INVITED REVIEW

ABSTRACT: Myotonia congenita is a hereditary chloride channel disorder characterized by delayed relaxation of skeletal muscle (myotonia). It is caused by mutations in the skeletal muscle chloride channel gene CLCN1 on chromosome 7. The phenotypic spectrum of myotonia congenita ranges from mild myotonia disclosed only by clinical examination to severe and disabling myotonia with transient weakness and myopathy. The most severe phenotypes are seen in patients with two mutated alleles. Heterozygotes are often asymptomatic but for some mutations heterozygosity is sufficient to cause pronounced myotonia, although without weakness and myopathy. Thus, the phenotype depends on the mutation type to some extent, but this does not explain the fact that severity varies greatly between heterozygous family members and may even vary with time in the individual patient. In this review, existing knowledge about phenotypic variability is summarized, and the possible contributing factors are discussed.

PHENOTYPIC VARIABILITY IN MYOTONIA CONGENITA

ESKILD COLDING-JØRGENSEN, MD

Department of Clinical Neurophysiology 19, Glostrup Hospital, University of Copenhagen DK-2600 Glostrup, Denmark

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Myotonia congenita is a hereditary muscle disorder characterized by impaired relaxation of skeletal muscle after voluntary contraction (myotonia). It was first described by the Danish physician Julius Thomsen, who himself suffered from the disease.106 In his description, Thomsen remarked upon the great variability between affected family members. Thus, myotonia was barely apparent in Thomsen’s mother and in her older brother, whereas his younger brother and sister were severely affected.106 Thomsen also described the gradual disappearance of myotonia through repetition of movements, often referred to as the “warm-up” effect. As other families with myotonia were subsequently described, it became clear that the phenotype also varied considerably between families. Whether the paramyotonia congenita of Eulenburg39 and the dystrophic variant (dystrophia myotonica or myotonic dystrophy) of Steinert97 and Curschmann31 were separate disease entities was debated for many decades.70,105

In 1977, Peter Emil Becker11 convincingly proved the existence of a recessive myotonia congenita variant with muscular manifestations, as it were, intermediate between those of Thomsen’s relatives and those of patients with dystrophia myotonica, although without the extramuscular abnormalities of the latter. Thus, myotonia congenita was divided into a recessive disorder (Becker’s disease) and a dominant disorder (Thomsen’s disease), which was further subdivided into a Thomsen type (type I) and some other types in which patients showed unusual features, such as fluctuating course or temperature dependence.11 When genetic testing became possible, many of the latter were shown to have sodium channel disorders,49,55,63,86 whereas the Becker55 and Thomsen44 phenotypes were shown to be caused by mutations in the skeletal muscle chloride channel gene CLCN1. This confirmed the chloride hypothesis advanced by Bryant and co-workers in the 1970s on the basis of experiments on isolated muscle fiber bundles from myotonic goats and patients with myotonia.4,22,65 Interestingly, the myotonia in myotonic dystrophy, although due to an entirely different genetic defect,18 can probably also be explained by reduced sarcolemmal chloride channel content.24

What remains is a dominant (Thomsen) phenotype characterized classically by early onset, mild to moderate myotonia, and slight muscular hypertrophy, and a recessive (Becker) phenotype characterized by later onset, moderate to severe myotonia, moderate muscular hypertrophy, transient weakness...
accompanying myotonia, and even permanent weakness and atrophy of forearm and neck muscles with myopathic electromyographic and histological changes in one fourth to one third of cases.\textsuperscript{11} The estimated prevalence of the dominant disorder in Germany is around twice (1 in 23,000) that of the recessive type (1 in 50,000), although recessive pedigrees are probably more frequent than dominant ones.\textsuperscript{11} Transient weakness\textsuperscript{88} was present on clinical testing in approximately 70\% (9 of 13) of patients with two mutated alleles,\textsuperscript{33} whereas it has not been reported as a clinical symptom or sign in patients with genetically verified dominant myotonia congenita. Thus, pronounced variation is observed in both dominant and recessive myotonia congenita, with approximately one third of recessive patients having a severe dystrophic phenotype, one third having transient weakness but no atrophy, and one third having relatively mild features indistinguishable from those of dominant myotonia congenita.

**GENERAL COMMENTS**

**The Chloride Channel.** The skeletal muscle chloride channel gene \textit{CLCN1} is located on chromosome 7 and codes for a subunit of the skeletal muscle chloride channel, CLC-1. CLC-1 is almost exclusively expressed in skeletal muscle,\textsuperscript{39} although low transcript levels are also found in kidney, heart, and smooth muscle,\textsuperscript{98} and its presence in intracellular organelles of glia cells has recently been reported.\textsuperscript{122} The bacterial homolog of CLC-1 is known to be a dimer consisting of two identical subunits each forming its own pore and each consisting of 18 helices.\textsuperscript{57} Although the biophysical properties of bacterial and human CLC channels markedly differ,\textsuperscript{1} it is widely held that the gross structural outline must be the same; that is, CLC-1 channels are dimers with two independent pores.\textsuperscript{52} This is supported by patch-clamp experiments of CLC-1 channels demonstrating the presence of two pores gated by a common slow gate and two individual fast gates.\textsuperscript{2,6,92}

**The Chloride Conductance.** The chloride conductance contributes 85\% to the resting membrane conductance of human muscle, ensuring its electrical stability.\textsuperscript{22} Whereas \( \text{Na}^+ \) and \( \text{K}^+ \) permeabilities undergo rapid time- and voltage-dependent changes during the action potential, the \( \text{Cl}^- \) permeability remains relatively constant.\textsuperscript{10} The chloride conductance is crucial for countering the depolarizing effect of \( \text{K}^+ \) accumulation in T-tubules: during muscle activity, action potentials are propagated radially into the fiber along elements of the T-tubular system. The efflux of \( \text{K}^+ \) associated with a single action potential increases its tubular concentration by 0.3 mM, and with multiple action potentials and no chloride conductance the cumulative effect would be a membrane depolarization of 10 mV or more.\textsuperscript{4,10} In normal muscle fibers, this depolarization is not reflected in the surface membrane potential because of the large stabilizing \( \text{Cl}^- \) conductance. However, in myotonic muscle fibers, \( \text{K}^+ \) accumulation in the T-tubular lumen depolarizes the surface membrane sufficiently to initiate self-sustaining action potentials causing a prolonged (myotonic) contraction.\textsuperscript{4,10} Further, large depolarizations (of 10–20 mV) may force enough sodium channels into the inactivated state as to render the membrane temporarily inexcitable. This explains the transient weakness sometimes observed in patients with recessive myotonia congenita.

How much, then, must the chloride conductance be reduced to cause myotonia? Using a simple mathematical model with parameters from frog sartorius muscle, Barchi\textsuperscript{9} showed that decreasing chloride permeability to 20\% would suffice to produce myotonic activity in response to a single stimulus. Further, applying myotonia-inducing drugs to human external intercostal muscle bundles, Kwiecinski et al.\textsuperscript{60} demonstrated that reduction of chloride conductance to 18\% was associated with a clear myotonic phenomenon, whereas reduction to 40\% only produced a small and variable prolongation of relaxation. Presumably, reduction in chloride conductance to 20\% is associated with severe myotonia congenita, whereas a reduction to 40\% may cause a mild or normal phenotype. A reduction to 50\% apparently never causes myotonia, because heterozygous carriers of nonfunctional (“recessive”) mutations are asymptomatic, although the tacit assumption of 1:1 allelic expression in such cases may not, in fact, be true.\textsuperscript{25}

**Estimating the Chloride Conductance.** Ideally, the chloride conductance should be measured in vivo, in the intact, functional muscles of myotonia patients. This has not been achieved. Lipicky et al.\textsuperscript{65} examined isolated fiber bundles of external intercostal muscle from 13 normal volunteers and 6 patients with myotonia congenita. By this procedure, they documented greater membrane resistance in myotonic muscle fibers than in normal fibers. However, the method is still far from the in vivo situation, and it is invasive and elaborate, so that some fiber damage is unavoidable during both the biopsy procedure and dissection.\textsuperscript{65}
With the identification of the \textit{CLCN1} gene it became possible also to identify the mutations responsible for myotonia in the individual patient. Thenceforth, chloride conductance has been studied exclusively in heterologous expression media such as \textit{Xenopus} oocytes or mammalian cell lines; for instance, with human embryonic kidney (HEK) cells, using artificially mutated CLC-1 RNA injected into, and expressed by, the cell in question. Such an approach gives valuable information about the effect of particular mutations on the biophysical properties of CLC-1 homodimers. Also, if a patient is homozygous for a given mutation (as are some of the patients with recessive myotonia congenita), the pathogenicity of the mutation can be confirmed in this way. However, studies of mutant homodimers give no information about the chloride conductance in heterozygotes: that is, patients with the dominant (Thomsen) phenotype, in whom—assuming 1:1 allelic expression and normal dimer formation—the chloride conductance will be effectively determined by the 25% normal (WT:WT) homodimers and the 50% abnormal (WT:MUT) heterodimers. The contribution of the 25% abnormal (MUT:MUT) homodimers, having almost zero conductance if functional at all, will be negligible. Thus, in order to simulate heterozygote conditions, the effect of equal amounts of normal and mutated RNA injected into \textit{Xenopus} oocytes has been studied in a number of cases. Further, for example, in the elegant study by Wu et al.,\textsuperscript{115} heterodimers were directly constructed and expressed in mammalian cells. For some recessive mutations, such as R496S\textsuperscript{80,82} or E291K,\textsuperscript{82} 1:1 expression results in 50% conductance, meaning either that heterodimers are not formed or that the normal (WT) subunit of heterodimer complexes is not impaired by its nonfunctional companion. For the dominant P480L or I290M mutations, 1:1 expression results in approximately 25% conductance,\textsuperscript{82,99} meaning that heterodimers are formed but that both pores have zero conductance at physiological voltages. In many cases, strong depolarization will open the pores of the heterodimers, but this is of limited clinical relevance because the chloride conductance is important for the resting potential, not the action potential.

The influence of other chloride channels, such as the ubiquitous CLC-2, CLC-6, and CLC-7 channels,\textsuperscript{15,104} on muscle membrane excitability is generally assumed to be negligible, but little is in fact known about the quantity and function of these channels in human skeletal muscle. CLC-2 homodimers are closed under normal resting conditions but, remarkably, CLC-1 and CLC-2 subunits may form heteromultimeric channels\textsuperscript{69} that have a linear current–voltage relation and thus must contribute somewhat to the resting chloride conductance. It should also be borne in mind, when interpreting the results of heterologous expression experiments as just described, that both \textit{Xenopus} oocytes and HEK cells have endogenous chloride currents. Thus, four different endogenous chloride conductances have been described in \textit{Xenopus} oocytes,\textsuperscript{3,23,57,76} and no less than five have been identified in HEK cells.\textsuperscript{123} Although the currents mediated by the latter are very small in nontransfected cells, some of the conductances may be strongly activated by the artificial expression procedure per se, as has in fact been described for the \textit{Xenopus} expression medium.\textsuperscript{23,57}

\textbf{Estimating Severity of Myotonia.} For expressing severity of myotonia, Becker\textsuperscript{11} applied a semiquantitative measure of general impairment based on the patient’s history. In the most severe grade (+ + +), myotonia is a great handicap in daily work, is immediately noticeable, and can be hidden from others only with difficulty. In the mild grade (+), the affected person is impeded only slightly, if at all, and does not suffer because of his muscle abnormality. Myotonia limited to one area of the body, for instance the legs, is also classified as mild grade. In spite of the evident drawbacks of such a subjective measure, the approach may be superior to any single evaluation of clinical, mechanical, or electromyographic parameters, because the test–retest reproducibility of the latter is usually very low. For instance, measuring the duration of electromyographic activity after percussion\textsuperscript{56} or voluntary contraction\textsuperscript{30} seems too capricious to be of any practical value. Twitch and tetanic relaxation parameters have better reproducibility,\textsuperscript{67,85} and in myotonic dystrophy mechanographical relaxation time correlates with leukocyte CTG repeat length.\textsuperscript{67} Functional tests, such as the time spent on fist opening or staircase climbing,\textsuperscript{12} may also be valuable, but no systematic studies or comparisons of these different methods have yet been performed. In the more severe cases, where sodium channels are inactivated during voluntary contraction or repetitive stimulation, the resulting amplitude decrement of the compound muscle action potential (CMAP) may be used as a quantitative measure of severity (Fig. 1). The reproducibility of this measure is high,\textsuperscript{7,19,30,119} but, unfortunately, a decrement is only found in a minority of patients with the dominant (Thomsen) phenotype.\textsuperscript{7,30,35} The decrement may be elicited by 10-Hz repetitive nerve stimulation\textsuperscript{30,35} or by voluntary mus-
cle activation for 10 seconds (short exercise test), whereas voluntary muscle activation for 5 minutes (long exercise test) rarely affects the CMAP.

The simplest genotype–phenotype relationship imaginable is that the phenotype directly reflects the degree of chloride conductance reduction, and that the degree of chloride conductance reduction in turn depends on the CLCN1 genotype only. As a hypothesis this is unsatisfactory because of the marked intrafamilial variation described, such as in the pedigree of Dr. Thomsen. It will probably be closer to the truth to speak of a range of severity grades (and a corresponding chloride conductance range) possible for a given CLCN1 genotype.

We can arbitrarily define five “severity grades”: (1) no symptoms, but unequivocal myotonia at examination; (2) mild (and/or fluctuating) symptoms; (3) pronounced myotonia, but no transient weakness; (4) pronounced myotonia with transient weakness, but without dystrophic features; and (5) pronounced myotonia, transient weakness, and dystrophic features. In this context, dominant myotonia may exhibit severity grades 1–3 (0–3 if cases of reduced penetrance are included), whereas recessive myotonia is usually of grades 4–5. However, approximately one third of patients with recessive myotonia exhibit lower grades of severity, causing significant overlap between the phenotypic spectra of the two conditions.

In the following sections, attention is focused on the component of phenotypic variation that is explained by different mutation types and the component that is not.

**VARIATION BETWEEN FAMILIES: THE SIGNIFICANCE OF THE MUTATION TYPE**

Today, 80 different mutations in the CLCN1 gene have been described (Table 1). The mutations are conveniently divided into dominant and recessive mutations. Dominant mutations are found in dominant pedigrees, and heterozygotes are usually symptomatic. Recessive mutations are found in recessive pedigrees, and heterozygotes do not have symptoms or clinical signs of myotonia. Sometimes inheritance is not clear, in which case 1:1 heterologous expression in oocytes or expression of heterodimeric constructs in mammalian cells may disclose the presence or absence of a dominant-negative effect, an important clue as to whether the mutation is likely to behave dominantly or recessively.

**Dominant Mutations with Apparentely Full Penetrance.**

The I290M mutation was found in two pedigrees; the R338Q mutation in one pedigree and in a single patient; the mutations M128V, E193K, T268M, P480L, fs872X were each found in one pedigree; and the mutations S132C, V286A, and S471F were found in single patients only.

**I290M.**

The mutation was found in one family with “typical” Thomsen myotonia,63 and in another family in which an 8-year-old boy heterozygous for the mutation was asymptomatic.56 Because the age of onset in the latter family ranged between 9 and 20 years, the lack of symptoms in the 8-year-old should not be considered evidence of reduced penetrance. A 1:1 expression of wild-type (WT) and mutant subunits in oocytes revealed a large shift (+45 mV) in the voltage dependence of the open probability curve, suggesting virtually nonfunctional het-
erodimers at physiological voltages (Fig. 1).82

**R338Q.** The mutation was found in a fully penetrant dominant pedigree.119 It had previously been described in a single compound heterozygous patient (R300X/R338Q).45 Expression of R338Q homodimers in HEK cells revealed a +38-mV shift of the open probability curve, whereas 1:1 WT:R338Q expression apparently yielded normal currents.120 The latter finding should be interpreted with caution, however, because it is impossible to control for cellular expression of two independent plasmids in transfected HEK cells.120

**P480L.** The mutation was found in Dr. Thom- sons's pedigree. The pedigree comprises nine generations of myotonia congenita patients, with probably almost 100 affected members altogether.11,106 Thomsen pointed out that affected family members had qualitatively similar symptoms, but that the symptoms were more prominent in one and less in another.11,106 Thus, stiffness was barely apparent in Thomsen’s mother and in her older brother, whereas Thomsen’s younger brother and sister were affected to a great degree.11,106 Variability of symptoms with periods of almost complete remission were also described in a 34-year-old woman.11,105 Three patients examined by Becker were unaware that they were affected at the time of examination.11 Thus, using the severity scale just proposed, severity ranges

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**Table 1. CLCN1 mutations (modified after Pusch83).**

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</tr>
<tr>
<td>2264delC</td>
<td>fs793X</td>
<td>R</td>
<td>89</td>
</tr>
<tr>
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<td>sd</td>
<td>R</td>
<td>102</td>
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<tr>
<td>2419C &gt; T</td>
<td>Q807X</td>
<td>R</td>
<td>27</td>
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<tr>
<td>c.2452 + 2T &gt; A</td>
<td>sd</td>
<td>R</td>
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<tr>
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<tr>
<td>2576Q &gt; A</td>
<td>G859D</td>
<td>R?</td>
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<tr>
<td>2680C &gt; T</td>
<td>R894X</td>
<td>D/R</td>
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<td>*2795C &gt; T</td>
<td>*P932L</td>
<td>D</td>
<td>77, 94</td>
</tr>
</tbody>
</table>

D, dominant; NR, sequence change not reported; R, recessive; sa, splice acceptor mutation; sd, splice donor mutation.

*Pathogenicity debated.
from grade 1 to grade 3 within the Thomsen kindred. The 1:1 WT:P480L expression in oocytes revealed an extreme (+90 mV) shift of the open probability curve, meaning that heterodimers are completely nonfunctional at physiological voltages (Fig. 1).8,99

M128V, E193K, T268M, and fs872X. The M128V,30 E193K,30 T268M,30 and fs872X52 mutations were found in dominant pedigrees with two to four affected members. A 1:1 expression of the M128V and E193K mutations in oocytes yielded moderately reduced currents at physiological voltages.46 Further, expression of the E193K mutation revealed a clearly shifted reversal potential, meaning that a substantial part of the current could be due to a cationic conductance (as described later for the G230E mutation). A sodium leak conductance could aggravate (or at least would be of no help in countering) the abnormal depolarization of contracting myotonic muscle fibers. Expression studies of the T268M and fs872X mutations have not been performed.

S132C, V286A, and S471F. The S132C,115 V286A,58 and S471F58 mutations were found in single patients only. Expression of WT:S132C heterodimeric constructs in mammalian cells revealed a drastically shifted voltage dependence of the open probability curve115 compatible with dominant inheritance. A 1:1 expression of WT:V286A in oocytes yielded a moderate (+37 mV) shift of the open probability curve, which is likewise compatible with dominant inheritance.58 Expression studies of the S471F mutation were not carried out, and the assumption of dominant inheritance rests solely on the fact that the patient was heterozygous for the mutation.53

Dominant Mutations with Reduced Penetrance. The mutations A313T and R317Q were each found in two pedigrees; the mutations P480T and I556N in one; and the mutations L198V, A218T, L283F, F307S, G482R, and F708L were found in pedigrees with unclear inheritance patterns or in single patients only.

A313T. Plasart-Schiess et al.81 described this mutation in two unrelated families (families A and J). In family A, a dominant inheritance pattern with incomplete penetrance in three generations was found. In family J, the index patient may have carried two mutations, but only the A313T mutation was identified. The mother, carrying the A313T mutation, was clinically and electrophysiologically normal. Although this is not incompatible with recessive inheritance, as claimed by the investigators, it more likely reflects dominant inheritance with incomplete penetrance as described in family A. Expression of A313T revealed a shift of the open probability curve and a predominant effect on the common gate of mutant channels.8,58

R317Q. The mutation was found in one clearly dominant pedigree.75 It was further described in homozygous and heterozygous members of another family.58 Of the three heterozygote family members, one had occasional muscle stiffness, whereas the other two were asymptomatic.58 This points towards dominant inheritance with reduced penetrance. A 1:1 expression in oocytes revealed a slight shift of the open probability curve, in agreement with the normal or mild phenotype (severity grades 0–2) of heterozygotes (Fig. 1).52

P480T. The mutation was found in a large dominant pedigree with 14 affected members in five generations.91 Of eight documented heterozygotes, one—a 45-year-old woman—was clinically and electromyographically unaffected, suggesting incomplete penetrance. No expression studies have been performed.

I556N. Plasart-Schiess et al.81 described a consanguineous family in which the mutation was dominantly inherited with reduced penetrance, because one male heterozygote was clinically and electrophysiologically unaffected, whereas two female heterozygotes had mild symptoms of late onset. A patient homozygous for the mutation had a more severe phenotype. A 1:1 expression in oocytes58 yielded currents only slightly different from wild-type currents (Fig. 1), in agreement with the mild or normal phenotype of heterozygotes.

L198V, L283F, and F307S. Mutations L198V94 and L283F115 were found in heterozygous form in single patients and their unaffected parents. A 1:1 expression of WT:L198V94 and expression of heterodimeric WT:L283F constructs115 supported the assumption of dominant inheritance. The F307S mutation was found in two unrelated patients,30,58 one of whom had unaffected parents.30 Dominant inheritance was suggested on the basis of expression studies.8,58

A218T, G482R, and F708L. The A218T mutation was found in a family with a dominant pattern of inheritance,52 but the mutation did not segregate with the disease and no expression studies were carried out. The G482R mutation was described in two unrelated patients studied by Meyer-Kleine et al.75 and was further described by Jou et al.53 in two heterozygotes of which one was completely unaffected. Expression studies were not undertaken. The F708L mutation was found in heterozygous form in
one patient and his asymptomatic father. Expression studies were not performed.

**Mutations Found in Both Dominant and Recessive Pedigrees.** The phenomenon that the same pathogenic mutation may be found in dominant and recessive pedigrees is a peculiar and almost unique feature of myotonia congenita, although it has also been described for the D90A mutation in the SOD1 gene causing a hereditary variant of amyotrophic lateral sclerosis. In the latter case, all recessive families were shown to share a common founder, and the existence of a tightly linked protective factor was suggested. In myotonia congenita, the G230E, A531V, and R894X mutations have been found in both dominant and recessive pedigrees.

**G230E.** The mutation was found in eight dominant pedigrees, and of more than 20 heterozygous family members only 1 (a 35-year-old woman) was asymptomatic. In one additional family recessive inheritance was reported because a compound heterozygote (G230E/R894X) had an unaffected mother and sister heterozygous for the G230E mutation. Expression of G230E channels in mammalian cells revealed increased cation permeability of mutant homodimers. A 1:1 WT:G230E expression in oocytes resulted in only a slight current reduction, but with a clearly shifted reversal potential (as described earlier for the E193K mutation), meaning that a substantial part of the current could be due to a cationic conductance.

**A531V.** The mutation was described in a recessive pedigree, and a pedigree with reduced penetrance. Due to the small number of families reported, the possibility cannot be excluded that the A531V mutation is simply a dominant mutation with strongly reduced penetrance. No expression studies have been performed.

**R894X.** This mutation has been found to segregate with the disease in four highly penetrant dominant pedigrees as well as in more than ten recessive pedigrees. Thus, the R894X mutation clearly has a dual-inheritance pattern. Although there is no proof of a common founder (such as that described for the SOD1 D90A pedigrees mentioned earlier), the presence of a tightly linked aggravating or protective genetic factor seems probable. It may be speculated that dominant or recessive pedigrees share a common founder, or that mutant channels may be particularly sensitive to some unknown genetic factor. The fact that 1:1 WT:R894X expression in oocytes yields only slightly reduced currents points towards the existence of an aggravating factor in the dominant pedigrees. Such aggravation is probably not mediated through differential allelic expression, because Dunø et al. reported that the allelic expression ratio found in heterozygous members of a dominant pedigree was identical to that found in members of recessive pedigrees.

**FIGURE 2.** Some phenotypic variability may be caused by differential allelic expression. (a) Pedigrees four families segregating the R894X mutation. (A) and (B) are recessive pedigrees, whereas (C) and (D) are dominant. A thick arrow identifies the proband in each pedigree. A thin arrow identifies individuals from whom a muscle biopsy was obtained for quantification of allelic expression (from Dunø et al.). (b) Relative expression of the R894X allele in selected heterozygotes from the four families. Note that two patients from the dominant family, D, had an almost twice as large R894X:WT expression ratio than the other heterozygotes (from Dunø et al.).
Dominant Mutations Causing Unusual Phenotypes.

Six dominant mutations have been reported to cause unusual phenotypes: G200R, Q552R, and T310M causing mild/fluctuating phenotypes;63,71,109,115 F428S causing a paramyotonia-like phenotype;112,115 T550M causing a phenotype with proximal weakness and dysphagia;115; and P932L causing a phenotype with distal myopathy.77

G200R, Q552R, and T310M. The G200R mutation was found in a family in which affected members had long symptom-free intervals.71,109 Female patients almost exclusively experienced symptoms during pregnancies. The Q552R mutation was found in a family with clinically mild myotonia.65 The T310M mutation was described in two unrelated sporadic patients of whom one had mild myotonia53 and another had symptoms almost exclusively during pregnancies.115 Except for the symptom-free intervals, which could simply be one manifestation of a mild phenotype, none of these patients exhibited unusual clinical or paraclinical features. The 1:1 expression of WT:G200R yielded a slight (+20 mV) shift of the open probability curve (Fig. 1).114 Likewise, Ryan et al.87 found a +28-mV shift for heterodimeric WT:Q552R constructs. Thus, in agreement with the mild phenotype, the G200R and Q552R mutations produce functional heterodimers with only slightly altered gating characteristics. The T310M mutant channels behave somewhat differently: Wu et al.115 described that WT:T310M constructs appeared normal in standard solutions, but that their voltage dependence of activation was markedly shifted at low internal chloride conditions, producing a strong reduction of conductance (corresponding to a +120-mV shift of the open probability curve) at physiologically relevant voltages.115 Thus, the T310M mutation produces highly chloride-sensitive homo- and heterodimers that probably have severely reduced conductance at physiological voltages and internal chloride concentrations.

F428S. In one patient, originally regarded as an unusual case of paramyotonia congenita,112 the missense mutation F428S was found in the CLCN1 gene, whereas no mutation was found in the skeletal muscle sodium channel gene SCN4A.115 The clinical and paraclinical features deserve attention. The patient was a 47-year-old woman with a long-standing history of intermittent hand stiffness, mild weakness, pain, and transient paresthesias of the hands. The symptoms worsened in cold weather and with exercise. Her neurological examination was entirely normal at room temperature, without clinical myotonia or myokymia. Myotonic discharges and peculiar repetitive bursts (burst duration 50 ms, repetition interval 250 ms) were present on electromyography at room temperature. Immersion of the hand in ice-water produced clinical myotonia, aggravated the myotonic discharges, and abolished the repetitive bursts. At this point, exercise for 1 minute resulted in pain, stiffness, and mild weakness graded 4+ on the Medical Research Council (MRC) scale. The patient’s father and daughter were reported to have similar symptoms with hand stiffness worsened by cold, and the daughter also had myotonic electromyographic discharges at room temperature, but the genotype of the family members was not reported. Upon expression in mammalian cells, the gating properties of the F428S ClC-1 channels were indistinguishable from those of WT channels at both high and low chloride concentrations, but the current density was reduced (from 6.9 nA to 1.5 nA for homozygous expression, and from 6.9 nA to 0.5 nA for heterozygous constructs).115 The effect of temperature on channel properties was not reported. Thus, although the F428S mutation is clearly pathogenic, it remains a possibility that the unique phenotype of this single patient is due to some additional unknown factor.

T550M. The mutation was found in a 62-year-old woman who had had myotonic stiffness since the age of 7 years. The patient later developed proximal muscle weakness and dysphagia. A muscle biopsy showed increased fiber size variability, angular fibers, and many central nuclei, often in longitudinal chains.115 The mutation was also found in her 24-year-old son, who had only mild and occasional muscle stiffness. Likewise, the patient’s brother and his two sons were heterozygous for the T550M mutation, but were reported to have occasional symptoms only. Heterodimeric WT:T550M constructs showed increased conductance at high internal chloride and only slightly decreased conductance at low internal chloride concentrations.115 In conclusion, the T550M mutation seems to cause a very mild phenotype in most cases, and it is thus possible that the proband developed a myopathy from some other cause.

P932L. A 14-year-old boy heterozygous for the P932L mutation had congenital agenesis of the left pectoralis muscle, club feet, and lid lag. Electromyographic studies revealed myotonic discharges without abnormal motor units.77 His grandmother (deceased) probably had the same genotype P932L/WT and, as judged from her history, may have had grip myotonia and distal muscle atrophy in the upper extremities.77 The mother and maternal uncle were compound heterozygotes F289X/P932L. Patient II-1 (uncle) had early-onset myotonia and later weakness and atrophy of distal forearm muscles as well as...
dysphagia and contractures at the knees and hips. Serum creatine kinase was elevated to 2480 IU/L, 14 times the upper limit of normal, an elevation never previously reported in myotonia congenita. Electromyography and muscle biopsy showed myopathic changes. Patient II-3 (mother) also had myotonia, contractures, dysphagia, a serum creatine kinase of 1671 IU/L, and myopathic electrophysiological and biopsy findings. Of two fs289X carriers, one was completely asymptomatic and the other had isolated elbow contracture without myotonia. The pathogenicity of the P932L mutation was challenged by Simpson et al., 94 who found that expression of the mutation in mammalian cells yielded normal (wild-type) currents. Thus, it seems probable that some other factor, perhaps myotonic dystrophy type 2, was the cause of the dystrophic phenotype of the adult patients and the less severe phenotype of the 14-year-old patient. The contractures did not segregate with the myotonia and remain unexplained.

Recessive Mutations. Recessive mutations are generally assumed to result in mutant channels that are nonfunctional or at least have reduced function in such a way that the normal wild-type pore in a mutant:WT heterodimer complex is minimally affected. Regarding the 18 recessive truncations described: Q68X, Q74X, E193X, fs231X, fs258X, fs289X, R300X, fs387X, fs429X, fs433X, Q445X, C481X, fs503X, Q658X, E717X, fs793X, Q807X, and fs840X, the early truncations can safely be regarded as nonfunctional. Later truncations may be functional, however, if the membrane-spanning components of the subunit are intact. Thus, two very late truncations, fs872X and R894X, have been described in dominant pedigrees, and R894X expresses well in oocytes with even homodimers having some chloride conductance.75 However, no expression studies of the 19 earlier truncations with reduced penetrance or mutations that might still be pathogenic, because the mutation alters the exonic guanosine residue immediately preceding the intronic splice donor site. For the Y150C and Y261C mutations, however, there is no such explanation. The mutations were found in a single patient with the recessive myotonia congenita phenotype, 71,114 but it remains a possibility that the patient’s myotonic condition was due to some other factor than the CLCN1 mutations described.

The Concept of “Latent Myotonia” in Heterozygote Carriers. Becker11 described four asymptomatic heterozygote carriers (belonging to recessive pedigrees) who had brief myotonic discharges on electromyography. The myotonic discharges were only of 100–500-ms duration, but showed the parameters of myotonic discharges. Other heterozygote carriers had remarkably brisk insertional activity, whereas myotonic discharges were not recorded. Becker concluded that heterozygotes could sometimes be identified with the help of electromyography.11 Zellweger et al.118 confirmed these findings and considered the electromyographic abnormalities important laboratory evidence of the heterozygote carrier state. Unfortunately, consensus is lacking as to whether brief myotonic discharges and brisk insertional activity should be considered abnormal at all. Thus, Streib and Sun110 argued that fibrillation potentials and short runs of positive waves are produced by needle movements in normal subjects, and cautioned against an overly enthusiastic search for—and interpretation of—such findings. Demonstration of myotonia at clinical examination may be difficult in mild cases, and electromyography may well be more sensitive than the clinical examination. Thus, myotonic bursts should sometimes be present in asymptomatic subjects heterozygous for certain mutations, including dominant mutations with reduced penetrance or mutations that can both be dominant and recessive. Accordingly, electromyographic abnormalities have been described in 1 A531V and 2 R894X asymptomatic heterozygotes,102 whereas 2 A531V102 and at least 11 R894X heterozygotes30,75,102,119 have been characterized as electromyographically normal. It is more difficult to comprehend that electromyographic abnormalities are also found in approximately half the heterozygote carriers of nonfunctional mutations such as Q74X, fs433X, fs503X, and Q658X.
Although this could be a manifestation of, for example, differential allelic expression, as discussed in what follows, it is disturbing that Mailänder et al. found electromyographic abnormalities in 5 of 11 heterozygotes (representing 5 of 7 different mutations) examined, whereas Papponen et al. reported absence of myotonic bursts in all 22 heterozygote carriers (of the F413C, A531V, and R894X mutations) examined. This seems to justify the skepticism of Streib and Sun; in any event, genetic testing now provides an alternative and simpler way of confirming the heterozygous carrier state of asymptomatic family members.

**VARIATION BETWEEN PATIENTS WITH IDENTICAL MUTATIONS: THE POSSIBLE EFFECT OF DIFFERENTIAL ALLELIC EXPRESSION**

Marked intrafamilial variation was reported in the dominant pedigree of Dr. Thomsen with phenotypes ranging from pronounced myotonia to such mild affection that myotonia was only revealed upon careful clinical examination. Although reduced penetrance (i.e., complete absence of clinical myotonia in heterozygotes) was not observed in Dr. Thomsen’s pedigree, it has been reported in several other dominant pedigrees in which the phenotypic spectrum of heterozygotes thus ranged from pronounced myotonia to no myotonia. This suggests a strong influence of other genetic factors, but no such factors have been identified so far, except for a slight effect of gender on myotonia severity in recessive myotonia congenita.

**Gender.** Neither Thomasen nor Becker found that men are more frequently affected than women. However, it is sometimes suggested that myotonic symptoms may be more pronounced in men. Using the severity scale of Becker, the gender difference is in fact statistically significant in recessive, but not in dominant, myotonia congenita.

Anecdotal evidence may also suggest a tendency towards more pronounced transient weakness and larger decrements in compound muscle action potentials (CMAPs) upon 10-Hz repetitive stimulation in male than female patients. Thus, Deymeer et al. described two related patients with recessive myotonia of approximately the same age (mutations A15V/A15V): a 38-year-old man with pronounced transient weakness and a CMAP decrement of 92%, and a 42-year-old woman with only slight transient weakness and a CMAP decrement of 33%. Similarly, Colding-Jørgensen et al. described two related patients with dominant myotonia (mutation P480L, Dr. Thomsen’s family): a 34-year-old man with a decrement of 69%, and a 25-year-old woman with a decrement of 30%. In conclusion, at least in recessive myotonia congenita, the severity of myotonia (and possibly of transient weakness) may be somewhat more pronounced in men than women.

**Differential Allelic Expression.** The possibility that, in a diploid organism, the two alleles may be expressed with an expression ratio far from the expected 1:1 has been known for some time, but the surprisingly high frequency of such differential expression in the human genome has only recently been elucidated. Thus, Lo et al. examined 602 genes from 7 individuals, and found that 326 genes (54%) showed preferential expression of one allele in at least 1 individual, and 170 of those showed a greater than fourfold difference between the two alleles. Until now, functional consequences of such differential expression have only been proven for the APC gene, the expression of which plays a critical role in the development of colon cancer. In theory at least, differential allelic expression may explain much of the phenotypic variation observed in dominant myotonia congenita. However, it cannot explain phenotypic variation between homozygotes as described by Deymeer et al. for 5 adult patients homozygous for the A415V mutation. In the latter case, the variation may be ascribed to gender differences and to the fact that some of the patients were treated with mexiletine, whereas others were not, but further investigations of homozygous patients are needed to clarify this point.

Differential allelic expression of the CLCN1 gene was recently reported. Allelic expression was studied in 5 patients and unaffected subjects homozygous for the R894X mutation (Fig. 2). Three patients belonged to the fully penetrant dominant pedigrees C and D, while a compound heterozygote (R894X/G285E) from the recessive pedigree A and an asymptomatic carrier from the recessive pedigree B were also included. The patients from pedigree D expressed almost twice as much R894X mRNA relative to the wild-type allele as did the heterozygotes of the other families (Fig. 2). Rather disappointingly, the heterozygous patient from the other dominant pedigree (C) showed an allelic expression ratio identical to that found in the asymptomatic carrier of pedigree B. Thus, differential allelic expression cannot explain the phenomenon that some mutations behave dominantly in some pedigrees and recessively in others. Nevertheless, the demonstration of differential allelic expression of the CLCN1 gene is of...
interest. For instance, a simple calculation shows that a nonfunctional mutation behaves recessively at 1:1 expression \((0.25 \times 1 + 0.50 \times 0.5 + 0.25 \times 0 = 0.5 \times \text{WT-current} \Rightarrow \text{no myotonia})\), whereas it may behave dominantly at 3:1 \((0.06 \times 1 + 0.38 \times 0.5 + 0.56 \times 0 = 0.25 \times \text{WT-current} \Rightarrow \text{myotonia})\) or even 2:1 expression \((0.11 \times 1 + 0.445 \times 0.5 + 0.445 \times 0 \Rightarrow 0.33 \times \text{WT-current} \Rightarrow \text{mild/fluctuating myotonia?})\). In conclusion, the phenomenon of differential allelic expression offers an attractive hypothesis explaining the intrafamilial variation observed in dominant pedigrees.

**VARIATION WITH TIME: PHYSIOLOGICAL AND ENVIRONMENTAL MODIFYING FACTORS**

Systematic studies of the influence of environmental factors have been carried out only with myotonic animals. Bryant et al.\(^{21}\) observed myotonia over a period of 7 months in a colony of eight myotonic goats. No correlation was found with variations in serum electrolyte levels, temperature changes, humidity, or atmospheric pressure. However, myotonic stiffness in the “scare response” was abolished in water-deprived animals and recurred when the water deprivation was discontinued.\(^{21,48}\) This phenomenon remains to be explained. In humans, thirst has not been reported as a factor influencing the degree of myotonia. Hunger, physical fatigue, and psychological stress are sometimes reported to aggravate myotonia,\(^{11,105}\) but by no means consistently, and objective evidence for such effects has not been reported. Of the countless drugs that have been reported to alleviate myotonia, sodium channel blocking drugs such as mexiletine may be the most effective, but drug effects have not been studied in detail. Phenolamine, for instance, has no known effect on chloride channels and probably simply dampens myotonic discharges by reversibly blocking sodium channels.

“Fluctuating myotonia” was described by Becker\(^{11}\) as a subtype of dominant myotonia congenita. Many of the patients were later shown to have sodium channel disorders. However, the dominant G200R mutation does seem to be associated with a fluctuating phenotype in the sense that the patients experience long symptom-free intervals.\(^{109}\) Female patients with the G200R mutation almost exclusively experience symptoms during pregnancies (see later). Although the G200R mutation probably causes an unusually mild (rather than an unusually fluctuating) phenotype, the G200R family nevertheless offers valuable evidence about the existence of long-term (weeks–months) fluctuations of symptoms in myotonia congenita.

**Age.** The age of onset is extremely variable. Onset of symptoms may occur in infancy as well as after the age of 40.\(^{11}\) Onset is generally later in recessive than in dominant myotonia congenita,\(^{11}\) although the recessive disorder has the more severe phenotype. This seems hard to explain but for the very fact that parents are unaffected, and thus unfamiliar with the symptoms, which may delay both the diagnosis and the reported age of onset. In general, at least some of the variability in age of onset may be ascribed to the heavy reliance of this parameter on the attentiveness and memory of patient and relatives. After onset, severity of symptoms does not systematically increase or decline with age.\(^{11}\) Thus, at the age of 76, Dr. Thomsen stated that “I still suffer from it as I have since the earliest memories of my childhood.”\(^{11,107}\) In agreement with this, Becker stated that severity of myotonia did not vary markedly during life in members of Dr. Thomsen’s kindred or in the majority of patients from other kindreds.\(^{11}\) However, in some cases aggravation was observed at puberty,\(^{11,79}\) and in others myotonia seemed to improve with increasing age, a phenomenon perhaps related to physiological involution of the musculature or decreased activity.\(^{11}\)

**Pregnancy.** Women often report that pregnancy worsens myotonia,\(^{45,47}\) and, in rare cases, patients almost exclusively experience symptoms during pregnancies,\(^{61,109,115}\) although electromyographic signs of myotonia are present between pregnancies.\(^{61,109}\) For myotonia in general, there are no systematic comparisons of myotonic stiffness in the pregnant and nonpregnant states. Muscle tissue of normal pregnant women has increased intracellular sodium and reduced intracellular chloride concentrations, and the electrolyte distribution corresponds to a slightly hyperpolarized calculated resting membrane potential in pregnant compared to nonpregnant women.\(^{42}\) Hyperpolarization has actually been measured in smooth muscle of pregnant rats and may reflect increased Na–K pump activity as was previously described in erythrocytes.\(^{96}\) The increased Na–K pump activity may in turn be brought about by increased levels of thyroid hormones during pregnancy.\(^{17}\)

There are no studies of electrolyte changes in pregnant patients with myotonia congenita. Because resting potentials are generally similar in myotonic patients and controls, it may be speculated that muscle membranes are also hyperpolarized in pregnant patients.
patients with myotonia. Paradoxically, this would be expected to decrease, not increase, myotonia. It is therefore an interesting finding that the conductance of T310M channels and heterodimeric WT: T310M constructs depends in a unique way on the intracellular chloride concentration. The T310M mutation was found in a woman with sporadic disease and symptoms appearing almost exclusively during pregnancies. Mutant channels appeared normal in standard solutions, but their voltage dependence of activation was markedly shifted at low internal chloride, producing strong reduction of open probability at physiologically relevant voltages. Thus, it may be speculated that, in patients with particularly chloride-sensitive mutations, pregnancy-induced lowering of internal chloride, in turn, lowers the chloride conductance sufficiently to produce the marked aggravation observed.

**Cold.** Some patients with myotonia congenita report that cold substantially aggravates their symptoms, but objective measures of myotonic stiffness fail to confirm this and may even suggest some improvement with cold. Thus, Ricker et al. examined 13 patients, including 8 with recessive myotonia congenita and 5 with myotonic dystrophy. The hand and forearm of each patient was put in a water bath, and the muscle temperature in the adductor pollicis was varied. The CMAP and the isometric force were recorded. As in healthy controls, twitch force decreased and CMAP amplitude increased as the temperature was lowered. The myotonic contraction was shorter upon cooling in both nondystrophic and dystrophic myotonia. This agreed with the finding of a decreased tendency to repetitive firing at low temperature in 9-AG–induced experimental myotonia, as well as with the theoretical prediction by the Adrian–Chandler–Hodgkin model of the membrane. Also, Brown reported a slight attenuation of the CMAP decrement at 10-Hz repetitive stimulation when the arm and hand were submerged in an ice-water bath. After 10 minutes, the skin temperature was 15°–20°C. At these temperatures, myotonic discharges lasted longer than at room temperature, and myotonic after-activity showed less liability to diminish after repeated contractions. Further, in one exceptional patient with the F428S mutation, cold produced weakness as well as aggravation of myotonia.

**Exercise: The Warm-Up Effect.** The warm-up effect is a conspicuous and hitherto unexplained feature of myotonia congenita. Repeated contractions of a muscle reduce or abolish myotonia. After a few moments of energetic exercise, patients with myotonia may successfully engage in physical activities. Indeed, some affected members of Dr. Thomsen’s family were able to make a living as trapeze acrobats. Thus, gymnastics as such may have beneficial long-term effects.

An attractive hypothesis is that the warm-up effect is due to a temporary increase in Na–K pump activity elicited by muscle activity. During each action potential, Na+ flows into the fiber and K+ accumulates outside the fiber, and this in turn causes a strong stimulation of Na–K pump activity. Increased Na–K pump activity will in itself hyperpolarize the membrane, because the pump is electrogenic (pumps three Na+ ions out for every two K+ ions pumped in). Thus, in normal muscle, the activity of the Na–K pump can generate 3–10 mV of hyperpolarization. Further, increased Na–K pump activity will quickly restore the extracellular K+ concentration to normal and even subnormal levels, thus also hyperpolarizing the membrane.

The main problem with the hypothesis is that blocking the Na–K pump does not abolish the warm-up effect either in vitro or in patients. Thus, Birnberger and Klepzig observed that the warm-up effect of experimental myotonia could not be prevented by blocking the pump with strophantin. Further, van Beekvelt et al. reported that the transient paresis of three patients with recessive myotonia congenita disappeared during isometric exercise in both the absence and presence of intraarterial ouabain. Another problem is that Na–K pump activators do not improve myotonia in patients. Although adrenergic and β-adrenergic agonists such as salbutamol are powerful activators of the pump, adrenaline injections may actually aggravate myotonia. Thus, Kennedy and Wolf stated that, clinically, adrenaline aggravated myotonia, and Lindsley and Curnen found that the electrical after-activity had a longer duration and was of greater amplitude after the use of adrenaline. Selective β-adrenergic agonists, when given intravenously in high doses to pregnant women, have also been reported to worsen myotonia in two cases, although the value of such anecdotal evidence is obviously slight, because the
β-antagonist propranolol has likewise been reported to worsen myotonia. Finally, in spite of the widespread use of β-adrenergic agonist inhalators in the treatment of asthma, improvement of myotonia after inhalations has not been reported. In the author’s experience, two patients treated with β-adrenergic agonist inhalations reported no improvement of myotonia, although absorption is rapid and maximal plasma concentration is reached after only 13 minutes.

Birnberger and Klepzig hypothesized that lowering of intracellular pH during exercise might be the mechanism behind the warm-up effect. Although they did not directly measure intracellular pH, they observed that lowering external pH to 6.8 would abolish experimental myotonia in vitro. In humans, intracellular pH does decline markedly during fatiguing ischemic exercise, and a small decline may be observed even in nonischemic fatiguing exercise. Recovery takes place over about 5 minutes, a time-dependence similar to that of the warm-up effect. However, there is no evidence documenting significant pH decline during such slight nonischemic nonfatiguing exercise that suffices to elicit the warm-up phenomenon in myotonia patients. Although intracellular pH may transiently decline during each single contraction, it returns rapidly to baseline, and tetanic stimulation of mouse single muscle fibers actually produces a slight (0.03 unit) alkalinization after 10 tetani. Thus, unless myotonia patients exhibit unusual degrees of intra- cellular acidification during exercise, an anomaly not observed in either paramyotonia or myotonic dystrophy, a decline in pH probably cannot explain the warm-up phenomenon, simply because pH does not drop.

The warm-up effect remains unexplained. It can hardly be due to a direct effect of exercise on mutated chloride channels, because in recessive myotonia congenita CLC-1 channels may not be expressed at all. Because the phenomenon is much less pronounced in the sodium channel disorders, it may be speculated that exercise temporarily activates a mechanism that either reduces tubular potassium accumulation or increases the membrane leak conductance.

CONCLUSIONS AND FUTURE PERSPECTIVES

The phenotypic spectrum of myotonia congenita ranges from very mild myotonia disclosed only by clinical examination to severe and disabling myotonia with transient weakness and myopathy. The most severe phenotypes are seen in patients with two mutated alleles (recessive myotonia congenita). Heterozygotes are often asymptomatic, but for some mutations (i.e., dominant mutations) heterozygosity is sufficient to cause pronounced myotonia, although without weakness and myopathy. Thus, severity somewhat depends on the mutation type, but the marked intrafamilial variability observed in, for example, the pedigree of Dr. Thomsen cannot be explained in this way. The recently discovered differential allelic expression of the CLCN1 gene offers an attractive explanation of the latter variability, as, in general, the concept of differential allelic expression offers a new and exciting approach to the study of monogenic disorders. Another phenomenon, the disappearance of myotonia upon brief exercise (the warm-up effect), also deserves attention, as elucidation of the underlying channel or transporter mechanism may prove a valuable basis for the development of an effective treatment.

REFERENCES

Myotonia Congenita