211	0.57
SF36: Vitality – Second (57)	51.9 (48.1, 55.5)	40.0 (35.1, 45.0)	11.9 (-0.307, 20.5)	1.43	0.06
SF36: Social Function – Overall (57)	47.1 (44.4, 49.8)	41.9 (38.5, 44.9)	5.27 (2.69, 7.85)	0.809	<0.001
SF36: Role Emotional - First (57)	46.2 (42.0, 50.3)	45.5 (41.2, 49.4)	0.764 (-5.68, 7.21)	0.102	0.81
SF36: Role Emotional - Second (57)	49.9 (46.2, 53.1)	39.1 (33.5, 45.0)	10.8 (-1.51, 21.6)	1.45	0.09
SF36: Mental Health - First (57)	47.3 (43.6, 51.0)	47.3 (43.7, 50.6)	0.016 (-5.24,5.27)	0.00258	0.99
SF36: Mental Health – Second (57)	53.3 (50.2, 56.2)	44.4 (39.8, 48.7)	8.84 (-0.572, 18.2)	1.42	0.07
SF36: Physical Composite – Overall (57)	44.8 (41.9, 47.4)	39.2 (35.9, 41.9)	5.58 (3.44, 7.72)	1.04	<0.001
SF36: Mental Composite - First (57)	47.4 (44.0, 50.2)	47.7 (44.2, 51.3)	-0.351 (-5.87, 5.17)	-0.0539	0.90
SF36: Mental Composite – Second (57)	53.1 (50.3, 55.8)	42.7 (36.8, 48.3)	10.4 (0.941, 20.6)	1.60	0.03

Table 5.37: Table of secondary outcome measures for the mexiletine study (cont.)

Endpoint – Period (No. of Participants)	Mean Mexiletine Treatment (95% CI)	Mean Placebo Treatment (95% CI)	Treatment Effect Estimate (95% CI)	Effect Size	p-value
INQoL: Weakness – Overall (35)	45.7 (37.7, 52.6)	49.3 (41.7, 57.3)	-3.56 (-9.54, 2.43)	-0.290	0.24
INQoL: Muscle Locking – Overall (43)	40.0 (33.1, 46.7)	53.8 (46.4, 61.1)	-13.7 (-20.4, -7.03)	-0.888	<0.001
INQoL: Pain – Overall (32)	39.9 (30.6, 49.0)	48.2 (39.2, 57.1)	-8.32 (-13.8, -2.87)	-0.782	0.004
INQoL: Fatigue – Overall (35)	48.4 (40.9, 56.6)	58.3 (50.6, 66.0)	-9.96 (-17.0, -2.93)	-0.678	0.007
INQoL: Activity – Overall (51)	34.2 (26.7, 43.0)	47.1 (40.1, 55.5)	-12.9 (-18.3, -7.43)	-0.950	<0.001
INQoL: Independence – Overall (51)	17.8 (12.3, 23.3)	22.5 (17.2, 28.1)	-4.74 (-8.14, -1.35)	-0.561	0.007
INQoL: Social Relations – Overall (51)	18.9 (13.5, 24.5)	25.9 (18.0, 35.2)	-7.02 (-13.4, -0.671)	-0.440	0.03
INQoL - Emotions – Overall (51)	27.7 (22.0, 34.4)	33.8 (27.1, 41.5)	-6.13 (-10.1, -2.15)	-0.619	0.003
INQoL: Body Image – Overall (51)	24.2 (17.3, 31.0)	29.4 (22.0, 36.5)	-5.27 (-10.4, -0.105)	-0.408	0.05
INQoL: QOL – Overall (51)	14.0 (11.6, 16.5)	16.7 (14.0, 19.4)	-2.69 (-4.07, -1.30)	-0.780	<0.001
INQoL: Perceived Treatment Effect – Overall (51)	36.6 (27.1, 45.8)	21.7 (12.7, 31.1)	14.9 (7.43, 22.3)	0.797	<0.001
INQoL: Expected Treatment Effect – Overall (51)	36.1 (26.9, 47.0)	23.1 (14.5, 33.6)	13.0 (4.18, 21.8)	0.585	0.005

Chapter 6

DISCUSSION

6.1 Prevalence Study of the Skeletal Muscle Channelopathies in England

6.1.1 Key Findings of the Prevalence Study

This prevalence study identifies the overall prevalence of skeletal muscle channelopathies in England. It estimates the minimum prevalence at 1.12/100,000, suggesting it is comparable to other rare neuromuscular disorders such as Pompe Disease (1.1/100,000) and Lambert-Eaton myasthenic syndrome (1.0/100,000) (www.orpha.net). The prevalence of the commonest channelopathy, MC was estimated at 0.52/100,000 and PP at 0.37/100,000.

6.1.2 Novel Findings Identified in the Prevalence Study

This is the first documented study investigating the prevalence of all of the skeletal muscle channelopathies as there are no other studies documenting the prevalence of HyperPP, ATS or SCM in any country. It is also the only study to estimate channelopathy prevalence for the whole of England. The only previous study identified prevalence for a part of northern England (Walton and Nattrass, 1954). This is the largest series of patients with genetically-defined skeletal muscle channelopathies reported to date and one of very few conducted since genetic testing has become widely available (Darin and Tulinius, 2000; Sun et al., 2001). It is also one of the only studies to use only genetically confirmed cases to try to accurately determine the minimum point prevalence (Sun et al., 2001).

6.1.3 Comparison with Previous Prevalence Studies

As this is the first study of prevalence of all skeletal muscle channelopathies it is difficult to compare the overall results, however the individual prevalences can be compared. The majority of literature available discusses the prevalence of MC. When all the previous literature is combined it estimates the prevalence of MC worldwide at approximately 0.67/100,000 with a range from 0.3-9.0/100,000 (Table 2.2, p 36). The prevalence of MC in this study is comparable to the lower end of the average and this is likely to be related to the fact that I have only used genetically confirmed cases. The prevalence for England is significantly lower when compared to the Scandinavian countries, which report prevalences between 7-9/100,000 (Baumann et al., 1998; Sun et al., 2001) but this is likely to be related to the founder effect and geographical restrictions of that region causing higher prevalences than is seen in other areas of the world (Baumann et al., 1998; Papponen et al., 1999; Sun et al., 2001).

In keeping with other reports of MC prevalence, the male to female ratio was 1.97 (Papponen et al., 1999) with a higher ratio in recessive MC than dominant MC (Becker et al., 1977) (Table 5.1, p 97). This may be due to the fact that females are more mildly affected than males and therefore there may be a selection bias towards male patients (Becker et al., 1977; Colding-Jorgensen, 2005). A possible explanation for the ratio being higher in recessive MC is that there have been reports of male patients with single recessive mutations being more likely to have myotonia on EMG, suggesting that there may be male manifesting carriers (Deymeer et al., 1999; Mailander et al., 1996). However, when the cases in this study were analysed there were only two heterozygous patients with recessive MC and although they were both male they would not be enough to account for the large difference here in the male to female ratio seen between dominant and recessive cases in this cohort. This suggests that there may be a hormone-related explanation for this (Fialho et al., 2008; Burge et al., 2013).

The prevalence of PMC from other studies ranges from 0.2-1.1/100,000 (Table 2.2). These studies do not make a distinction between SCM and PMC and therefore it is likely that the term PMC was used to encompass both diseases. This study estimates the prevalence of PMC and SCM together at 0.22/100,000 and therefore matches the lower end of other studies.

The prevalence of HypoPP from other studies ranges from 0.4-1.7/100,000 but all studies pre-date genetic testing (Table 2.2). The overall estimate for PP from this study is 0.37/100,000 with HypoPP specifically at 0.17/100,000. This difference is likely to be related

to the clinical overlap between the different types of periodic paralysis as well as the overlap between HypoPP and other neuromuscular disorders causing an overestimate and possible misclassification of cases if genetic testing is not done. This problem is more common in the diagnosis of PP than in NDM as the main objective clinical test, the long exercise test, is only positive in approximately 60% of cases. This is unlike in NDM, when myotonia on EMG can provide an objective marker of disease with a high negative predictive value as only a handful of cases do not have myotonia.

There is likely to be an underestimate in cases in this study as only genetically confirmed cases were counted. This is especially problematic in PP as it is known that approximately 20% of cases remain genetically unconfirmed despite full sequencing of known channel genes (Sternberg et al., 1993; Jurkat-Rott and Lehmann-Horn, 1993). If this is corrected for in the prevalence estimate then the prevalence for PP in England would be closer to 0.46/100,000 which is in keeping with other studies but still at the lower end of other estimates. The male to female ratio for PP in this study was strikingly high at 3.52 (Figure 5.2, p 98). It has been noted in many other studies that the ratio is high in HypoPP and this is thought to be related to reduced penetrance in women but other studies have only reported a ratio of between 1.23-1.63 (Elbaz et al., 1995; Miller et al., 2004). The high ratio was primarily in patients with the Arg528His mutation, at 5.8 and it is unclear why the ratio overall is still much higher. It may be related to a greater selection bias due to under-reporting in milder females as this study follows an epidemiological design rather than a genetic study design in which all family members would be sequenced.

6.1.4 Genetic Mutations Identified in the Prevalence Study

The spectrum and frequency of genetic alterations observed in patients with NDM and PP were similar to those reported in the literature (Feero et al., 1993; Fouad et al., 1997; Miller et al., 2004; Donaldson et al., 2004; Vicart et al., 2005; Lossin and George, 2008). A large number of private mutations were found in patients with MC and ATS. However, only a limited number of mutations accounted for most cases: 15 CLCN1 mutations accounted for disease in 83% of all patients with MC, 11 SCN4A mutations in 86% of PMC and SCM pedigrees (Figure 5.4, p 101) and three KCNJ2 mutations in 42% of ATS pedigrees.

6.1.4.1 Myotonia Congenita

Whilst previous surveys have suggested a predominance of recessive over dominant MC, I found similar percentages of dominant and recessive pedigrees (Baumann et al., 1998; Sun et al., 2001; Becker et al., 1977). Studies in areas with high prevalence of MC have shown that more than 85% of affected individuals were compound heterozygous or homozygous for CLCN1 mutations, suggesting recessive inheritance in most cases, even in families with apparent dominant transmission (Papponen et al., 1999; Sun et al., 2001). This may be explained by a high carrier frequency of certain mutations in those areas, probably resulting from a founder effect and geographical isolation (Papponen et al., 1999; Sun et al., 2001). In this study, most patients with a dominant family history were heterozygous, with variations that are known to exhibit a dominant-negative effect, supporting a true dominant inheritance (Figure 5.5, p 103). One possible bias in this study data could be that there is more likely to be accurate family history data in dominant MC pedigrees therefore making it easier to classify these patients compared with recessive MC giving the appearance of higher numbers of dominant versus recessive cases. Also, patients with a dominant family history may exhibit a reporting bias as they know what signs are associated with the disease. When family history was excluded the total proportion of heterozygous patients (59%) was higher than that of patients with two or more mutated alleles (41%) suggesting that this may be a true difference in the British population.

In this study, the breakdown of mutations in MC patients and the frequency with which they are associated with second mutations was assessed. It is thought that certain mutations cause dominant-negative inhibition of the ClC-1 homodimer resulting in dominant MC and dominantly inherited disease. However, it has been observed that some mutations in CLCN1 can cause both dominantly and recessively inherited disease (George et al., 1994; Meyer-Kleine et al., 1995; Zhang et al., 1996; Sun et al., 2001) although the mechanism for this is not understood. This study allowed a closer look at the inheritance of a large number of mutations. It demonstrates that the majority of heterozygous mutations were associated with dominant MC (95%). Of the two patients with recessive MC and a single mutation, one had a novel mutation, Val321Leu, and the other carried Phe413Cys which, although is commonly associated with recessive MC, has been identified heterozygously in another study (and is seen here heterozygously in two other cases with unknown family histories) (Sun et al., 2001). The makes it more likely that in these cases there may be a second mutation that has

yet to be identified.

When a mutation-specific analysis is done, however, the the issue of variable inheritance is more evident. For instance the Gly285Glu mutation is expressed with a second mutation in only 65% (20) of cases. In the remaining 11 cases the family history could not be fully identified so it is not clear if in those cases it is associated with the dominant MC. A similar case is seen for Phe167Leu and Val327Ile when 63%(5) and 57%(4) of cases were associated with a second mutation. However in all the single mutation cases, again the inheritance was unknown. So although the data here supports the possibility of variable inheritance for particular mutations it must be interpreted with caution as the family data was not available.

The other mutation which is commonly discussed in the literature as being associated with dominant and recessive MC is Arg894X. It is commonly associated with recessive MC (George et al., 1994) but cellular electrophysiology studies for this mutation have been inconclusive, demonstrating both a large reduction in chloride currents and a weak dominant-negative effect in co-expression studies consistent with both recessive or dominant inheritance (Meyer-Kleine et al., 1995). In this study 14 (78%) patients had a second mutation expressed with it but three of these patients came from dominant pedigrees. The family history was unknown for the four patients who only carried Arg894X. When the data is critically assessed there is insufficient evidence for true duality in MC mutations.

6.1.4.2 PMC & SCM

This study demonstrates an interesting pattern for mutations causing PMC and SCM. As has been found by others, all the cases with Thr1313Met and mutations at the Arg1448 locus clinically had PMC (McClatchey et al., 1992b; Ptacek et al., 1993; Hayward et al., 1996; Yang et al., 1994) (Table 5.4b, p 104). However, although previously mutations at the Gly1306 locus have traditionally been thought to only cause SCM (Mitrović et al., 1995; Ricker et al., 1994; Lerche et al., 1993; Colding-Jorgensen et al., 2006) this study demonstrates that they can also be associated with PMC, especially Gly1306Val which was associated with PMC in 6/8 patients (Table 5.4b). Some of this data was initially published by our group suggesting that there were 3 atypical cases but since then we have found numerous others (Matthews et al., 2008b).

The Val1589Met mutation is well documented as being associated with both PMC and with SCM (Mitrović et al., 1994; Ferriby et al., 2006; Heine et al., 1993); and there were almost equal numbers of patients in this study (14:13) with each type of disease (Table 5.4b).

Another 2 mutations which have previously been documented as being associated with only PMC, Ile693Thr (Plassart-Schiess et al., 1998; Plassart et al., 1996; Hayward et al., 1999) or only SCM, Val1293Ile (Green et al., 1998; Koch et al., 1995) were found to be associated with both forms of the disease (Table 5.4b). This evidence demonstrates that there is more phenotypic variability within different SCN4A genotypes than previously described and how difficult it is to direct genetic testing based on current disease classification alone. This suggests that further work may be needed to better differentiate between these two diseases.

6.1.4.3 Periodic Paralysis

Amongst patients with HypoPP, 82%(78) had HypoPP1 and 18%(17) HypoPP2. This is higher than the 10-12% that has been previously documented in other studies (Sternberg et al., 2001; Matthews et al., 2009) which may be related to the fact that previous studies did not look for mutations in the whole of SCN4A and only sequenced specific exons. In this study 6/17 of mutations in SCN4A were found outside of exons 12 and 18 and therefore would have been missed by routine sequencing (Sternberg et al., 1993). In HypoPP1 the majority of patients had either Arg1239His (50%) or Arg528His (44%) mutations. In HypoPP2 there was a more even spread of mutations although they did cluster around exons 12 and 18 (65%).

Amongst HyperPP patients, 75% (53) had mutations at Thr704Met and 20%(14) had mutations at Met1592Val which is consistent with the numbers found in the large series by Miller *et al* (Miller et al., 2004).

In ATS, I observed that the majority of mutations were personal to specific pedigrees as described elsewhere (Donaldson et al., 2004) although the Arg218Trp and Arg67Trp were each observed in four different UK families.

6.1.5 Limitations of the Prevalence Study

Due to the study design there are some limitations in the data. To ensure that only definite cases were counted, only genetically-confirmed, clinically affected patients were included. Whilst this gives a more accurate calculation of the minimum prevalence it makes it more likely that this may be an underestimate of the true prevalence. There are other aspects of the study design which increase the possibility of incomplete case ascertainment. The study had a service-based, epidemiological design, so only affected individuals seeking medical attention who were referred to our centre were identified. However, as the genetic diagnostic service

for skeletal muscle channelopathies in England is centralised, this means all genetically confirmed cases in England should have been captured. There are however, a small number of patients who have been sending DNA samples to Germany as part of their free research service and therefore they may not be captured unless they then seek intervention in England following diagnosis. The common difficulty with these types of studies is that those who are mildly affected and do not seek medical advice and those that may be part of large affected families with a well established diagnosis, may choose not to have a formal genetic diagnosis and therefore are not captured.

6.1.6 Further Work to Determine Prevalence

To take this work further it would be useful to repeat this study with a survey-based design to see if a similar prevalence rate is identified, including approaching many of the charities to distribute information and advertising on social media. This would reduce the problem of incomplete ascertainment especially in mildly affected patients who are genetically unconfirmed. It would also be useful to repeat the study including looking for patients with mutations in the recently identified KCNJ18 gene (Ryan et al., 2010).

6.2 Clinical Phenotype Study of Patients with PP and NDM

To date the only large phenotype studies in muscle chanelopathies in the UK cohort have been in MC (Fialho et al., 2007). There have been some studies of HypoPP and HyperPP patients in other countries (Miller et al., 2004; Charles et al., 2013) but no phenotype studies of these diseases in the UK cohort. Again in ATS there have not been any large phenotype studies in the UK cohort. Most strikingly, however, there have been no large studies internationally comparing PMC and SCM patients. As part of this thesis, I therefore attempted to carefully phenotype the UK cohort of patients with PMC, SCM, HypoPP, HyperPP and ATS to establish the key features of these diseases in those with genetically confirmed disease.

There have also been no studies investigating the effect of anaesthetics and pregnancy in NDM and PP patients and therefore I have attempted to ascertain key aspects of these two conditions in patients compared with the general population.

6.2.1 Clinical Phenotypes of PMC & SCM

Traditionally patients with sodium channel mutations and prominent myotonia have been divided into PMC and SCM (or PAMs). Patients with PMC have been described as having myotonia with episodes of weakness. They typically have paramyotonia, worsening myotonia with exercise, and are usually markedly cold-sensitive (Eulenburg, 1886). Patients with SCM have been described as being potassium-sensitive with no episodes of weakness. They may have warm-up, are not always cold-sensitive and may have delayed myotonia following exercise (Trudell et al., 1987; Ricker et al., 1990; Lerche et al., 1993; Matthews et al., 2010). As demonstrated in the prevalence study in the previous chapter, individual mutations were associated with both diseases and some patients have a mixed presentation making clinical diagnosis difficult. There are no large studies comparing the phenotypes of these two diseases and little information published on the general phenotype of patients with SCM. This study aimed to be the first to carefully phenotype patients with PMC and SCM and identify key differentiating aspects between the two groups and improve diagnostic clarity. However, when I analysed the data from a large cohort of genetically confirmed patients it did not illustrate two clinically distinct groups. This suggests that these two conditions are part of a spectrum of a single disease rather than two distinct diseases and it would be more accurate to consider them as one disease.

6.2.1.1 Key Differences between PMC and SCM

PMC and SCM are traditionally clinically differentiated by the presence or absence of weakness. They are also thought to be distinguished by neurophysiology type, with PMC associated with a Fournier type I pattern and SCM associated with a Fournier type III pattern. I therefore first looked at how many patients, diagnosed by the assessing clinician with PMC and SCM, had weakness and discovered that despite the traditional classification, not all patients with PMC had weakness and some patients with SCM had episodes of weakness (Figure 5.8, p 109). This suggested that there were flaws in the classification. This led me to split the cohort according to the presence and absence of weakness rather than by clinician diagnosis to see if this distinction produced two clinically distinct groups. As illustrated in Table 5.6 (p 110), the only difference between these groups of over 80 patients, was the severity of myotonia and that was not significant once multiple corrections were accounted for. Surprisingly, none of the patients without episodes of weakness reported potassium sensitivity as would be expected in SCM, which is traditionally composed of the "potassium-aggravated" myotonias. This may either be because the incidence of potassium sensitivity is lower than first thought or more likely because, since the advent of genetic testing, we no longer perform potassium loading tests and so have no objective measure of potassium sensitivity.

To investigate if neurophysiology classification was a better differentiator of patients with PMC and SCM, I analysed the data according to Fournier type. This again did not show any significant differences apart from in interictal potassium which was high in patients with type III pattern of neurophysiology (associated with SCM). As type III is commonly associated with PAM, the marginally higher interictal potassium may explain the increased sensitivity to potassium in those patients. As previously reported it is however still within the normal range for potassium in both groups (Miller et al., 2004) and the difference seen was not significant once corrected for multiple comparisons.

6.2.1.2 Key Genotype-Phenotype Correlations in PMC and SCM

I then looked at the phenotypes of specific variations to see if specific genotypes were associated with distinct phenotypes. This analysis was limited due to the relatively small numbers but despite this, a number of interesting trends were revealed. Although all patients with variations at position Arg1448 and those with the Thr1313Met variation were diagnosed

as PMC, there was a mix of SCM and PMC diagnoses in patients with variations at position Gly1306 and Val1589Met variations which may be why an arbitrary comparison of PMC and SCM may lead to two similar phenotypic groups.

Overall this study found that Gly1306 variations were associated with older onset, warm-up and more severe pain, which correlates with other publications (Ricker et al., 1994). Patients tended to be both heat and cold-sensitive, which may be why others have reported patients not being as cold-sensitive as seen in other variations (McClatchey et al., 1992b). Patients were less likely than in other variations to have attacks of weakness but weakness was still seen in 50% of the UK cohort, contrary to previous reports that this mutation was primarily associated with SCM and no attacks of weakness (McClatchey et al., 1992b; Ricker et al., 1994). Attacks, however, tended to be shorter in duration and these patients frequently had type III neurophysiology as previously reported (Fournier et al., 2006).

Arg1448 variations tended to have a younger age of onset also with warm-up. The eyes were the most severely affected part and patients were more likely to have episodes of weakness as described in the literature for classical PMC. In this cohort, patients had type I neurophysiology, but this was only apparent after cooling. Electrical myotonia commonly increased with cooling, suggesting electrical cold sensitivity. This is in contrast to previous reports that suggest that Arg1448 variations are associated with a type I pattern without the need for cooling (Fournier et al., 2004, 2006).

Thr1313Met patients had a younger onset of symptoms and were more sensitive to exertion than the other mutations. They also more commonly had episodes of weakness consistent with reports that they were commonly associated with a PMC phenotype (McClatchey et al., 1992b; Ptacek et al., 1993). Attacks were more frequent and longer duration than the other mutations. In keeping with this they had type I neurophysiology, abnormal long exercise testing and myotonia reduced by cold.

The Val1589Met mutations had an older age of onset with eyes markedly affected. They were often heat as well as cold sensitive and had limb hypertrophy on examination. They were also noted to have a high CK and type III pattern of neurophysiology.

6.2.1.3 Suggestions for a Change in Nomenclature

Analysing the data from a large number of patients with sodium channel mutations, it appears that the traditional distinction of patients as having PMC or SCM according to presence or absence of weakness is arbitrary and there is no significant phenotypic difference

between these two groups. This also holds true when dividing patients according to their neurophysiology pattern. Overall, cold sensitivity was very common amongst all patients, being seen in 93% and therefore not restricted to the PMC phenotype. Warm-up was also more common than previously thought and reported in 35% of all patients. Painful myotonia was also common amongst patients, reported in 78% but not particularly associated with SCM or patients with no weakness as has been previously suggested (Trudell et al., 1987). As originally observed by Eulenburg, I also found face and eyelid myotonia were very common.

The similarity of the phenotypes of PMC and SCM may explain why there have been many reports of patients with the same mutation, both from different pedigrees and even occasionally within the same pedigree, that may present with either PMC or SCM (Plassart et al., 1996; Matthews et al., 2008b). This finding would be better explained by these conditions being part of the same disease. I did however, find specific genotype-phenotype correlations in this cohort suggesting that particular variations predispose an individual to being at a particular end of the sodium channel disease spectrum. I therefore propose that it may be more clinically useful to diagnose patients as having "Sodium channel disease" with further clinical advice being given according to the specific genotype identified on genetic testing.

6.2.2 Phenotypic Differences in HyperPP versus PMC & SCM

Since dividing PMC and SCM patients did not yield any differences, I set out to confirm that the UK cohort of PMC and SCM patients was still phenotypically different from those with HyperPP. The data showed significant differences between these two groups, especially related to painful myotonia and the nature of the attacks of weakness (Figure 5.13, p 121). Those with PMC/SCM tended to have shorter more frequent attacks of milder severity. These were more likely to be focal and occur at any time of day compared with the primarily night-time, generalised attacks seen in HyperPP.

The nature of the myotonia in these two groups, however was very similar although, as with the episodes of weakness it tended to be more generalised in HyperPP and focal in PMC/SCM with greater cold sensitivity. These results are in keeping with those published in the large comparative study conducted by Miller *et al* (Miller et al., 2004), validating these results.

6.2.3 Clinical Phenotype of Periodic Paralysis

There is one large comparative phenotype study of patients with HypoPP and HyperPP conducted in 2004 (Miller et al., 2004) that included genetically unconfirmed cases, but since then no further studies have been published. There are no published studies that compare ATS and the other types of PP and individual studies in ATS have looked at fewer than 25 patients. I therefore attempted to phenotype a large number of genetically confirmed PP patients referred to our centre to identify key phenotypic patterns, identify genotype-phenotype correlations and further understand these diseases in the UK cohort.

The age of onset in HyperPP and HypoPP was similar to what has previously been reported, with HyperPP cases having a significantly lower age of onset than HypoPP patients (Miller et al., 2004) (Figure 5.15, p 126). The ratio of males to females was high as has been suggested in other studies although this cohort had a higher ratio than was previously reported in both HypoPP and HyperPP patients. This may suggest a lower penetrance in females in this cohort than has previously been documented or may be related to ascertainment bias as mildly affected females are less likely to be referred for genetic testing and assessment (Sternberg et al., 2001; Charles et al., 2013). Others have also suggested that there is a difference in age of onset between males and females (Sternberg et al., 1993), finding that females tended to have two to three years earlier age of onset. I found an even more striking difference with the median age of onset for women, 6.5 years younger than that for men (12 years versus 5.5 years) in HypoPP. In HyperPP and ATS however, the age of onset was much more similar between genders (HyperPP, 5 versus 4 years and ATS, 10 versus 7.5 years). The incidence of sporadic cases in this cohort was similar to that recently reported in HyperPP of less than 10% (Charles et al., 2013) although previously it was thought that the incidence was higher (Venance et al., 2006). This is likely to be because genetically unconfirmed cases were included in previous estimates.

6.2.3.1 Key Phenotypic Features in HypoPP

To improve the knowledge of the HypoPP phenotype and identify key features present in the UK cohort, I investigated key elements of the attacks of weakness (Table 5.10, p 124). I found that almost all patients (48/49) had attacks of complete paralysis involving arms, legs and neck which is similar to what has been reported previously (Sternberg et al., 1993). To investigate attack frequency and duration, I split them into major and minor attacks to

encompass the significant differences in types of attack commonly reported by patients. This aimed to improve accuracy of reporting, as patients find it easier to report the worst ever attack length than try to estimate an average length of their very variable attacks. Severe attacks of complete paralysis occurred, on average, once a month whereas minor attacks, either focal attacks or attacks with only partial paralysis, were on average 9.9 times per month. Severe attacks lasted on average, 109 hours compared to 52 hours for minor attacks. Previous papers have not made this distinction in types of attacks but I felt it was important to do so as, after talking to patients, there is extensive variability in attack length in each individual. Previous estimates of attack frequency range from 7 to 9 attacks per month (Miller et al., 2004) which matches my estimate for minor attacks.

The average duration of attacks in many studies are poorly defined and suggest it may be hours to days. The only papers to fully quantify it suggested it was on average 20 hours (Miller et al., 2004; Venance et al., 2006; Sternberg et al., 1993). I found the average attack duration, even of minor attacks to be much higher suggesting under-reporting of average attack length previously although it could alternatively represent over-reporting in this cohort (Table 5.10). However, given that this higher attack duration is seen across all the UK patients it may be an accurate representation. As a marker of severity I also looked at the number of patients who required hospitalisation during severe attacks and in HypoPP this was as high as 82%. This has not been previously reported but gives an important insight into the associated morbidity of the disease. Attacks primarily occurred at night or on waking (72%). Ictal potassium was noted to be low in 93% of patients with a mean of 2.39mmol/L.

I investigated key precipitants and relievers of attacks in HypoPP and, as has been reported throughout the literature, carbohydrates and strenuous exercise were the two commonest precipitants (Miller et al., 2004; Venance et al., 2006; Sternberg et al., 1993; Kantola and Tarssanen, 1992) (Table 5.10). Potassium supplementation helped relieve attacks in the majority of cases (89%). Acetazolamide, dichlorphenamide and potassium sparing diuretics were all effective in improving symptoms in the majority of patients who tried them. Two patients reported worsening with dichlorphenamide, both of whom had HypoPP2 and one reported worsening with a potassium-sparing diuretic.

Progressive weakness was noted in half of participants with an average age of onset of 36.7 years. There has been no systematic study investigating this but Links *et al* suggested that it commonly occurs in the fourth to fifth decades independent of the attack frequency

and severity (Links et al., 1990). This study therefore suggests that the age of onset may be earlier than initially thought. There was also no correlation between attack frequency, duration or age of onset of weakness and the age of onset of progressive weakness. Despite only 20 patients reporting proximal weakness, 25 patients were found to have some proximal weakness on examination suggesting that it may be under-reported by patients (Table 5.10).

The EMG was myopathic in 43%, with normal short exercise tests in all patients and abnormal long exercise tests in only 67% of patients suggesting that it cannot be relied on to identify all patients who are likely to be mutation positive. There was no correlation between percentage decrement on long exercise testing and severity of attacks (p=0.634). 9 patients had biopsies, all of which were abnormal, most commonly with vacuoles although one had tubular aggregates similar to previous reports (Links et al., 1990) (Table 5.10).

There has only been one study of MRI in HypoPP in 6 patients which demonstrated a difference in oedema compared with controls (Nagel et al., 2011). I found the MRI was abnormal in 12 out of 14 patients with primarily fatty infiltration seen in the thighs. Those without fatty infiltration in the thighs had oedema either in the calves or the thighs. 4 out of the 12 patients had severe changes on MRI and all reported: progressive weakness, severe to moderately severe attacks of weakness, fixed upper limb and lower limb weakness and elevated CK. No patients with only mildly abnormal or normal MRI scans had fixed weakness and all had normal or only mildly elevated CK (maximum 363IU/L). Two patients had mild arm or leg weakness on examination and there was significant variation in attack severity between the patients. Not only did MRI severity correlate with progressive weakness but there was also a difference in the average age between those with severe to moderate changes (43 years) and those with mild or normal changes (32 years). These findings suggest that MRI findings may be a good marker of clinical severity and may be useful in prognosticating for patients.

Phenotypic Differences in HypoPP1 versus HypoPP2

I investigated the key differences in phenotypes between HypoPP caused by mutations in sodium versus calcium channels. There is conflicting evidence in previous studies as to differences between these two groups. The two main studies only had a small number of patients with HypoPP2, as was also the case in this study, making it difficult to make strong conclusions about these two phenotypes (Miller et al., 2004; Sternberg et al., 2001).

Miller *et al* suggested that those with HypoPP1 had an earlier age of onset and longer

duration of attacks compared to HypoPP2 (Miller et al., 2004). However the original study in which the HypoPP2 mutation, Arg672Gly was identified suggests that the age of onset is lower than Arg528His mutations and similar to Arg1239His mutations (Sternberg et al., 2001). This study had marginally more patients sampled (n=11) compared to the Miller *et al* paper (n=7) but also found that the age of onset for HypoPP2 cases was younger (although not significantly so), especially compared to the Arg528His patients. The Miller *et al* study suggested that the duration of attacks in HypoPP2 were significantly shorter (1 hour versus 20 hours) (Miller et al., 2004), however I found attack length was much longer (153 hours versus 29 hours) but the UK data is skewed by two patients with very prolonged attacks of two to three weeks long and so is questionable. Both groups had 43% of patients with attacks lasting less than 24 hours suggesting that when the outliers are removed the attack duration may be similar to other studies.

As was found in the Miller *et al* paper, common precipitants were very similar between the two groups. There was a marked difference in age of onset of progressive weakness, with HypoPP2 patients in this cohort having an earlier age of onset of weakness, greater than could be accounted for by their earlier age of onset of disease alone. This is also reflected by the markedly poorer functional status of these patients with almost half needing to mobilise with a stick or worse compared with only 7% of patients with HypoPP1. More HypoPP2 patients also had myopathic EMGs which would correlate with this. There was however, no significant difference in power grading or presence of muscle atrophy possibly because these measures may not be sensitive enough to determine a difference and further quantitative measures of muscle strength are needed. It does seem from this evidence that, in the UK cohort, HypoPP2 patients were more severely physically affected.

There have been a number of publications suggesting that patients with HypoPP2 have poorer response to acetazolamide and in some cases a deleterious effect (Sternberg et al., 2001; Bendahhou et al., 2001; Miller et al., 2004; Kim et al., 2007). One study reviewing all the case literature found that only 3 out of 19 patients had a response to acetazolamide and 11 had a deleterious effect (Matthews et al., 2011a). This is compared to 31 out of 55 patients with HypoPP1. I also found a significant difference in response to acetazolamide with only 2 out of 6 patients responding. Interestingly none of the patients reported a deleterious effect. The UK cohort however, did have a much higher percentage of patients with HypoPP1 that had improvement with acetazolamide, 83% compared to the reported

56% in other studies (Matthews et al., 2011a). The results for dichlorphenamide were also contrasting, in HypoPP1 all patients reported an improvement and in other studies it has been demonstrated to have a significant effect compared to placebo (Tawil et al., 2000). However, only three UK patients with HypoPP2 found it improved symptoms and two patients found that it worsened their symptoms, something which has not been reported in the literature previously. In this cohort, potassium was beneficial in 96% of HypoPP1 but only 60% of patients with HypoPP2. This has not been investigated in other studies.

Overall the evidence from this cohort suggests that HypoPP2 patients were more severely physically affected than HypoPP1 patients, supported objectively by myopathy on EMG. However, these results should be interpreted with some caution given the small numbers. I also found that HypoPP2 patients were not as responsive to acetazolamide, may be worsened by dichlorphenamide and may not be as responsive to potassium, a finding which has not been previously reported.

Genotype-Phenotype Correlations in HypoPP Mutations

Previous studies have looked at the differences between specific genotypes. They found that patients with Arg1239His mutations have a younger age of onset than Arg528His and were more sensitive to exercise (Miller et al., 2004; Sternberg et al., 2001; Jurkat-Rott et al., 2009). Arg528His patients also tended to be more sensitive to carbohydrates, have more severe weakness and have a more myopathic EMG (Miller et al., 2004). The data from this cohort concurs with existing evidence, demonstrating a significantly lower age of onset of disease with Arg1239His patients. The duration and frequency of attacks were not significantly different as found by Miller *et al* (Miller et al., 2004). Contrary to Miller *et al* however, I found that all patients with Arg1239His were sensitive to carbohydrates and rates of sensitivity to exercise were similar between the two groups. I also found that Arg1239His patients were more sensitive to stress and illness than Arg528His patients. Age of onset of proximal weakness was also markedly lower in Arg1239His and associated with a more myopathic EMG which is contrary to what was found by Miller *et al* but their study had smaller numbers for comparison suggesting they may not be representative.

Previous studies have suggested that the response to acetazolamide was similar in both groups (Sternberg et al., 2001; Jurkat-Rott et al., 2009; Matthews et al., 2011a) and this study had similar findings, although I found overall there was a greater response rate noted amongst these patients. Ictal potassium level has been variably reported with some groups suggesting

that levels are higher in Arg1239His (Sternberg et al., 2001; Jurkat-Rott et al., 2009) and others reporting it higher in Arg528His (Miller et al., 2004). This study supports a lower ictal potassium level in Arg1239His mutations but it is difficult to ascertain the accuracy of this as the data was not taken in a controlled way and the time it was taken during an attack varied between patients and may account for the variability between different studies.

Overall I found that Arg528His and Arg1239His mutations were very similar. The key differences were that patients with Arg1239His appeared to be more severely affected with an earlier age of onset, earlier age of progressive weakness and myopathic EMG.

6.2.3.2 Key Phenotypic Features in HyperPP

As part of this comparative study, I looked at the phenotype data for HyperPP patients. During this data analysis period a large scale phenotype study of HyperPP was also published (Charles et al., 2013), but prior to this the only comparative study published was in 2004 (Miller et al., 2004).

Other reports suggested that onset of HyperPP is in the first decade of life, which is consistent with the findings in this study. There is some variability between other studies, with Miller *et al* suggesting an age of onset of 2 years $\pm$ 4 (Miller et al., 2004) and Charles *et al* finding that 25% of patients had their first attacks in the second decade of life (Charles et al., 2013). This study's findings correlate to some extent with these papers. The mean age of onset was 7 years $\pm$ 5 years but I found that a third of patients had onset in the second decade of life agreeing with the recent suggestion that many patients do have a later onset than previously thought.

Patients with HyperPP are traditionally described as having relatively mild attacks of paralysis, tending to have more focal attacks with high frequency and duration of approximately 24 hours (Amato and Russell, 2008; Miller et al., 2004). The types of attack have not been quantified previously but in this cohort a surprisingly large number of HyperPP patients (76%) had severe attacks of paralysis from the neck down. I found that 42% had 1-3 attacks per month which is a much higher proportion than described (Charles et al., 2013). The duration of HyperPP attacks were longer than the 24 hours suggested in early reports (Miller et al., 2004; Venance et al., 2006). The Charles *et al* study also suggested that 22% of their subjects had attacks lasting over 2 days (Charles et al., 2013). I found that 56% of patients had major attacks lasting over 2 days and only 14% had minor attacks lasting over 2 days. 43% of patients in the study had minor attacks lasting less than 2 hours consistent

with the earlier literature. The time of attacks clustered around night-time and early morning with 27% of patients having attacks at night, 27% on waking and 36% in the morning which is similar to the ratios reported previously (Charles et al., 2013). During attacks the ictal potassium was thought to be normal or high and I found that the mean ictal potassium was mildly elevated at 5.16mmol/L (upper limit of normal 5.0) with four out of seven cases having a high potassium and three having a normal potassium. This value is marginally lower than 5.4mmol/L previously reported (Miller et al., 2004).

Put together this data suggests that patients with HyperPP may have two distinct types of attack, the minor more focal attacks which are short and frequent in duration and major attacks which are infrequent but much more prolonged.

In HyperPP, rest after exercise, immobility and exercise were the commonest precipitants. Exercise only precipitated events in 45% compared to the 80% previously reported but in that study they did not separate it with rest after exercise and so may have encompassed both groups (Miller et al., 2004). Rest after exercise in this group was a precipitant in 68%, very similar to the 67% reported by Charles *et al* (Charles et al., 2013). Surprisingly, in this study, high potassium only triggered attacks in 7% of patients in contrast to the previously reported 20-35% (Miller et al., 2004; Charles et al., 2013). This may be explained by the fact that many patients avoid potassium and so do not report it as a common trigger and the fact we no longer perform potassium provocation testing.

Proximal weakness was reported commonly in this group of patients with estimates varying from 37–60%, tending to occur in the fifth decade (Miller et al., 2004; Charles et al., 2013). I identified it in 54% of patients, with a mean age of onset of 41.6 years and two thirds of those over 40 years reporting some progressive weakness, compared to 85% reported elsewhere (Charles et al., 2013). It has been noted to be unrelated to attack frequency which is what was found in this study. I did, however, find a strong correlation with age, both with patient-reported weakness ($p=0.010$) and weakness on examination ($p<0.001$) and all patients over 52 years had fixed weakness (Bradley et al., 1990; Plassart et al., 1994; Miller et al., 2004).

Myotonia was previously thought to be uncommon in HyperPP with initial reports suggesting less than 20% were affected clinically (Plassart et al., 1994). More recent studies, however, have reported myotonia in approximately 55-74% of cases and I detected it in 71% of cases. Electrical myotonia has been reported in 50-75% of individuals (Plassart et al.,

1994; Miller et al., 2004), and I identified it in 81% of patients.

Traditionally acetazolamide or dichlorphenamide have been used for treatment. One report suggested that just under three quarters of patients notice an improvement with acetazolamide but there are no randomised controlled studies investigating its efficacy (Miller et al., 2004; Sansone et al., 2008). The results in this study suggest that acetazolamide was effective in 84% of patients and did not worsen any patients but, given the study design, this improvement was not quantifiable. Dichlorphenamide has been shown to reduce attack frequency in a RCT in HyperPP (Tawil et al., 2000), and I found that 67% of patients had improved with the medication and one out of six patients worsened. Patients also trialled thiazide diuretics and salbutamol following evidence of efficacy in some case reports (Ricker et al., 1983; Hanna et al., 1998) and these were effective in 86% and 80% of patients respectively. A small number of patients were also on mexiletine for the treatment of their myotonia which was effective in 80% of cases.

Investigation results were in keeping with the current literature. However in this cohort the CK was markedly high with a mean of 776IU/L. This did not correlate with attack duration or frequency but inversely correlated with age (p=0.010) which may be related to the observation that patients with frequent attacks often have a high CK and are younger whereas older patients have less frequent attacks and more proximal weakness. However, there was no correlation with attack frequency but this may be because of the small sample sizes and the fact that the maximum attack frequency is recorded rather than current average attack frequency.

Neurophysiology testing has been reported as having either a normal or mild increase in CMAP on short exercise testing and a significant decrement on long exercise testing (Fournier et al., 2004). Only one out of ten of the patients had an increment during short exercise testing. Interestingly three of the patients developed a significant decrement on cooling, all three of which also had electrical myotonia more suggestive of a cross-over with PMC. 77% of patients had an abnormal long exercise test and 40% of patients had a myopathic EMG, all of whom were over the age of 50 years. The numbers of patients with biopsies and MRI of the lower limbs were too small to draw any meaningful conclusions.

Genotype-Phenotype Correlations in HyperPP Mutations

To investigate the phenotype-genotype correlations in HyperPP I looked at the two common mutations, Thr704Met and Met1592Val. There is only one large study comparing genotype

and phenotype in HyperPP by Miller *et al* and they had larger numbers of patients in their cohort than in this study. They identified a marked difference in age of onset between Thr704Met and Met1592Val patients which was also noted in this cohort (Miller et al., 2004)

The frequency of attacks reported by Miller *et al* were higher for Thr704Met compared to Met1592Val, however I did not note such a significant difference (Miller et al., 2004). This data for Thr704Met patients was skewed by one very high result and when excluded the frequency of attacks was similar to other reports, with Thr704Met patients having 1.5 attacks per month and Met1592Val patients having just over two attacks per month. Data from both Miller's study and this one had very small numbers which may account for the differences reported. Duration of attacks, however were significantly different in both this study (p=0.032) and the previous study. Both studies demonstrated much longer attacks for Met1592Val patients, although in this study there were even longer attacks than found by Miller (Miller et al., 2004). Attack severity and distribution of weakness were very similar between the two genotypes.

Looking at attack triggers the most marked difference was in strenuous exercise with Met1592Val patients being very sensitive to it (p=0.009), they were also more sensitive to illness than Thr704Met patients but the numbers were very small. Miller *et al* noted the largest differences to be between sensitivity to cold, finding that Thr704Met mutations were more cold-sensitive. However I did not note a significant difference in this cohort.

Patients with Thr704Met were more likely to have progressive weakness although the numbers were not sufficient to reach significance and this was mirrored by a higher proportion of those with reduced power proximally on examination, poorer functional status, significantly more limb atrophy and more patients with myopathic EMG.

Both studies noted a higher incidence of myotonia both electrically and clinically in patients with Met1592Val mutations although not statistically significant (p=0.074) (Miller et al., 2004). In Thr704Met the myotonia was more prominent in the eyes than in Met1592Val.

Response to acetazolamide was previously thought to be poorer in Thr704Met, with only 50% of patients with that mutation responding compared to 75% overall (Miller et al., 2004). Although response in this study was poorer (77% versus 100%), it was not significantly different.

6.2.3.3 Key Phenotypic Features in ATS

ATS is characterised by the presence of three key features: episodes of paralysis, ventricular arrhythmias and skeletal abnormalities. The reported prevalence of episodes of paralysis in other studies in patients varies from 57-83% (Tristani-Firouzi et al., 2002; Donaldson et al., 2003; Haruna et al., 2007; Kimura et al., 2012). The prevalence in this UK cohort was much higher at 100% which probably demonstrates ascertainment bias as patients have all been seen in a specialist periodic paralysis clinic which therefore selects for those with episodes of paralysis. Cardiac abnormalities have been reported in 67-84% of patients (Tristani-Firouzi et al., 2002; Haruna et al., 2007; Kimura et al., 2012). In this study it was in a slightly lower proportion, 61%, but again this is likely to be due to ascertainment bias with the majority of the published cases being reported by cardiologists making it likely that they see a higher proportion of cardiac abnormalities. Finally skeletal abnormalities have been reported with a huge variation in incidence from 36-78%. I identified two or more dysmorphic features in all of the UK patients, many of which were subtle but this suggests that features may have been under-reported in other studies.

The features of attacks of paralysis in ATS have been poorly documented in previous publications, often because the studies have concentrated on the cardiac aspects of the disease. The one study that includes some data is a UK study and does contain some of the patients described in this cohort (Davies et al., 2005). I found paralysis to be the presenting symptom in 88% of patients with onset between the first and second decades (mean 9.9 years) which corresponds with reports in the literature (Davies et al., 2005; Venance et al., 2006). Patients reported an average attack frequency of just under seven per month, with attacks lasting, on average, almost a week (163 hours). There has been no systematic survey of attack duration in ATS but most papers have suggested attacks normally last one to two days (Donaldson et al., 2003; Venance et al., 2006; Sansone and Tawil, 2007; Davies et al., 2005). I did not find any correlation between age and attack frequency.

Ictal potassium ranged between hypokalaemic, normokalaemia and hyperkalaemic (2.5-5.12mmol/L) with 67% of patients having low potassium during attacks but not to the level commonly seen in HypoPP. This is very similar to what was reported by Tristani-Firouzi *et al* where they found 55% of patients had hypokalaemic attacks with an overall average potassium of 3.6mmol/L (Tristani-Firouzi et al., 2002). The commonest precipitants for attacks were strenuous exercise and rest after exercise with immobility and carbohydrates

being the next most common which is similar to what others have reported (Haruna et al., 2007; Tristani-Firouzi et al., 2002; Venance et al., 2006).

Proximal weakness is thought to be a common feature (Donaldson et al., 2003; Venance et al., 2006) and it was identified in 62% of this cohort. Age of onset for fixed weakness was younger than expected at 31 years and did not correlate with attack severity, frequency or duration. Despite having such a young age of onset, unlike in HyperPP, being an older age did not determine the presence of fixed weakness, with the oldest patient (63 years) not having any fixed weakness. This may be partly related to the marked variation of phenotype between patients.

Treatment with acetazolamide was effective in 91% of patients with none reporting any worsening. Dichlorphenamide was effective in 67%, but fewer patients had tried it (6 patients). Both drugs have been reported as having benefit in ATS patients (Tawil et al., 1994; Sansone et al., 1997). Some patients with hypokalaemic attacks also improved with oral potassium or potassium-sparing diuretics and one patient also noted benefit with a thiazide diuretic. These were, however, always used with caution to avoid precipitating hyperkalaemic attacks or cardiac arrhythmias.

Neurophysiology testing in this ATS cohort demonstrated myopathic EMG in only 32% but all apart from one had fixed proximal weakness. Short exercise testing was normal in all patients and long exercise testing was positive in 18 out of 20 cases which is similar to previous reports (Katz et al., 1999; Davies et al., 2005). Biopsies were performed in eight patients, six of which were abnormal. Tubular aggregates were seen in two patients which have been reported in other ATS patients, the rest having myopathic changes or vacuoles (Tristani-Firouzi et al., 2002; Davies et al., 2005; Haruna et al., 2007). MRI was performed in three severely affected patients, all of which were abnormal with fatty infiltration in the thighs.

Cardiac symptoms are often the most concerning in ATS as they are associated with a risk of cardiac arrest and sudden death and therefore many published studies on ATS have focused on that. In this study, cardiac symptoms tended to follow the onset of episodes of paralysis with an average age of onset of under 13 years. Whilst it is acknowledged that cardiac symptoms are noted after episodes of paralysis (Donaldson et al., 2003), few studies have mentioned age of onset of cardiac symptoms, possibly because patients may often be asymptomatic despite having cardiac abnormalities. I found the commonest symptoms to

be palpitations (94%) with only one patient having had a cardiac arrest and two requiring an implantable cardioverter defibrillator. This matches the observation by others that the arrhythmias associated with ATS are less commonly malignant than other long QT syndromes (Tristani-Firouzi et al., 2002).

Although only 61% of patients were symptomatic, 85% had abnormalities on ECG. This included the presence of abnormal U waves (81%) and premature ventricular contractions (32%). Surprisingly only one patient had a prolonged QTc although the average QTc was at the upper limit of normal for both male and female patients. Although these values appear much lower than some of the original reports (Tristani-Firouzi et al., 2002), they do correlate with more recent papers which encompass some of the less severely affected cases (Haruna et al., 2007; Kimura et al., 2012). The incidence of premature ventricular contractions in this cohort was still considerably lower and this may be a reflection on having a paralysis-predominant phenotype rather than cardiac-predominant phenotype. Three patients had abnormalities on echocardiogram but all were valvular abnormalities, unlikely to be related to ATS. None had a dilated cardiomyopathy that has been reported in one family (Schoonderwoerd et al., 2006). 50% of patients were on cardiac treatment, of which 83% were on a beta blocker. A quarter required dual therapy.

Skeletal abnormalities were very common in this cohort. The commonest findings being micrognathia and low set ears which is similar to findings in other centres (Tristani-Firouzi et al., 2002; Donaldson et al., 2003). Scoliosis and cleft palate were relatively rare. There has been debate as to whether short stature should be included in the selection of associated features (Canún et al., 1999) but it was noted in over a third of patients suggesting it is a relative common finding.

Aside from the skeletal abnormalities, this study highlighted a number of other interesting observations. There was a higher incidence of learning difficulties (27%), memory impairment (36%) and depression (45%) amongst this cohort than would be expected for the normal population. Learning difficulties have only been reported in a couple of studies and were not previously thought to be common but there has been anecdotal evidence of a higher incidence (Sansone et al., 1997; Davies et al., 2005). It was more formally investigated along with memory impairment in a group of 10 patients matched with their normal siblings (Yoon et al., 2006). The study found no overall difference in scoring but some reduction in executive function and on tests of general ability. This, combined with evidence from the phenotype

study, suggests that further testing needs to be done to investigate the cognitive problems in ATS. Memory impairment in this cohort was not related to age with the mean age of those with memory impairment being 31 years. It occurred in a range of different genotypes and could potentially be related to the expression of KCNJ2 in the hippocampus (Karschin et al., 1996). Depression tended to occur in older patients (p=0.051) but was not related to attack severity. It is unclear if it is related to the disease itself or the impact of the disease on the individual's life. The rates were much higher than seen in patients with HyperPP and HypoPP which may suggest it is related to the underlying disease rather than solely the impact of the disease on lifestyle alone. It would be useful to systematically perform SF-36 and INQoL questionnaires in patients to further determine the relevance of these findings. Higher incidence of memory impairment and depression have not been recorded previously in the literature and so is a novel finding.

Previous studies have suggested a high incidence of *de novo* mutations with 30-37% of kindreds having sporadic mutations (Donaldson et al., 2003; Haruna et al., 2007). Only 3 out of 20 sporadic cases (15%) were present suggesting it may not be as common as previously suggested.

Genotype-Phenotype Correlation in ATS

Due to the small numbers of patients, I was unable to perform a meaningful analysis of genotype-phenotype correlations in ATS. I therefore looked at a small number of key factors and split genotype according to whether mutations were known to affect phosphatidylinositol-3,4-bisphosphate (PIP_2) binding or known to cause disease by another mechanism. I also looked at the two commonest mutations in this cohort (Arg82Gln and Arg218Trp) to see if there were any phenotype-genotype trends. As ATS is commonly caused by personal mutations there was not a large enough number of patients with other mutations to draw conclusions.

A number of mutations affect PIP_2 binding and therefore it is thought to be one of the key pathomechanisms in inhibiting functioning of Kir2.1 (Tristani-Firouzi et al., 2002; Donaldson et al., 2003). Other mechanisms include alterations in membrane trafficking and effects on the pore forming region of the channel. As found by others, there was no significant difference in the phenotype between those with PIP_2 binding altering mutations and other mutations (Donaldson et al., 2003). When I looked at the phenotypes of the two commonest mutations, the only difference was in proximal weakness, with Arg218Trp

being more likely to have proximal weakness (83% versus 20%, p=0.036) and abnormal early milestones (p=0.025) but these findings need confirmation in a larger study. There was no difference in age of onset, severity of symptoms or ECG findings.

6.2.3.4 Phenotypic Differences between HypoPP, HyperPP and ATS

Although in the past HypoPP and HyperPP have been compared there has never been a comparison including ATS patients. I set out to identify any key distinguishing features between these diseases as some cases do not present with the typical triad of signs and understanding more about the nature of attacks relative to the more common types of PP may inform management. The first marked difference in type of attack was in age of onset, with HyperPP patients having the youngest age of onset and HypoPP having the oldest, with a significant difference between the two (p=0.004). This was also reported by Miller *et al* (Miller et al., 2004). ATS had a more intermediate age of onset, possibly matching with the fact that patients may have hypokalaemic, hyperkalaemic or normokalaemic attacks. As previously documented, patients with HyperPP were less likely to have severe attacks of complete paralysis, tending to have more focal attacks (Amato and Russell, 2008). This has not been quantified previously but a surprisingly large number of HyperPP patients (76%) had severe attacks of paralysis from the neck down. Consistent with other groups, almost all patients with HypoPP had episodes of complete paralysis (Sternberg et al., 1993). When comparing the frequency of all types of attacks the three diseases were very similar which is contrary to previous comparisons of HyperPP and HypoPP. However, when specifically comparing frequency of major attacks there were significantly more attacks in HyperPP than HypoPP which does correspond to what has previously been reported (Miller et al., 2004). It is also striking that ATS patients have much fewer attacks compared to HyperPP and HypoPP. There is no published data to compare with but this may be related to the prolonged duration of attacks experienced by ATS patients. Attack duration was longer in HypoPP than HyperPP as has been previously reported (Miller et al., 2004). Attacks in ATS were of significantly longer duration than HypoPP and HyperPP attacks which may explain why patients develop proximal weakness at a younger age, however there was no significant correlation between duration and age of onset of progressive weakness in these patients as the numbers were too small.

As reported extensively in the literature there was a significant difference between ictal potassium in HypoPP and HyperPP, with ATS patients again having an intermediate result at

the lower limit of normal values (Miller et al., 2004; Tristani-Firouzi et al., 2002). Severity of attacks did not demonstrate any significant differences but severity scores are naturally graded relative to other patients with the same disease and therefore is not suited to comparison between diagnoses. The number of patients who were hospitalised during attacks however, was 82% in HypoPP compared with only 33% in HyperPP suggesting that major attacks may be more severe in these patients.

I investigated key precipitants and relievers for each of the three diseases. Exercise was a key precipitant in all three as has been commonly reported (Miller et al., 2004; Tristani-Firouzi et al., 2002; Venance et al., 2006). The most significant difference however, was in carbohydrates which again were a very common trigger in HypoPP, a rare trigger in HyperPP and an intermediate trigger in ATS. Immobility was a much more frequent trigger in HyperPP and ATS although this was not significant after Bonferroni correction. Acetazolamide and dichlorphenamide had similar efficacy in all three groups contrary to previous reports that acetazolamide is less effective in HyperPP cases (Miller et al., 2004).

Interestingly, there was no significant difference between proximal weakness in each of the diseases, however it was more common and occurred at a lower age of onset in patients with ATS which may account for the disability that is associated with this condition. This was also illustrated by differences in functional status. Although functional status was similar between groups, a larger proportion of patients with ATS had difficulty with stairs compared to a very small proportion of HypoPP and HyperPP cases who had impaired function which corresponds to the higher incidence of proximal weakness in ATS patients.

On examination there was no significant difference in proximal limb strength between the diseases, probably because MRC scoring is not sensitive to subtle differences in power. Limb appearance however was different, especially the rate of atrophy seen in HyperPP. Interestingly, 27% of patients with ATS had hypertrophy which is striking since these patients do not have myotonia and the mechanism for this is unclear. These patients do however, often complain of stiffness and it would be interesting to investigate if they have increased muscle membrane excitability during episodes of high potassium that may account for it. CK was higher in patients with HyperPP than HypoPP and ATS patients had intermediate levels. Interictal potassium levels were similar throughout. There was no significant difference in short or long exercise testing, biopsy or MRI results.

Overall when comparing the three diseases, HyperPP and HypoPP lie at either end

of the spectrum of periodic paralysis with ATS patients having an intermediate phenotype. This is somewhat expected given that ATS patients can have hypokalaemic, hyperkalaemic and normokalaemic attacks. When treating ATS patients it may therefore be useful to identify whether they have predominantly hyperkalaemic or hypokalaemic attacks and treat accordingly.

6.2.3.5 Overall Findings and Limitations of the Clinical Phenotypes Study

This large phenotype study was done retrospectively, utilising data collected from patients over many years and is therefore subject to recall bias. It may not fully capture all the data as it is reliant on accurate documentation at the time of assessment and means that some data collection was incomplete. It does, however, set the groundwork for a larger, prospective study which would be very valuable if done in a systematic way with clearly defined questionnaires. If coupled as part of a natural history study over 10 years it would aid in determining patient prognosis more fully. The other key problem was the number of patients recruited. Despite this being one of the largest studies to date, numbers for analysis of genotype-phenotype correlations were small and meant that, after correction for multiple comparisons, the number of statistically significant findings was diminished. It did however still allow for trend analysis. Despite these limitations in the study a number of novel findings were identified.

Overall I identified that in a large group of patients with PMC and SCM there is little phenotypic difference between the two groups suggesting that this is actually a spectrum of clinical phenotypes within one disease. HyperPP continues to be a distinct phenotypic entity with marked differences compared to patients with PMC and SCM.

My findings when investigating HyperPP patients was similar to the two previously published studies. I did however note that HyperPP patients tended to have two types of attacks, not only the focal attacks that are traditionally described but also longer generalised attacks. These are important findings as previously we have always advised patients that HyperPP is associated primarily with short, focal, less disabling attacks and so are often treated less aggressively. It may be that more aggressive treatment may lead to better outcomes in these patients in the long term and it is therefore important to investigate this further.

My findings when investigating HypoPP patients was also similar to the published literature, although the age of onset of proximal weakness in this cohort appears to be earlier than previously reported. I also noted that MRI severity correlated with progressive weakness and CK and therefore may be a promising objective marker of severity for clinical trials. A systematic study with quantitative measures of strength and MRI changes would be useful to further evaluate this as a clinical tool and is currently underway.

The ATS study gave similar results to previous studies, although this cohort had a higher rate of episodes of paralysis than other cohorts. This is the first study comparing the phenotype of ATS with HypoPP and HyperPP and I found that the nature of attacks was in the intermediate range between the types of attacks seen in HyperPP and HypoPP. This is unsurprising as patients often have a mixture of types of attacks and will be useful for guiding treatment in these patients.

In all diseases I found interesting genotype-phenotype correlations despite the small numbers of patients, highlighting the importance of genetic diagnosis in directing patient treatment and advice. It also suggests the importance of large specific genotype-phenotype studies to inform clinical practice.

6.2.4 Pregnancy and Anaesthetics in Channelopathy Patients

6.2.4.1 Anaesthetics in Channelopathy Patients

Very little data exists on the effect of anaesthetics in patients with channelopathies, with all the published data consisting mainly of case studies. I surveyed patients to try and assess the incidence of problems with anaesthetics in this population.

General Anaesthetics in Channelopathy Patients

There were two main forms of complications associated with general anaesthesia. Firstly exacerbation of symptoms and secondly MH-like reactions. The evidence for MH-like reactions in NDM is sparse and based on case studies that pre-date genetic testing and therefore may not be indicative of a problem directly related to the disease. It has also been suggested that the previously reported reactions represent generalised muscle spasms with increased temperature rather than true MH and have been misdiagnosed (Lehmann-Horn and Iaizzo, 1990; Gronert, 1995). The data from this study concurs with this with none of the patients with NDM having MH-like reactions. The other group of patients in which MH has been reported are in HypoPP where one patient with an RYR1 mutation and episodes of

paralysis had MH (Marchant et al., 2004). Others have been from patient-reported studies. However, given that a CACNA1S mutation has been associated with MH, and RYR1 (the gene commonly associated with MH susceptibility) is coupled to CACNA1S it is possible that there is a true association with HypoPP. We, however, did not have any patients with MH reactions.

The most significant problem reported with general anaesthetics in this cohort has been the exacerbation of symptoms. In MC there have been reports of depolarising agents causing severe stiffness and masseter spasm affecting intubation and ventilation (Thiel, 1967). In PMC and HyperPP there are reports of patients developing paralysis for several hours after an anaesthetic, related to hypothermia and respiratory depression associated with the anaesthetic (Egan and Klein, 1959; Klingler et al., 2005). Specifically in HyperPP there have been reports of potassium-releasing drugs such as succinylcholine inducing weakness (Weller et al., 2002) or spasm (Paterson, 1962; Cody, 1968). In HypoPP there have also been reports of attacks following anaesthesia related to hypokalaemia and hyperglycaemia (Lema et al., 1991).

In this cohort overall 17% (10 out of 59) of patients reported a worsening of symptoms following general anaesthetic which was unrelated to duration of procedure. It was most common in HyperPP (29%) and least common in PMC/SCM (6%), however the difference was not significant between the groups possibly because of the small numbers. The higher frequency in HyperPP patients does match the literature, which has several reports of patients with attacks of weakness following anaesthetic and may be related to the use of potassium-releasing agents. However, because patients are not given details of what anaesthetics were used the exact details of this could not be collected. Interestingly, nine patients also reported a prolonged recovery after general anaesthetic, the majority of whom had NDM. It is unclear what the mechanism for this is. It has not been previously reported in the literature as a specific problem related to channelopathies. One possibility is that some of the cases of prolonged recovery may indicate a worsening of symptoms. The designation of a 'prolonged' recovery is based on a judgement by the patient as to what is considered prolonged. At baseline each individual will have differing recovery rates depending on the anaesthetic agent used, length of surgery and other comorbidities so it is difficult to draw extensive conclusions from this information without more clinical information. There was no relationship between those with prolonged recovery and a longer length of surgery. Those with prolonged recovery had a variety of procedures from tooth

extractions to cholecystectomies and none had extensive surgery.

Local Anaesthetics in Channelopathy Patients

There are no reports in the literature of problems with local anaesthetics, although the use of adrenaline (which may be mixed with lidocaine) is contraindicated as it can exacerbate myotonia (Klingler et al., 2005). Although local anaesthetics are known to improve myotonia when given systemically this is via their effect on the NaV1.4 channel not the other sodium channels (NaV1.8 and 1.9) or the potassium channels that are blocked in the skin on injection so problems with local anaesthetic injection would not be expected. Interestingly however, over 20% of patients reported that local anaesthetic was ineffective. Again this is difficult to quantify as it would vary for a number of different reasons from patient to patient. How much was given to each patient is not known, if they had any local factors which would reduce efficacy and whether it was correctly injected to provide an effective blockade. There was no difference between NDM and PP patients or those with SCN4A mutations compared to other mutations suggesting that this is unlikely to be a specific problem related to channel excitability. In the normal population local anaesthetic for dental procedures is effective in 75-90% of patients and this is much lower in infected or inflamed tissue suggesting that the incidence in this population is likely to be similar to the normal population (Meechan, 2002). Only two patients reported worsening of symptoms and therefore it is more likely to be unrelated to the local anaesthetic per se although it could be related to the stress of the procedure precipitating an attack. Four patients reported prolonged effects of local anaesthetic and again it is difficult to know whether this is a true effect or confounded by other factors such as quantity injected and location.

Overall findings of Anaesthetics in Channelopathy Patients

This study on anaesthetics was a patient survey and therefore information collected was limited by the patients' knowledge of the procedure and any anaesthetic complications. It is also subject to recall bias by the patient and without a control arm it is difficult to know the normal occurrence rate of these problems. To fully investigate these differences a further prospective study looking at the full anaesthetic notes is needed. This was not possible for this study as all the procedures done were performed at other hospitals where I did not have access to the notes. Investigating these aspects in a rare disease is complex as although patients are all seen at one centre for disease management, management of any other problems are at multiple different sites across the country.

Overall, local anaesthetic is unlikely to cause any significant problems in channel patients. It is possible that it is associated with reduced efficacy but it is not possible to be certain if this is confounded by other factors. The data collected on general anaesthetics suggests that around a sixth of patients have worsening of symptoms with general anaesthetics but patients may also notice a prolonged recovery time.

6.2.4.2 Pregnancy in Channelopathy Patients

There is no extensive published information on pregnancy in muscle channelopathy patients. The little data available suggests that symptoms can be exacerbated in pregnancy, most often in HyperPP and less frequently in PMC and MC (Charles et al., 2013; Trivedi et al., 2013). In some cases of HyperPP, however, patients did report improvement of symptoms (Charles et al., 2013).

The average completed family size (in females aged 45 years and over) for this population was 1.89, very similar to the national average of 1.91 (Office for National Statistics, 2012). The rate of childlessness of those at the end of their childbearing years (45 years) was only 11%, lower than the national average of 19% (Office for National Statistics, 2012). This suggests that the fertility amongst channelopathy patients is similar to the general population. The incidence of miscarriages in this population was 21%, similar to the 17-22% miscarriage rate reported in the normal population (García-Enguídanos et al., 2002).

21% of pregnancies had complications during the pregnancy and 21% had complications during delivery of which 42% of patients had problems in both pregnancy and delivery. 8% of patients had gestational hypertension, compared to approximately 10% of the population (Duley, 2009). 4% of patients developed pre-eclampsia during pregnancy compared to 2–8% of the normal population (Duley, 2009). 11% of patients required emergency caesarean sections which is lower than the average rate of 14.8% in England (Health & Social Care Information Centre, 2013). Assisted vaginal delivery was necessary in 11% of patients which is lower than the average in England of 12.8% (Health & Social Care Information Centre, 2013). Overall complication rates in this population appear to be lower than the normal population in England. Unfortunately I do not have data for the rate of elective caesarean section rate and it would be interesting to identify if this rate is higher amongst channelopathy patients, taken as a precaution because of their condition.

Postnatal problems were noted in four patients overall, three of whom had PMC/SCM. This correlates with reports of paediatric phenotypes of neonatal hypotonia, neonatal stridor

and laryngospasm which have been documented in patients, often with *de novo* mutations in Gly1306Glu (Lion-Francois et al., 2010; Caietta et al., 2013) but also with Ile693Thr (Matthews et al., 2008a), Thr1313Met (Matthews et al., 2011b), Asn1297Lys (Gay et al., 2008) and Arg799Ser (Lion-Francois et al., 2010). The patients with children with neonatal problems had Gly1306Glu, Thr1313Met and Ile693Thr variations which were then identified in those children. This highlights the importance of genetic counselling of neonatal risks in patients with these variations but also in all those who have SCN4A mutations.

The most interesting data is that for the change in symptoms during pregnancy. I noted a much higher percentage worsened in pregnancy than the previously reported figures of 25 to 44% in NDM (Trivedi et al., 2013), with over 75% worsening and only 16% noting some improvement. This was most evident in patients with PMC/SCM where 19 patients noted worsening, most commonly of the stiffness. HypoPP patients least commonly reported worsening with only 50% (3 patients) noting a difference. The one patient who had ATS was not worsened by pregnancy but the numbers were too small to draw any conclusions. The percentages for worsening of symptoms during menstruation had a similar distribution with PMC/SCM patients being most commonly affected but overall worsening was less frequent than in pregnancy.

Although this is the first study of a significant number of patients with muscle channelopathies, this data has to be treated with caution as the numbers were still small as this is such a rare disease. The data also relied on patients recall of events as well as the patients' understanding of any problems in pregnancy or delivery which may lead to some inaccuracies. Unfortunately there was insufficient data to draw any conclusions regarding patients with ATS. This not only related to the rarity of the condition but also to the observation that only a small number of female patients tried to conceive. This may be partly related to the severity of symptoms but also to the cognitive implications of this disease. To draw more concrete conclusions it would be useful to repeat a prospective study with a larger multi-centre design to collect a larger data set. It would also be more informative to correlate this data with patients' obstetric notes. This was not possible in this study as I did not have access to patient records from other hospitals.

Overall the data suggests that patients with channelopathies do not have a higher complication rate or infertility rate than the normal population. There is however, a high proportion of patients who experience worsening during pregnancy which has not been

previously reported. The most interesting aspect is that this worsening in pregnancy is present in almost all patients with PMC or SCM and therefore it is important to make these patients aware of this possibility.

6.3 Genetic Diagnosis in Skeletal Muscle Channelopathies

To try and improve the genetic diagnosis rate in patients with skeletal muscle channelopathies, I utilised a number of approaches to identify the "missing" causative variations in undiagnosed channelopathy patients. I selected three main cohorts of patients: those with unusual phenotypes, some of whom may have had a variation identified but had an unusual presentation; those with a single CLCN1 loss of function variation from a recessive pedigree and those with no pathogenic variations identified despite full sequencing of the known channelopathy genes.

Overall 182 patients were carefully phenotyped. Of these, 85 were selected for more detailed testing. All patients identified for targeted sequencing had likely causative variations identified. 7% of patients selected for MLPA analysis had large scale rearrangements identified. 7 patients selected for next generation sequencing and aCGH had likely causative variations identified, suggesting a hit rate of 70% for carefully selected patients. Overall, 25% of unconfirmed cases were genetically diagnosed, improving the genetic diagnosis rate (Figure 4.1, p 77).

6.3.1 Targeted Sequencing of Unusual Cases

6.3.1.1 Case 1

Case 1 demonstrates that one possible explanation for phenotypic variability can be the presence of "double trouble", 2 pathogenic variations in different related genes. This family is a very interesting example as there was marked phenotypic variability between the two branches of a family which was independent of their gender. I showed that the more severe phenotype in one branch of the family was associated with the presence of dominant-acting mutations in both the sodium and chloride channels whereas the mildly affected branch only had a single dominant sodium channel variation

Clinically, the presence of two variations is likely to account for the earlier age of onset, the generalised muscle hypertrophy and the unusual pattern on neurophysiology seen in the proband. The short exercise test for the proband did not demonstrate a pattern that fits either dominant MC or PMC but a mixture of both. She had both paramyotonia after trials three and four of exercise (as seen in PMC) but then warm-up following the fifth and sixth trials (as seen in MC) (Fournier et al., 2004) (Figure 5.26, p 148). She was sensitive to cold, as would be expected in PMC but following rewarming, rather than demonstrating paramyotonia with

subsequent trials she showed warm-up as would be seen in MC. However, unlike what is seen in MC, she did not fully recover her decrement in CMAP after rewarming (Fournier et al., 2006). This results in a mixed Fourner type I and II pattern. So it appears that the interplay of these two variations gives rise to a cold-sensitive myotonia that initially worsens with exercise but may improve with further exercise giving a more delayed warm-up. In distinct contrast, the proband's cousin, who only had the Gly1306Val variation, had neurophysiology consistent with an SCM phenotype with increment rather than decrement on exercise.

The branch of the family with the milder phenotype only carried the sodium channel mutation which manifests as a pure myotonic phenotype with marked worsening in pregnancy and a characteristic Fournier type III pattern on testing. The striking features in both branches of the family was the marked exacerbation in pregnancy which is likely related to the Gly1306Val variation and correlates with the findings of the pregnancy survey, suggesting that patients with sodium channel mutations commonly worsen during pregnancy.

It is interesting to speculate about the pathophysiology of disease in the individuals with the two variations. It was thought that two variations in SCN4A would be lethal given the importance of the role of the channel in muscle excitability. However, there have been a few cases of homozygosity of dominant mutations in SCN4A not being lethal but causing a severe phenotype with marked weakness in the homozygous, compared to heterozygous carriers. This is thought to be due to reduced penetrance of those particular variants (Arzel-hézode et al., 2010). Although the patients in Case 1 with two variations did not have marked weakness as in that case, they did demonstrate a marked drop in CMAP following cooling and subsequent exercise compared to the individuals with a single variation, as was seen when comparing homozygotes and heterozygotes in the reported case (Arzel-hézode et al., 2010).

In this particular case, as dominant MC variations would only be expected to cause a maximum of 75% reduction of chloride current and because chloride current only contributes to 85% of the resting membrane potential (Bryant and Morales-Aguilera, 1971), the combination of a dominant chloride variation and a sodium channel variation may be expected to give the phenotype of a recessive MC. They would not have the lethality of two SCN4A mutations as 50% of the sodium channels are still able to function. The variation in the sodium channel, however, does confer the susceptibility to cold and a predominance of symptoms in the eyes and face. It would be interesting to see electrically if the warm-up and paramyotonia in the eyes compared to the hands differ as PMC and MC have different

predispositions to each of these areas.

This case illustrates the value of neurophysiology in guiding diagnosis and the importance of investigating cases of marked phenotypic variability further.

6.3.1.2 Case 2

Case 2 illustrates again how the variation in clinical phenotype within a family may be due to the presence of a second variation. In this case there was a marked change in clinical severity and phenotype seen with the combination of a ZNF9 expansion and homozygous CLCN1 mutations compared with family members with only CLCN1 mutations.

DM2 is a multi-system disorder, typically presenting in the third decade with myotonia and proximal weakness. Patients may also develop cardiac conduction defects, cataracts, insulin sensitivity and cognitive problems (Turner and Hilton-Jones, 2010). Although patients may present with a similar myotonic phenotype to MC the mechanism behind the disease is markedly different. It is caused by an expansion of between 75 and 11,000 of the micro-satellite repeat CCTG in intron 1 of the ZNF9 gene (Udd et al., 2003). This leads to abnormal splicing of a number of genes including CLCN1, resulting in myotonia, and RYR1 possibly causing muscle weakness.

A number of studies have identified heterozygous CLCN1 mutations co-segregating in patients with DM2 (Sun et al., 2001; Suominen et al., 2008; Lamont, 2004). This includes a large study looking at 200 DM2 patients that identified a higher frequency of patients with specific heterozygous CLCN1 mutations than in the normal population (Suominen et al., 2008). This was thought to be due to ascertainment bias as patients were thought to be more likely to have myotonia and therefore more likely to have disease. However, a worsening in clinical severity of these patients has not been described.

In this case the father and two of his children both had DM2 expansions and were homozygous for the Ala566Thr mutation. The children who carried both the DM2 and MC variations had a severe phenotype with a younger age of onset than is characteristically seen in DM2, with not only severe grip myotonia, but also significant proximal weakness, cardiac arrhythmias and daytime somnolence. It appears in this case that the presence of both variations modifies the onset of DM2 symptoms as well as the myotonic symptoms. Commonly in DM2, the average age of onset is the third decade. The earliest sign is often myotonia with the youngest report being at 13 years but other symptoms such as weakness and arrhythmias occurred later, with none reporting it before 18 years (Day et al., 2003).

The age of onset of myotonia in this family was much younger than reported in DM2 alone, occurring within the first decade but this is often seen in recessive MC. However, the daughter of the proband (VI.I) also noted a very early onset of arrhythmias and weakness which is hard to explain pathophysiologically as the CLCN1 variation would not be expected to modify abnormal splicing of genes outside of CLCN1. One possible explanation is the presence of observer bias, because the father is affected the symptoms are looked for more carefully in affected children at an earlier age. This bias has been reported in other family groups with DM2 (Day et al., 2003).

The children who were only homozygous for the CLCN1 mutation have less severe myotonia and no other significant symptoms supporting the suggestion that the combination of the two variations worsens the severity of symptoms. This is interesting because the homozygous variation would be expected to cause a loss of function of both copies of the ClC-1 protein, supported by the fact that expression studies show no current when the variation is expressed homozygously (Figure 5.30, p 156). This means that problems with splicing would not be expected to have any further effect on the severity as the protein is already non-functional. It suggests that there may either be some low level function of the ClC-1 and so once splicing defects occur no protein is produced, or that the splicing abnormalities of RYR1 may contribute to the exacerbated symptoms. Without analysis of biopsy samples, which the family declined, it will be difficult to ascertain the true reason.

This family illustrates how dual variations modify severity and again highlight the importance of assessing other genes in cases of atypical severity and presentation.

6.3.1.3 Case 3

Case 3 is an example of a family with symptoms consistent with a sodium channelopathy including stiffness and episodes of weakness but with atypical features. These included developmental delay and severe weakness which resolved over time. The electrophysiology demonstrated an abnormal long exercise test and abnormalities in short exercise testing suggestive of abnormal muscle membrane excitability. However, sequencing of the skeletal muscle channel genes was normal. Repeated EMG identified abnormal decrement and jitter and so the search was focused on variations in channels associated with congenital myasthenic syndromes and compound heterozygous variations were found in DOK7. The c.1124_1127dupTGCC;p.Leu375fs variation was a known pathogenic mutation, and the c.533-37_c.533-11del27 variation encompassed a consensus branch point and is predicted

to lead to aberrant splicing.

DOK7 encodes docking protein 7, which is essential for neuromuscular synaptogenesis through interaction with MuSK. Pathogenic variations were found to be associated with a limb girdle-type phenotype (Beeson et al., 2006). It is associated with delayed milestones, although most children are walking by 4 years with frequent falls and difficulty rising from the floor as is seen in these two patients. If weakness is present it is primarily proximal and, unlike in this case, often associated with ptosis and restricted eye movements. There are reports of patients having variability and prolonged periods of worsening as was seen in this family. Feeding difficulties and respiratory problems are also common, especially early in life (Klein et al., 2013). The phenotype of DOK7 mutations is very variable and the phenotype of this family does fit with it. It is interesting that it causes alterations in muscle membrane excitability despite being a neuromuscular junction phenomenon and suggests it is important to investigate for possible neuromuscular junction impairment in patients with atypical muscle channelopathy phenotypes.

6.3.1.4 Case 4

Case 4 was a patient with a very severe phenotype with onset in childhood of impaired mobility and later developing episodes of paralysis with supportive electrophysiology. He also had additional problems with respiratory muscle weakness, swallowing compromise and ophthalmoplegia which is not usually seen in typical periodic paralysis. There was also fatiguability with an associated mild decrement on repetitive stimulation suggestive of a neuromuscular junction defect, however this was not felt to be significant given the extent of ocular weakness.

The likely causative pathogenic variations were found in RYR1. RYR1 mutations are associated with a number of different congenital myopathies with both dominant and recessive inheritance. This includes the dominantly-inherited central core disease and MH susceptibility and the recessive diseases, multi-minicore disease, congenital fibre-type disproportion, centronuclear myopathy and King Denborough syndrome (Klein et al., 2012). Central core disease is typically associated with hip girdle weakness and scoliosis and not usually bulbar or respiratory weakness whereas multi-minicore disease can be associated with severe rigidity and respiratory involvement. Histopathological analysis shows central ‘cores’, which are devoid of oxidative enzyme activity (Zhou et al., 2007).

The resulting phenotype is related to the location of variations in RYR1, with those

in the N-terminal or central regions (amino acids 35-614 and 2163-2458 respectively) being associated with a MH susceptibility phenotype, compared to those with C-terminal variations being associated with a central core disease phenotype (amino acids 4550-4940) (Zhou et al., 2007). Recessive-acting mutations however appear to be more distributed throughout the gene (Zhou et al., 2007). This patient had multiple variations including the c.208C>T;p.Gln70X variation which is predicted to cause loss of function of one of the RYR1 alleles. The c.325C>T;Arg109Trp variation in combination with c.1453A>G;p.Met485Val, has been previously reported in two different families and associated with minicore disease either in isolation (Jungbluth et al., 2005), or with another loss of function mutation (Zhou et al., 2006; Klein et al., 2012). The combination of these pathogenic variations is likely to be the underlying cause of the patient's unusual phenotype.

The reports of the Arg109Trp mutation with Met485Val in isolation were in a family with minicore myopathy (Jungbluth et al., 2005). The affected siblings had the characteristic proximal muscle weakness and wasting affecting the shoulder girdle more than the hip girdle, external ophthalmoplegia predominantly affecting the upward and lateral gaze and worsening of weakness in the cold, all similar to this case. Their neonatal history, however was more fraught with reduced fetal movements, hydrops, hypotonia, feeding and respiratory difficulties. They also had a reduced FVC of 58% & 80% and needed a gastrostomy tube. The patient in Case 4 had a similarly low FVC but only required his gastrostomy during attacks of weakness. The biopsy in the published case demonstrated variable fibre size and increased central nucleation with isolated nemaline rods and had both multiple and single core lesions. The MRI findings were consistent as well with marked involvement of the soleus and sparing of the gastrocnemius and relative involvement of the tibialis anterior. However, this group of patients had some involvement of the peroneal group of muscles which is spared in the proband (Jungbluth et al., 2005). In the published case, despite analysing the whole RYR1 coding sequence, variations were only identified in one allele, the Arg109Trp *in cis* with Met485Val but they were homozygous in cDNA, suggesting that the second allele was either not expressed or deleted (Jungbluth et al., 2005).

The other report was a family with the c.325C>T;Arg109Trp and c.1453A>G; p.Met485Val that was later found to have a premature stop codon, c.4485G>A;p.Tyr1495X on the other allele (Zhou et al., 2006; Klein et al., 2012). This patient also had onset before 10 years with delayed motor milestones, with difficulty with stairs and rising from the floor,

similar to this case. However, he presented with muscle weakness, hypotonia and feeding difficulties at birth, which the proband did not have. He also had ophthalmoplegia, as in this case and marked axial weakness and needed gastrostomies in childhood due to poor feeding, something the proband had only required during his attacks of paralysis in later life. Respiratory muscle weakness was also present although mild, as in this patient, although his was most significant during attacks (Zhou et al., 2006). The published case did not have episodes of MH and the biopsy demonstrated type 1 fibre predominance, cores and minicores. Functional studies were done which demonstrated that there was only expression of the allele with the Arg109Trp and Met485Val variations and not the allele carrying the stop codon (Jungbluth et al., 2005). When they expressed the Arg109Trp variation homozygously it affected calcium transport and ryanodine binding causing dysregulation of calcium (Jungbluth et al., 2005). Given that the patient in this case has a non-functional second allele due to the premature stop codon (c.208C>T;p.Gln70X), only the allele with Arg109Trp would be expected to be expressed and would therefore act as if it were homozygously expressed as well.

This case had some marked differences compared to the reported cases. The key differences being the episodic paralysis and the MH episodes. MH reactions have been reported in a few cases of multi-minicore disease (Koch et al., 1985; Osada et al., 2004) and minicores have been seen in families with MH susceptibility (Guis et al., 2004). It is also more commonly associated with variations in the N-terminal hotspot which correlates with the location of the patient's variations. Therefore in this case it is likely to be related to the patient's multi-minicore disease. There has been one previous report of a patient with multi-minicore disease and atypical episodes of PP (Zhou et al., 2010). This patient had a very similar presentation to Case 4 with some motor developmental delay and difficulties rising from supine and climbing stairs. She also experienced exercise-induced myalgia and cold-induced stiffness and had distinct episodes of paralysis, starting in the legs and ascending to the arms for several days from 18 years of age. She did not however, have respiratory or bulbar compromise and tended to have episodes triggered by cold. She had no MH reactions. On examination she did have facial weakness and ophthalmoplegia, affecting primarily up-gaze and abduction. She had axial and neck flexion weakness with more pronounced shoulder girdle than hip girdle weakness. She had normal FVC and cardiac function and her MRI demonstrated diffuse involvement of primarily the vasti, the gastrocnemius and the

soleus. She had myopathic changes on EMG and cold immersion testing which triggered profound weakness of the hands with normally preserved CMAP responses both pre- and post-cooling, suggesting cold-induced electron-contraction uncoupling. Her long exercise test was negative but demonstrated an increase in CMAP amplitude and area post-exercise and oral potassium loading triggered profound paralysis with a normal ictal potassium level. She was found to have a heterozygous missense variation in exon 57 in RYR1 c.8816G>A;p.Arg2939Lys and a premature stop mutation, c.6721C>T;p.Arg2241X on the other allele which resulted in only the first allele being expressed in muscle. However functional studies did not demonstrate abnormal functioning of RYR1 with the Arg2939Lys variation and the exact mechanism is not yet fully understood for that case.

The phenotype in Case 4 is very similar to the reported patient with a proximal weakness and ophthalmoplegia phenotype, later developing episodes of paralysis. However, the patient in Case 4 did not demonstrate uncoupling of excitation and contraction and had electrophysiological features more typical of classic PP. It suggests that there may be a further mechanism at work that may be causing the unusual mixed phenotype which needs further investigation. It may be, as many of the multi-minicore disease patients are children, they have not yet developed the episodes of paralysis and as more survive into adulthood we may begin to see this phenotype more commonly. It may also be that other patients have such severe weakness and respiratory compromise that the episodes of paralysis are not noted as distinct episodes and instead attributed to natural variation of the disease day to day. It would be useful to look at this patient's muscle protein expression and further determine the possible pathomechanisms involved.

6.3.2 Diagnosing Patients with Single Mutations in CLCN1 using MLPA

I utilised MLPA to demonstrate that exonic deletions and duplications were present in approximately 6% of this MC patient cohort with a single identifiable recessive mutation (Figure 4.2, p 79). The deletions and duplications found are likely to be pathogenic. Although it was not possible to do detailed break point mapping in all cases, it is probable that the alterations are contiguous and cause disruption of the reading frame resulting in nonsense mediated decay of the resultant mRNA. It is possible, though unlikely, that the alterations are in-frame causing production of either a severely truncated or elongated protein which

may alter insertion of the protein into the membrane or impair channel function. I did not identify any deletions or duplications in 124 control chromosomes and none were identified in CLCN1 in the Database of Genomic Variants (Zhang et al., 2006).

6.3.2.1 Large Scale Rearrangements Identified

Patient 5 had a contiguous heterozygous deletion of exons 8 to 10. If in-frame it would disrupt 3 of the 18 α helices and eliminate a number of highly conserved domains (Fialho et al., 2007) making it likely to be pathogenic. The patient was also heterozygous for the previously described recessive intronic point mutation, c.1167-10T>C;p.? This has been reported as a predicted splice-site acceptor (Trip et al., 2008) but according to prediction software it has a low probability of affecting splicing. Muscle was not available from this patient to confirm the effect of this mutation on splicing empirically. However, it has been reported to segregate with disease in multiple families and we have observed it in homozygous form in two other unrelated patients making it likely to be pathogenic.

Patient 6 had a large contiguous deletion of exons 17 to 22. If in-frame this would affect both of the channel's CBS domains, which are highly conserved regions essential for channel opening and chloride channel subunit interactions (Estevez et al., 2004; Scott et al., 2004). The patient was also heterozygous for the common mutation, Arg894X, in the final exon of the gene, which has previously been described in many recessive pedigrees (George et al., 1994) and is predicted to cause a 95 amino acid truncation of the carboxyl-terminus of the protein (George et al., 1994). Previous cellular electrophysiology cell studies have been inconclusive, demonstrating both a large reduction in chloride currents and a weak dominant-negative effect in co-expression studies consistent with both recessive and dominant inheritance (Meyer-Kleine et al., 1995). However, the observation that the patient's clinically unaffected mother carried the Arg894X mutation, is consistent with recessive inheritance in this family.

Patients 7 and 8 exhibited a similar very severe myotonic phenotype with onset in infancy. These patients both harboured the novel homozygous sequence variant Pro744Thr. Our detailed expression studies in HEK293T cells showed no difference in cell currents compared to the WT channel suggesting it was non-pathogenic and therefore not responsible for the patients' phenotype. MLPA studies in these patients showed peak ratios greater than 1.8 encompassing exons 8 to 14, indicating the presence of four copies of these exons. Parental DNA was unobtainable to confirm if this was the result of a homozygous

duplication (two copies on each allele) or a heterozygous triplication (three copies on one allele and one copy on the other). However, as both individuals were severely affected and came from consanguineous pedigrees it is most likely that the rearrangement detected represents a contiguous homozygous duplication of exons 8-14. If this was the case and these duplications caused a frameshift, they would render the downstream homozygous variation, Pro744Thr, redundant. As both patients were of the same ethnic origin, have the same apparent homozygous duplication, sequence variant and intragenic SNPs, it suggests that the duplication is a founder mutation. However, this could not be confirmed as additional family members were not available for haplotype analysis.

6.3.2.2 Limitations of MLPA and Further Work

MLPA is a commonly used method for identifying genomic deletions and duplications. Although it has been very useful in identifying copy number changes in this cohort it has limitations. It cannot detect inversions and translocations that do not result in relative copy number changes. Also, as the MLPA probes cover a small region of each exon it will not detect small deletions and duplications within an intron, or 5' and 3' UTRs. The probability of a normal sample incorrectly having an abnormal peak ratio is statistically small but if the MLPA probe sequence lies over a polymorphism, point mutation, small deletion or insertion in the sample DNA this can influence the ligation or destabilise the binding of the probe oligonucleotides giving a false positive. This is illustrated by my finding in the patient with a homozygous 14 base pair deletion and apparent probe ratio of zero in the same exon. This occurrence can be minimised by cross-checking the sample DNA sequence with the probe sequence. False positives are however extremely unlikely in cases with multiple contiguous exon copy number changes as was seen in these four cases but caution must always be taken when interpreting such results.

Useful further work would include confirming the effects of these changes on chloride channel function and expression by analysing patient muscle mRNA and performing functional studies of mutated channels. This would give a more extensive understanding of the consequences of these changes and confirm the findings identified by MLPA.

6.3.2.3 Key findings of MLPA

This study demonstrates that CLCN1 exon deletions or duplications are an important genetic mechanism in patients with recessive MC. In particular it shows that large scale alterations

may explain some apparent recessive pedigrees with single loss of function mutations. Genetic counselling in this group of patients is difficult and causes uncertainty, so MLPA analysis may be a relatively quick and cost-effective way of improving diagnostic accuracy in these cases. There remains a significant number of cases that cannot be not explained by such rearrangements indicating that there are additional, as yet unidentified genetic mechanisms.

6.3.3 Whole exome sequencing and aCGH

6.3.3.1 Case 9

Case 9 was a patient with symptoms and signs consistent with MC from a recessive pedigree. He had the characteristic generalised hypertrophy, onset of stiffness in the first decade of life and primarily hand and limb myotonia (Fialho et al., 2007; Trivedi et al., 2013). His electrophysiology showed unequivocal evidence of myotonia and short exercise testing was characteristic of recessive MC (Figure 5.41, p 173) (Fournier et al., 2004). His MRI demonstrated marked fatty infiltration in the gastrocnemii and milder fatty infiltration elsewhere. The gastrocnemii involvement was more severe than typically seen in recessive MC patients, although he was 65 years old and we have seen a marked correlation with age and severity of MRI changes in recessive MC patients (Figure 5.42, p 174) (Morrow et al., 2013). The rest of his MRI pattern was consistent with our findings in other MC patients.

Sanger sequencing did not identify any pathogenic variations in CLCN1 or SCN4A or expansions in DMPK or ZNF9. aCGH did not reveal any related CNVs. I did, however, identify a novel homozygous variation, c.1-59$C > A$;p?, in the 5'UTR region of CLCN1, that may be responsible for the patient's phenotype. This variation has not been documented in dbSNP, 1000 Genomes or the Exome Variant Server. It was also not present in 504 chromosomes from a mixture of ethnically matched and unmatched samples. This homozygous variant would fit with the autosomal recessive inheritance.

The 5'UTR region of genes are important in the translation of the gene and therefore variations in that region may be responsible for causing disease. In this particular case the variation in the 5'UTR region is at a base which is not highly conserved making it harder to be certain of its relevance. Splicing prediction software, NetGen2 and RegRNA (A Regulatory RNA Motifs and Elements Finder), was used to identify if this region was at a predicted splice site or other regulatory motif but none were identified. These did not reveal any predicted role for this location. We are therefore in the process of establishing a minigene assay to try and

determine the pathogenicity of this variation. Unfortunately we were unable to get DNA from the affected and unaffected siblings to confirm segregation of the homozygous variation.

As the variation in CLCN1 did not have definitive evidence in favour of its pathogenicity, I went on to look at other variations found in known muscle channel genes to ensure there were no more likely pathogenic variations. Although variations in three different genes were identified, the variation in the dysferlin gene was discounted as two affected alleles are required to cause the autosomal recessive LGMD2B. I did identify two variations in TTN, a protein which is associated with autosomal dominant tibial muscular dystrophy (with mutations in the final exon), cardiomyopathy and with autosomal recessive LGMD2J. These variations were novel and since the patient had an autosomal recessive pedigree, I looked further into the possibility him having LGMD2J. The two variations identified were predicted to be polymorphisms making them less likely to be pathogenic. The phenotype for LGMD2J is also quite different from the phenotype in Case 9. At its most severe, it is associated with severe childhood onset of proximal muscle disease that progresses to wheelchair confinement in 20 years (Udd et al., 2005). In the more recently described less severe form, it is still associated with early onset of proximal weakness that gradually progresses through life (Evilä et al., 2014) making it very unlikely that it is the underlying cause of this patient's phenotype. Most importantly it is not associated with myotonia which is the predominant feature in this case.

The final possible variant I investigated was the heterozygous variant in BAG3. BAG3 is associated with autosomal dominant myofibrillar myopathy type 6 (Selcen et al., 2009). Myofibrillar myopathies have been associated with myotonic discharges on EMG (Selcen et al., 2004; Hanisch et al., 2013), however these myotonic discharges were not associated with clinical myotonia. Myotonia has also not been specifically reported in BAG3 mutations. Although electrical myotonia is present in this patient, the other aspects of BAG3-associated disease, with early onset of severe and progressive muscle weakness with cardiomyopathy and respiratory insufficiency, were not present. MFM type 6 is seen specifically with the c.626C>T;p.Pro209Leu variation which was not the variation identified in this patient. Pathogenicity prediction software also predicted this to be a polymorphism making less likely that this variation is responsible for the patient's disease phenotype.

It therefore seems likely, given the patient's very characteristic recessive MC phenotype, that the variation in the 5'UTR region is responsible for his disease although we are

currently undertaking further studies to investigate this further (Table 6.1). This illustrates the importance of looking at the 5'UTR and 3'UTR regions when sequencing CLCN1.

6.3.3.2 Case 10

Case 10 is of a patient with initially rare episodes of weakness in his late teens, which progressed significantly to regular episodes in his 30s. These attacks were characteristic of PP although they were not improved by acetazolamide. In his 50s however, attacks started to tail off and he began to have fixed weakness which slowly progressed. His electrophysiology confirmed changes consistent with PP with a positive long exercise test. His MRI had features similar to other patients with HypoPP of the same age in the phenotype study with marked fatty infiltration. His biopsy was also consistent with other HypoPP cases with tubular aggregates, although this is not a specific finding and is seen in many other types of myopathy (Rosenberg et al., 1985; Miller et al., 2004). He had a dominant family history with at least one affected daughter.

I identified two possible causative variations. The first was previously reported as a pathogenic variation, c.406$G > A$;p.Gly154Ser, in αB-crystallin and causing a myofibrillar myopathy (Reilich et al., 2010). The published patient had late onset distal leg weakness and finger flexor weakness. His MRI demonstrated symmetrical fatty degeneration and atrophy of the vastus, rectus femoris, tensor fascia lata and sartorius. There was also marked fatty replacement of the medial gastrocnemius and tibialis anterior. The pattern of MRI changes was similar to those seen in Case 10 although the pattern in the calf from the published case was much more severe (Figure 6.1). The published case, however, had anti-desmin and anti-αB-crystallin positive aggregates whereas Case 10 only had tubular aggregates. There were therefore a number of distinct findings from Case 10. This coupled with the fact that the variation has also been reported in dbSNP as uncertain significance makes it unclear if this variation is causative. Given that there were some similarities it does suggest that this variation may show reduced penetrance and be responsible for the later onset fixed myopathy that the patient developed. It is, however, unlikely to account for his episodes of paralysis.

The second variation was in SCNN1B, c.1307_1310delAGAG;p.Arg438PfsX17. This gene encodes an amiloride-sensitive sodium channel. Autosomal dominant mutations in this gene are known to cause Liddle's syndrome. All the described mutations have been frameshifts, stop codons or missense variations affecting the PY motif in the gene. This proline-rich region is important for binding Nedd4-2, which regulates the turnover and

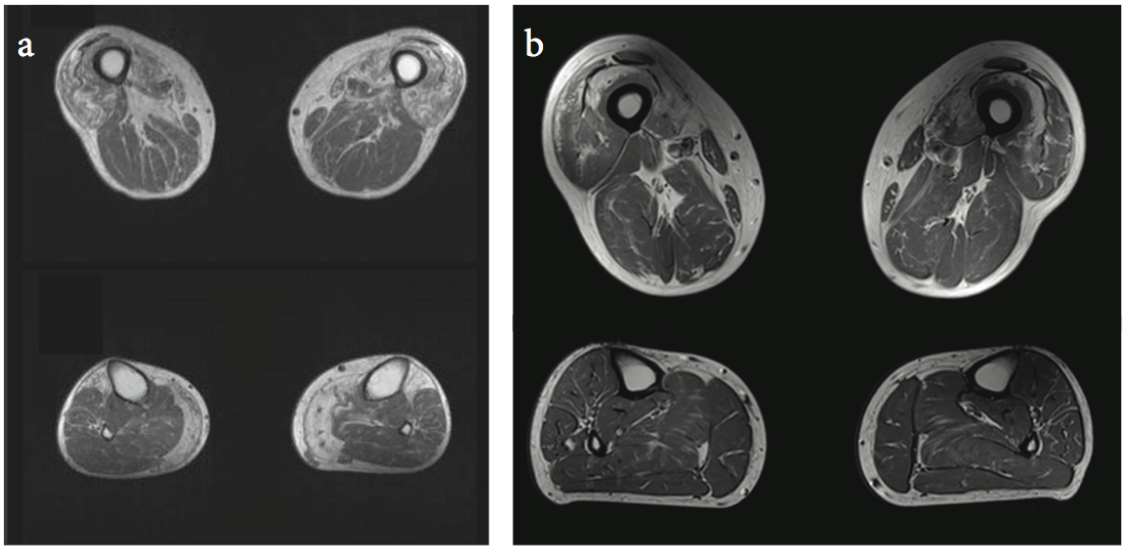


Figure 6.1: MRI images of case 10 and corresponding images from Reilich *et al* study from patient with the same variation Gly154Ser in CRYAB gene a) T1 weighted MRI of thigh and calf from patient with Gly154Ser in CRYAB from (Reilich et al., 2010). b) T1 weighted MRI of thigh and calf from Case 10 who also carries the Gly154Ser

internalisation of the sodium channel. If Nedd4-2 is unable to bind it leads to reduced internalisation and an increase in the number of sodium channels resulting in a gain of function. This is associated with a syndrome of young onset hypertension, usually in the third or fourth decade, with hypokalaemia and metabolic alkalosis (Shimkets et al., 1994). The previously reported frameshift mutations all lie from codon 579 onwards (Yang et al., 2014). The frameshift mutation I identified was located from codon 438 creating a truncation in the protein 17 codons downstream. This is earlier in the protein than has been previously described in cases of Liddle's syndrome but would be expected to have the same effect as it removes the PY motif and therefore would prevent binding of Nedd4-2. What is unclear, however, is if this would give rise to a functional protein or if the mRNA would be subject to nonsense mediated decay due to the premature termination. The location of the termination would truncate the channel before the second helical region and therefore would be expected to lead to a non-functioning protein. If the transcript did not translate to a functional protein then it would be unlikely to cause Liddle's syndrome as it is a gain of function disease.

Looking at the patient's analysis, he had a young onset of hypertension with poor control despite anti-hypertensives. He did have a very low aldosterone which is in keeping with the disorder. His renin however was normal. This may be because he was on a calcium

channel blocker for treatment of his blood pressure which is documented to cause a selective rise in renin levels without affecting aldosterone. At the point I tested him he did not have hypokalaemia or metabolic alkalosis but at that stage his episodes of paralysis had burnt out which may be related to the treatment of hypertension managing his Liddle's syndrome. There have been a handful of case reports from Asian journals of patients with Liddle's syndrome with episodes of HypoPP. None of these reports however, had genetic confirmation of the condition (Kayal et al., 2013; Kang et al., 1998; Boorugu et al., 2009). It does however suggest that there is evidence of Liddle's causing secondary PP. As this is secondary HypoPP it would be expected that the symptoms would settle when the Liddle's syndrome was treated.

Interestingly, the patient's possibly affected 35 year old daughter had both the CRYAB and SCNN1B variations. She had some early signs on MRI of fatty infiltration in a similar pattern to her father although no evidence of Liddle's syndrome. The patient's other daughter, who declined testing, was known to have early hypertension in her 30s and episodes of paralysis. These findings combined do suggest that it is possible that Case 10 has a combination of the consequences of both Liddle's disease leading to secondary HypoPP prior to treatment and as he has become older has developed a proximal myopathy related to the CRYAB mutation (Table 6.1).

To try and verify this further we are currently trying to establish the pathogenicity of the SCNN1B frameshift mutation by expressing it in HEK293T cells. The pathogenicity of the CRYAB variation will be harder to establish and it will be interesting to see how the patient's phenotype develops over time since the previously reported case had a late onset of his marked symptoms.

6.3.3.3 Case 11

Case 11 was a patient with a phenotype suggestive of ATS. She had prolongation of QTc, mild dysmorphic features and episodes of paralysis supported by an initially positive long exercise test. Her sequencing of KCNJ2 and MLPA of the gene were normal which led me to perform whole exome sequencing. There were a number of atypical features in her phenotype. Firstly, she had onset of attacks in the third decade, which is extremely unusual in ATS as attacks most commonly occur in the first decade or early teens. Her attacks were also atypical, as it is very unusual to see PP attacks that only occur in the day and never at night. Her dysmorphic features were subtle and could be unrelated. Interestingly, her MRI was normal which again is unusual compared to the other ATS cases in the phenotype study who all had, at the very

least, fatty infiltration of the calves. However objectively, she had a prolonged QT interval and a positive long exercise test at presentation done at another centre.

Array CGH revealed only one possibly relevant CNV encompassing an extra copy of the gene MRPL44. This is a nuclear gene that encodes the large subunit of the mitochondrial ribosome. Homozygous variations have been documented as causing a childhood onset hypertrophic cardiomyopathy (Carroll et al., 2013). More recently there has also been evidence of a case with hemiplegic migraine, Leigh-like lesions on MRI, renal insufficiency and hepatopathy (Distelmaier et al., 2015). A single gain of copy number may disrupt the open reading frame for the MRPL44 gene on one allele, assuming it is out of frame and close to the start codon for the gene. If it were in-frame or was distant enough from the beginning of the gene it would not be expected to cause disruption as the extra copy includes the entire gene. Even if the CNV did disrupt production of the protein as there is still one functioning allele it would be unlikely to cause disease without a pathogenic variation on the second allele. The phenotype of affected patients is also different as Case 11 did not have hypertrophic cardiomyopathy making it unlikely that this variation is causative.

The whole exome sequencing did identify a known pathogenic variation in a Long QT gene, KCNQ1 (previously KvLQT1). This variation has been reported in a number of cases (Donger et al., 1997; Ackerman et al., 1999; Murray et al., 2002). It is primarily associated with a mildly prolonged QT interval, can be associated with asymptomatic carriers and does not commonly lead to sudden death except in homozygous carriers. Expression analysis suggests it only prevents channel function in the homozygous state and does not cause a dominant negative effect in the heterozygous state (Murray et al., 2002). Given that it has been reported in many patients with Long QT and is present in the affected sister it is very likely that it is responsible for the prolongation of the QT interval in this case. It does not however, explain the episodes of weakness in this patient. As none of the reported cases or the affected sister have reported episodes of weakness and because KCNQ1 is not significantly expressed in skeletal muscle (Uhlén et al., 2015), it makes it unlikely that it is also the cause of the episodes of weakness.

As no other likely variations were identified, it seems in this particular case that I can only explain the long QT in this patient and her sister with a genetic cause but not the episodes of weakness.

6.3.3.4 Case 12

Case 12 was a patient with typical episodes of paralysis associated with hypokalaemia starting in his teens and an affected father. He also had a reaction to volatile anaesthetics. He had an abnormal long exercise test but only subtle abnormalities on MRI of the lower limbs. Unusually he also had a single ragged red fibre on muscle biopsy.

The most notable novel variation identified on sequencing was in RYR1. The c.3128G>A;p.Arg1043His has been previously described in the literature in one study as being possibly pathogenic (Brandom et al., 2013). This case was a patient with MH, a family history of the condition and positive caffeine and halothane contracture tests, which are used to identify MH susceptibility. A variation affecting the same codon, c.3127C>T;p.Arg1043Cys has also been described in two cases of MH (Robinson et al., 2006; Levano et al., 2009). In one of these cases it was expressed with Arg2336His on the same chromosome and this variation was shown to significantly alter intracellular calcium levels in the presence of 4-chloro-m-cresol or caffeine which is thought to indicate susceptibility to MH (Levano et al., 2009). In that particular case it was concluded that the Arg2336His was the pathogenic variation and they did not do further functional analysis on Arg1043Cys. None of the reported cases, however, had documented episodes of weakness and as the patient's affected father was deceased I was unable to demonstrate if the variation segregated with disease.

Codon 1043 lies in a highly conserved region and is predicted by SIFT, PolyPhen2 and Mutation taster to be pathogenic. It lies in the the recently described P1 domain of RYR1, a phosphorylation hotspot that is intertwined with the SPRY domains (Yan et al., 2015). The SPRY domains have been postulated to be important in coupling to CaV1.1 (Lau and Van Petegem, 2014). However, the interaction between the two channels is still poorly understood and the role of the P1 region is not established. Interestingly other pathogenic malignant hyperthermia variations are located in this region. Even more interestingly the only other reported periodic paralysis variation in RYR1 lies in the P2 region which is functionally similar to P1 (Zhou et al., 2010). In that particular case however, there was decoupling of CaV1.1 and RYR1 as the episodes of paralysis were associated with a normal CMAP on long exercise testing rather than a decrement. The other case of a patient with an RYR1 mutation with episodes of paralysis (Case 4), however, also demonstrated a lack of electron-contraction uncoupling with a positive McManis test suggesting that it may not be necessary to cause a

PP phenotype in RYR1 mutations.

The other variations identified in muscle disease-causing genes were all point variations in genes only associated with disease in the presence of deletions or triplet repeat expansions. The one variation found in an ion channel gene, although highly likely to produce an abnormal protein, encoded a chloride permeable channel and therefore would be unlikely to cause attacks of weakness.

Another possible explanation for this case is that he has a secondary cause of periodic paralysis, given the low potassium recorded during attacks, however, this was investigated fully suggesting that it is unlikely to be the underlying cause. This suggests that the the variation in RYR1 could be underlying his disease and at the very least may be responsible for his reaction to volatile anaesthetics (Table 6.1). It is unclear, however, why it would cause a PP phenotype in him but not in other reported cases. One possibility is reduced penetrance. Another alternative is that he carries a polymorphism that, in combination with the RYR1 variation, leads to susceptibility to episodes of paralysis. One way of investigating this further would be to get DNA from affected and unaffected family members which was not possible in this case. Another option would be to perform functional studies with RYR1 and its interaction with CACNA1S but given the size of the gene and the current lack of understanding of exactly how the two channels couple this is currently a complex task.

6.3.3.5 Case 13

Case 13 was of a young girl with problems since birth with cleft palate, scoliosis, conductive hearing loss, short stature, ventricular tachycardias, reduced ventilatory function, lower than normal IQ and episodes of weakness. She also had distinctive facial abnormalities including micrognathia, syndactyly, clinodactyly and nail dysplasia. Her muscle biopsy revealed a number of COX negative fibres which suggested a mitochondrial disease process but with normal mitochondrial respiratory chain enzyme analysis. Despite this collection of symptoms her KCNJ2 and mitochondrial genome and rearrangement analysis were all negative.

Array CGH suggested the presence of an extra copy of exons 112 to 117 with flanking intronic regions in SYNE1. This encodes the spectrin nuclear envelope protein 1. Homozygous variations in this gene are related to SCA1 with a late onset ataxia phenotype which was not present in this case. However heterozygous missense mutations in this have been associated with Emery-Dreifuss muscular dystrophy type 4 (Zhang et al., 2007). The published phenotype of these cases is of onset of progressive muscle weakness in childhood,

cardiac arrhythmias, cardiomyopathy and early contractures which is similar to the phenotype in this case although there have not been significant contractures noted. Muscle biopsy in the reported cases confirmed a muscular dystrophy with normal immunohistochemistry, although there have not been reports of COX negative fibres as was found in Case 13. Some caution has to taken with these results. The SYNE1 gene consists of 536117 bases and 146 exons and the 1.4M assay covers it with 330 probes. (The coverage of this region is similar in the 720K array). The call of the presence of an extra copy was based only on the presence of 3 probes with an average ratio of 1.77. Given the sparse coverage of this region it is difficult to be certain whether this is a true CNV. Repeating the test with the 720K exon focused array would not improve coverage of this particular region and therefore has not been done. There is currently no MLPA kit designed for this gene, therefore further verification will be sought using Sanger sequencing and PCR of exons 111 to 118 to try and confirm the presence of an extra copy.

Whole exome sequencing did not reveal any variations in known channelopathy or nuclear mitochondrial genes. However, two novel variations were identified in the nebulin gene, c.5567$G > A$;p.Arg1856Glu and c.10383$G > C$;p.Met3461Ile. Variations in the nebulin gene are associated with nemaline myopathy 2 which is an autosomal recessive condition associated with proximal weakness, occasionally respiratory muscle weakness and dysmorphic features including micrognathia and scoliosis. However, these patients do not typically have cardiac abnormalities and commonly have nemaline rods on muscle biopsy which was not noted in this patient. Both of the variations identified are present in isolation in dbSNP in two separate cases, however this in itself does not confirm they are polymorphisms as they would only be expected to cause disease when expressed with a second pathogenic variation. It therefore appears to be the most likely explanation (Table 6.1). The other alternative explanation is that, given the presence of COX negative fibres on biopsy, that this is a mitochondrial disease and the variation has been missed on initial sequencing. We are therefore in the process of resequencing the mitochondrial genome to ensure this is not the case.

6.3.3.6 Case 14

Case 14 was of a 23 year old man with marked painful episodes, which also involved episodes of weakness. He had some history of autonomic symptoms with a positive tilt table test suggestive of postural tachycardia syndrome. He had abnormal long exercise testing, a

reduced left sural SNAP and one episode of raised CK during an attack.

Array CGH was not informative. Exome sequencing identified one variation of note which was located in SCN11A, c.2776G>A;p.Val926Ile. *In silico* analysis suggested that this was likely to be a polymorphism, however variations in this gene have been associated with a number of neuropathic phenotypes. The first is a gain of function mutation which results in congenital inability to perceive pain with marked autonomic dysfunction. These patients had self-mutilation, slow healing wounds and multiple painless fractures (Leipold et al., 2013). The second phenotype is a gain of function which is associated with a painful neuropathy (Huang et al., 2014). The phenotype of those with SCN11A variations included cramping of the legs, orthostatic dizziness, palpitations and hyperhydrosis. This is a very similar presentation to this patient. The only difference is the initial episodes of weakness and positive long exercise test which could not be easily explained by a variation in SCN11A. To further confirm this we intend to perform voltage clamp analysis of the mutated SCN11A channel to assess its impact on channel function and determine if it may underlie the patient's phenotype (Table 6.1).

6.3.3.7 Cases 15-18

The remaining four cases analysed with next generation sequencing did not have any likely causative variations found in whole exome or aCGH testing. There are a number of possible explanations for this. This includes the possibility that the causative variation has been missed by whole exome sequencing, that it lies in a gene which was not identified in my targeted analysis approach or that the patient has a non-organic or secondary cause of disease that is not genetic.

Cases 15 and 16 only had very limited clinical information from the clinicians and patients were not contactable for further information. They were included in the study because both had documented evidence of low potassium in the context of weakness which suggested there was some objective evidence of disease. However, in these cases it was not possible to rule out secondary causes of hypokalaemia and both patients had a significantly older age of onset than would be expected in primary HypoPP, which commonly presents in the teenage years to the early twenties.

In case 15 the only novel variation in an ion channel gene was in CACNA2D3. This gene encodes the $\alpha 2\delta 3$ calcium channel subunit. When knocked out of Drosophila and mice it alters thermal sensitivity to pain and SNPs within this gene are thought to modify

human thermal pain sensitivity (Neely et al., 2010). More recently a role for $\alpha 2\delta 1$ and $\alpha 2\delta 2$ have been identified in synaptic transmission in the control of the abundance of presynaptic calcium channels and the probability of exocytosis (Hoppa et al., 2012). Neither of these characteristics would be expected to lead to episodes of paralysis in the presence of hypokalaemia making the variation identified unlikely to be related to the clinical picture.

Case 16 only had a novel variation identified in CLCN1. Variations in CLCN1 have never been documented as causing episodes of profound weakness and are unlikely to cause disease in the absence of myotonia. This patient did not complain of stiffness and did not have clinical or electrical myotonia suggesting that this variation is unrelated to the clinical picture.

Case 17 was already known to have the homozygous intronic variant c.1930+6T>G in CLCN1. He had a typical recessive MC phenotype with onset at 8 years, profuse myotonia on EMG and short exercise testing suggestive of recessive MC. However, the variation does not appear to lie at a predicted splice site and therefore its pathogenicity was uncertain. I therefore performed whole exome sequencing. This did not reveal any possible causative variants suggesting that the homozygous intronic variant was likely to be pathogenic in this case (Table 6.1), however, further expression studies with a minigene assay are needed to confirm this.

Finally case 18 had atypical attacks of weakness with an abnormal CK but objective evidence of altered muscle membrane excitability from a positive long exercise test and fatty infiltration in thighs and calf on MRI. Despite having a distinctive phenotype, the only variation identified was a heterozygous variation in TTN which is predicted to be a polymorphism. Variations in TTN are associated with autosomal dominant tibial muscular dystrophy. However, this is associated with variations in the final exon (191) and this variation is in exon 186. The common phenotype is of progressive distal weakness in later life, very different to this patient's presentation. It therefore seems very unlikely to be related. In this particular case I have excluded secondary causes of HypoPP and this therefore suggests that the correct gene responsible for this patient's phenotype has not been identified. As there were no other affected family members and the patient has lost contact with his father this makes trio sequencing to identify the likely pathogenic variant more difficult. However, it would be the next step to identify the underlying cause in this case.

6.3.3.8 Limitations of Whole Exome Sequencing and aCGH

Whole exome sequencing and aCGH is a useful technique for sequencing a large number of genes quickly and cost effectively. In this study it has provided a useful method of identifying possible causative variations in a number of patients. This approach to diagnosing cases does, however, have limitations. Firstly these techniques have an inherent false negative rate that is higher than seen in Sanger sequencing. This is because of a combination of, low coverage in particular areas, missing sequencing in areas that are not covered by the targeted capture technology (like deep within introns) and poor sequencing of GC-rich areas. These aspects may partly explain why at least 30% of the cases remain undiagnosed. One way to minimise these problems would be to repeat the exome sequencing in negative cases using different capture technology to see if that reveals any other variations missed with the first run of sequencing.

Secondly variations that are detected may still be missed following filtering. This technique generates a large number of variations per patient and it is difficult to know which is the likely causative variation. To narrow down the search, different levels of filtering were used but this means that causative variations can be missed or ignored, especially if they lie in novel genes whose function is currently unknown. Another problem arises if variations detected by other groups are wrongly entered into variation databases such as dbSNP as non-pathogenic when they actually are pathogenic. It is difficult to manage this aspect as a balance must be struck between the large volume of sequencing and identifying a meaningful result. One technique, which we are currently trying in the negative cases is trio sequencing. This involves sequencing of the patient with both parents (or in some cases with an affected and closely related unaffected family members). This increases the likelihood of identifying the causative variation especially in novel genes. This was not possible initially in this study because of lack of samples from family members. Another useful approach would be to perform muscle biopsies in undiagnosed patients and perfom mRNA expression profiling to see if this could direct sequencing further. Trying this approach in this cohort was limited by difficulties getting patient consent for muscle biopsy.

With the aCGH no likely pathogenic rearrangements were identified. This may be for a number of reasons. Firstly it may be that, given the nature of these diseases, none of the patients had rearrangements causing disease. This seems to be a likely explanation but it was still important to use the technique to exclude these types of variation. Alternatively it may be

that this technique was not sensitive enough to pick up causative rearrangements. Although a combination of both 720K and 1.4M arrays were used to try and cover the most likely affected areas, small rearrangements would be missed. One option to tackle this would be to create a targeted aCGH array with closer spaced probes covering the ion channels genes.

This study was designed based on the theory that these patients were most likely to have variations in known ion channel genes or genes expressed in skeletal muscle. Whilst this remains the most likely answer in most cases, it relies heavily on correct identification of all genes in the human genome and an understanding of the function of the protein that they code for. It may be that variations in unexplained cases lie in genes that are unidentified, wrongly identified or in accessory genes that code for proteins that interact with ion channels. As our understanding of the genome and gene function develops it will be useful to rescreen the results to try and identify genes that may have been initially discounted.

Finally, the results of this study were limited by information available about specific variations. In the cases described I have presented the evidence available for suggesting that particular variations are responsible for causing disease. In many cases, further proof of the deleterious nature of these variations is required using minigene assays and channel expression studies. These are currently being established in our department to provide the final evidence of the pathogenicity of these variations.

6.3.3.9 Summary of Whole Exome Results

Overall of the 10 cases assessed by whole exome sequencing and in some cases array CGH, possible explanations have been reached for seven of them (Table 6.1). Two cases had intronic variations identified in CLCN1 which are likely to be responsible for the disease. One case is likely to be a genetic cause of secondary periodic paralysis on the background of a late onset myofibrillar myopathy. The others appear to be atypical presentations of other muscle diseases, one of an atypical RYR1 disease and other a possible nemaline myopathy. Finally, the other two cases appear to have a non-muscle pathology, one with a long QT syndrome mutation to explain their cardiac phenotype and the other a SCN11A phenotype that may explain their painful neuropathic and autonomic phenotype. This illustrates the variety of possible explanations for genetic disease in this small cohort.

This genetic study shows the value of next generation sequencing in the diagnosis of carefully phenotyped patients. These results suggest that RYR1 should be considered as a muscle channelopathy gene given there are now 3 cases of patients with episodes of

paralysis and variations in this gene. For patients with myotonia it appears that variations in unsequenced regions of CLCN1 or large scale rearrangements may underlie the few undiagnosed cases and so full sequencing of exonic and intronic regions of CLCN1 should be considered.

Table 6.1: Summary of likely causative variations and diagnoses in whole exome cases

Case	Clinical Phenotype	Key Investigations	Likely causative variation	Associated disease	Supportive evidence
Case 9	Hand and limb myotonia from 1st decade Generalised hypertrophy	EMG myotonia Ex test: Marked decrement with warm-up MRI: fatty infiltration of gastrocnemii	**CLCN1** c.1-59C>A;p? homozygous in 5'UTR region	**Recessive myotonia congenita**	Not seen in controls or dbSNP. Phenotype characteristic of recessive MC
Case 10	Episodes of weakness from teens. Fixed weakness from 50s	Positive McManis MRI: fatty replacement of vasti with ring of oedema, fatty infiltration of tibialis anterior and soleus Biopsy: tubular aggregates, two rimmed vacuoles of unknown significance	**CRYAB** c.406G>A;p.Gly154Ser & SCNN1B c.1307_1310delAGAG; p.Arg438PfsX17	**Myofibrillar myopathy** **Liddle's syndrome**	Variation reported in another patient with late onset distal myopathy Low aldosterone level. Likely to be pathogenic as frameshift mutation but unclear if it would cause a gain of function
Case 11	Mild dysmorphic features, episodes of paralysis	Positive McManis Prolonged QTc MRI: normal	**KCNQ1** c.805G>A;p.Gly269Ser	**Long QT**	Variation reported in other cases of long QT. Sister also carries variation and has long QT
Case 12	Episodes of paralysis with associated K+ 2.31mmol/L. Possible MH Dominant family history	Positive McManis MRI: mild fatty infiltration in posterior compartment of thighs and calves Biopsy:single COX –ve ragged red fibre	**RYR1** c.3128G>A;p.Arg1043His	**Atypical periodic paralysis & malignant hyperthermia**	Predicted to be pathogenic. Variations at this position reported in other cases with MH.
Case 13	Dysmorphic, scoliosis, developmental delay, ventricular tachcardias, episodes of paralysis and fixed weakness	Biopsy: COX negative fibres, normal mitochondrial respiratory chain enzymes	**NEB** c.5567G>A;p.Arg1856Glu & c.10383G>C;p.Met3461Ile	**Possible nemaline myopathy type 2**	One variation predicted to be pathogenic and other tolerated. Variations reported in dbSNP but only in isolation. However no nemaline rods on biopsy
Case 14	Pain and episodes of weakness from childhood. Postural syncope and palpitations	Positive McManis Reduced sural SNAP MRI: mild fatty infiltration of semimembranosus and long head of biceps	**SCN11A** c.2776G>A;p.Val926Ile	**Possible painful small fibre neuropathy**	Variation predicted to be a polymorphism.
Case 17	Stiffness and myotonia from childhood. Generalised muscle hypertrophy	EMG: myotonia Ex test: immediate decrement with warm-up	**CLCN1** c.1930+6T>G;p? homozygous	**Recessive myotonia congenita**	Variation not in controls or dbSNP Phenotype characteristic of recessive MC

6.4 Efficacy of Mexiletine in Treating NDM

6.4.1 Key Findings of the Mexiletine Study

The mexiletine study is the first study in patients with NDM to provide level I evidence for the efficacy of any drug in the treatment of NDM. The efficacy of mexiletine in this group was demonstrated, not only in the primary outcome measure of patient-reported stiffness, but also in the majority of secondary outcome measures. This included patient-reported weakness, pain and tiredness, quality of life assessments and the more objective measures of clinical myotonia. It also demonstrated a marked reduction in electrical myotonia which is an objective electrical correlate to clinical myotonia. The drug was well tolerated and side effects were mild, with primarily gastrointestinal discomfort which in all cases but one was not severe enough to require withdrawal from in the study. There were no study-related serious adverse events.

The majority of effect sizes were greater than 0.5 which, in the literature, corresponds to a moderate effect but there were a number of outcome measures, including the the primary outcome measure, that had effect sizes of greater than 0.8 corresponding to a large effect (de Vet et al., 2006; Husted et al., 2000; Kazis et al., 1989; Liang et al., 1985). This also included IVR measures of stiffness, weakness and pain; SF-36 physical composite score, clinical eye closure myotonia and EMG myotonia.

6.4.2 Comparison to other Treatment Studies

Prior studies of mexiletine in NDM have been limited to case studies and a single-blind trial as described earlier (Pouget and Serratrice, 1983; Leheup et al., 1986; Kwiecinski et al., 1992; Jackson et al., 1994). These studies included myotonic dystrophy patients and had no washout period and therefore did not provide objective, good quality evidence of efficacy. The more recent study from Logigian *et al* however, demonstrated safety and efficacy for mexiletine in myotonia in DM1 patients (Logigian et al., 2010) in a randomised-controlled double-blinded trial, which supports the evidence from this study. That trial however, was specific to DM1 and did not look at any patient-reported outcomes or quality of life measures to demonstrate whether the improvement to stiffness had any significant impact on patient health. In DM1 patients, the significant problems are rarely due to myotonia but to the systemic effects of the disease (Turner and Hilton-Jones, 2010), whereas in the NDM population the primary symptoms are related to myotonia (Statland et al., 2011). Therefore a medication that

specifically targets this symptom is more likely to have a significant impact on patient health, as suggested by these results.

6.4.3 Carry-over effect in Cross-over Studies

An important consideration in cross-over studies is the possible carry-over effect of a drug from one treatment period to another. To identify the presence of this the Wald test was applied to all data. It was noted in the patient-reported outcomes there was a significant increase in treatment effect when comparing mexiletine and placebo in treatment period 2. A significant Wald test would normally be explained by carry-over effect of the drug, however the result was the inverse of what would be expected if this was the case. Those patients on mexiletine in period 1 had greater stiffness in period 2 than those that had placebo in period 1. Conversely those who had mexiletine in period 2 had a greater mean improvement of stiffness than those who were on mexiletine in period 1. None of the patients had any mexiletine detected in serum samples following wash-out, making it unlikely that this was due to a direct chemical drug effect. The groups were well balanced apart from the group of placebo followed by mexiletine having a larger proportion of males to females (67% compared to 45% in the other group). This could potentially affect the results as men are reported to be more severely affected than women but the overall mean IVR stiffness between the two groups was not significantly different suggesting this difference in gender did not impact on the stiffness scores overall. If gender were to have an effect it would be expected to worsen the IVR placebo scores in period 1 compared to period 2 when in fact the placebo scores for period 2 were worse. When the differences between the groups were eliminated by calculating the treatment effect within each group (by calculating the change for the first group from when they were on mexiletine to when they were on placebo and the change in the second group from when they were on placebo to when they were on mexiletine) then there was no significant difference between the two groups (treatment effect 2.74 vs 2.61). This again suggests that the groups were balanced.

A possible explanation for this difference between the two treatment periods is that unblinding has occurred. This could be an unintentional unblinding due to a marked treatment effect caused by the efficacy of mexiletine or due to the distinctive side effects of mexiletine making it apparent which medication the patient was on. This means that when patients entered into period 2 they may have known whether they were on treatment or placebo therefore biasing their responses on the IVR. It is evident from the patient survey at the

end of the study that there was some unblinding as over 79% of patients correctly identified the drug they were on in period 2.

An alternative explanation for this is that patients without medication have some compensation mechanisms in place to manage their stiffness on a day to day basis, including avoiding particular tasks and using tricks to warm-up the stiffness. When a patient is on mexiletine, due to its efficacy, they stop needing to put these compensation processes in place and therefore when the medication is suddenly stopped the stiffness initially appears more exaggerated than before starting the medication. Another explanation could be that prolonged use of mexiletine causes an up-regulation of sodium channel expression in skeletal muscle myocytes, as this has been demonstrated in rat cardiac myocytes (Duff et al., 1992). If this is the case, when the patient stops mexiletine in the initial period the myotonia is worse than if they had been on no drug as there are more abnormal channels expressed with their persistent inward current. This effect would be expected to settle as the channel expression down-regulates again. If either of these two explanations were valid then I would expect the stiffness in the group which was on mexiletine and then placebo, to be significantly worse in the first two weeks after stopping mexiletine compared to the second two weeks whilst on placebo but in fact the results were the other way round with worsening of stiffness in the second set of two weeks when on placebo (4.63 vs 4.97; p=0.02). Average stiffness severity may also be expected to be worse in those that had placebo second compared to those that had placebo first but there was no significant difference in stiffness in the first two weeks on placebo between the two treatment groups (p=0.28).

This analysis suggests that the most likely mechanism for the difference is unblinding bias. Despite this there was still a significant difference when data was analysed with adjustments for the carry-over effect. The fairest interpretation may therefore be that the estimate of treatment effect lies between the 2 values for the 2 treatment periods, 1.68-3.68.

6.4.4 Limitations of the Mexiletine Study

One of the main limitations of this study has been the short duration of treatment. Patients were only on treatment for four weeks and so it is not known whether the improvement would have been sustained over a longer period of time. This therefore warrants further investigation. This short treatment phase, limited the time frame for adverse events and the small study number also limited the power for detecting adverse events. The inclusion of both chloride and sodium channel mutation patients for analysis also made data analysis more

complex and it would have been more robust to investigate these groups separately. However, given the rarity of these diseases the inclusion of both groups were necessary to power the study appropriately. This was felt to be a reasonable approach as the final mechanism for myotonia in both these diseases is the same and mexiletine specifically acts on this final mechanism.

The unblinding of this study is another limitation which is difficult to prevent given the side effect profile and efficacy of the drug but does limit some of the interpretation of the study. It would not, however, be ethical to redesign the study to give patients the same side effects when on placebo and therefore this needs to be borne in mind when analysing the data.

6.4.5 Further Work in Treatment Trials

The data from this study should ideally be extended further to include a larger number of patients and over a longer duration to identify the long term efficacy and tolerability of the drug. It would be very interesting to extend it with a large enough number of sodium and chloride channel patients to determine any difference in effect between the two groups. It would also be useful to compare its efficacy with some of the other available treatments such as carbamazepine and disopyramide to determine its relative efficacy.

Chapter 7

CONCLUSIONS

This thesis set out to increase the understanding of skeletal muscle channelopathies. It has done this by looking at a number of different aspects of the skeletal muscle channelopathies and identifying a number of novel findings.

I performed a prevalence study of the minimum point prevalence of all the skeletal muscle channelopathies in England. This is the first study to identify total prevalence in England and the first study worldwide to identify prevalence estimates for SCM, HyperPP and ATS. This study also identified the common mutations present in the UK population in the individual channelopathies.

I performed a comprehensive clinical phenotype study of PMC, SCM and periodic paralysis. There have been no previous published phenotype studies comparing PMC and SCM and this is the largest phenotype study of PMC patients documented. This study indicated that PMC and SCM are actually part of a single disease rather than two separate entities as traditionally thought, suggesting that a change in nomenclature may be appropriate. This is also the first comparative study of ATS with HypoPP and HyperPP, showing the intermediate nature of ATS attacks when compared to these two other diseases. It also identified correlations between MRI imaging and disease severity suggesting a further role for work in this area. As part of the clinical phenotype study I also performed the first study on anaesthetics and pregnancy in skeletal muscle channelopathies. All previous literature has been based on individual case studies and this is the first patient-based survey. This revealed that no patients had malignant hyperthermia but worsening of symptoms were seen in 17% after general anaesthetic. In pregnancy the most significant finding was the marked worsening in symptoms seen in the majority of patients and in almost all patients with PMC and SCM.

I performed a genetic diagnosis study to improve the genetic diagnosis rate in genetically

unconfirmed patients. This utilised a combination of targeted Sanger sequencing, MLPA, arrayCGH and whole exome sequencing with a focused analysis of muscle disease and ion channel genes. This is the first study to demonstrate large scale rearrangements in CLCN1 in MC patients. It is also the first study using whole exome and aCGH to identify variations in channelopathy patients. This study revealed that in atypical or undiagnosed periodic paralysis, RYR1 should be sequenced as this is associated with episodes of paralysis in two cases from this cohort and one previously published case. It also revealed that variations in intronic, 5'UTR and 3'UTR regions may underlie the undiagnosed cases of recessive MC and should be routinely checked in these patients. I also identified a likely case of inherited secondary periodic paralysis with a patient with a variation consistent with Liddle's syndrome. This suggests that undiagnosed patients should be screened for possible genetic causes of secondary periodic paralysis when a common cause is not identified.

Finally I recruited and performed the data collection and some of the statistical analysis for the UK part of an international phase II randomised double-blinded placebo-controlled trial in the efficacy of mexiletine in NDM. This is the first level I evidence available showing the efficacy of any drug in the treatment of NDM. This demonstrated efficacy in the primary outcome of patient-reported stiffness and the majority of the secondary outcomes, including both quality of life questionnaires and objective electrical and clinical measures of myotonia.

Overall this thesis has identified a number of novel findings. It has given new perspectives on the prevalence of channelopathies. It has changed our understanding of clinical phenotypes and increased our knowledge of pregnancy and anaesthetics in this group of diseases. It has expanded the spectrum of known variations and disease genes associated with channelopathy phenotypes and provided evidence for the efficacy of treatment with mexiletine in NDM.

Appendix A

PUBLICATIONS ARISING FROM THIS THESIS

A.1 Research Articles

- Morrow, J. M., Matthews, E., **Raja Rayan, D. L.**, Fischmann, A., Sinclair, C. D. J., Reilly, M. M., Thornton, J.S., Hanna, M.G., Yousry, T. A. (2013). Muscle MRI reveals distinct abnormalities in genetically proven non-dystrophic myotonias. Neuromuscular Disorders, 23(8), 637-646.

- Horga, A., **Rayan, D. L. R.**, Matthews, E., Sud, R., Fialho, D., Durran, S. C. M., Burge, J.A., Portaro S., Davis, M.B., Haworth, A., Hanna, M. G. (2013). Prevalence study of genetically defined skeletal muscle channelopathies in England. Neurology, 80(16), 1472-1475.

- Hehir, M. K., Logigian, E., **Raja Rayan, D. L.**, Ciafaloni, E. (2013). Double trouble in a patient with myotonia.. BMJ case reports, 2013.

- Trivedi, J. R., Bundy, B., Statland, J., Salajegheh, M., **Rayan, D. R.**, Venance, S. L., Wang, Y., Fialho, D., Matthews E., Cleland, J., Gorhm, N., Herbelin, L., Cannon, S. Amato, A., Griggs, R.C., Hanna, M.G., Barohn, R.J., CINCH Consortium. (2013). Non-dystrophic myotonia: prospective study of objective and patient reported outcomes.. Brain, 136(Pt 7), 2189-2200.

- Statland, J. M., Bundy, B. N., Wang, Y., **Rayan, D. R.**, Trivedi, J. R., Sansone, V. A., Salajegheh, M. K., et al. (2012). Mexiletine for symptoms and signs of myotonia in nondystrophic myotonia: a randomized controlled trial. JAMA: the journal of the American Medical Association, 308(13), 135765

- Statland, J. M., Bundy, B. N., Wang, Y., Trivedi, J. R., **Raja Rayan, D.**, Herbelin, L, et al and the CINCH Consortium. (2012). A quantitative measure of handgrip myotonia in non-dystrophic myotonia.. Muscle Nerve, 46(4), 482-489.

- **Raja Rayan DL**, Haworth A, Sud R, Matthews E, Fialho D, Burge J, Portaro S, Schorge S, Tuin K, Lunt P, et al. A new explanation for recessive myotonia congenita: Exon deletions and duplications in

CLCN1. Neurology 78(24):1953-1958 12 Jun 2012

A.2 Review Articles

- **Raja Rayan, D.L** and Hanna, M.G Skeletal Muscle Channelopathies: Non-Dystrophic Myotonias and Periodic Paralysis. Current Opinion in Neurology 2010 Oct;23(5):466-76.

- **Raja Rayan, D.L**, Parton, M. Clinical Introduction to Muscle Diseases. Ebrain online teaching resource for Neurology trainees. http://www.ebrainjnc.com/

- **Raja Rayan, D.L.** and Hanna, M.G. (October 2010) Ion Channels and Human Disorders. In: ENCYCLOPEDIA OF LIFE SCIENCES 2010, John Wiley and Sons, Ltd: Chichester http://www.els.net/

A.3 Published abstracts

- **Rayan, D. R.**, Haworth, A., Sud, R., McCall, S., Tan, S. V., Durran, S., . . . Hanna, M. G. (2012). Identifying the Cause of Phenotypic Variability in a Family with Non-dystrophic Myotonia. In Journal of Neurology Neurosurgery and Psychiatry Vol. 83.

- **Rayan, D. L. R.**, Rajakulendran, S., Barreto, G., Tan, S. V., Dewar, L., Griggs, R. C., . . . CINCH Grp. (2011). Genotype-phenotype correlation and longitudinal study of Andersen-Tawil Syndrome in the UK. In Neuromuscular Disorders Vol. 21 (pp. S14).

- **Rayan, D. L. R.**, Matthews, E., Rajakulendran, S., Barreto, G., Tan, S. V., Dewar, L., . . . CINCH Grp. (2011). Genotype-phenotype correlation and longitudinal three year natural history study in the non-dystrophic myotonias in the UK. In Neuromuscular Disorders Vol. 21 (pp. S14).

- Durran, S., Matthews, E., **Rayan, D. L. R.**, Sud, R., Polke, J., Haworth, A., . . . Hanna, M. G. (2011). Genetic heterogeneity and mechanisms of phenotypic variability in human skeletal muscle channelopathies - a new S4 mutation not associated with HypoPP. In Neuromuscular Disorders. Vol. 21 (pp. S13).

- **Rayan, D. R.**, Matthews, E., Barreto, G., Tan, S. V., Dewar, L., Burge, J., . . . Hanna, M. G. (2010). Double-blind placebo controlled cross-over study to investigate the efficacy of mexiletine in patients with non-dystrophic myotonia in the UK. In Neuromuscular Disorders. Vol. 20 (pp. S17).

Appendix B

GENE TABLES FOR WHOLE EXOME SEQUENCING

Table B.1: List of known genes associated with muscle disease phenotype used for filtering whole exome data (LGMD = Limb girdle muscular dystrophy)

Gene	Name	Associated Muscle Disease
ACAD9	Acyl-CoA Dehydrogenase 9	Mitochondrial Complex I Deficiency
ACTA1	Alpha-Actin	Nemaline Myopathy Type 3, Congenital Myopathy With Cores, Congenital Myopathy With Fibre Type Disproportion
AGL	Amylo-1,6-Glucosidase & 4-Alpha-Glucanotransferase	Glycogen Storage Disease III
AGRN	Agrin	Congenital Myasthenia
AMPD1	Myoadenylate Deaminase	Myopathy Due To Myoadenylate Deaminase Deficiency
ANO5	Anoctamin 5	LGMD 2L, Miyoshi Muscular Dystrophy 3
AR	Androgen Receptor	Spinal And Bulbar Muscular Atrophy Of Kennedy
ATP2A1	Calcium-Transporting ATPase	Brody Myopathy
ATXN1	Ataxin 1	Spinocerebellar Ataxia 1
BAG3	BCL2-Asociated Athanogene 3	Myofibrillar Myopathy 6
BIN1	Bridging Integrator 1	Centronusclear Myopathy
CACNA1A	P/Q Type Voltage Gated Calcium Channel	Spinocerebellar Ataxia 6, Episodic Ataxia, Type 2
CACNA1S	L Type Voltage Gated Calcium Channel	Hypokalemic Periodic Paralysis
CAPN3	Calpain-3	LGMD2A
CAV3	Caveolin-3	LGMD1C, Distal Myopathy, Rippling Muscle Disease
CFL2	Cofilin-2	Nemaline Myopathy 7
CHAT	Choline Acetyltransferase	Congenital Myasthenia
CHRNA1	Nicotinic Acetylcholine Receptor, Alpha Subunit	Congenital Myasthenia
CHRNB1	Nicotinic Acetylcholine Receptor, Beta Subunit	Congenital Myasthenia
CHRND	Nicotinic Acetylcholine Receptor, Delta Subunit	Congenital Myasthenia
CHRNE	Nicotinic Acetylcholine Receptor, Epsilon Subunit	Congenital Myasthenia
CLCN1	Muscle Chloride Channel 1	Myotonia Congenita
CNTN1	Contactin 1	Congenital Myopathy
COL6A1	Collagen Type VI, Alpha 1	Bethlem Myopathy, Ulrich Congenital Muscular Dystrophy
COL6A2	Collagen Type VI, Alpha 2	Bethlem Myopathy, Ulrich Congenital Muscular Dystrophy
COL6A3	Collagen Type VI, Alpha 3	Bethlem Myopathy, Ulrich Congenital Muscular Dystrophy
COLQ	Collagenic Tail Of Endplate Acteylcholinetserase	Congenital Myasthenia
CRYAB	Alpha-B-Crystallin	Myofibrillar Myopathy
DAG1	Dystrophin-Associated Glycoproteins	A-Dystroglycanopathy
DES	Desmin	LGM1E, Scapuloperoneal Syndrome

Table B.1: List of known genes associated with a muscle disease phenotype (cont.)

Gene	Name	Associated Muscle Disease
DMD	Dystrophin	Duchenne Muscular Dystrophy, Becker Muscular Dystrophy
DMPK	Dystrophia Myotonica Protein Kinase	Myotonic Dystrophy Type 1
DNAJB6	DNAJ/HSP40 Homolog	LGMD1E
DNM2	Dynamin 2	Centronuclea Myopathy, CMT Type 2M
DOK7	Downstream Of Tyrosine Kinase 7	Congenital Myasthenia
DPM3	Dolichol-Phosphate-Mannosyltransferase 3	Congenital Disorder Of Glycosylation
DYSF	Dysferlin	LGMD2B, Distal Myopathy, Miyoshi Muscular Dystrophy 1
EMD	Emerin	Emery-Dreifuss Muscular Dystrophy 1
ENO3	Enolase 3	Glycogen Storage Disease XIII
FHL1	Four And A Half LIM Domanins 1	Emery-Dreifuss Muscular Dystrophy 6, Scapuloperoneal Myopathy, Reducing Body Myopathy
FKBP14	FK506-Binding 14	Ehlers-Danlos Syndrome With Myopathy
FKRP	Fukutin-Related Protein Gene	LGMD1I
FKTN	Fukutin	LGMD2M
FLNC	Filamin C	Myofibrillar Myopathy Type 5, Distal Myopathy
GAA	Alpha-1,4-Glucosidase	Glycogen Storage Disease II
GBE1	Glycogen Branching Enzyme	Glycogen Storage Disease IV
GFPT1	Glutamine:Fructose-6-Phosphate Amidotransferase	Congenital Myasthenia
GMPPB	GDP-Mannose Pyrophosphorylase B	Muscular Dystrophy-Dystroglycanopathy
GNE	UDP-N-Acetylglucosamine 2-Epimerase/ N-Acetylmannosamine Kinase	Inclusion Body Myositis, Nonaka Myopathy
GYG1	Glycogenin-1	Glycogen Storage Disease XV
GYS1	Glycogen Synthase	Glycogen Storage Disease
HMBS	Porphobilinogen Deaminase	Acute Intermittent Porphyria
HNRNPDL	Heterogeneous Nuclear Ribonucleoproteins	LGMD1G
HSPB1	Heat-Shock Protein 27	Charcot-Marie-Tooth Disease
HSPG2	Perlecan	Schwartz-Jampel Syndrome
IGHMBP2	Immunoglobulin Mu-Binding Protein 2	Charcot-Marie-Tooth Disease
ISPD	Isoprenoid Synthase Domain-Containing Protein	Muscular Dystrophy-Dystroglycanopathy
KBTBD13	Kelch Repeat And BTB/POZ Domains-Containing Proteins 13	Nemaline Myopathy 6
KCNJ18	Inward Rectifying Potassium Channel 18	Susceptibility To Thyrotoxic Periodic Paralysis
KCNJ2	Inward Rectifying Potassium Channel	Andersen Tawil Syndrome
KIF1B	Kinesin Family Member 1B	Charcot-Marie-Tooth Disease
LAMA2	Laminin 2	Congenital Merosin-Deficient Muscular Dystrophy
LAMP2	Lysosome-Associated Membrane Protein B	Danon Disease
LARGE	Acetylglucosaminyltransferase-Like Protein	Muscular Dystrophy-Dystroglycanopathy

Table B.1: List of known genes associated with a muscle disease phenotype (cont.)

Gene	Name	Associated Muscle Disease
LDB3	LIM Domain-Binding 3	Myofibrillar Myopathy 4
LDHA	Lactate Dehydrogenase A	Glycogen Storage Disease XI
LIFR	Leukemia Inhibitory Factor Receptor	Schwartz-Jampel Type 2 Syndrome
LMNA	Lamin A/C	Emery-Dreifuss Muscular Dystrophy, LGMD1B
MATR3	Matrin 3	Distal Myopathy, Amyotrophic Lateral Sclerosis
MEGF10	Multiple Epidermal Growth Factor-Like Domains 10	Myopathy, Areflexia, Respiratory Distress, And Dysphagia
MFN2	Mitofusin 2	Charcot-Marie-Tooth Disease
MPZ	Myelin Protein Zero	Charcot-Marie-Tooth Disease
MSTN	Myostatin	Muscle Hypertrophy
MTM1	Myotubularin	Myotubular Myopathy
MUSK	Muscle-Specific Kinase	Congenital Myasthenia
MYF6	Myogenic Factor 6	Centronuclear Myopathy
MYH2	Myosin Heavy Chain 2	Proximal Myopathy And Ophthalmoplegia
MYH3	Myosin Heavy Chain 3	Arthrogryposis
MYH7	Myosin Heavy Chain 7	Myosin Storage Myopathy, Scapuloperoneal Syndrome, Laing Distal Myopathy
MYOT	Myotilin	LGMD1A, Myofibrillar Myopathy
NEB	Nebulin	Nemaline Myopathy
NEFL	Neurofilament Protein, Light Polypeptide	Charcot-Marie-Tooth Disease
PABPN1	Pokyadenylate –Binding Protein, Nuclear 1	Oculopharyngeal Muscular Dystrophy
PGAM2	Phosphoglycerate Mutase 2	Glycogen Storage Disease
PGK1	Phosphoglycerate Kinase 1	Phosphoglycerate Kinase 1 Deficiency
PGM1	Phosphoglucomutase 1	Congenital Disorder Of Glycosylation, Type It
PHKA1	Alpha Subunit Phosphorylase Kinase	Muscle Glycogenosis
PHKB	Beta Subunit Phosphorylase Kinase	Phosphorylase Kinase Deficiency Of Liver And Muscle
PLEC1	Plectin 1	LGMB2Q
PMP22	Peripheral Myelin Protein 22	Charcot-Marie-Tooth Disease
POLG	DNA Polymerase Gamma	Progressive External Ophthalmoplegia, Alpers, MNGIE
POMGNT1	Protein O-Mannose Beta-1,2-N-Acetylglucosaminyltransferase	Muscular Dystrophy-Dystroglycanopathy
POMK	Protein-O-Mannose Kinase	Muscular Dystrophy-Dystroglycanopathy
POMT1	Protein O-Mannosyltransferase 1	Muscular Dystrophy-Dystroglycanopathy
POMT2	Protein O-Mannosyltransferase 2	Muscular Dystrophy-Dystroglycanopathy
PTRF	RNA Polymerase I And Transcript Release Factor	Congenital Lipodystrophy 4
PYGM	Muscle Glycogen Phosphorylase	Mcardle Disease
RAPSN	Receptor-Associated Protein Of The Synapse	Congenital Myasthenia Syndrome
RMD1	Rippling Muscle Disease 1	Rippling Muscle Disease
RYR1	Ryanodine Receptor 1	Central Core Disease, Multi-Minicore Disease, Malignant Hyperthermia Susceptibility
SCN4A	Voltage-Gated Sodium Channel Type 4	HyperPP, PMC, SCM, HypoPP2

Table B.1: List of known genes associated with a muscle disease phenotype (cont.)

Gene	Name	Associated Muscle Disease
SEPN1	Selenoprotein N1	Congenital Myopathy, Rigid Spine Muscular Dystrophy
SGCA	Alpha Sarcoglycan	LGMD2D
SGCB	Beta Sarcoglycan	LGMD2E
SGCD	Delta Sarcoglycan	LGMD2F
SGCG	Gamma Sarcoglycan	LGMD2C
SIL1	Homolog S.Cerevisiae	Marinesco-Sjogren Syndrome
SLC25A4	Solute Carrier Family 25, Member 4	Progressive External Ophthalmoplegia
SMCHD1	Structural Maintenance Of Chromosomes Hinge Domain-Containing Protein 1	Fascioscapulohumeral Muscular Dystrophy 2
SMN1	Survival Of Motor Neuron 1	Spinal Muscular Atrophy
SMN2	Survival Of Motor Neuron 2	Spinal Muscular Atrophy
SOD1	Superoxide Dismutase 1	Amyotrophic Lateral Sclerosis
SYNE1	Spectrin Repeat-Containing Nuclear Envelope Protein 1	Emery-Dreifuss Muscular Dystrophy, Spinocerebellar Ataxia
SYNE2	Spectrin Repeat-Containing Nuclear Envelope Protein 2	Emery-Dreifuss Muscular Dystrophy
TARDBP	TAR DNA Binding Protein	Amyotrophic Lateral Sclerosis
TAZ	Tafazzin	Barth Syndrome
TCAP	Titin-CAP	LGMD2G
TGFB1	Transforming Growth Factor, Beta-1	Camurati-Engelmann Disease
TK2	Thymidine Kinase 2	Mitochondrial DNA Depletion Syndrome
TNNT1	Troponin T1 Slow	Nemaline Myopathy
TNPO3	Transportin 3	LGMD1F
TPM2	Tropomyosin 2	Nemaline Myopathy, CAP Myopathy
TPM3	Tropomyosin 3	Nemaline Myopathy, CAP Myopathy, Congenital Myopathy
TRAPPC11	Trafficking Protein Particle Complex, Subunit 11	LGMD2S
TRIM32	Tripartite Motif-Containing Protein 32	LGMD2H
TTN	Titin	LGMD2J, Tibial Muscular Dystrophy
VCP	Valosin-Containing Protein	Inclusion Body Myositis, Amyotrophic Lateral Sclerosis
WDM	Welander Distal Myopathy	Welander Distal Myopathy
ZC4H2	Zinc Finger C4h2 Domain-Containing Protein	Wieacker-Wolff Syndrome
ZNF9	Zinc Finger Protein 9	Myotonic Dystrophy Type 2

Table B.2: List of known ion channel genes used for filtering whole exome data with skeletal muscle expression levels

Gene	Gene description	Skeletal muscle Expression
CACNA1S	calcium channel, voltage-dependent, L type, alpha 1S subunit	High
CACNG1	calcium channel, voltage-dependent, gamma subunit 1	High
CLCN1	chloride channel, voltage-sensitive 1	High
KCNA2	potassium channel, voltage gated shaker related subfamily A, member 2	High
KCNJ11	potassium channel, inwardly rectifying subfamily J, member 11	High
SCN3B	sodium channel, voltage gated, type III beta subunit	High
TRPC6	transient receptor potential cation channel, subfamily C, member 6	High
CACNA1S	calcium channel, voltage-dependent, L type, alpha 1S subunit	High
ASIC4	acid sensing (proton gated) ion channel family member 4	Medium
AQP4	aquaporin 4	Medium
CACNA1G	calcium channel, voltage-dependent, T type, alpha 1G subunit	Medium
CACNB4	calcium channel, voltage-dependent, beta 4 subunit	Medium
CACNG3	calcium channel, voltage-dependent, gamma subunit 3	Medium
CACNG5	calcium channel, voltage-dependent, gamma subunit 5	Medium
CACNG8	calcium channel, voltage-dependent, gamma subunit 8	Medium
CLCA2	chloride channel accessory 2	Medium
CLCN5	chloride channel, voltage-sensitive 5	Medium
CLCNKA	chloride channel, voltage-sensitive Ka	Medium
CLIC5	chloride intracellular channel 5	Medium
CNGA3	cyclic nucleotide gated channel alpha 3	Medium
HCN2	hyperpolarization activated cyclic nucleotide gated potassium channel 2	Medium
KCNA7	potassium channel, voltage gated shaker related subfamily A, member 7	Medium
KCNC1	potassium channel, voltage gated Shaw related subfamily C, member 1	Medium
KCNE1	potassium channel, voltage gated subfamily E regulatory beta subunit 1	Medium
KCNE2	potassium channel, voltage gated subfamily E regulatory beta subunit 2	Medium
KCNE4	potassium channel, voltage gated subfamily E regulatory beta subunit 4	Medium
KCNF1	potassium channel, voltage gated modifier subfamily F1	Medium
KCNG4	potassium channel, voltage gated modifier subfamily G, member 4	Medium

Table B.2: List of known ion channel genes (cont.)

Gene	Gene description	Expression
KCNH5	potassium channel, voltage gated eag related subfamily H, member 5	Medium
KCNIP4	Kv channel interacting protein 4	Medium
KCNJ14	potassium channel, two pore domain subfamily J, member 14	Medium
KCNK13	potassium channel, two pore domain subfamily K, member 13	Medium
KCNK18	potassium channel, two pore domain subfamily K, member 18	Medium
KCNN2	potassium channel, calcium activated intermediate/small conductance subfamily N alpha, member 2	Medium
KCNQ5	potassium channel, voltage gated KQT-like subfamily Q, member 5	Medium
KCNU1	potassium channel, subfamily U, member 1	Medium
RYR3	ryanodine receptor 3	Medium
SCN1A	sodium channel, voltage gated, type I alpha subunit	Medium
SCN7A	sodium channel, voltage gated, type VII alpha subunit	Medium
SCN8A	sodium channel, voltage gated, type VIII alpha subunit	Medium
TRPC3	transient receptor potential cation channel, subfamily C, member 3	Medium
TRPM3	transient receptor potential cation channel, subfamily M, member 3	Medium
TRPM7	transient receptor potential cation channel, subfamily M, member 7	Medium
TRPV3	transient receptor potential cation channel, subfamily V, member 3	Medium
CACNA1C	calcium channel, voltage-dependent, L type, alpha 1C subunit	Low
CACNA1E	calcium channel, voltage-dependent, R type, alpha 1E subunit	Low
CACNA2D1	calcium channel, voltage-dependent, alpha 2/delta subunit 1	Low
CACNA2D2	calcium channel, voltage-dependent, alpha 2/delta subunit 2	Low
CACNB1	calcium channel, voltage-dependent, beta 1 subunit	Low
CACNB3	calcium channel, voltage-dependent, beta 3 subunit	Low
CLCN4	chloride channel, voltage-sensitive 4	Low
CLCNKB	chloride channel, voltage-sensitive Kb	Low
CLIC2	chloride intracellular channel 2	Low
CNGA2	cyclic nucleotide gated channel alpha 2	Low
CNGB3	cyclic nucleotide gated channel beta 3	Low
KCNA4	potassium channel, voltage gated shaker related subfamily A, member 4	Low
KCNA5	potassium channel, voltage gated shaker related subfamily A, member 5	Low

Table B.2: List of known ion channel genes (cont.)

Gene	Gene description	Expression
KCNC3	potassium channel, voltage gated Shaw related subfamily C, member 3	Low
KCNC4	potassium channel, voltage gated Shaw related subfamily C, member 4	Low
KCND1	potassium channel, voltage gated Shal related subfamily D, member 1	Low
KCND3	potassium channel, voltage gated Shal related subfamily D, member 3	Low
KCNE3	potassium channel, voltage gated subfamily E regulatory beta subunit 3	Low
KCNH1	potassium channel, voltage gated eag related subfamily H, member 1	Low
KCNJ13	potassium channel, inwardly rectifying subfamily J, member 13	Low
KCNJ18	potassium channel, two pore domain subfamily J, member 18	Low
KCNJ2	potassium channel, two pore domain subfamily J, member 2	Low
KCNK10	potassium channel, two pore domain subfamily K, member 10	Low
KCNK12	potassium channel, two pore domain subfamily K, member 12	Low
KCNK15	potassium channel, two pore domain subfamily K, member 15	Low
KCNK4	potassium channel, two pore domain subfamily K, member 4	Low
KCNK6	potassium channel, two pore domain subfamily K, member 6	Low
KCNMA1	potassium channel, calcium activated large conductance subfamily M alpha, member 1	Low
KCNMB1	potassium channel subfamily M regulatory beta subunit 1	Low
KCNN4	potassium channel, calcium activated intermediate/small conductance subfamily N alpha, member 4	Low
KCNQ3	potassium channel, voltage gated KQT-like subfamily Q, member 3	Low
KCNS1	potassium voltage-gated channel, modifier subfamily S, member 1	Low
KCNV1	potassium channel, voltage gated modifier subfamily V, member 1	Low
SCN10A	sodium channel, voltage gated, type X alpha subunit	Low
SCN1B	sodium channel, voltage gated, type I beta subunit	Low
SCN4A	sodium channel, voltage gated, type IV alpha subunit	Low
SCN4B	sodium channel, voltage gated, type IV beta subunit	Low
SCNN1A	sodium channel, non voltage gated 1 alpha subunit	Low

Table B.2: List of known ion channel genes (cont.)

Gene	Gene description	Expression
TPTE2	transmembrane phosphoinositide 3-phosphatase and tensin homolog 2	Low
TRPA1	transient receptor potential cation channel, subfamily A, member 1	Low
TRPC7	transient receptor potential cation channel, subfamily C, member 7	Low
TRPM5	transient receptor potential cation channel, subfamily M, member 5	Low
TRPV2	transient receptor potential cation channel, subfamily V, member 2	Low
TRPV4	transient receptor potential cation channel, subfamily V, member 4	Low
ASIC1	acid sensing (proton gated) ion channel 1	None
ASIC2	acid sensing (proton gated) ion channel 2	None
ASIC3	acid sensing (proton gated) ion channel 3	None
ASIC5	acid sensing (proton gated) ion channel family member 5	None
ALG10	alpha-1,2-glucosyltransferase	None
ANKRD36C	ankyrin repeat domain 36C	None
ANO1	anoctamin 6	None
ANO10	Anoctamin 10	None
ANO2	Anoctamin 2	None
ANO3	Anoctamin 3	None
ANO4	Anoctamin 4	None
ANO5	Anoctamin 5	None
ANO6	Anoctamin 6	None
ANO7	Anoctamin 7	None
ANO8	Anoctamin 8	None
ANO9	Anoctamin 9	None
ANXA9	annexin A9	None
APP	amyloid beta (A4) precursor protein	None
AQP1	aquaporin 1	None
BEST1	Bestrophin 1	None
BEST2	Bestrophin 2	None
BEST3	Bestrophin 3	None
BEST4	Bestrophin 4	None
CACNA1A	Calcium channel, voltage-dependent, P/Q type, α1A subunit	None
CACNA1B	calcium channel, voltage-dependent, N type, α1B subunit	None
CACNA1D	calcium channel, voltage-dependent, L type, α1D subunit	None
CACNA1F	calcium channel, voltage-dependent, L type, α1F subunit	None
CACNA1H	calcium channel, voltage-dependent, T type, α1H subunit	None
CACNA1I	calcium channel, voltage-dependent, T type, α1I subunit	None
CACNA2D3	calcium channel, voltage-dependent, alpha 2/delta subunit 3	None

Table B.2: List of known ion channel genes (cont.)

Gene	Gene description	Expression
CACNA2D4	calcium channel, voltage-dependent, alpha 2/delta subunit 4	None
CACNB2	calcium channel, voltage-dependent, beta 2 subunit	None
CACNG2	calcium channel, voltage-dependent, gamma subunit 2	None
CACNG4	calcium channel, voltage-dependent, gamma subunit 4	None
CACNG6	calcium channel, voltage-dependent, gamma subunit 6	None
CACNG7	calcium channel, voltage-dependent, gamma subunit 7	None
CALHM1	calcium homeostasis modulator 1	None
CAPN2	calpain 2, (m/II) large subunit	None
CATSPER1	cation channel, sperm associated 1	None
CATSPER2	cation channel, sperm associated 2	None
CATSPER3	cation channel, sperm associated 3	None
CATSPER4	cation channel, sperm associated 4	None
CDH5	cadherin 5, type 2	None
CFTR	cystic fibrosis transmembrane conductance regulator (ATP-binding cassette sub-family C, member 7)	None
CHRFAM7A	CHRNA7 (cholinergic receptor, nicotinic, alpha 7, exons 5-10) and FAM7A (family with sequence similarity 7A, exons A-E) fusion	None
CHRNA1	cholinergic receptor, nicotinic, alpha 1 (muscle)	None
CHRNA10	cholinergic receptor, nicotinic, alpha 10 (neuronal)	None
CHRNA2	cholinergic receptor, nicotinic, alpha 2 (neuronal)	None
CHRNA3	cholinergic receptor, nicotinic, alpha 3 (neuronal)	None
CHRNA4	cholinergic receptor, nicotinic, alpha 4 (neuronal)	None
CHRNA5	cholinergic receptor, nicotinic, alpha 5 (neuronal)	None
CHRNA6	cholinergic receptor, nicotinic, alpha 6 (neuronal)	None
CHRNA7	cholinergic receptor, nicotinic, alpha 7 (neuronal)	None
CHRNA9	cholinergic receptor, nicotinic, alpha 9 (neuronal)	None
CHRNB1	cholinergic receptor, nicotinic, beta 1 (muscle)	None
CHRNB2	cholinergic receptor, nicotinic, beta 2 (neuronal)	None
CHRNB3	cholinergic receptor, nicotinic, beta 3 (neuronal)	None
CHRNB4	cholinergic receptor, nicotinic, beta 4 (neuronal)	None
CHRND	cholinergic receptor, nicotinic, delta (muscle)	None
CHRNE	cholinergic receptor, nicotinic, epsilon (muscle)	None
CHRNG	cholinergic receptor, nicotinic, gamma (muscle)	None
CLCA3P	chloride channel accessory 3, pseudogene	None
CLCN2	chloride channel, voltage-sensitive 2	None
CLCN3	chloride channel, voltage-sensitive 3	None
CLCN6	chloride channel, voltage-sensitive 6	None
CLCN7	chloride channel, voltage-sensitive 7	None
CLIC1	chloride intracellular channel 1	None
CLIC3	chloride intracellular channel 3	None
CLIC4	chloride intracellular channel 4	None
CLIC6	chloride intracellular channel 6	None

Table B.2: List of known ion channel genes (cont.)

Gene	Gene description	Expression
CNGA1	cyclic nucleotide gated channel alpha 1	None
CNGA4	cyclic nucleotide gated channel alpha 4	None
CNGB1	cyclic nucleotide gated channel beta 1	None
CTNNB1	catenin (cadherin-associated protein), beta 1	None
CYBB	cytochrome b-245, beta polypeptide	None
DLG2	discs, large homolog 2	None
DLG4	discs, large homolog 4	None
ENKUR	enkurin, TRPC channel interacting protein	None
ENSA	endosulfine alpha	None
FKBP1B	FK506 binding protein 1B	None
FXYD1	FXYD domain containing ion transport regulator 1	None
FXYD2	FXYD domain containing ion transport regulator 2	None
FXYD3	FXYD domain containing ion transport regulator 5	None
FXYD4	FXYD domain containing ion transport regulator 6	None
FXYD5	FXYD domain containing ion transport regulator 6 pseudogene 3	None
FXYD6	FXYD domain containing ion transport regulator 7	None
FXYD6P3	FXYD domain containing ion transport regulator 1	None
FXYD7	FXYD domain containing ion transport regulator 2	None
GABRA1	gamma-aminobutyric acid (GABA) A receptor, alpha 1	None
GABRA2	gamma-aminobutyric acid (GABA) A receptor, alpha 2	None
GABRA3	gamma-aminobutyric acid (GABA) A receptor, alpha 3	None
GABRA4	gamma-aminobutyric acid (GABA) A receptor, alpha 4	None
GABRA5	gamma-aminobutyric acid (GABA) A receptor, alpha 5	None
GABRA6	gamma-aminobutyric acid (GABA) A receptor, alpha 6	None
GABRB1	gamma-aminobutyric acid (GABA) A receptor, beta 1	None
GABRB2	gamma-aminobutyric acid (GABA) A receptor, beta 2	None
GABRB3	gamma-aminobutyric acid (GABA) A receptor, beta 3	None
GABRD	gamma-aminobutyric acid (GABA) A receptor, delta	None
GABRE	gamma-aminobutyric acid (GABA) A receptor, epsilon	None
GABRG1	gamma-aminobutyric acid (GABA) A receptor, gamma 1	None
GABRG2	gamma-aminobutyric acid (GABA) A receptor, gamma 2	None
GABRG3	gamma-aminobutyric acid (GABA) A receptor, gamma 3	None
GABRP	gamma-aminobutyric acid (GABA) A receptor, pi	None
GABRQ	gamma-aminobutyric acid (GABA) A receptor, theta	None
GABRR1	gamma-aminobutyric acid (GABA) A receptor, rho 1	None
GABRR2	gamma-aminobutyric acid (GABA) A receptor, rho 2	None
GABRR3	gamma-aminobutyric acid (GABA) A receptor, rho 3 (gene/pseudogene)	None
GLRA1	glycine receptor, alpha 1	None
GLRA2	glycine receptor, alpha 2	None
GLRA3	glycine receptor, alpha 3	None
GLRA4	glycine receptor, alpha 4	None
GLRB	glycine receptor, beta	None

Table B.2: List of known ion channel genes (cont.)

Gene	Gene description	Expression
GNB2L1	guanine nucleotide binding protein (G protein), beta polypeptide 2-like 1	None
GPR89B	G protein-coupled receptor 89B	None
GRIA1	glutamate receptor, ionotropic, AMPA 1	None
GRIA2	glutamate receptor, ionotropic, AMPA 2	None
GRIA3	glutamate receptor, ionotropic, AMPA 3	None
GRIA4	glutamate receptor, ionotropic, AMPA 4	None
GRID1	glutamate receptor, ionotropic, delta 1	None
GRID2	glutamate receptor, ionotropic, delta 2	None
GRIK1	glutamate receptor, ionotropic, kainate 1	None
GRIK2	glutamate receptor, ionotropic, kainate 2	None
GRIK3	glutamate receptor, ionotropic, kainate 3	None
GRIK4	glutamate receptor, ionotropic, kainate 4	None
GRIK5	glutamate receptor, ionotropic, kainate 5	None
GRIN1	glutamate receptor, ionotropic, N-methyl D-aspartate 1	None
GRIN2A	glutamate receptor, ionotropic, N-methyl D-aspartate 2A	None
GRIN2B	glutamate receptor, ionotropic, N-methyl D-aspartate 2B	None
GRIN2C	glutamate receptor, ionotropic, N-methyl D-aspartate 2C	None
GRIN2D	glutamate receptor, ionotropic, N-methyl D-aspartate 2D	None
GRIN3A	glutamate receptor, ionotropic, N-methyl-D-aspartate 3A	None
GRIN3B	glutamate receptor, ionotropic, N-methyl-D-aspartate 3B	None
HCN1	hyperpolarization activated cyclic nucleotide gated potassium channel 1	None
HCN3	hyperpolarization activated cyclic nucleotide gated potassium channel 3	None
HCN4	hyperpolarization activated cyclic nucleotide gated potassium channel 4	None
HTR3A	5-hydroxytryptamine (serotonin) receptor 3A, ionotropic	None
HTR3B	5-hydroxytryptamine (serotonin) receptor 3B, ionotropic	None
HTR3C	5-hydroxytryptamine (serotonin) receptor 3C, ionotropic	None
HTR3D	5-hydroxytryptamine (serotonin) receptor 3D, ionotropic	None
HTR3E	5-hydroxytryptamine (serotonin) receptor 3E, ionotropic	None
HVCN1	hydrogen voltage gated channel 1	None
IAPP	islet amyloid polypeptide	None
ITPR1	inositol 1,4,5-trisphosphate receptor, type 1	None
ITPR2	inositol 1,4,5-trisphosphate receptor, type 2	None
ITPR3	inositol 1,4,5-trisphosphate receptor, type 3	None
KBTBD13	kelch repeat and BTB (POZ) domain containing 13	None
KCNA1	potassium channel, voltage gated shaker related subfamily A, member 1	None
KCNA10	potassium channel, voltage gated shaker related subfamily A, member 10	None
KCNA3	potassium channel, voltage gated shaker related subfamily A, member 3	None

Table B.2: List of known ion channel genes (cont.)

Gene	Gene description	Expression
KCNA6	potassium channel, voltage gated shaker related subfamily A, member 6	None
KCNAB1	potassium channel, voltage gated subfamily A regulatory beta subunit 1	None
KCNAB2	potassium channel, voltage gated subfamily A regulatory beta subunit 2	None
KCNAB3	potassium channel, voltage gated subfamily A regulatory beta subunit 3	None
KCNB1	potassium channel, voltage gated Shab related subfamily B, member 1	None
KCNB2	potassium channel, voltage gated Shab related subfamily B, member 2	None
KCNC2	potassium channel, voltage gated Shaw related subfamily C, member 2	None
KCND2	potassium channel, voltage gated Shal related subfamily D, member 2	None
KCNG1	potassium channel, voltage gated modifier subfamily G, member 1	None
KCNG2	potassium channel, voltage gated modifier subfamily G, member 2	None
KCNG3	potassium channel, voltage gated modifier subfamily G, member 3	None
KCNH2	potassium channel, voltage gated eag related subfamily H, member 2	None
KCNH3	potassium channel, voltage gated eag related subfamily H, member 3	None
KCNH4	potassium channel, voltage gated eag related subfamily H, member 4	None
KCNH6	potassium channel, voltage gated eag related subfamily H, member 6	None
KCNH7	potassium channel, voltage gated eag related subfamily H, member 7	None
KCNH8	potassium channel, voltage gated eag related subfamily H, member 8	None
KCNIP1	Kv channel interacting protein 1	None
KCNIP2	Kv channel interacting protein 2	None
KCNIP3	Kv channel interacting protein 3, calsenilin	None
KCNJ1	potassium channel, inwardly rectifying subfamily J, member 1	None
KCNJ10	potassium channel, inwardly rectifying subfamily J, member 10	None
KCNJ12	potassium channel, inwardly rectifying subfamily J, member 12	None
KCNJ15	potassium channel, two pore domain subfamily J, member 15	None

Table B.2: List of known ion channel genes (cont.)

Gene	Gene description	Expression
KCNJ16	potassium channel, two pore domain subfamily J, member 16	None
KCNJ3	potassium channel, two pore domain subfamily J, member 3	None
KCNJ4	potassium channel, two pore domain subfamily J, member 4	None
KCNJ5	potassium channel, two pore domain subfamily J, member 5	None
KCNJ6	potassium channel, two pore domain subfamily J, member 6	None
KCNJ8	potassium channel, two pore domain subfamily J, member 8	None
KCNJ9	potassium channel, two pore domain subfamily J, member 9	None
KCNK1	potassium channel, two pore domain subfamily K, member 1	None
KCNK16	potassium channel, two pore domain subfamily K, member 16	None
KCNK17	potassium channel, two pore domain subfamily K, member 17	None
KCNK2	potassium channel, two pore domain subfamily K, member 2	None
KCNK3	potassium channel, two pore domain subfamily K, member 3	None
KCNK5	potassium channel, two pore domain subfamily K, member 5	None
KCNK7	potassium channel, two pore domain subfamily K, member 7	None
KCNK9	potassium channel, two pore domain subfamily K, member 9	None
KCNMB2	potassium channel subfamily M regulatory beta subunit 2	None
KCNMB3	potassium channel subfamily M regulatory beta subunit 4	None
KCNMB4	potassium channel, calcium activated large conductance subfamily M alpha, member 1	None
KCNN1	potassium channel, calcium activated intermediate/small conductance subfamily N alpha, member 1	None
KCNN3	potassium channel, calcium activated intermediate/small conductance subfamily N alpha, member 3	None
KCNQ1	potassium channel, voltage gated KQT-like subfamily Q, member 1	None
KCNQ2	potassium channel, voltage gated KQT-like subfamily Q, member 2	None

Table B.2: List of known ion channel genes (cont.)

Gene	Gene description	Expression
KCNQ4	potassium channel, voltage gated KQT-like subfamily Q, member 4	None
KCNS2	potassium voltage-gated channel, modifier subfamily S, member 2	None
KCNS3	potassium voltage-gated channel, modifier subfamily S, member 3	None
KCNT1	potassium channel, sodium activated subfamily T, member 1	None
KCNT2	potassium channel, sodium activated subfamily T, member 2	None
KCNV2	potassium channel, voltage gated modifier subfamily V, member 2	None
LIN7B	lin-7 homolog B (C. elegans)	None
LRRC26	leucine rich repeat containing 26	None
LYN	LYN proto-oncogene, Src family tyrosine kinase	None
MCOLN2	Mucolipin 2	None
MCOLN3	Mucolipin 3	None
MFSD11	major facilitator superfamily domain containing 11	None
MLC1	megalencephalic leukoencephalopathy with subcortical cysts 1	None
MMD	monocyte to macrophage differentiation-associated	None
MTCH1	mitochondrial carrier 1	None
MYOCD	myocardin	None
NALCN	sodium leak channel, non selective	None
NOX1	NADPH oxidase, EF-hand calcium binding domain 1	None
NOX5	NADPH oxidase, EF-hand calcium binding domain 5	None
OPN4	opsin 4	None
ORAI1	ORAI calcium release-activated calcium modulator 1	None
ORAI3	ORAI calcium release-activated calcium modulator 3	None
P2RX1	purinergic receptor P2X, ligand gated ion channel, 1	None
P2RX2	purinergic receptor P2X, ligand gated ion channel, 2	None
P2RX3	purinergic receptor P2X, ligand gated ion channel, 3	None
P2RX4	purinergic receptor P2X, ligand gated ion channel, 4	None
P2RX5	purinergic receptor P2X, ligand gated ion channel, 5	None
P2RX5-TAX1BP3	P2RX5-TAX1BP3 readthrough (NMD candidate)	None
P2RX6	purinergic receptor P2X, ligand gated ion channel, 6	None
P2RX6P	purinergic receptor P2X, ligand gated ion channel, 6 pseudogene	None
P2RX7	purinergic receptor P2X, ligand gated ion channel, 7	None
PANX1	pannexin 1	None
PANX2	Pannexin 2	None
PANX3	Pannexin 3	None
PDZD3	PDZ domain containing 3	None

Table B.2: List of known ion channel genes (cont.)

Gene	Gene description	Expression
PHPT1	phosphohistidine phosphatase 1	None
PICK1	protein interacting with PRKCA 1	None
PIEZO1	piezo-type mechanosensitive ion channel component 1	None
PIEZO1P1	piezo-type mechanosensitive ion channel component 1 pseudogene 1	None
PIEZO2	piezo-type mechanosensitive ion channel component 2	None
PKD1L2	polycystic kidney disease 1-like 2 (gene/pseudogene)	None
PKD1L3	polycystic kidney disease 1-like 3	None
PKD2	polycystic kidney disease 2 (autosomal dominant)	None
PKD2L2	polycystic kidney disease 2-like 2	None
PKDREJ	polycystin (PKD) family receptor for egg jelly	None
PLLP	plasmolipin	None
PLP2	proteolipid protein 2 (colonic epithelium-enriched)	None
PNPLA8	patatin-like phospholipase domain containing 8	None
RAPGEF3	Rap guanine nucleotide exchange factor (GEF) 3	None
RSC1A1	regulatory solute carrier protein, family 1, member 1	None
RYR1	ryanodine receptor 1	None
RYR2	ryanodine receptor 2 (cardiac)	None
SCN11A	sodium channel, voltage gated, type XI alpha subunit	None
SCN2A	sodium channel, voltage gated, type II alpha subunit	None
SCN2B	sodium channel, voltage gated, type II beta subunit	None
SCN3A	sodium channel, voltage gated, type III alpha subunit	None
SCN5A	sodium channel, voltage gated, type V alpha subunit	None
SCN9A	sodium channel, voltage gated, type IX alpha subunit	None
SCNN1B	sodium channel, non voltage gated 1 beta subunit	None
SCNN1D	sodium channel, non voltage gated 1 delta subunit	None
SCNN1G	sodium channel, non voltage gated 1 gamma subunit	None
SIRT2	sirtuin 2	None
SLC26A9	solute carrier family 26 (anion exchanger), member 9	None
SLC4A11	solute carrier family 4, sodium borate transporter, member 11	None
SLC9A11	solute carrier family 9, sodium borate transporter, member 11	None
SLC9A3R2	solute carrier family 9, subfamily A (NHE3, cation proton antiporter 3), member 3 regulator 2	None
SRC	SRC proto-oncogene, non-receptor tyrosine kinase	None
SRF	serum response factor (c-fos serum response element-binding transcription factor)	None
STX1A	syntaxin 1A (brain)	None
STX1B	syntaxin 1B (brain)	None
TMEM37	transmembrane protein 37	None
TPCN1	two pore segment channel 1	None
TPCN2	two pore segment channel 2	None

Table B.2: List of known ion channel genes (cont.)

Gene	Gene description	Expression
TPTE	transmembrane phosphatase with tensin homology	None
TRPC1	transient receptor potential cation channel, subfamily C, member 1	None
TRPC4	transient receptor potential cation channel, subfamily C, member 4	None
TRPC5	transient receptor potential cation channel, subfamily C, member 5	None
TRPM1	transient receptor potential cation channel, subfamily M, member 1	None
TRPM2	transient receptor potential cation channel, subfamily M, member 2	None
TRPM4	transient receptor potential cation channel, subfamily M, member 4	None
TRPM6	transient receptor potential cation channel, subfamily M, member 6	None
TRPM8	transient receptor potential cation channel, subfamily M, member 8	None
TRPV1	transient receptor potential cation channel, subfamily V, member 1	None
TRPV5	transient receptor potential cation channel, subfamily V, member 5	None
TRPV6	transient receptor potential cation channel, subfamily V, member 6	None
TTYH1	tweety family member 1	None
TTYH2	tweety family member 2	None
TTYH3	tweety family member 3	None
UNC93A	unc-93 homolog A (C. elegans)	None
UNC93B1	unc-93 homolog B1 (C. elegans)	None
YES1	YES proto-oncogene 1, Src family tyrosine kinase	None
ZACN	zinc activated ligand-gated ion channel	None

Table B.3: List of known genes associated with long QT with associated phenotype

Gene	Gene Description	Disease
ABCC9	ATP-Binding Cassette, Subfamily C, Member 9	Familial AF, Dilated Cardiomyopathy
ACADM	Acyl-CoA dehydrogenase, medium chain	Deficiency of Acyl-CoA dehydrogenase, medium chain (causing longQT)
ACE	Angiotensin I-converting enzyme	Long QT in ESRF
ADRA2C	Alpha-2c-Adrenergic Receptor	
ADRB1	Beta-1-Adrenergic Receptor	
ADRB2	Beta-2-Adrenergic Receptor	
AKR1B1	Aldo-Keto Reductase Family 1, Member B1	
ALG10B	alpha-1,2-glucosyltransferase	Long QT
ANK2	Ankyrin 2	Long QT 4
AR	androgen receptor	
ATP6	ATP synthase 6	Mitochondrial complex V deficiency
ATP8	ATP synthase 8	Mitochondrial complex V deficiency
C2	Complement component 2	C2 defieciency
CACNA1C	calcium channel, voltage-dependent, L type, alpha 1C subunit	Brugada syndrome, Timothy syndrome
CACNA1D	calcium channel, voltage-dependent, L type, alpha 1D subunit	Sinoatrial node dysfunction
CACNB3	calcium channel, voltage-dependent, beta 3 subunit	
CALM1	Calmodulin 1	Long QT 4, polymorphic VT
CALM2	Calmodulin 2	Long QT 15
CALM3	Calmodulin 3	
CAV1	Caveolin 1	
CAV3	Caveolin 3	Long QT 9
CDH15	Cadherin 15	
CDKN2B	Cyclin-Dependent Kinase Inhibitor 2b	
EDA	Ectodysplasin A	
ELN	Elastin	Supravalvar aortic stenosis
FBN1	Fibrillin 1	Marfan Syndrome
FBXW4	F-Box And Wd40 Domain Protein 4	
GAS	Gastrin	
GJA8	Gap Junction Protein, Alpha-8	
GJB3	Gap Junction Protein, Beta-3	Deafness
GJB6	Gap Junction Protein, Beta-6	Deafness
GPR133	G Protein-Coupled Receptor 133	
HAND2	Heart- And Neural Crest Derivatives-Expressed 2	
HCN4	Hyperpolarization-Activated Cyclic Nucleotide-Gated Potassium Channel 4	Brugada syndrome
HFE	HFE gene	Haemochromatosis

Table B.3: List of genes associated with Long QT (cont.)

Gene	Gene Description	Disease
HLA-DRB3	Major Histocompatibility Complex, Class Ii, Dr Beta-3	
HMI	Hypomelanosis of Ito	
HMOX1	Heme Oxygenase 1	Heme oxygenase-1 deficiency
HRAS	V-Ha-Ras Harvey Rat Sarcoma Viral Oncogene Homolog	Congenital myopathy
IGLV2-18	Immunoglobulin lambda variable 2-18	
IGLV2-8	immunoglobulin lambda variable 2-8	
IKZF1	Ikaros Family Zinc Finger 1	
IL10	Interleukin 10	
INS	Insulin	
KCNA4	potassium channel, voltage gated shaker related subfamily A, member 4	
KCNA5	potassium channel, voltage gated shaker related subfamily A, member 5	Familial Atrial fibrillation
KCNB1	potassium channel, voltage gated Shab related subfamily B, member 1	
KCND2	potassium channel, voltage gated Shal related subfamily D, member 2	
KCND3	potassium channel, voltage gated Shal related subfamily D, member 3	Spinocerebellar ataxia 19
KCNE1	potassium channel, voltage gated subfamily E regulatory beta subunit 1	Long QT 5
KCNE1L	Potassium Channel, Voltage-Gated, Isk-Related Family, Member 1-Like	
KCNE2	potassium channel, voltage gated subfamily E regulatory beta subunit 2	Long QT 6
KCNE3	potassium channel, voltage gated subfamily E regulatory beta subunit 3	Brugada syndrome
KCNH2	potassium channel, voltage gated eag related subfamily H, member 2	Long QT 2
KCNJ11	potassium channel, two pore domain subfamily J, member 11	
KCNJ2	potassium channel, two pore domain subfamily J, member 2	Andersen Tawil Syndrome
KCNK3	potassium channel, two pore domain subfamily K, member 3	
KCNN4	potassium channel, calcium activated intermediate/small conductance subfamily N alpha, member 4	
KCNQ1	potassium channel, voltage gated KQT-like subfamily Q, member 1	Long QT1
KCNQ2	potassium channel, voltage gated KQT-like subfamily Q, member 2	Epileptic encephalopathy, seizures

Table B.3: List of genes associated with Long QT (cont.)

Gene	Gene Description	Disease
KCNQ4	potassium channel, voltage gated KQT-like subfamily Q, member 4	Deafness
LDLR	low density lipoprotein receptor	
LQT1	Long QT Syndrome 1	Long QT1
MAPK3	mitogen-activated protein kinase 3	
MEA1	Male-enhanced antigen 1	
MINK1	Misshapen/Nik-Related Kinase 1	
MMP9	Matrix Metalloproteinase 9	Metaphyseal anadysplasia 2
MRPL28	Mitochondrial Ribosomal Protein L28	
MTND1	Complex 1, Subunit ND1	MELAS, Leber optic atrophy
MTND2	Complex 1, Subunit ND2	Mitochondrial Complex 1 Deficiency, Leber optic atrophy
MTND4	Complex 1, Subunit ND4	Leber optic atrophy
MTND5	Complex 1, Subunit ND5	MELAS, Leber optic atrophy, Mitochondrial Complex 1 Deficiency
MTND6	Complex 1, Subunit ND6	Leber optic atrophy
MUC2	Mucin 2	
MYBPC3	Cardiac myosin-binding protein C	Hypertrophic cardiomyopathy, Dilated cardiomyopathy
MYH7	Myosin, Heavy Chain 7	Hypertrophic cardiomyopathy, Hypertrophic cardiomyopathy, Myopathy
NOS1AP	Nitric Oxide Synthase 1 (Neuronal) Adaptor Protein	
NXT1	NTF2-Like Export Factor 1	
OR6K3	Olfactory Receptor, Family 6, Subfamily K, Member 3	
PELI1	Drosophila homolog of Pellino 1	
PKP2	Plakophilin 2	Arrhythmogenic right ventricular dysplasia 9
POMC	Proopiomelanocortin	
PTEN	Phosphatase And Tensin Homolog	
PTRF	RNA Polymerase I And Transcript Release Factor	Lipodystrophy
RYR2	Ryanodine Receptor 2	Arrhythmogenic right ventricular dysplasia 2
SCD	Stearoyl-CoA desaturase	
SCN4B	Sodium Channel, Voltage-Gated, Type Iv, Beta Subunit	Long QT10, Atrial fibrillation
SCN5A	Sodium Channel, Voltage-Gated, Type V, Alpha Subunit	Long QT3, Atrial Fibrillation, Brugada syndrome 1, Cardiomyopathy
SLC18A2	Solute Carrier Family 18 (Vesicular Monoamine), Member 2	
SLC22A5	Solute Carrier Family 22 (Organic Cation Transporter), Member 5	Carnitine deficiency

Table B.3: List of genes associated with Long QT (cont.)

Gene	Gene Description	Disease
SLN	Sarcolipin	
SNTA1	Alpha 1 syntrophin	Long QT12
SPESP1	Sperm Equatorial Segment Protein 1	
SUB1	S. Cerevisiae, Homolog Of SUB1	
TF	Transferrin	Atransferrinemia
TGFB3	Transforming Growth Factor, Beta-3	Arrhythmogenic right ventricular dysplasia 1
TIMP2	Tissue Inhibitor Of Metalloproteinase 2	
TLX1NB	TLX1 Divergent Gene	

Appendix C

PREGNANCY AND ANAESTHETICS QUESTIONNAIRE

MRC Centre for Neuromuscular Diseases

University College London Hospitals NHS
NHS Foundation Trust

Anaesthetics and Pregnancy in Channelopathy Patients

There is very little information available about the effect of anaesthetics and pregnancy in muscle channel patients. We are therefore trying to gather data on all our patients so that we can give better advice and support in the future. We of course need your help to do this.

Please fill out all the applicable sections of this form as fully as possible.

Name: _____

Gender: **Male / Female**

Date of Birth: _____

Your diagnosis: (if known) _____

Have you had a positive genetic test? **Yes / No**

At what age did you first get symptoms? _____

1. Anaesthetics

a) Have you ever had a local anaesthetic? **Yes / No**
(e.g dental procedures/ stitches)

If yes: What did you have the local anaesthetic for? (please give full details)

_____
_____
_____

Did you have any problems with it? **Yes / No**

If yes: What problems did you have?

It was ineffective ☐
Its effect was prolonged ☐
Other: _____

b) **Have you ever had a spinal anaesthetic or epidural?** **Yes / No**

If yes: What did you have it for? (please give full details)

_____
_____
_____

Did you have any problems with it? **Yes / No**

If yes: What problems did you have?

It was ineffective. ☐
Its effect was prolonged ☐
Other: _____

c) Have you ever had a general anaesthetic? **Yes / No**

If yes: What procedure/s did you have it for?
(please give details of all surgeries and the treating hospital)

_____
_____
_____

Did you have any problems with the anaesthetic? **Yes / No**

If yes, what problems did you have? (please give full details)

_____
_____
_____

2. Pregnancy

Male & Female patients please complete this section

a) Have you ever tried to conceive? **Yes / No**

If yes: Have you ever had problems trying to conceive? Yes / No

How long did it take you to conceive?_____

b) How many children have you had? _____

Female patients ONLY need to complete this section

d) How many pregnancies have you had?_____

c) Have you ever had a termination of pregnancy? **Yes / No**

If yes: How many terminations have you had?

_____

d) Have you ever had a miscarriage? **Yes / No**

If yes: How many miscarriages have you had?_____

At what stage of the pregnancy was your miscarriage/s?

_____
_____
_____

What was the cause of your miscarriage/s?

_____
_____
_____

e) Did you have any problems during your pregnancies? Yes / No

If yes: What problems did you have:

- Pre-eclampsia ☐
- Hypertension ☐
- Gestational diabetes ☐
- Placenta previa ☐
- UTI ☐
- Other: _____

Please give details of any problems you had in pregnancy:

_____
_____
_____
_____
_____
_____

f) Did you have any problems during delivery? Yes / No

If yes: What problems did you have?

- Emergency C-section ☐
- Forceps delivery ☐
- Ventouse delivery ☐
- Placenta previa ☐
- Post-partum haemorrhage ☐
- Breech baby ☐
- Other: _____

Please give details of any problems you had during delivery:

_____
_____
_____
_____
_____
_____

g) At how many weeks were each of you children born and were there any post-natal problems?

_____
_____
_____
_____
_____
_____

Your channelopathy during pregnancy

h) Did the pregnancy make your disease: **Better/ Worse / No change**

If your disease changed during pregnancy which symptoms changed?

_____

If your disease changed did the symptoms settle after birth? Yes / No

If yes how long did it take to settle?

_____

i) Does Menstruation make your disease: **Better/ Worse / No change**

Any other comments or details you would like to add?

_____
_____
_____
_____
_____
_____
_____
_____
_____

Thank you for completing this form.

Please return the form by post, email or fax to the address below.

If you have any questions or comments about this form please feel free to contact me or another member of our team:

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