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Channelopathies: Their Contribution to Our Knowledge About Voltage-Gated Ion Channels

Frank Lehmann-Horn and Reinhardt Rüdel

Since 1990, many mutations in genes encoding voltage-dependent sodium, potassium, calcium, and chloride channels have been discovered to cause disorders of heart, skeletal muscle, brain, or kidney. Study of the defective gene products has furthered our knowledge not only of pathology but also of ion-channel function.

Excitation of nerve and muscle cells is mediated by voltage-gated ion channels, proteins that may conduct sodium, calcium, potassium, or chloride ions across the cell membrane. Most of these channels are characterized by a high selectivity for one of the ion species. The channels can assume either of three major conformational states, closed, open, or inactivated, and usually respond to membrane depolarization by a transition from the closed to an open state

followed by an intrinsic inactivation. Channel activation and inactivation are the basis of the action potential, the signal that allows the cells to conduct information along nerve axons and muscle fibers. Nonexcitable cells may also exert their specific function by voltage-gated ion channels. For instance, in the tubular cells of the distal nephron of the kidney, secretion is controlled by chloride channels that react to slow alterations of the resting potential.

Most of our knowledge about the conformational changes that lead to the transitions between the different states stems from electrophysiological evidence gathered from current

"Channel activation and inactivation are the basis of the action potential. . . ."

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TABLE 1. Human ion-channel diseases

Sodium channelopathies
  Skeletal muscle sodium channel (Skm-1)
    Hyperkalemic periodic paralysis
    Paramyotonia congenita
    Potassium-aggravated myotonia
  Heart muscle sodium channel (hH1)
    Long Q-T syndrome type 3
Potassium channelopathies
  Heart muscle potassium channels (KVLQT1, HERG)
    Long Q-T syndrome type 1
    Long Q-T syndrome type 2
  Neuronal potassium channel (KCNA1)
    Episodic ataxia with myokymia
Calcium channelopathies
  Skeletal muscle L-type calcium channel
     (dihydropyridine receptor)
    Hypokalemic periodic paralysis
P/Q-type calcium channel
  Episodic ataxia
  Familial hemiplegic migraine
  Spinocerebellar ataxia
Chloride channelopathies
  Skeletal muscle chloride channel (ClC-1)
    Dominant myotonia congenita (Thomsen type)
    Recessive myotonia congenita (Becker type)
  Kidney chloride channel (ClC-5)
    Hereditary nephrocalcinosis

". . . sodium channels . . . are composed of two or three subunits of which the α-subunit constitutes the major functional part. . . ."

measurements obtained in both the whole cell and single-channel modes of voltage clamping. When the corresponding cDNA is cloned and sequenced, such experiments are nowadays carried out with the channels expressed in a heterologous expression system, such as *Xenopus* oocytes or human embryonic kidney cells.

Understanding of channel structure-function relationships was furthered greatly by engineered mutagenesis, the aimed exchange of certain amino acids in the channel protein, and investigation of the alterations that such a replacement effects. Usually, such studies are performed with a specific preconception of the experimenter as to what the interesting function of a certain protein domain may be. Completely unexpected corrections of these preconceptions are sometimes provided by "experiments of nature," namely, naturally occurring mutations in channel genes that are not lethal but lead to hereditary diseases. The growing number of rare diseases that are recognized as being caused by mutations in genes encoding voltage-gated ion channels has recently been designated as "channelopathies" (7).

The desire to understand the pathomechanism of these diseases (listed in Table 1) has indeed spurred many scientists to find new ion-channel genes and to understand the function of their products. A whole group of ion channels, the ClC family of voltage-gated chloride channels, would perhaps still be unknown (13) had not the

pathology of the symptom of myotonia (a transient stiffness in skeletal muscles occurring after muscle rest, as in the classic disease of myotonia congenita or "Thomsen's disease") challenged pharmacologists, physiologists, and molecular biologists to search for the substrate of the rather high resting chloride conductance of the sarcolemma that has long been known to be substantially reduced in Thomsen's disease.

Three clinical variants of skeletal muscle sodium channelopathies shed new light on channel inactivation

The first myotonic disease, as a matter of fact, the first hereditary disease recognized as being caused by natural mutations affecting voltage-gated ion channels, was, however, not classic myotonia congenita. Before Thomsen's disease was established as a chloride channelopathy in 1992 (9), disease-causing mutations were discovered in the gene encoding the muscle sodium channel.

The channels conducting the sodium current during the action potential are surprisingly tissue specific, i.e., a whole family of closely related genes exists that code specifically for the sodium channels in nerve, skeletal muscle, and heart. These different sodium channels have in common that they are composed of two or three subunits of which the α-subunit constitutes the major functional part; it contains the pore, the voltage sensor, and the gates. All disease-causing sodium-channel mutations concern α-subunit genes.

Before the gene encoding the α-subunit of the human adult skeletal muscle sodium channel, *SCN4A*, was cloned, an extensive electrophysiological survey, carried out with excised muscle specimens of all kinds from myotonia patients, had suggested that in two rare hereditary conditions the function of the sodium channels might be defective (10). These diseases were hyperkalemic periodic paralysis with myotonia, characterized by episodes of muscle weakness that in severe cases might develop into a fullfledged generalized paralysis, and paramyotonia congenita, characterized by a very unpleasant stiffening of the muscles occurring whenever they have to do work in the cold. Studies of large families quickly revealed that both diseases are indeed linked to *SCN4A* and that the different symptoms are caused by different mutations in the same gene (see Ref. 7).

These findings caused the experts to summon all their known affected families for confirmation and search of possible further mutations in *SCN4A*. Nineteen disease-causing amino acid

changes have since been identified (Fig. 1A), and, unexpectedly, a new nosological entity was revealed during this search. The new disease, called potassium-aggravated myotonia, is characterized by muscle stiffness that is most prominent when the muscles are used after a period of rest (12). It is thus symptomatically very similar to classic myotonia congenita. Most patients who were recognized as having the new disease had, in fact, been misdiagnosed before as having "peculiar forms" of Thomsen's myotonia. Clinically, the major difference of what is now distinguished as a form of sodium-channel myotonia from the latter is that the muscle stiffness is very much aggravated when the patients ingest potassium-rich food. Pathogenetically, the two diseases differ by the mutated gene (SCN4A vs. CLCN1).

The α-subunit of the sodium-channel protein is known to be composed of four very similar domains (called repeats I–IV) that are connected by intracellular amino acid chains ("linkers"). Each repeat contains six membrane-spanning segments (S1 to S6) that are connected, in turn, by extracellular and intracellular "interlinkers." Certain functions have been ascribed to the various parts of the channel protein. The extracellular interlinkers between segments S5 and S6 of all four repeats are thought to constitute the lining of the pore, whereas the intracellular linker between repeats III and IV is supposed to mediate inactivation. In each repeat, segment S4 contains sequences of charged residues. These segments could thus act as voltage sensors and were consequently supposed to be mainly involved in channel activation (3).

The above-mentioned electrophysiological studies on excised muscle specimens of patients having paramyotonia congenita or hyperkalemic periodic paralysis (10) had already revealed the incomplete inactivation of sodium currents as the pathogenetic basis of the two apparently opposite symptoms of these diseases. A persistent, tetrodotoxin-sensitive sodium current leads to either small membrane depolarization and generation of repetitive action potentials, i.e., myotonia, or, if larger, to severe membrane depolarization causing inexcitability, i.e., paralysis. Heterologous expression of mutant human sodium-channel cDNA in human embryonic kidney cells and patch-clamp studies of the resulting currents now confirmed these early results and allowed us to further specify the alterations that the various natural mutations effect in channel inactivation. In general, a combination of several features of channel inactivation is changed, such as the speed of current decay after a depolarization step, the

current fraction that persists after the decay, the speed of the recovery from inactivation, the position of the steady-state inactivation curve, and/or the degree of uncoupling of inactivation from activation.

As it turned out, 14 of the 19 substitutions are situated in the "inactivating" linker between repeats III and IV ($n = 5$) or in repeat IV ($n = 9$), in particular, in the "voltage-sensing" segment S4 ($n = 3$). What was learned from these substitutions about channel function? Three of the mutations in the III/IV linker (Gly-1306-Glu, Ala,Val) effect different amino acid substitutions for one of a pair of glycines proposed to act as the "hinge" for the inactivation gate. They all cause potassium-aggravated myotonia, and, interestingly, the more the substituting amino acid differs from the physiological Gly-1306 (longer side chains and/or greater charge), the more enhanced is the membrane excitability and the more severe are the clinical symptoms. Glutamic acid, an amino acid with a long side chain, causes permanent myotonia, the most severe form of the disease; valine, having a side chain of intermediate size, causes moderate exercise-induced myotonia; alanine, with a short side chain, results in a benign, often "subclinical" form of myotonia. Thus the natural mutations affecting Gly-1306 confirmed our notion of the III/IV interlinker being involved in channel inactivation and provided evidence that increased rigidity of the amino acid chain at the position of the highly conserved pair of glycines increasingly hampers channel inactivation (12). Unexpectedly, in addition to channel inactivation, mutations at this hinge site also altered activation and deactivation.
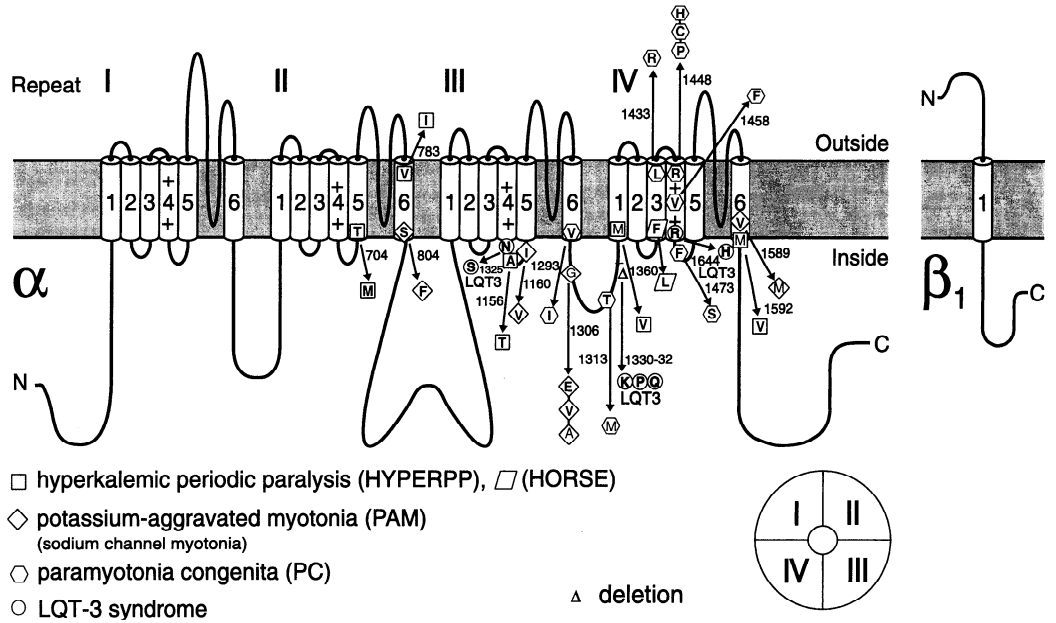
A similar correlation between the difference in physiological and mutant amino acid on one side and the severity of clinical symptoms on the other exists for three paramyotonia-causing substitutions located in an identical position (Arg-1448-Cys,His,Pro) near the extracellular face of IVS4. This finding led to the systematic application of site-directed mutagenesis in this supposed channel-activation domain. The mutations primarily affected channel inactivation (4). Therefore, it was hypothesized that depolarization-induced movements in IVS4 concern both the inactivation gate and the docking site for the inactivation particle (15).

An elegant method was used to probe the charged amino acids in segment IVS4 for their accessibility from the intra- or extracellular space. The amino acids were replaced by cysteines, and the access to these was then tested by specific cysteine-modifying reagents. Reduction of the sodium current by the reagent revealed

> ". . . in addition to channel inactivation, mutations at this hinge site also altered activation and deactivation."
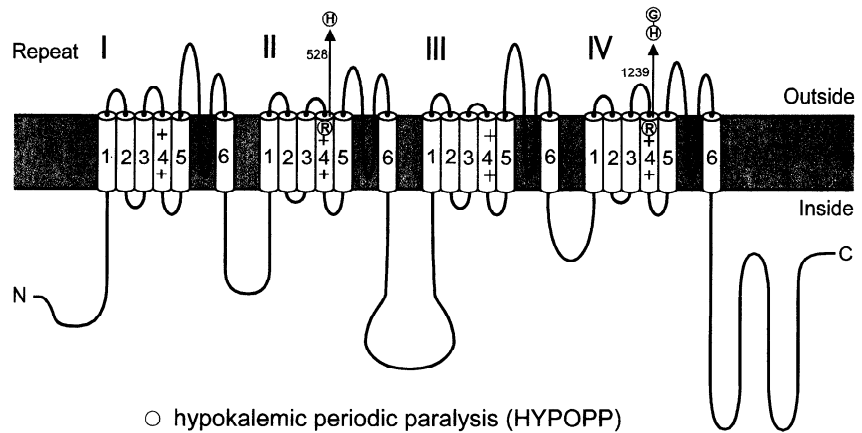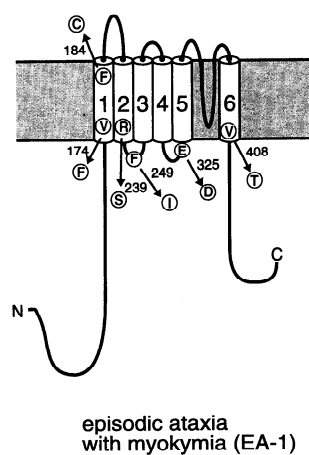
A

Muscle Na⁺ channel α-subunit (hSkm-1, hH-1)

☐ hyperkalemic periodic paralysis (HYPERPP), ⬜ (HORSE)

◇ potassium-aggravated myotonia (PAM)
(sodium channel myotonia)

◯ paramyotonia congenita (PC)

○ LQT-3 syndrome

△ deletion

B

Skeletal muscle Ca²⁺ channel α1-subunit

○ hypokalemic periodic paralysis (HYPOPP)

C

Neuronal K⁺ channel Kv1.1

episodic ataxia
with myokymia (EA-1)

Cardiac K⁺ channel KVLQT1

LQT-1 syndrome
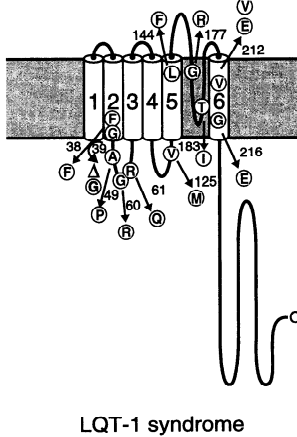
Cardiac K⁺ channel HERG

LQT-2 syndrome
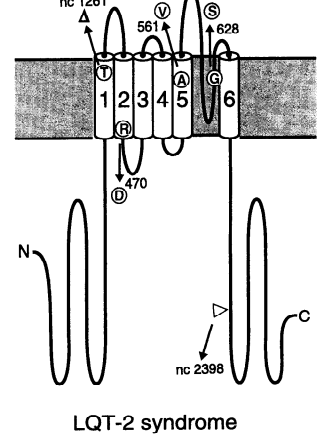
△ splice
▲ deletion

that two of the charged residues in IVS4 and presumably also the hydrophobic residues between them move from a position that is accessible from the intracellular space to one that is accessible from the outside (15).

Heart diseases trigger the search for cardiac ion-channel genes

The first heart diseases to be recognized as cardiac muscle channelopathies are the various forms of long Q-T syndrome (LQT) in which, as the name suggests, patients present with a prolonged Q-T interval of the electrocardiogram. The plateau of the cardiac action potential is physiologically maintained by a delicate balance between inward (sodium and calcium) and outward (potassium) currents. Membrane repolarization begins when outward current prevails over the inward current. Sustained inward current or reduced outward current may prolong the action potential and thus increase the Q-T interval.

LQT is rare and is a serious condition because it may lead to sudden death when defective membrane repolarization results in ventricular arrhythmia. The different types of LQT stem from genetic heterogeneity, most of them being inherited as autosomal dominant traits. Three gene loci have been identified for these dominant forms by the use of candidate gene approach.

Already in 1991, the gene responsible for the most common (50–60% of cases) LQT type 1 (LQT1) was mapped to the short arm of chromosome 11. Positional cloning revealed that the mutated gene, termed *KVLQT1*, has 30% identity with the *Shaker* potassium-channel gene (14). It encodes a potassium channel with six putative membrane-spanning regions that are most likely involved in cardiac repolarization. Point mutations were discovered in the intracellular loop between segments S4 and S5, the supposed acceptor for the inactivation particle, and in the sixth transmembrane domain (Fig. 1*B*).

LQT type 2 (LQT2) is also a potassium channelopathy (Fig. 1*B*). *HERG*, the mutated gene, is located on chromosome 7q35-36 and is related to a *Drosophila* gene with the funny name *ether-a-go-go*. The *HERG* product is a voltage-gated potassium channel that is also involved in cardiac repolarization. Functional expression of *HERG* in *Xenopus* oocytes demonstrated that the channel has properties that resemble the delayed rectifier potassium channel in cardiac myocytes (5).

Finally, the most rare form, LQT type 3 (LQT3), was found to be a sodium channelopathy. The mutated gene *SCN5A*, located on chromosome 3p21-24, codes for the α-subunit of the major cardiac sodium channel (hH1). Analysis on the molecular level revealed mutations in domains that are homologous to those found mutated in skeletal muscle channelopathies: a deletion of three highly conserved amino acids (Lys, Pro, Gln) in the cytoplasmic linker between repeats III and IV, the inactivation gate, as well as point mutations in the interlinkers between segments 4 and 5 of repeats III and IV (Fig. 1*A*). Expression of the mutated gene in *Xenopus* oocytes revealed that the mutant channels conduct a persistent inward current that would explain the prolonged plateau phase of the cardiac action potential (2).

With LQT now being more or less understood at the molecular level, there should be rapid progress in its treatment. Preliminary results suggest the use of sodium-channel blockers that have also proven successful in the management of muscle sodium channelopathies such as paramyotonia congenita.

Mutant neuronal potassium channels were found responsible for ataxia and myokymia

Mutations in potassium-channel genes concern not only the heart but also the nervous system. The first neuronal channelopathy to be detected was episodic ataxia with myokymia. This autosomal dominant disease is characterized by episodes of failing excitation of cerebellar neurons and by permanent hyperexcitability of the second motoneurons. The latter feature results in myokymia, the sustained twitching of motor units. The symptom responds to anticonvulsants such as carbamazepine. Exercise may provoke brief attacks of atactic gait, and this can be prevented by acetazolamide.

The disease is linked to *Kv1.1* (located on chromosome 12p13), the first of seven *Shaker*-

"LQT . . . may lead to sudden death when defective membrane repolarization results in ventricular arrhythmia."

FIGURE 1. Disease-causing amino acid substitutions in voltage-gated cation channels. Channels are structurally homologous in that they are all either made up of 4 (I–IV) similar repeating domains of 6 membrane-spanning segments (1–6), as in the case of skeletal (hSkm-1) and cardiac muscle (hH-1) Na^+ channels (A) and skeletal muscle Ca^{2+} channel (B), or by 4 identical units of 6 membrane-spanning segments (1 6), as in the case of various K^+ channels (C). Channel proteins are drawn unfolded for better clarity. Domains I–IV should be imagined as being assembled to form a central pore, as in channel head-on view in *bottom right* corner in *A*. Conventional 1-letter abbreviations are used for wild-type and substituted amino acids, the latter also being characterized by their position numbers. Different symbols characterize various resulting Na^+-channel diseases (and also a corresponding disease of horse), as explained in *bottom left* corner in *A*. LQT-1, LQT-2, and LQT-3, long Q-T syndrome types 1, 2, and 3, respectively. Cartoons modified from Catterall (3).

related genes Kv1.1 to Kv1.7. Their products, KCNA1 to KCNA7, are voltage-gated potassium channels that inactivate at different rates (fast N-type and slow C-type inactivation). Heterotetramers show fast inactivation if at least one of the four domains, present in KCNA1, carries the inactivation ball within its NH_2 terminus. Some of these channels are involved in neuronal excitability; others are specifically expressed in "inexcitable" cells such as lymphocytes and pancreatic islet cells.

Six point mutations have been detected in Kv1.1 (Fig. 1B). When the corresponding mutant cDNAs were expressed in Xenopus oocytes, four of the six products did not form functional channels (1). Because the wild-type allele is also expressed in vivo, the overall result of these mutations would just be a reduced potassium conductance. In the remaining two cases, the mutant products formed channels with faster kinetics and increased C-type inactivation (Val-408-Ala) or with a shifted voltage dependence of activation (Phe-184-Cys). In all cases, the results are compatible with the notion that the affected nerve cells fire repetitively because reduction of the potassium current slows membrane repolarization.

Mutations in the skeletal muscle L-type calcium-channel gene cause defective excitation

A rare disease that still poses queries both for clinicians and basic scientists is hypokalemic periodic paralysis. The disease, first described in 1727, is transmitted as an autosomal dominant trait. Clinically, it very much resembles hyperkalemic periodic paralysis described above as a sodium channelopathy. The major symptoms of both diseases, i.e., episodes of generalized paralysis, may occur more frequently and be, on average, of shorter duration in hyperkalemic periodic paralysis, but there are many cases where a differential diagnosis requires considerable skill of the physician. The two diseases may, of course, be discerned by the level of serum potassium during a paralytic attack, which may fall below 2 mmol/l in the former, whereas in the latter, it may rise beyond 4.5 mmol/l. The alterations of serum potassium are, however, not always very distinct. Diagnosis is confounded (although treatment is facilitated) by the clinical experience that in both diseases the frequency and severity of attacks may be reduced by acetazolamide.

The gene responsible for hypokalemic periodic paralysis was detected in a systematic genome-wide search. It encodes the α_1-subunit of the muscle "dihydropyridine receptor," the L-type calcium channel that has so far mainly been associated with excitation-contraction coupling but not with excitation (8).

The skeletal muscle dihydropyridine receptor is located in the membrane of the transverse tubular system and consists of five subunits: α_1, α_2/δ, β, and γ (3). The α_1-subunit (Fig. 1C) contains the receptor for dihydropyridines and other calcium-current antagonists and the ion-conducting pore. It is assumed to possess a dual function as a calcium channel and as a voltage sensor for the control of calcium release from the sarcoplasmic reticulum, mediating contraction. Disease-causing point mutations were detected that result in substitutions of histidine for arginine in segments IIS4 and IVS4 (8).

The study of the functional consequences of these mutations has just begun. They resemble those of corresponding mutations in the sodium-channel gene in that myotubes from patients have inactivation but not activation of the calcium currents changed (see Ref. 7).
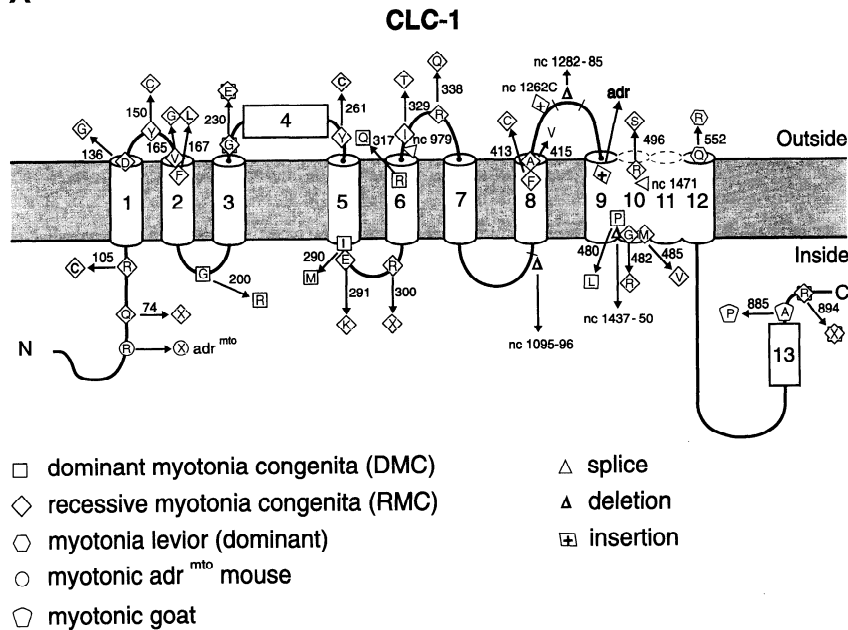
Despite these detailed findings, it is still a mystery how the changed inactivation of the L-type calcium current relates to the hypokalemia-induced attacks of muscle weakness that, after all, gave the starting signal for these investigations.

Search for the defect in myotonia congenita led to the detection of voltage-gated chloride channels

In resting nerve, the potassium conductance is by far the largest component. In contrast, in resting skeletal muscle, the chloride conductance is four times larger than the potassium conductance. The sarcolemmal density of the channels responsible for this large conductance must be very high because the conductance of a single channel is only 1 pS. It has been known since the 1960s that in myotonia congenita, a disease transmitted in some families as a dominant and in others as a recessive trait, the chloride conductance may be substantially reduced. On the other hand, artificial reduction of the chloride conductance by means of chloride-channel blockers has shown that this results in repetitive activity of the sarcolemma, the pathomechanism underlying the symptom of muscle stiffness (see Ref. 7).

Soon after the gene encoding this chloride channel, CLCN1, was cloned in 1991, it was established that both dominant and recessive myotonia are linked to this gene (9). Seventeen mutations and two deletions in various exons of CLCN1 have been discovered to date (Fig. 2A),

A

CLC-1

☐ dominant myotonia congenita (DMC)
◇ recessive myotonia congenita (RMC)
○ myotonia levior (dominant)
○ myotonic adr^{mto} mouse
○ myotonic goat

△ splice
▲ deletion
⊞ insertion

B

CLC-5

☐ X-linked recessive nephrolithiasis (XRN)
◇ X-linked recessive hypophosphatemic rickets (XLRH)
○ dent's disease
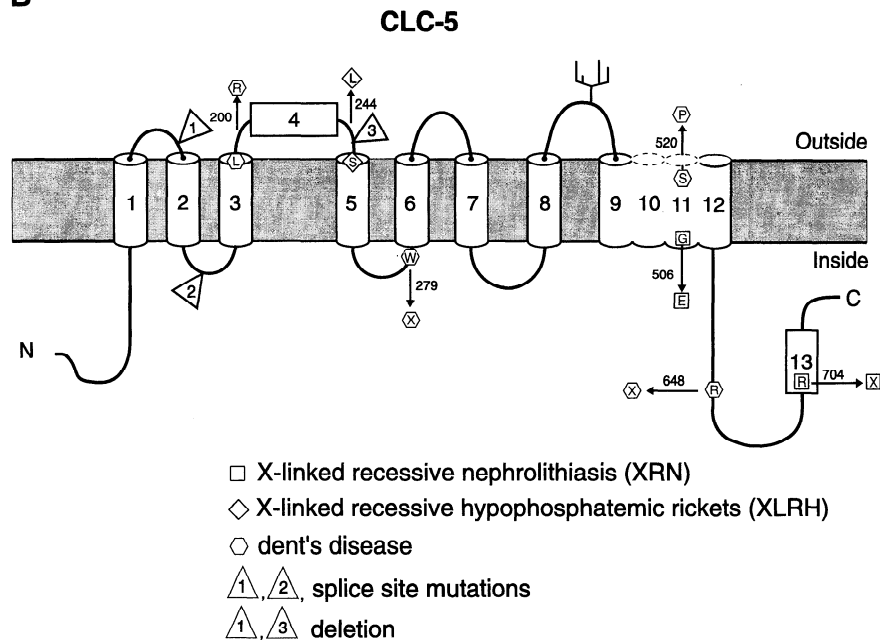△1,△2, splice site mutations
△1,△3 deletion

FIGURE 2. Disease-causing amino acid substitutions, splice variants, deletions, and insertions in voltage-gated muscle (A) and kidney (B) chloride channels. The 2 homologous channels are structurally completely different from cation channels shown in Fig. 1. Different symbols in CLC-1 characterize resulting different types of myotonia congenita in humans, mouse, and goat, as explained in *bottom left* corner in A. Splice variants, deletions, and insertions detected so far all cause recessive myotonia congenita. Cartoons modified from Pusch and Jentsch (13).

among them Dr. Thomsen's mutation (he had the disease himself!). An intriguing outcome of these studies was the hypothesis as to why, in a disease like myotonia congenita, the mode of inheritance may be dominant or recessive (13). Most likely, the *CLCN1* gene product forms dimers. The effect of a particular *CLCN1* mutation on the inheritance pattern depends on the ability of the mutant product to interact with other monomers. A mutant product that is unable to polymerize,

e.g., a severely truncated protein, allows normal monomers (expressed by the other allele) to form normal complexes, although reduced in number (50%). If there are no effective compensatory mechanisms, heterozygous carriers of such a mutation would have one-half the normal muscle chloride conductance, which is sufficient for electrical stability of the membrane. Thus the effect of this mutation would be recessive. On the other hand, a mutant product that is able to

polymerize with normal monomers could impair the function of the channel complex; the effect of such a mutation would be dominant. Mutations causing complete loss of function, as well as mutations causing a change in function, have been discovered.

Our knowledge of the structure-function relationship of the various domains of the chloride-channel protein is still very limited compared with that of voltage-gated cation channels. The currents through ClC-1 channels expressed in human embryonic kidney cells recorded at varying extracellular and intracellular chloride concentrations and pH values were used to develop a model for the gating process in these channels. The missense mutation (Asp-136-Gly), leading to recessive myotonia congenita, considerably affects voltage-dependent gating without altering permeation properties. Thus a disease-causing mutation led to the suggestion that the aspartic acid in position 136 is the voltage sensor of ClC-1 (6).

Nephrolithiasis may be caused by loss of renal voltage-gated chloride channels

Recently, even kidney stones were found to be connected with mutant channels. Three variants of hypercalciuric nephrolithiasis, Dent's disease, X-linked recessive nephrolithiasis, and recessive hypophosphatemic rickets, are inherited as an X-chromosomal trait. When Dent's disease was discovered to be linked to *CLCN5*, another member of the same gene family encoding voltage-gated chloride channels, this came as a big surprise because the disease had hitherto been considered a dysfunction of renal calcium transport. Meanwhile, the other two variants were recognized to be caused by allelic mutations, all of them leading to a loss of function of the channels (Fig. 2*B*) (11).

These results opened a host of new questions, e.g., why does the functional loss of a renal chloride channel cause hypercalciuria? Why does it cause defective tubular transport such as proteinuria in Dent's disease or loss of amino acids, uric acid, or glucose in X-linked recessive nephrolithiasis? How do three allelic loss-of-function mutations cause three clinically different disorders, one of them characterized by bone abnormalities (rickets)? Hopefully, the future will show!

". . . why does the functional loss of a renal chloride channel cause hypercalciuria?"

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