Genotype-Phenotype Correlations in Human Skeletal Muscle Sodium Channel Diseases

Reinhardt Rüdel, PhD; Kenneth Ricker, MD; Frank Lehmann-Horn, MD

Background: Over the past 3 years, the genetics of the myotonic diseases have been substantially elaborated. Three genetically different groups of myotonic disease can be discerned: (1) the chloride channel myotonias, (2) the adynamia-paramyotonia complex, and (3) myotonic dystrophy.

Methods and Results: Electrophysiology has suggested and molecular biology has proven that the diseases belonging to the adynamia-paramyotonia complex, ie, paramyotonia congenita, hyperkalemic and normokalemic periodic paralysis, and some rare forms of myotonic disease, are caused by point mutations in the gene encoding the α subunit of the sodium channel in adult human skeletal muscle, located on chromosome 17q23. Thirteen different mutations have been described by various groups in the United States and Germany. The various mutations causing a particular form of the complex are not located in the gene in a predictable or easily understandable regular manner.

Conclusions: Further study of the genotype-phenotype correlations should not only increase our understanding of the variability of signs in this group of diseases, it could also provide us with a deeper insight in the function of the various regions of the sodium channel protein.

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OR MANY years, the pathomechanism of myotonia was exclusively associated with a defect of the skeletal muscle chloride conductance. Since Bryant's¹ first demonstration of the stunning similarities of the properties of muscles from myotonic goats with those of normal goat muscles bathed in low-chloride saline, it was shown that, indeed, the sarcolemmal chloride conductance is significantly reduced in skeletal muscles from myotonic goats² and in rats treated with the myotonia-inducing drug 20,25diazacholesterol.³ The finding of a reduced chloride conductance in muscles from myotonia congenita patients4 firmly established the "low-chloride conductance hypothesis of myotonia,"5 proved by molecular genetics for Thomsen and Becker myotonia congenita.6,7

And yet, for another classic human hereditary disorder associated with myotonia, Eulenburg's⁸ paramyotonia congenita, an electrophysiologic search for a reduced chloride conductance, did not lead to the expected finding.⁹ Rather, abnormalities of the sodium conductance were noted, yielding the first hints that myotonia is not necessarily connected with a reduced chloride conductance and that the sodium channels might be affected by genetic abnormalities to a degree that was not incompatible with life function, although it led to myotonialike symptoms.

The symptoms of paramyotonia congenita, cold-aggravated stiffness and weakness of the voluntary muscles, are sometimes associated with the symptoms of another rare hereditary muscle disease, hyperkalemic periodic paralysis. This disease, separated from the more common hypokalemic periodic paralysis by Gamstorp,¹⁰ was originally given the name *adynamia episodica hereditaria*. For quite some time it was listed in textbooks as a metabolic disease,¹¹ although early investigators postulated that in fact paramyotonia and hyperkalemic periodic paralysis were a nosologic entity.¹²

The possibility that the pathomechanism in hyperkalemic periodic paralysis might indeed be related to that of paramyotonia congenita, ie, that the defect in this disease is located in the skeletal muscle fiber membranes rather than being associated with one of the classic metabolic defects, led to the electrophysiologic investigation of the sarcolemmal component conductances in this disease.^{13,14}

From the Department of Physiology, University of Ulm (Germany). Indeed, even the muscles from hyperkalemic periodic paralysis patients that showed no signs of myotonia displayed abnormalities of the sodium currents that closely resembled those seen in paramyotonia congenita, while abnormalities of the chloride conductance were not seen.

When the gene encoding the α subunit of the sodium channel in adult skeletal muscle (SCN4A) was cloned, a new road was opened to test the electrophysiologic hints. The evidence for linkage of hyperkalemic periodic paralysis with SCN4A¹⁵ was the first proof for the existence of human sodium channel disease. Not much later, three groups showed independently that paramyotonia congenita is also linked to SCN4A.¹⁶⁻¹⁸ At the time of completion of this review (May 1993), no less than 13 mutations are known in SCN4A that lead to the group of diseases that we like to call the "adynamia-paramyotonia complex."¹⁹

At a recent workshop on nondystrophic myotonias and periodic paralyses, the term *sodium channel diseases* (sodium channelopathies) was officially used for the first time by an international consortium.²⁰ On the basis of extensive clinical studies, we suggest the following classification of this group: hyperkalemic and normokalemic periodic paralysis, paramyotonia congenita, and sodium channel myotonia (myotonia fluctuans).

All diseases belonging to this group are inherited as an autosomal dominant trait. Attacks of muscle weakness are the key symptom of hyperkalemic periodic paralysis. The key symptom of paramyotonia is cold- and work-induced muscle stiffness giving way to muscle weakness. Only with the advent of molecular biologic methods has it become clear that there is a third sodium channel disease and that patients having this disease had been mistaken for having myotonia congenita Thomsen. The feature that most distinguishes sodium



Figure 1. Cytoplasmic view of the sodium channel depicting the open state. The cytoplasmic loop between repeats III and IV is shown as a ball and chain. (Modified from Caldwell and Schaller.³³)

channel myotonia from chloride channel myotonia is a fluctuation of the degree of muscle stiffness. In contrast to hyperkalemic periodic paralysis and paramyotonia congenita, muscle weakness is not a remarkable feature, and there is no substantial cold-dependence of the symptoms.²¹

Although the symptoms overlap considerably in the sodium channel diseases, the above differentiation makes sense from a clinical point of view. Preventive measures are different for muscle stiffness and weakness.²² Only hyperkalemic periodic paralysis implies a possible prognosis of progressive permanent weakness. At any rate, the diseases of this group should be clearly separated from the chloride channel myotonias (Thomsen- and Becker-type myotonia congenita) and from myotonic dystrophy (Steinert's disease).

In this review, we will first characterize the human skeletal muscle sodium channel, then differentiate the sodium channel diseases. Finally, we describe the mutations found so far in the sodium channel gene and try to correlate them with their phenotypes.

THE STRUCTURE OF THE ADULT HUMAN SKELETAL MUSCLE SODIUM CHANNEL

As in all excitable cells, the fast depolarization of the membrane characterizing the upstroke of the action potential in human skeletal muscle fibers is mediated by the inflow of sodium from the extracellular to the intracellular space through special membrane-spanning proteins, the sodium channels. In fully differentiated human skeletal muscle, the tetrodotoxin (TTX)–sensitive adult channel is the only type expressed. This adult sodium channel consists of two subunits called α and β . The α subunit is the essential part that contains all characteristic properties including the ion-conducting pore (**Figure 1**). The functions of the β subunit are an increase of the current amplitude and an influence on the speed of inactivation, which is markedly increased by its presence.²³

The α subunit is a large, approximately 260 000–dalton polypeptide containing 1820 to 2020 amino acids and 25% to 30% carbohydrate by weight. The sequence shows four regions of internal homology each encompassing 225 to 325 amino acids. Each of these four so-called repeats (1 to IV) reveals six hydrophobic segments (S1 to 56), putative transmembrane helices located within highly conserved regions in each domain. The S4 helix contains a repeating motif with a positively charged amino acid at every third position. The high charge density of this helix suggests that it may function as a voltage sensor. The charges could, eg, shift in response to depolarization, thus playing an essential part in voltage-dependent activation of the channel.²⁴

Between segments S5 and S6 of each repeat, an interlinker is found consisting of an extracellular part and a sequence that dips into the membrane. As in the model of shakerrelated potassium channels, these four intramembrane loops are thought to form the lining of the channel pore. The orifice on the intracellular side of the pore or its surrounding protein parts may act as acceptor of the inactivation gate. The part of the protein most likely responsible for the inactivation of the channel was determined by enzymatic removal experiments. According to the "ball and chain" model first developed by Armstrong and Bezanilla²⁵ on the basis of electrophysiologic experiments, the inactivation gate is comparable to a ball attached to the channel by a chain on the inner side. In the closed state, the ball is away from the pore, and, on activation of the channel, the ball swings into the mouth to inactivate the pore. Mutation experiments on neuronal rat sodium channels expressed in *Xenopus* oocytes provided evidence for the cytoplasmic region between repeats III and IV being critical for inactivation, suggesting that the essential amino acid that might exert the "ball" function is phenylalanine,²⁶ which corresponds to phenylalanine 1311 of the human protein.

High conservation of the transmembrane segments and the intracellular loop between repeats III and IV of the channel protein suggests that the exact sequence of amino acids is essential for the proper function of the channel.

The location of the gene for the human α subunit was accomplished by homology screening.¹⁵ The gene contains 24 exons distributed over about 30 kilobases of chromosome 17q23. As with many genes, the genomic structure becomes more condensed toward the 3' end, with at least 30% of the coding sequence appearing in a single exon.²⁷⁻²⁹ Intron-exon boundaries are known; primer sets consisting of intron sequences for amplification of all 24 exons by use of polymerase chain reaction are available.³⁰

The expression of the human sodium channel α subunit in *Xenopus* oocytes has been successful but the expression system was not satisfactory because of an abnormally slow inactivation of the normal channel. When transfected into mammalian cells, eg, human embryonic kidney cells (HEK), inactivation was normal.^{31,32}

Data on other human sodium channel genes are available. About 10 different, but closely related, sodium channel α subunit genes are known, most of them expressed in the brain, peripheral nerves, and muscle.^{27,33} While hSkM1, the TTX-sensitive SCN4A product, is only expressed in adult human skeletal muscle, another two distantly related α subunits, both with low TTX sensitivity, were found in fetal and denervated skeletal muscle and in adult cardiac muscle (hH1) as well as in myometrium and in fetal skeletal muscle (hH2).^{34,35} No diseases have so far been linked to any so-dium channel gene other than to SCN4A.

CLINICAL FEATURES OF THE SODIUM CHANNEL DISEASES

Familial hyperkalemic periodic paralysis was the first disease for which linkage to SCN4A was shown.^{15,18,36,37} The disease clinically resembles primary hypokalemic periodic paralysis, although the latter is not linked to the sodium channel α subunit gene.³⁸ In contrast to hypokalemic periodic paralysis, the serum potassium rises rather than falls during an attack. The disease is transmitted with complete penetrance in both sexes, although incomplete penetrance was reported for two rare mutations.^{20,39} Sporadic cases have also been reported,^{40,41} and the real existence of a de novo mutation was proven in a patient whose genetically confirmed father and mother did not carry the defective gene.⁴²

As described in many reviews, attacks of generalized weakness usually begin to occur in the first decade of life. They commonly start in the morning and last 15 minutes to an hour, then disappear spontaneously. Immobility or intake of potassium, eg, during a provocative test, may provoke an attack of weakness, and prior strenuous work aggravates the attack. The generalized weakness is usually accompanied by a significant increase in the serum potassium concentration (up to 5 to 7 mmol/L). Sometimes, the level of serum potassium remains within the upper range of normal, and only rarely rises so high that the function of the heart is impaired. The recovery can be improved by moderate exercise. Slight weakness may continue for several days. Between attacks, the serum potassium concentration is normal. Usually the frequency of attacks declines during the second half of life.

Electromyography reveals the existence of a variant with myotonia and a variant in which not only clinical but also electrical myotonia is absent.^{13,43,44} There is also a paramyotonic variant of hyperkalemic periodic paralysis, a condition also called *paralysis periodica paramyotonica*.^{45,46} In some patients, a chronic progressive myopathy⁴⁷ may develop that seems to be genetically determined. It might be specifically linked to the most common mutation.^{48,49}

Naturally occurring animal models are the hyperkalemic dog⁵⁰ and quarter horse.^{51,52} As in man, hyperkalemic periodic paralysis in the horse is caused by a sustained membrane depolarization.⁵³ Genetic and molecular biologic data suggest an identical pathogenesis.⁵⁴

The very rare condition of *normokalemic periodic paraly*sis resembles hyperkalemic periodic paralysis in many respects, but differs from it in that even during serious attacks of paralysis the serum potassium level is not increased. Its existence as a nosologic entity of its own was questioned because some patients with normokalemic periodic paralysis are sensitive to oral potassium salts.⁵⁵ Large doses of sodium improve the weakness, but glucose administration has no effect. There are no consistent changes in serum electrolytes, but increased sodium excretion and potassium retention occur during the attacks. The urinary potassium retention, the lack of a beneficial effect of glucose, and failure of the serum potassium level to increase in attacks distinguish this disease from primary hyperkalemic periodic paralysis.

The resistance to potassium loading distinguishes another kinship⁵⁶ from the first one investigated by Poskanzer and Kerr.⁵⁵ Thus heterogeneity may exist for this condition.

Paramyotonia congenita is transmitted with complete penetrance. Linkage to SCN4A has been established.¹⁶⁻¹⁸ The hallmarks of the disease, as first described by Eulenburg⁸ and independently by Rich,⁵⁷ and later specified in a large number of families by Becker,⁵⁸ are (1) paradoxical myotonia, ie, a muscle stiffness that appears during muscle exercise and

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increases with continued exercise; (2) severe aggravation of this exercise-induced stiffness by cold; (3) a predilection of the myotonia for face, neck, and the long muscles of the hands; and (4) in most cases, weakness after protracted exercise and exposure to cold. Some paramyotonia patients also show attacks of hyperkalemic muscle weakness (paramyotonic periodic paralysis). This condition does not seem specifically related to a particular mutation; as in large paramyotonia families it is always encountered in one or the other patient.

Paramyotonic symptoms are present at birth and remain basically unchanged for the entire lifetime, though the attacks of weakness and hyperkalemia begin to appear in adolescence, if at all. In the cold, the face may appear masklike, and the eyes cannot be opened for several seconds. Working in the cold makes the fingers so stiff that the patient is unable to move them within a few minutes. The stiffness quickly gives way to weakness. After warming, the hands may not regain strength for several hours. As a rule, the legs are less affected. Under warm conditions many patients have no complaints. Muscle pain as well as muscle atrophy or hypertrophy are not typical for the disease.

Sodium channel myotonia, as mentioned in the introductory section, is a relatively new classification.⁵⁹ Becker⁴⁶ had investigated many families with nondystrophic dominant myotonia and proposed many subtypes for what he thought was myotonia congenita. Reinvestigation of a number of these families revealed that these "subtypes" were, in fact, paramyotonia, but not the classic type characterized by cold-induced weakness, but by cold- and exercise-induced stiffness (M. C. Koch, MD, and K.R., unpublished observation, 1993). Other families supposed to having myotonia congenita had an unusual degree of fluctuation in the tendency to experience muscle stiffness. Afflicted persons never experienced muscle weakness. Detailed testing revealed that they were also not substantially sensitive to cold in regard to muscle stiffness.

Their muscle stiffness was sometimes provoked by exercise but more often during rest after heavy exercise. The stiffness would then last 30 minutes to 2 hours. On many days or even for weeks they experienced no muscle stiffness at all. Ingestion of potassium sometimes caused severe myotonia but no weakness. Because of these features, the condition was named myotonia fluctuans.^{21,60} Meanwhile, four such families have been analyzed by us on the molecular biologic level, three of them showing the same mutation on SCN4A resulting in a substitution of alanine for glycine 1306. The fourth family, with identical clinical features, had another mutation on SCN4A.

Another substitution for glycine 1306 results in a similar condition in which the muscle stiffness is permanently much more severe than in myotonia fluctuans. The electromyogram reveals continuous myotonic activity. Two such nonrelated patients were found to have the same mutation on SCN4A. In one of them, a young boy, the myotonia was so unusually severe that the diagnosis of Schwartz-Jampel syndrome had been suggested.⁶¹ The other patient had experienced most severe attacks of generalized myotonic stiffness that impaired her breathing, before treatment with tocainide relieved her successfully. Because of the persisting myotonia, the disease was described as "permanent myotonia."59

A third substitution for glycine 1306 causes paradoxic myotonia with little cold aggravation. In a German family presenting with this condition, stiffness was aggravated by oral intake of potassium but not by cooling. Muscle weakness never occurred.⁵⁹ Of two American families with similar symptoms, only one had myotonia aggravated by cold.62 In yet another condition linked to SCN4A, the mutation awaits detection.63,64 Here, muscle stiffness occurs even in a warm environment and, in addition, muscle pain is induced by exercise. The stiffness is not induced or aggravated by oral potassium, but both stiffness and pain are reduced by acetazolamide, hence the name atypical (acetazolamide-responsive) myotonia.64,65

PATHOMECHANISMS

Patch clamp studies of sodium channels in native muscle preparations, cultured cells, and expression systems revealed an increased number of openings and/or an increase in the time constant of fast inactivation, no matter whether the patient's diagnosis was hyperkalemic periodic paralysis, paramyotonia congenita, or sodium channel myotonia.31,32,59,66,67 The shift in the inactivation pattern toward a more frequent occurrence of late sodium channel openings explained the sustained membrane depolarization and the hyperexcitability of the muscle fiber membrane discovered in many earlier electrophysiologic tests,9,13,14,68 although it should be mentioned that similar alterations in the inactivation of the sodium channels have also been detected in various myotonic diseases where the mutation is clearly not in SCN4A.69

Microelectrode recordings of the membrane potential mirrorimaged the different clinical features in vitro in that the depolarization could be most readily elicited by cooling when the muscle was from a paramyotonia patient and by increased extracellular potassium when the muscle was from a patient having hyperkalemic periodic paralysis. A possible explanation for the long-lasting depolarization in hyperkalemic periodic paralysis could be the following. Since in the patients one of the SCN4A alleles is mutated and the other is normal, two kinds of sodium channels are expressed in their muscle fiber membranes, one that inactivates (ie, closes) normally, and one that fails to inactivate properly. The physiologic effect of hyperkalemia induced by potassium intake or by work is a slight depolarization of the muscle fiber membrane. In the defective muscle, this depolarization causes the abnormally inactivating sodium channels to open. 14,66,67 Sodium ions continue to enter the muscle fibers through the noninactivating channels, thus reinforcing and sustaining the depolarization. This, in turn, causes inactivation of the normally functioning sodium channels (ie, those expressed by the normal gene), leading to inexcitability of the muscle fibers, which is the immediate reason for the paralysis.14,70 In association with this influx of sodium, water is shifted into the muscle fibers. This causes hemoconcentration, and the increase in the serum potassium concentration that occurs will also affect muscle fi-

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Genotype-Phenotype Correlation in Patients With a Muscle Sodium Channel Disease*					
Genotype	Channel Part	Predicted Substitution	Exon No.	Phenotype	Reference No
TRANSPORT		Dis	8ase		
C2188T	liss,	Thr704Met	13	mHyperPP myopathy nmHyperPP myopathy	37, 71 49
C2411T	lis6	Ser804Phe	14	SCM	39, 76
G3466A	(IIIS4/5),	Ala1156Thr	19	HyperPP	39
G3917T	(111/17);	Gly1306Val	22	SCM	59, 62
G3917A	(111/17)	Gly1306Glu	22	SCM	59
G3917C	(III/IV),	Gly1306Ala	22	SCM	59
C3938T	(III/IV)	Thr1313Met	22	PC	62
A4078G	IVS1	Met1360Val	23	HyperPP	20
T4298G	IVS3	Leu1433Arg	24	PC	72
C4342T	IVS4	Arg1448Cys	24	PC	74
C4343A	IVS4	Arg1448His	24	PC	74
G4765A	IVS6	Val1589Met	24	. SCM	75
A4774G	IVS6 _i	Met1592Val	24	HyperPP	42
		Polym	orphism		
607A/G	IS3	203Met/Val	4	Normal	11
2578G/A	(II/III),	860Glu/Lys	14	Normal	11
2580G/T	(11/111),	860Glu/Asp	14	Normal	31
2794G/C	(11/111) _i	932Asp/His	14	Normal	24
4126G/A	IVS1/S2,	1376/Asp/Asn	23	Normal	12
4817A/G	IVS6	1606Glu/Gly	24	Normal	30

*I to IV indicates number of sodium channel repeats; S1 to S6, number of the transmembrane segment within each repeat; i, intracellular loop or inactivation-gate acceptor at the intracellular pore lining; e, extracellular loop; PC, paramyotonia congenita; HyperPP, hyperkalemic periodic paralysis; mHyperPP, myotonic HyperPP; nmHyperPP, nonmyotonic HyperPP; SCM, sodium channel myotonia; Thr, threonine; Met, methionine; Ser, serine; Phe, phenylalanine; Ala, alamine; Gly, glycine; Val, valine; Glu, glutamine, Leu, leucine; Arg, arginine, Cys, cysteine; His, histidine; Asp, aspartic acid; and Asn, asparagine.

bers that had high resting potential. This, then, may result in paralysis of the whole muscle. It is most likely ended by increased excretion of potassium by the kidneys. Furthermore, the sodium-potassium pump may be maximally activated by the increased concentration of intracellular sodium and extracellular potassium. When serum potassium returns to normal values, the defective sodium channels are likely to close allowing the membrane to repolarize and become excitable again.

In paramyotonia and in sodium channel myotonia, the mechanism must be rather similar. The opposite clinical signs of muscle stiffness and/or paralysis are determined by a relatively small difference in the membrane potential, as a small permanent sarcolemmal depolarization may cause hyperexcitability, and a moderate depolarization may cause inexcitability. Obviously, the variability of signs between family members is not only determined by the altered channel function but also by a number of accidental physiologic conditions.

MUTATIONS OF THE SCN4A GENE AND THEIR CLINICAL PHENOTYPES

The number of different mutations detected in SCN4A has increased to 13 (**Table**) since 1991, and more detections can be expected to follow. In addition, six polymorphisms of SCN4A were found as a by-product of the search for mutations in affected families (Table). Four of the 13 mutations comprise families with hyperkalemic periodic paralysis, four of the mutations lead to paramyotonia congenita, and five other ones to sodium channel myotonias. The various mutations causing a particular disease are not distributed over the gene in a predictable or easily understandable regular manner (**Figure 2**). Obviously, not only the site of replacement but also the properties of the replaced and the replacing amino acids play a very important role. This is best seen in amino acid 1306, where three known replacements lead to three different clinical pictures.

The mentioned first detected mutation⁴² is second in frequency of all *hyperkalemic periodic paralysis* families.^{71,72} It causes an A4774G transversion predicting replacement of a neutral hydrophobic amino acid by another neutral hydrophobic amino acid (Met1592Val) in the membrane-spanning segment S6 of repeat IV. As a consistent finding, it results in the form of hyperkalemic periodic paralysis that is associated with myotonia and does not lead to permanent weakness. The site of the change of the amino acids is close to the intracellular part of the S5/S6 interlinker, a region hypothesized to act as acceptor of the inactivation gate. It is therefore conceivable that the structural change alters the inactivation of the channel, in agreement with the electrophysiologic findings.

The most frequent mutation leading to hyperkalemic periodic paralysis was found in three families presenting myotonic signs and chronically progressing myopathy⁴⁸ and in all four investigated families with the nonmyotonic form that also disposes to permanent weakness.⁴⁹ It leads to replacement of the polar amino acid threonine 704 by the hydrophobic amino acid methionine in segment S5 of repeat II.

Again, the changed amino acids are close to the intracellular part of another S5/S6 interlinker, also belonging to the hypothesized acceptor region of the inactivation gate. Both the A4774G and the C2188T mutations are situated in regions of the gene that are highly conserved in evolution, and the relatively mild clinical consequences may be explained by the fact that the properties of the replacing amino acids are very similar to those of the amino acids they replace.

A third mutation concerns an interlinker directly. The mutation G3466A, found so far in only one family, causes hyperkalemic periodic paralysis with incomplete penetrance. The mutation results in the replacement Ala1156Thr in the S4/S5 interlinker of repeat III.³⁹

In contrast to the first three mutations, a fourth mutation, A4078G, is not closely related to the interlinker regions. It results in the replacement Met1360Val in segment S1 of repeat IV. In the affected family, clinical manifestation occurred only in one male subject. Females carrying the mutation never experienced attacks of weakness, although the finding of electrical myotonia in the electromyogram supported the presence of defective sodium channels.²⁰

An interesting, as yet not explained, finding is that a family that was convincingly diagnosed as having hyperkalemic periodic paralysis did not show linkage to SCN4A, and sequencing of the cDNA encoding the α subunit of the sodium channel revealed no mutation. A possible explanation is genetic heterogeneity.⁷³

Only one family with the clinical diagnosis of *normokalemic periodic paralysis* was subject to molecular biologic studies so far. Interestingly, the common Thr704Met substitution that was found⁴⁹ lead to hyperkalemic periodic paralysis in other families. This finding suggests that the disease is not a nosologic entity but a variant of the hyperkalemic form caused by additional factors not yet understood. A molecular biologic study of the original family described by Poskanzer and Kerr⁵⁵ should clarify this case.

It seems worth mentioning that the peculiar disease of

periodic paralysis in quarter horses is caused by yet another mutation located in the homologous horse gene.⁵⁴ The mutation causes the replacement Phe1421Leu in segment S2 of repeat IV, not very far from the Leu1433Arg replacement that causes paramyotonia congenita in man. The horse disease is associated with myotonia, and the persisting muscle activity gives rise to a desirable muscle hypertrophy. For this reason, the mutated gene was inadvertently disseminated by breeders so that up to 5% of this popular breed (3 000 000 currently registered in the United States) are affected.

In all families having paramyotonia congenita that were studied, point mutations were detected in fairly different parts of SCN4A: (1) a C3938T transit causing a substitution of threonine 1313 by methionine was discovered in the gene region encoding the cytoplasmic loop between repeats III and IV that is supposed to act as the inactivation gate.^{24,26} In the five families with this mutation, affected persons suffer from muscle stiffness aggravated by cold. 62,72 (2) Two mutations at the adjacent nucleotide sites 4342/4343 were detected in a gene region encoding the transmembrane segment S4 of repeat IV. The segments S4 are supposed to act as voltage sensors for the mechanism of channel activation.²⁴ One of the mutations causes a CGT \rightarrow TGT transversion that predicts a substitution of arginine 1448 by cysteine.⁷⁴ This mutation, in which a positive charge is replaced by a neutral polar amino acid, was found in a family in which affected members suffer from cold-induced stiffness (without weakness) and spontaneous episodic attacks of weakness. At the same codon, a CGT \rightarrow CAT exchange predicting a replacement of arginine 1448 by the weakly positive histidine was found in two families having cold-induced and potassiuminduced stiffness combined with spontaneous episodic attacks of weakness.72,74 Since electrophysiology has shown that inactivation is altered in paramyotonia congenita, it is likely that this S4 segment is not only involved in channel



Figure 2. Scheme of the primary structure of the α subunit of the adult skeletal muscle sodium channel consisting of four repeats that contain six transmembrane segments each. Amino acids are shown for which substitutes were deduced from point mutations found in hyperkalemic periodic paralysis, paramyotonia congentia, and in sodium channel myotonia (T indicates threonine 704; S, serine 804; A, alanine 1156; G, glycine 1306; T, threonine 1313; M, methionine 1360 and 1592; L, leucine 1433; R, arginine 1448; and V, valine 1589).

activation but also inactivation. Comparable mutations that also neutralize S4 charges have been generated by site-directed mutagenesis of potassium channel genes, and experiments with products from such mutated genes expressed in *Xenopus* oocytes indeed indicate coupling between channel activation and inactivation. (3) Another point mutation was discovered in a typical paramyotonia congenita family characterized by muscle stiffness exacerbated in the cold, the stiffness giving way to flaccid paralysis when the cooled muscles were exercised. The transversion T4298G predicts an amino acid substitute arginine for leucine 1433 located in segment S3 of repeat IV.⁷²

As to sodium channel myotonia, investigation of a family characterized by cold- and potassium-induced stiffness as well as by absence of weakness showed a G4765A transversion predicting the substitution Val1589Met in segment S6 of repeat IV.⁷⁵ Interestingly, this substitution is located right next to the helical position of the vice-versa substitution Met1592Val, the latter causing hyperkalemic periodic paralysis.⁴²

Two different mutation loci, both resulting in amino acid replacements in intracellular loops, have also been described as being responsible for sodium channel myotonia. One of these loci is closely located to the Thr1313Met substitution in the cytoplasmic loop between repeats III and IV that leads to paramyotonia congenita. It is particularly remarkable because three different point mutations were discovered leading to three different replacements for one and the same nucleotide. These mutations result in different amino acid substitutes for one (1306) of a pair of glycines 1306/07 that are supposed to render the flexibility of the inactivation gate because of their lack of side chains. Length, ramification, and charge of the side chains in the substitutes correlate well to both the degree of membrane hyperexcitability and the clinical phenotype: glutamic acid, ie, an amino acid with a long side chain, was the substitute in two sporadic patients with permanent myotonia, the most severe form of sodium channel myotonia^{59,61}; valine, an amino acid with a side chain of intermediate size, was the substitute in patients with moderate exercise-induced myotonia^{59,62}; and alanine, distinguished by a short side chain, resulted in myotonia fluctuans, one of the most moderate forms of sodium channel disease. The latter substitution was found in three families. In a family presenting myotonia fluctuans, a C2411T transversion was found resulting in a substitution of serine 804 by threonine.⁷⁶ Another family with this same mutation was diagnosed as having paramyotonia congenita, 39 but from the clinical signs reported we would not exclude the diagnosis of myotonia fluctuans.

CONCLUSIONS

Molecular biology has contributed much to our understanding of the variability of the clinical signs among the diseases belonging to the group of myotonias and periodic paralyses. The existence of defects in the skeletal muscle sodium channels, predicted by electrophysiology, was proven, and it has become clear that allelic mutations in SCN4A cause the clinically defined symptoms of hyperkalemic paralysis and paramyotonia. A major breakthrough in our understanding of rare phenotypes was generated by molecular biology by evidencing the existence of sodium channel myotonia. This condition resembles the chloride channel disease of myotonia congenita so much that previously it was mistaken for it.

Apart from enlarging our understanding of the variations in these diseases, further detailed correlations of the clinical symptoms with the naturally occurring mutations may provide us with a deeper understanding of the function of the various regions of the sodium channel protein than can ever be obtained from established biological methods, ie, site-directed mutagenesis with subsequent study of the gene products in expression systems.

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Reprint requests to Abteilung für Allgemeine Physiologie der Universität Ulm, HD-89060, Ulm, Germany (Dr Rüdel).

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