The non dystrophic myotonias: molecular pathogenesis, diagnosis and treatment

Running Title: The non dystrophic myotonias

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Abstract

The non-dystrophic myotonias are an important group of skeletal muscle channelopathies electrophysiologically characterised by altered membrane excitability. Many distinct clinical phenotypes are now recognized and range in severity from severe neonatal myotonia with respiratory compromise through to milder late-onset myotonic muscle stiffness. Specific genetic mutations in the major skeletal muscle voltage gated chloride channel gene (CLCN-1) and in the voltage gated sodium channel gene (SCN4A) are causative in most patients. Recent work has allowed more precise correlations between the genotype and the electrophysiological and clinical phenotype. The majority of patients with myotonia have either a primary or secondary loss of membrane chloride conductance predicted to result in reduction of the resting membrane potential. Causative mutations in the sodium channel gene result in an abnormal gain of sodium channel function that may show marked temperature dependence.

Despite significant advances in the clinical, genetic and molecular pathophysiological understanding of these disorders, which we review here, there are important unresolved issues we address; 1. Recent work suggests that specialized clinical neurophysiology can identify channel specific patterns and aid genetic diagnosis in many cases however, it is not yet clear if such techniques can be refined to predict the causative gene in all cases or even precise genotype. 2. Although clinical experience indicate these patients can have significant progressive morbidity, the detailed natural history and determinants of morbidity have not been specifically studied in a prospective fashion. 3. Some patients develop myopathy, but its frequency, severity and possible response to treatment remains undetermined. Furthermore, the pathophysiological link between ion channel dysfunction
and muscle degeneration is unknown. There is currently insufficient clinical trial evidence to recommend a standard treatment. Limited data suggests that sodium channel blocking agents have some efficacy. However, establishing the effectiveness of a therapy requires completion of multi-centre randomised controlled trials employing accurate outcome measures including reliable quantitation of myotonia. More specific pharmacological approaches are required and could include those which might preferentially reduce persistent muscle sodium currents or enhance the conductance of mutant chloride channels. Alternative strategies may be directed at preventing premature mutant channel degradation or correcting the mis-targeting of the mutant channels.

**Keywords:** ion channels, neuromuscular, genetics, EMG,
The non dystrophic myotonia (NDMs) are skeletal muscle ion channel disorders traditionally considered to be distinct from myotonic dystrophy because of the absence of progressive weaknesses and systemic features. The NDMs are now known to be caused by dysfunction of key skeletal muscle ion channels and include myotonia congenita, paramyotonia congenita and the sodium channel myotonias. The worldwide prevalence of non-dystrophic myotonia has been estimated to be approximately 1 in 100,000 (Emery, 1991). However, the prevalence seems to vary considerably between geographical regions. For example, myotonia congenita alone was estimated to have a prevalence of between 7-10 in 100,000 in Scandinavia (Baumann et al., 1998; Sun et al., 2001).

The major clinical manifestation of the NDMs is muscle stiffness as a consequence of the myotonia. Additional common symptoms include pain, weakness, and fatigue (Walsh et al., 2007; Trivedi et al., 2008; Wang et al., 2008c). Patients with NDM do not exhibit extra-muscular systemic clinical features which may be observed in myotonic dystrophy. Myotonia can be demonstrated on examination as delayed muscle relaxation following muscle contraction or following mechanical stimulation such as percussion. The underlying muscle membrane hyper-excitability manifests neurophysiologically as repetitive muscle fibre after-discharges on electromyography (EMG). Recent studies have revealed a wide range of clinical phenotypes which may present diagnostic difficulty. Importantly, it has also been observed that patients with DM2 may present with a clinical phenotype that is difficult to distinguish from myotonia congenita (Fialho et al., 2007). It
is now clear that the clinical severity of these disorders can range from a neonatal life
threatening presentation through to mild late-onset symptoms. The application of
specialized electrophysiological protocols can reveal gene-specific patterns which can be
used to direct DNA-based diagnosis (Fournier et al., 2004).

Myotonia congenita (MC) is caused by mutations in the skeletal muscle chloride channel
gene CLCN-1 and inherited in a dominant or in a recessive fashion. Paramyotonia
congenita (PMC) and the sodium channel myotonias (SCMs) are allelic, autosomal
dominant disorders caused by point mutations in the skeletal muscle sodium channel gene
SCN4A. Hyperkalaemic periodic paralysis is also caused by mutations in SCN4A and
may be accompanied by myotonia in some cases, although episodic paralysis is usually
the dominant feature [Reviewed (Venance et al., 2006)] .

**Clinical Features**

Myotonia Congenita

MC is the most common inherited skeletal muscle channelopathy. The autosomal
dominant form was first described in the 19th century by the Danish physician Julius
Thomsen in himself and his family (Thomsen J., 1876). In the 1970’s the German
physician P.E.Becker fully documented the existence of the recessive form of MC
(Becker PE., 1977). In both forms muscle stiffness is most pronounced during rapid
voluntary movements following a period of rest but improves with repeated activity - the
so called “warm-up” phenomenon (Walsh et al., 2007; Trivedi et al., 2008; Wang et al.,
2008c). Some clinical findings are more common in the recessive than in the dominant
form but considerable overlap exists. Recessive MC tends to be more severe, is more
frequently associated with muscle hypertrophy and with depressed tendon reflexes (Becker PE., 1977; Fialho et al., 2007). Patients with recessive MC typically experience a peculiar transient weakness on initiating an action, which is only rarely seen in dominant MC. Although Becker found that most patients with recessive MC presented between the ages 4-12 years while the dominant form usually manifested before the age of 3 years (Becker PE., 1977), we found no difference in the age of onset (Fialho et al., 2007).

Myotonic dystrophy types I and II (DM1 and DM2) can often be differentiated from MC by the presence of systemic features. However, cases of DM2 in whom myotonia is the predominant complaint without any overt systemic features have been described (Fialho et al., 2007) and can lead to diagnostic difficulty.

Paramyotonia Congenita

Eulenburg first used the term paramyotonia congenita in 1886 to describe a syndrome of episodic muscle cramps and paralysis profoundly exacerbated by cold and exercise in six generations of a German family (Eulenburg., 1886). The inheritance is autosomal dominant and symptoms usually manifest in the first decade of life. The facial, tongue, and hand muscles are predominantly affected and the lower limbs are generally only mildly affected (Miller et al., 2004). The myotonia can last seconds to minutes but the weakness may persist for hours and occasionally days. Paradoxical myotonia that worsens with exercise can be demonstrated at the bedside in most patients (Trivedi et al., 2008). Muscle hypertrophy is less frequent than in myotonia congenita but in our recent series we found it to be present in approximately 30% of patients (Matthews et al., 2008b).
Sodium Channel Myotonia

In 1987, prior to the availability of genetic testing, it was observed that there was a group of myotonic patients who seemed clinically distinct from either MC or PMC. The first kindred reported exhibited autosomal dominant inheritance of a phenotype characterized by cold insensitive painful myotonia that was markedly exacerbated by potassium ingestion. None of the affected family members reported attacks of weakness but all experienced a significant improvement in myotonic symptoms with acetazolamide treatment. The term “acetazolamide-responsive” myotonia congenita was coined to describe this family (Trudell et al., 1987; Ptacek et al., 1994). Subsequent reports described patients with a cold insensitive pure myotonic phenotype who did not experience weakness but whose myotonia fluctuated dramatically and was profoundly worsened by potassium ingestion. Notably the myotonia tended to occur with a more delayed (10-30 min) onset after exercise rather than with the initiation of movement after rest as seen in MC, or within seconds of exercise as seen in PMC. This phenotype was classified as myotonia fluctuans (Ricker et al., 1990; Lennox et al., 1992; Ricker et al., 1994). The term myotonia permanens was introduced to describe patients with a third clinical variant characterized by very severe persistent myotonia which significantly impaired respiration (Lerche et al., 1993; McClatchey et al., 1992b). These three purely myotonic disorders shared the potassium aggravation and the absence of sensitivity to cold. Together they have become known as the Potassium Aggravated Myotonias (PAM). Additional pure myotonic phenotypes have been described but these differ from the PAM phenotypes in that they have been reported to be cold-sensitive (Heine et al., 1993;
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Wu et al., 2001; Koch et al., 1995). All these pure myotonic phenotypes have now been shown to be caused by allelic point mutations in the gene encoding the voltage gated muscle sodium channel - SCN4A.

We consider that from a practical clinical viewpoint a simplified classification of sodium channel myotonic disorders into two broad groups based on the presence or absence of episodic weakness is helpful:

Group 1 Paramyotonia congenita- characterized by a marked worsening of myotonia by cold and by the presence of clear episodes of weakness;

Group 2 Sodium channel myotonia -notable for the absence of episodic weakness but may have cold sensitivity. This includes all the pure myotonic phenotypes, including the potassium aggravated myotonias (Fournier et al., 2004; Fournier et al., 2006).

Distinguishing chloride from sodium channel myotonias is often possible on clinical grounds alone as indicated in table 1. However, difficulty may arise as some cases with sodium channel myotonia may have clinical features that are very similar to those seen in some cases of dominant MC (see Table 1). For example, SCM may exhibit the presence of the warm up phenomenon, have minimal or absent sensitivity to cold, and have an upper limb/facial distribution of myotonia that is indistinguishable from dominant MC (see Table 1).
In such cases the presence of transient weakness would point to dominant MC whereas the presence of eyelid myotonia is more suggestive of sodium channel myotonia (Trip et al., 2009b). In addition specialized electrophysiological protocols can be helpful although have their limitations.

Myopathy

Myopathy may develop in some patients with NDM (Plassart et al., 1996; Nagamitsu et al., 2000; Becker PE., 1977). In a series of 49 genetically confirmed PMC cases “myopathic biopsy findings” were reported in 33% of those biopsied although full clinical details of the degree of weakness were not available (Miller et al., 2004). Permanent severe myopathy seems to be more common in patients with periodic paralysis than in the NDMs (Miller et al., 2004). In periodic paralysis it has been postulated that the severity of myopathy may not relate to paralytic attack frequency (Buruma et al., 1978; Links et al., 1990) but the exact relationship remains unclear. There is some evidence that the severity of myopathy associated with periodic paralysis does correlate with increasing age (Links et al., 1990; Plassart et al., 1994). It is not known if a similar relationship between age and severity of myopathy exists in the NDMs or if symptom frequency or severity has a direct influence on the development of myopathy. In periodic paralysis there is some evidence that the frequency of paralytic attacks may decline with age (Miller et al., 2004). However, it is not established if myotonia severity alters over time in NDM patients. Importantly, there are no published studies to provide accurate detailed data on the natural history of the NDMs in order to address the above
questions. Such a large natural history study is currently in progress as part of the CINCH consortium-[http://rarediseasesnetwork.epi.usf.edu].

Mechanisms of Muscle Degeneration

Patients with periodic paralysis have been frequently reported to exhibit vacuoles and/or tubular aggregates on muscle biopsy. However, the myopathological findings in NDMs are not defined well and often reported to be non-specific (Miller et al., 2004). Furthermore, with the characteristic clinical history and examination findings coupled with the recent advances in electrophysiological techniques a diagnosis of NDM is usually apparent and it is now rare that a muscle biopsy will be performed in such patients other than as a research procedure.

It is clear muscle damage can occur in the NDMs but its pathomechanism and frequency are unknown. It has been postulated that the abnormally prolonged intramuscular influx of sodium that is known to occur via the mutant sodium channels may be responsible for muscle degeneration (Bradley et al., 1990) but the specifics of such a mechanism have not been shown. However, it is of note that there is evidence for increased intracellular sodium contributing to cell necrosis in the mouse model of duchenne muscular dystrophy (Hirn et al., 2008). One recent study has employed ultrasound to assess permanent muscle changes in the NDMs. Using ultrasound measurements of eight muscles, (four upper limb and four lower limb) in a group of 63 genetically confirmed NDM patients an increase in the mean echo intensity compared with controls from all muscles examined except the rectus femoris was observed. The ultrasound changes were considered to indicate structural muscle damage such as fatty infiltration or fibrosis. This change was
most marked in the forearm flexors where the increased echogenicity correlated negatively with muscle power. There was no positive correlation between echo intensity and age for individual muscles except the rectus femoris although the sum of the scores did show a significant positive correlation (Trip et al., 2009c).

Recently, a mouse model of hyperkalaemic periodic paralysis has been engineered by introducing the murine equivalent of the SCN4A mis-sense mutation M1592V (Hayward et al., 2008). This mutation causes both myotonia and paralysis in humans (Rojas et al., 1991; Kelly et al., 1997) and was demonstrated to produce these same symptoms in the mouse verifying its use as a reasonable model. It is of interest to note that at a few months of age the mice heterozygous for this mutation already displayed subtle myopathic changes. In those that were homozygous significant muscle abnormalities were seen including an increase in fibre size variability, frequent internal nuclei and large scattered vacuoles. These early changes were present at a few months of age before any spontaneous episodes of paralysis had been observed. Furthermore, they were shown to increase with age in the heterozygotes while muscle force generation declined (Hayward et al., 2008). This seems to support the clinical observations that myopathy increases with age (Links et al., 1990; Plassart et al., 1994) and may be independent of paralytic attacks in the periodic paralyses (Buruma et al., 1978; Links et al., 1990).

Although this animal model is of a hyperkalaemic periodic paralysis genotype the same symptoms of myotonia and muscle weakness occur in the allelic disorders PMC and SCM. The pathomechanism is also a gain of function of the sodium channel in both. It is likely that future insights into muscle degeneration gained from the study of this model will also have implications for our understanding of PMC and SCM. The possibility that
myopathy develops independently of symptom frequency or severity may influence future approaches to therapy which is currently aimed at relieving symptoms. As such, many patients with minimal or manageable symptoms decline pharmacological treatment.

Morbidity in the NDMs

Very little is known about the impact of NDMs on quality of life and these disorders have often been regarded as benign. A single study has recently examined this in a group of 62 genetically confirmed NDM patients and found painful myotonia and fatigue to be the best predictors of poor general health perception and physical functioning (Trip et al., 2009a). In this study painful myotonia was reported in 28% of those with myotonia congenita and 57% with sodium channelopathy. In addition, there are numerous case reports where pain which is often severe, is described in the NDMs (Vicart et al., 2004; Rosenfeld et al., 1997; Ptacek et al., 1994; Colding-Jørgensen et al., 2006; Fialho et al., 2007; Walsh et al., 2007; Wang et al., 2008c). This suggests that pain is a frequent symptom that may have been previously under-recognised and possibly undertreated in the NDMs.

Clinical Electrophysiology

Recently, specialized clinical neurophysiology protocols have aided precise diagnosis in muscle channelopathies by directing genetic testing based on channel-specific electrophysiological patterns. Sarcolemmal excitability can be measured indirectly as the variability of the compound muscle action potential (CMAP) following different stimuli. The CMAP size varies in skeletal muscle channelopathies in response to short
(10-20 seconds) or long (3-5 minutes) exercise tests (Streib EW., 1982; McManis et al., 1986). Using these exercise protocols in combination with muscle cooling distinct electrophysiological patterns, termed patterns I, II and III are now recognized for each of the NDM groups (Fournier et al., 2004; Fournier et al., 2006). For clinical diagnosis the repeat short exercise test with muscle cooling is of great value in the NDMs.

Patients with chloride channel myotonia can show one of two patterns. The most common is pattern II (Fournier et al., 2004) in which at room temperature there is an immediate CMAP decrement after exercise which recovers quickly and diminishes with repetition, reflecting the transient weakness observed clinically (Fig 1). This pattern is most frequently seen in recessive MC but can be observed in any muscle ion channel disorder in which there is a loss of sarcolemmal chloride conductance. It is therefore also seen in dominant MC and in both DM1 and DM2. That this pattern may be seen in DM1 and DM2 is not unexpected as there is now clear evidence that the myotonia in DM1 and DM2 is secondary to reduced chloride conductance (Charlet et al., 2002). In recessive MC cooling has little further effect (Fig 1). However, in dominant MC the CMAP decrement may be worsened or only seen with cooling (Fournier et al., 2006) making it essential to perform the short exercise test at both room temperature and with the muscle cooled (Fig 2). Occasional patients with dominant MC show a normal response (pattern III (Fournier et al., 2006)) to all provocative tests, even with muscle cooling which is indistinguishable electrophysiologically from sodium channel myotonia (Fig 3).

Patients with paramyotonia congenita typically have a gradual and prolonged decrement in CMAP after exercise, termed pattern I (Fournier et al., 2004). This decrement is exacerbated with repeat testing and muscle cooling (Fig 4) reflecting the clinically
observed cold- and exercise-induced weakness. Some genotypes only display this typical pattern when the short exercise test is performed with the muscle cooled (Fournier et al., 2006) again emphasizing the importance in diagnosis of performing a SET at both room temperature and with the muscle cooled.

The sodium channel myotonias are separated clinically from PMC by their lack of weakness. This is illustrated by pattern III, normal responses to all provocative tests (Fig 3) and EMG myotonia is usually the only positive electrophysiological finding. This is the characteristic finding in SCM but is not absolute and there are some variations for certain genotypes (Fournier et al., 2006). It is notable that this is the same pattern observed in a significant minority of cases with dominant MC.

The similarities between SCM and dominant MC can lead to difficulty in prioritizing genetic testing. Clinical history and examination considered in conjunction with EMG findings (see Table 1) can improve the ability to distinguish between the two and guide genetic analysis but in some cases screening of both the CLCN-1 and SCN4A genes will be required.

Variability exists in the response of the NDM subgroups to exercise testing and where muscle cooling has already proven useful in improving diagnosis, repetitive nerve stimulation may have a future role to play in distinguishing the sub-types of NDM. There is some evidence that a reduction in CMAP may be provoked by repetitive nerve stimulation in certain cases of recessive MC where exercise testing even with the muscle cooled has failed to produce any such decrement (Michel et al., 2007). In this way repetitive nerve stimulation may become an additional future tool to guide the genetic analysis towards recessive MC in cases that may otherwise be thought to be dominant.
MC or SCM. There is currently no distinguishing electrophysiological test for DM2 and this diagnosis should be considered for patients with a myotonic disorder in whom no mutations are found in CLCN1 or SCN4A.

**Genetics**

Skeletal Muscle Chloride Channel

Recessive and dominant myotonia congenita are caused by mutations in the voltage gated chloride channel gene on chromosome 7q35. (Koch et al., 1992) (George, Jr. et al., 1993). The functional chloride channel exists as a dimeric structure with two gating pores. To date over 100 missense, nonsense, insertions, deletions and splice site mutations have been identified throughout CLCN1. Many patients carry ‘private’ mutations. It is a particular feature of MC that several mutations have been reported to be inherited in both an autosomal dominant and autosomal recessive manner in different families (Papponen et al., 1999; Sun et al., 2001; George, Jr. et al., 1994; Meyer-Kleine et al., 1995; Zhang et al., 1996). The advent of molecular genetic testing has demonstrated that familial non-dystrophic myotonia with dominant inheritance is often caused by missense mutations in SCN4A. Those CLCN-1 mutations that do cause dominant MC seem to cluster in exon 8 of the gene (Fialho et al., 2007)

More recently it has been observed that there is a higher frequency of recessive CLCN1 mutations in DM2 patients from Finnish and German populations. In the small number of individuals identified the co-segregation of the CCTG expansion in the first intron of ZNF9 and a CLCN1 mutation seemed to produce a greater distribution and severity of myotonia than is commonly encountered in DM2 although the number of cases was too
small for this finding to be statistically significant (Suominen et al., 2008). It is unclear if the presence of a CLCN1 mutation universally affects the severity of a DM2 phenotype but these findings do suggest these DM2 patients may have more striking myotonia which ultimately increases the likelihood of a diagnosis of DM2 being considered and genetically confirmed.

Skeletal Muscle Sodium Channel

Hyperkalaemic periodic paralysis was the first of the sodium channel disorders to be linked to the SCN4A gene on chromosome 17 which encodes the skeletal muscle voltage gated sodium channel Nav1.4 (Fontaine et al., 1990; Ptacek et al., 1991c; Koch et al., 1991b). Given some of the shared clinical features of HyperPP and PMC along with the recognition of abnormal sodium conductance in both (Lehmann-Horn et al., 1981; Lehmann-Horn et al., 1987b; Lehmann-Horn et al., 1987a) it was proposed and subsequently confirmed that PMC and HyperPP were allelic disorders (Ptacek et al., 1991b; Koch et al., 1991a; Ptacek et al., 1991a; Rojas et al., 1991; McClatchey et al., 1992a; McClatchey et al., 1992b). Later the phenotypes grouped together as the potassium aggravated myotonias were also shown to be sodium channel disorders (Lerche et al., 1993; Ricker et al., 1994; Ptacek et al., 1994)

All the skeletal muscle sodium channelopathies are autosomal dominant conditions and de novo mutations can occur. In a proportion of patients with a phenotype typical for PMC no mutation has been identified in SCN4A raising the possibility of further genetic heterogeneity (Miller et al., 2004). Virtually all described mutations are missense with the only exception being a three base pair deletion (Michel et al., 2007).
Over forty different mutations including those responsible for periodic paralysis have been reported in the SCN4A gene. Exons 22 and 24 are recognized as “hot spots” for PMC, particularly the T1313M mutation and amino acid substitutions at the R1448 position (Matthews et al., 2008b). The most common SCM mutations are V1589M and those at the G1306 position (Vicart et al., 2005).

Although there are many clinical and electrophysiological indicators to help prioritize genetic testing in the NDMs the difficulties described in distinguishing SCM from dominant MC indicate that a proportion of patients will require screening of both the CLCN1 and SCN4A genes (Trip et al., 2008). In some cases of recessive MC, only one CLCN-1 mutant allele has been identified despite analysis of all coding exons (Trip et al., 2008). This indicates that mutations may be present in deeper intronic regions or possibly in promoter regions although none have been reported. Another possibility is that there may be as yet unidentified large scale deletions in CLCN-1. Although it is recognized that some cases diagnosed clinically as non-dystrophic myotonia will have DM2 it is not known how frequent this is. Considering the importance of appropriate cardiac evaluation in DM2 we suggest that if CLCN1 and/or SCN4A screening is negative, ZNF9 analysis should be undertaken.

**Genotype-Phenotype Correlations**

Marked phenotypic heterogeneity is common in the skeletal muscle channelopathies even within individual kindreds. Clinical and electrophysiological findings can help to
distinguish between dominant and recessive MC but since the same mutation can be inherited in a dominant or recessive manner this doesn’t necessarily narrow the possible genotypes. From a genetic point of view, nonsense mutations, small deletions and insertions leading to frameshift or mutations interrupting splice sites are usually associated with recessive MC. However, missense mutations can lead to either recessive or dominant MC depending on their location and the effect of the amino acid substitution on channel gating. Dominant mutations are clustered around the dimer interface of the channel (Duffield *et al.*, 2003; Fialho *et al.*, 2007) but are also found in other regions of the channel. R894X is the most studied mutation causing recessive and dominant MC in different families. It is a nonsense mutation within the C-terminus of the channel. Due to its location within the last exon of the CLCN1 gene the m-RNA does not undergo the usual nonsense mediated decay typically induced by earlier premature stop-codons. Duno *et al.* compared two families with dominant MC and two families with recessive MC carrying the R894X mutation. There was no direct relation between levels of CLC-1 mRNA and inheritance type excluding differential allelic expression as an explanation for the varying inheritance mode. However, the most severely affected dominant case expressed more than twice the amount of mutant mRNA compared to the recessive families raising the possibility that this may contribute to phenotypic variability particularly within dominant pedigrees (Duno *et al.*, 2004).

PMC and SCM can usually be reliably distinguished from each other by the presence or absence of weakness from clinical and EMG findings. This narrows down the likely genotype to a certain degree but within each group there are still a number of
The non dystrophic myotonias possibilities. **Table 2 supplemental data** outlines the phenotypes reported for each mutation.

There is now evidence that certain SCN4A genotypes may be associated with a more severe form of neonatal phenotype in some cases. A fatal case of myotonia with significant respiratory muscle involvement was described in an infant with the de novo N1297K mutation (Gay *et al.*, 2008). We observed the I693T mutation linked to spontaneously resolving neonatal hypotonia with variable feeding and respiratory difficulties in four unrelated families (Matthews *et al.*, 2008a). The recognition that sodium channelopathies present in such a way is crucial in order to provide both appropriate pre-natal advice for mothers known to carry these mutations and neonatal care for their children.

One of the difficulties in accurately defining genotype-phenotype correlations is the wide phenotypic variability that may occur in the sodium channelopathies. An example of this is the G1306E mutation. The original phenotype reported with this was so severe the individual suffered permanent myotonia that included the respiratory muscles and which lead to hypoxia and acidosis requiring ventilatory support (Lerche *et al.*, 1993). In contrast, a more recent report observed that although affected individuals had relatively severe myotonia they did not exhibit respiratory involvement and were able to carry out daily activities including work without treatment (Colding-Jorgensen *et al.*, 2006). See **Table 2 supplemental data** for more detail of phenotypes reported in different kindreds with the same SCN4A mutation.

Another limitation in identifying genotype-phenotype correlations has been the availability of sufficient numbers of individuals carrying each mutation. The large
multicentre natural history trial currently being run by the CINCH group aims to address these issues.

**Molecular Pathophysiology**

In a normal muscle fibre a single nerve stimulus depolarizes the sarcolemma propagating a single action potential that results in a single muscle contraction followed by rapid relaxation. Myotonia results from an increased excitability of the muscle fibre membrane such that a single electrical stimulus triggers a repetitive train of action potentials. In myotonia congenita the enhanced excitability is due to reduced sarcolemmal chloride conductance as initially demonstrated in muscles of myotonic goats (Lipicky et al., 1966; Bryant., 1969) and later in humans (Lipicky et al., 1971). Compared with other excitable cells, skeletal muscle has an unusually high chloride conductance, accounting for up to 85% of the resting membrane conductance (Bryant et al., 1971). The high chloride conductance is especially important in view of the large size of the muscle fibers which require the T-tubule system to propagate an action potential into the depth of the cell to initiate a simultaneous contraction. Although T-tubules are directly connected to the extracellular space, they represent a significant diffusion barrier. Consequently with repeated membrane discharges a build-up of potassium ions within the t-tubule system due to the repolarising K+-currents increases the probability of additional spikes. The membrane depolarization as a consequence of K+ accumulation in the T-tubules is normally counteracted by the chloride conductance.
The chloride channel is an antiparallel assembled homodimer consisting of two identical subunits each with their own ion conducting pore (Fig 5A). There are two main gating modes referred to as the fast gate, which can open and close the two pores independently, and a slow gating mechanism or ‘common gate’ which causes deactivation of both pores simultaneously. While all chloride channel mutations lead to loss of function, recessive mutations usually exert their effect by loss of function of the mutated subunit, while the mutant subunit in dominant disease tends to have an adverse effect on the function of the co-expressed wild–type (WT) subunit, i.e. a dominant negative effect (Pusch et al., 1995). The majority of dominant MC mutations shift the voltage dependence of CLC-1 to more positive voltages (Pusch et al., 1995; Kubisch et al., 1998). Using a mathematical model (Barchi., 1975) showed that decreasing the chloride conductance to 20% is sufficient to trigger myotonic discharges following a single stimulus. A similar hyperexcitability threshold of 25% was predicted by graded pharmacological inhibition of muscle CLC-1 conductance (Kwiecinski et al., 1988). Clinically, a reduction to 50% does not seem to cause myotonia as evidenced by the majority of asymptomatic carriers of recessive MC mutations. However, this assumes a 1:1 allelic expression in these cases, which may not always be the case (Chen et al., 1997). Table 3 supplemental data outlines details of known functional effects of all reported CLCN1 mutations.

In contrast to the chloride channel, the voltage gated skeletal muscle sodium channel comprises a single ion conducting pore formed by the interaction between four homologous domains (Fig 5B). All of the mutations associated with PMC and sodium channel myotonia produce “gain of function” defects either by impaired inactivation or
enhanced activation of the Nav1.4 channel (see Table 2 supplemental data). Impaired inactivation can either involve delayed inactivation or incomplete inactivation. Delayed inactivation of the skeletal muscle sodium channel causes increased excitability of the muscle fibre membrane and myotonia (Yang et al., 1994). The increased availability of sodium channels immediately after an action potential, renders the fibre susceptible to sustained trains of repetitive discharges (Cannon., 2000), the electrophysiological hallmark of myotonia. In contrast to the repetitive firing seen in myotonia, the paralytic attacks experienced in PMC and in the allelic disorder hyperkalaemic periodic paralysis, are caused by episodic loss of fibre excitability. This sustained depolarization of the resting potential, is due to sodium channels that do not inactivate completely, thereby conducting a persistent inward sodium current that depolarizes the fibre. (Cannon., 2006; Bendahhou et al., 2002).

Sodium channels also undergo a second, mechanistically distinct form of inactivation on a much slower time scale of seconds termed slow inactivation. Defects of slow inactivation increase the propensity for depolarization-induced attacks of weakness, and missense mutations of SCN4A that disrupt slow inactivation always result in a paralytic phenotype (Hayward et al., 1999).

Animal Models

There are several animal models of myotonia congenita. The myotonic goat is thought to have been bred by Dr H H Mayberry in Tenessee in the 1800s. The myotonic goat has been shown to harbour an alanine to proline substitution in the carboxyl terminus of the
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chloride channel (Beck et al., 1996). Other animal models include the arrested
development of righting mouse (ADR) which is caused by an insertion of a transposon
element (Steinmeyer et al., 1991). More recently two different myotonic canine models
have been described; the Miniature Schnauzer and the Australian Cattle Dog with
recessive missense mutations in CLCN1 (Rhodes et al., 1999; Finnigan et al., 2007). The
small size of the myotonic mouse makes it a difficult model upon which to undertake in
vivo electrophysiology. Muscles from myotonic goats were very useful in the early
experiments to elucidate the pathophysiology of MC. However, probably because of the
substantial resource implications of goat care, goats are not ideal as a potential model for
therapeutic trials. The maintenance of the myotonic dog models is also a major
undertaking, but given that many other canine models of disease have been extensively
studied, greater facilities and technical experience are available suggesting that myotonic
dogs may be a more attractive model for future trials.

Hyperkalaemic periodic paralysis has been described in quarter horses. A single point
mutation has been identified in the equine skeletal muscle sodium channel gene that
substitutes a phenylalanine for a leucine in the DIV/S3 segment of the protein(Rudolph et
al., 1992). Recently a mouse model of hyperkalaemic periodic paralysis was successfully
engineered by introducing the equivalent of the common human M1592V mutation into
the murine SCN4A gene(Hayward et al., 2008). The mouse was shown to display similar
clinical and biopsy findings to those seen in human cases of hyperkalaemic periodic
paralysis validating it as a reasonable animal model. New insights into the beneficial
effects of elevated extracellular calcium levels and detrimental effects of impairing the
Na/K pump in the pathophysiology of hyperPP have already been determined(Hayward
The non dystrophic myotonias et al., 2008). This knock in mouse offers potential for developing an increased understanding of the pathophysiology of hyperPP and possibly the development of future therapies.

Although hyperPP is allelic to PMC and SCM, no such phenotypes have been described in animal models. Myotonia does occur in horses but in conjunction with multisystem defects the phenotype seems more like one of equine myotonic dystrophy (Reed et al., 1988).

Treatment

For those patients with mild symptoms no specific drug treatment may be needed although it is important to provide advice regarding the avoidance of precipitating factors such as cold exposure or strenuous exercise. In those patients with significant symptoms and disability from myotonia a variety of agents have been suggested. In early studies, procainamide, quinine and steroids were employed. A small randomized double blind trial compared the efficacy of each of these treatments in relation to placebo in 20 individuals with myotonic disorder (16 myotonic dystrophy, 4 myotonia congenita). The diagnosis was made on a clinical basis without genetic confirmation. The trial lasted 12 weeks, all participants receiving each of the four treatments for a three week period with no washout period. An end-point of at least a 50% reduction in the duration of hand grip myotonia measured by EMG and timed clinically was employed. Using this endpoint, 6/20 participants taking quinine, 15/20 taking procainamide, 15/19 taking prednisone
(one patient did not receive prednisone) and 0/20 taking placebo showed improvement (LEYBURN et al., 1959). This study although imperfect illustrated a low efficacy of quinine. Despite the suggested benefits of procainamide and prednisone the side effect profile of both these drugs restricts their use and they are no longer recommended as therapeutic agents in the NDMs.

The carbonic anhydrase inhibitor acetazolamide is commonly used in the periodic paralyses and has been reported to be beneficial in the NDMs (Trudell et al., 1987; Ferriby et al., 2006). A small series of nine patients with myotonia, seven diagnosed clinically with MC and two with PMC, reported a subjective and objective (timed measurements of myotonia) improvement in myotonia in all cases, although one individual with PMC developed quadrapareses 12 hours after the ingestion of acetazolamide (Griggs et al., 1978). Larger studies of acetazolamide use in the NDMs have not been performed, and while there is evidence of some benefit, it not generally considered as a first line agent for the treatment of myotonia.

Anti-convulsants, local anaesthetics and anti-arrhythmic drugs which block sodium channels are the most frequently used agents in the treatment of myotonia. There are currently no drugs which specifically act on the chloride channel CLC-1 (Verkman et al., 2009). Phenytoin has been shown to improve the righting time of myotonic mice turned onto their backs (Aichele et al., 1985). Ricker et al. reported subjective improvement in muscle stiffness and an improved timed walk in one patient with myotonia congenita and a dose dependant improvement in isometric force in another (Ricker et al., 1978). The lignocaine derivative tocainide gave encouraging results initially (Rudel et al., 1980; Streib., 1987) but was eventually withdrawn from the market due to the risk of potentially
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fatal agranulocytosis (Volosin et al., 1985). Synthesis of tocainide analogues has been attempted in vitro and it may be possible such agents are developed for future study as anti-myotonic agents if the efficacy and side effect profiles are favourable (Catalano et al., 2008).

Recently, class I anti-arrhythmics have offered potential for treatment. Flecainide a class Ic anti-arrhythmic, has been shown to be effective in vitro (Aoike et al., 2006) although its use in clinical practice as an anti-myotonic agent is rarely reported (Rosenfeld et al., 1997). An improvement in clinical symptoms and cold induced EMG findings with propafenone, another class Ic anti-arrhythmic has been reported in a single case of paramyotonia congenita (Alfonsi et al., 2007). The class 1b anti-arrhythmic mexiletine is generally considered to be the first-line treatment of choice by myologists but a randomized controlled trial is required. It is usually well tolerated with only minor side effects reported. Importantly it has pro-arrhythmic potential and therefore pre and post treatment ECGs are essential. More extensive cardiac evaluation prior to commencement is important if there is an abnormal baseline ECG or a history of cardiac disease. Single case reports have shown that mexiletine is effective in treating myotonia in both sodium and chloride channel disorders (Jackson et al., 1994; Ceccarelli et al., 1992). However, a recent Cochrane review highlighted the lack of adequate randomized double blind placebo controlled trials to prove efficacy (Trip et al., 2006). The ability to conduct such trials is partly hampered by the difficulty in quantitating myotonia (Torres et al., 1983; Hammaren et al., 2005; Logigian et al., 2005; Moxley, III et al., 2007; Hogrel., 2009) and in recruiting adequate numbers of patients to achieve statistical power. A recent study employed trunk sway analysis to measure the warm up phenomenon in recessive
myotonia congenita and proposed that with further evaluation this may offer an alternative potential end-point for therapeutic trials (Horlings et al., 2009).

Sodium MRI has also recently been proposed as a possible outcome measure in patients with sodium channel diseases. An increase in intramuscular sodium content was demonstrated to accompany muscle weakness following exercise of cooled muscles in PMC. In a small group of patients this increase was significantly reduced following treatment with mexiletine (Weber et al., 2006). It is possible this technique could be used to monitor response to treatment in both a clinical and research setting. The Consortium of Clinical Investigation of Neurologic Channelopathies is currently performing a double-blind, placebo-controlled cross-over study of mexiletine for nondystrophic myotonias (see clinicaltrials.gov) and will study 60 subjects with PMC and MC. The primary endpoint measurement is patient stiffness as reported in the interactive voice response system (Wang 2008c). The interactive voice response was chosen as the primary endpoint for this study as quantification of myotonia using handgrip devices has not consistently demonstrated the delayed relaxation phenomenon in patients with non-dystrophic myotonia (Wang 2008c). It is hypothesized that patients’ self-reported responses of stiffness will be a more consistent and reliable endpoint measurement in order to determine efficacy of drug response.

In vitro studies continue to identify pharmacological agents that preferentially block sodium channels in the open state, thereby targeting persistent sodium currents (Wang et al., 2008b; Wang et al., 2008a). These studies may identify future therapies.

No drugs are available which specifically act on the CLC-1 channel. A number of experimental approaches may have future implications for the treatment of myotonia
The non dystrophic myotonias (Cleland et al., 2008). It has been shown that alternative splicing of the CLCN-1 gene contributes to the myotonia in DM1 and that directed anti-sense morpholino oligonucleotides to skip exon 7a restores chloride channel function and abolishes myotonia in a mouse model of DM1 (Wheeler et al., 2007). It is possible that the development of this technique and its delivery could have implications for those cases of MC due to splice site mutations.

Trans-splicing is a natural form of RNA processing where exons from two separate RNA transcripts are joined together. This can be manipulated to restore normal RNA processing of a mutant transcript. Such a technique has been employed using a trans-splicing ribozyme to restore a mutant chloride channel transcript in a cellular model. Although a good recovery of chloride channel function could be seen in individual cells the efficiency of RNA repair in the cell culture as a whole was only 1.2% (Rogers et al., 2002).

Defective protein transport from the endoplasmic reticulum to the Golgi apparatus of the CLC-1 channel protein has also been shown for the F413C and A531V CLCN-1 mutations associated with recessive MC (Papponen et al., 2008). Functional expression of the F413C mutation in vitro showed only a minimal shift in chloride conductance (Zhang et al., 2000) suggesting that if the protein could be restored to the muscle membrane this may result in an at least partial restoration of chloride conductance.

Whether pharmacological therapies can be developed that would achieve this or build on the partial success of trans-splicing techniques safely in patients remains to be seen.

Some patients with NDM develop a fixed myopathy although the frequency of such a myopathy and its pathogenesis are unknown. At present, it is therefore not possible to
accurately advise if prophylactic treatment is justified to prevent myopathy in NDM patients.

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**Figure Legends**

FIG 1: AUTOSOMAL RECESSIVE MYOTONIA CONGENITA: SHORT EXERCISE TEST (MEAN CMAP AMPLITUDES) AT ROOM TEMPERATURE AND AFTER COOLING

FIG 2: AUTOSOMAL DOMINANT MYOTONIA CONGENITA: SHORT EXERCISE TEST (MEAN CMAP AMPLITUDES) AT ROOM TEMPERATURE AND AFTER COOLING

FIG 3: POTASSIUM AGGRAVATED MYOTONIA: SHORT EXERCISE TEST (MEAN CMAP AMPLITUDES) AT ROOM TEMPERATURE AND AFTER COOLING

FIG 4: PARAMYOTONIA CONGENITA SHORT EXERCISE TEST (MEAN CMAP AMPLITUDES) AT ROOM TEMPERATURE AND AFTER COOLING

FIG 5: DIAGRAMATIC REPRESENTATION OF CLC-1 AND NAV1.4 CHANNELS
Appendix

This report summarizes the findings presented at the International Conference on Non-Dystrophic Myotonias (Kansas, June 2007). The conference was generously supported by a National Institutes of Health conference grant [R13 NS057995]. Participants of the meeting listed alphabetically:

Anthony Amato (Brigham and Women’s Hospital), Marianne Arzel-Hezode (Pitié-Salpêtrière, France), Tetsuo Ashizawa (University of Texas), Richard Barohn (University of Kansas), Brian Bundy (DTCC, Florida), Steve Cannon (University of Texas Southwestern), Yoon-Hee Cha (UCLA), James Cleland (University of Rochester), John Day (University of Minnesota), Robert Dirksen (University of Rochester), Christoph Fahlke (Institute of Neurophysiology, Hannover, Germany), Doreen Fialho (Institute of Neurology, UK), Bertrand Fontaine (Pitié-Salpêtrière, France), Alfred L George (Vanderbilt University, Nashville), Tracey Graves (Institute of Neurology, UK), Robert Griggs (University of Rochester), Angelika Hahn (London Health Science Centre, Canada), Michael G Hanna (Institute of Neurology, UK), Laura Herbelin (University of Kansas), Barbara Herr (University of Rochester), Jeffrey Krischer (DTCC, Florida), Robert Layzer (University of California), Emma Matthews (Institute of Neurology, UK), Giovanni Meola (University of Milan, Italy), Richard T Moxley III (University of Rochester), Shree Pandya (University of Rochester), Ming Qi (University of Rochester), Sanjeev Rajakulendran (Institute of Neurology, UK), Jeffrey Ralph (University of California), Mohammed Salajegheh (Brigham and Women’s Hospital), David Saperstein (University of Kansas), Patty Smith (University of Rochester), Damien Sternberg (Pitié-Salpêtrière, France), Rabi Tawil (University of Rochester), Charles Thornton (University of Rochester), Susie Tomlinson (Institute of Neurology, UK), Jaya Trivedi (University of Texas Southwestern), Paul Twydell (University of Rochester), Shannon Venance (London Health Science Centre, Canada), Julio Vergara (University of California), Savine Vicart (Pitié-Salpêtrière, France), Ronan Walsh (Brigham and Women’s Hospital), Yunxia Wang (University of Kansas), Thurman Wheeler (University of Rochester).