Phenotype variation and newcomers in ion channel disorders

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Ion channels are part of a large family of macromolecules whose functions include the control and maintenance of electrical potential across cell membranes, secretion and signal transduction. Close inspection of the physiological processes involved in channel function and the secondary structure of various ion channels has served as a basis for subdividing ion channels into a number of superfamilies. The voltage-gated ion channels are one of these superfamilies. Recent work has shown that mutations in various ion channel genes are responsible for a number of neuromuscular and neurological disorders. Correlation of the various mutations with the clinical phenotype is providing us with insight into the pathophysiology of these channel proteins. Interestingly, different mutations within the same gene may cause quite distinct clinical disorders, while mutations in different channel genes may result in very similar phenotypes (genetic heterogeneity). Examples of phenotypic variation and genetic heterogeneity are presented in the context of the periodic paralytic disorders of skeletal muscle, episodic ataxia, migraine, long QT syndrome and paroxysmal dyskinesia. Some of these disorders are known to be caused by mutations in ion channel genes, while in the episodic movement disorders, ion channel genes are considered excellent candidate genes.

INTRODUCTION

The superfamily of voltage-gated K⁺, Na⁺ and Ca²⁺ channels are evolutionarily related, sharing a fundamental design consisting of a set of six potentially membrane spanning segments (S1–S6). The six segments comprise a domain which is present only once in the K⁺ channels, but is repeated four times within the α₁ subunit of the Na⁺ and Ca²⁺ channels. Segment four is thought to function as the voltage sensor and contains basic residues at every third or fourth position (1,2). The amino acid sequences of individual channels are highly conserved across species, with some regions being conserved between humans and Drosophila. Conserved regions are also observed between the different ion channels. Overall, the high degree of sequence conservation has been interpreted as evidence of strong selective pressure on these channels. Mutations in these voltage-gated ion channel genes have been shown to cause or have been implicated in a number of episodic disorders including periodic paralysis, episodic ataxia, migraine, long QT syndrome and paroxysmal dyskinesia.

PERIODIC PARALYSIS

The periodic paralytic disorders of skeletal muscle and the non-dystrophic myotonias are diseases which are due to mutations in voltage-gated ion channel genes. Nowhere is disease-based phenotypic variation more pronounced than in the gene that encodes the α-subunit of the skeletal muscle sodium channel gene (SCN4A). Hyperkalemic periodic paralysis (HyperPP), paramyotonia congenita, combined HyperPP and paramyotonia congenita, acetazolamide responsive myotonia and myotonia permanens/fluctuans are all clinically distinct autosomal dominant disorders which are due to mutations in the α₁-subunit of the skeletal muscle sodium channel, SCN4A (3–8) (Table 1, Fig. 1). To date, all of these disorders are inherited in an autosomal dominant manner although the penetrance may vary.

Hypokalemic periodic paralysis (MIM 170500) is characterized by attacks of generalized weakness which usually occur during the first decade of life. Commonly, attacks start in the morning and last between 15 min and 1 h and then remit spontaneously. Resting after strenuous work or potassium intake can provoke an attack of weakness, which is usually accompanied by a significant increase in serum potassium levels. A major factor in the precipitation of a paralytic attack in individuals with hyperkalemic periodic paralysis is an increase in extracellular potassium. Rarely does the level of serum potassium rise to a point which can cause cardiac problems. Moderate exercise can hasten recovery although slight muscle weakness may persist for a couple of days. When the potassium concentration surrounding the muscle fibre is lowered to normal levels following membrane depolarization, the sodium channel fails to inactivate and remains open with delayed repolarization of the muscle plasma membrane.
In this state the muscle is paralysed. After some delay, the normal resting membrane potential is restored. Interestingly, the frequency of attacks declines after the patient reaches mid-thirties/early forties, a phenomenon also noted in episodic ataxia. Complete penetrance is normally the rule for HyperPP, as incomplete penetrance was reported for only two rare mutations (9,10). In addition, HyperPP has been shown to be genetically heterogeneous (11); however, a second locus has yet to be identified.

Table 1. Skeletal muscle sodium channel (SCN4A, 17q23–25) mutations

<table>
<thead>
<tr>
<th>Disease</th>
<th>Amino acid mutation</th>
<th>Domain/segment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myotonia fluctuans</td>
<td>Ser804Phe</td>
<td>D2/S6</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Gly1306Ala</td>
<td>ID3–4</td>
<td>15,16</td>
</tr>
<tr>
<td>Myotonia permanens</td>
<td>Gly1306Glu</td>
<td>ID3–4</td>
<td>16</td>
</tr>
<tr>
<td>Acetazolamide responsive</td>
<td>Ile1160Val</td>
<td>D3/S4–S5</td>
<td>8</td>
</tr>
<tr>
<td>Hyperkalemic paralysis</td>
<td>Thr704Met</td>
<td>D2/S5</td>
<td>6</td>
</tr>
<tr>
<td>(HYKPP)</td>
<td>Val783Ile</td>
<td>D2/S6</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>Met1360Val</td>
<td>D4/S1</td>
<td>9,82</td>
</tr>
<tr>
<td></td>
<td>Met1592Val</td>
<td>D4/S6</td>
<td>3,83</td>
</tr>
<tr>
<td>Paramyotonia congenita (PC)</td>
<td>Val1293Ile</td>
<td>D3–4</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Gly1306Val</td>
<td>ID3–4</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Thr1313Met</td>
<td>ID3–4</td>
<td>5,83</td>
</tr>
<tr>
<td></td>
<td>Leu1433Arg</td>
<td>D4/S3</td>
<td>83</td>
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<tr>
<td></td>
<td>Arg1448His</td>
<td>D4/S4</td>
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<tr>
<td></td>
<td>Arg1448Cys</td>
<td>D4/S4</td>
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<td></td>
<td>Arg1448Pro</td>
<td>D4/S4</td>
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<tr>
<td></td>
<td>Val1458Phe</td>
<td>D4/S4</td>
<td>9</td>
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<tr>
<td></td>
<td>Phe1473Ser</td>
<td>D4/S4–S5</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Val1589Met</td>
<td>D4/S6</td>
<td>4</td>
</tr>
<tr>
<td>HYKPP/PC</td>
<td>Ser804Phe</td>
<td>D2/S6</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Ala1156Thr</td>
<td>D3/S4–S5</td>
<td>10</td>
</tr>
<tr>
<td>Hypokalemic paralysis</td>
<td>Arg669His</td>
<td>D2/S4</td>
<td>27</td>
</tr>
</tbody>
</table>

*Formerly called paralysis periodica paramyotonia.

Paramyotonia congenita (MIM 168300) is characterized by muscular stiffness which appears during exercise and worsens with continued exercise or exposure to cold. The myotonia primarily affects the neck, face and long muscles of the hand. In most cases muscle weakness occurs after exercise or exposure to cold. The symptoms are present at birth and persist throughout life. Ten different mutations have been identified to date, all of which occur between DIII/S6 and DIV/S6 (Fig. 1). In cases where an individual exhibits both paramyotonia congenita combined with hyperkalemic periodic paralysis, symptoms of the latter usually appear in the second decade. The phenotype of paramyotonia congenita without paralysis on exposure to cold (MIM 168350) represents yet another variant of paramyotonia congenita. Koch et al. (12) described three unrelated German kindreds with this condition and in all three families the disorder was due to the same mutation resulting in the amino acid substitution Val1293Ile.

The sodium channel myotonias, namely, myotonia fluctuans, myotonia permanens and acetazolamide responsive myotonia, differ from the non-paralytic paramyotonia variant described above as these disorders are potassium, but not cold, sensitive. Mitrovic et al. (13) included the cold-sensitive cases within this group whereas Hudson et al. (14) classified them as atypical paramyotonia preferring to wait until the significance of the temperature-sensitivity was understood. Myotonia fluctuans is a fluctuating myotonic condition appearing in adolescence that spontaneously varies in severity and, like hyperkalemic paralysis, increases markedly following potassium loading. Cold has no effect on the myotonia and paralysis is not a feature. Myotonia is induced by exercise but has a delayed onset (15–17). Many patients have almost constant fibrillation-like activity. Myotonia permanens is very similar to myotonia fluctuans except myotonia is permanent and much more severe. There is continuous myotonic activity on the EMG (9,17). Pulmonary compromise can occur due to stiffness of the respiratory muscles.

Acetazolamide-responsive myotonia (18) has clinical onset in the first decade and resembles Thomsen’s disease, a skeletal muscle chloride channel disorder, in appearance except muscular stiffness is painful (8). Moreover, myotonia is provoked by fasting and oral potassium and relieved by carbohydrate consumption similar to hyperkalemic paralysis although paralysis is not a feature. Cold only mildly enhances the myotonia. Acetazolamide, a carbonic anhydrase inhibitor, tends to provide dramatic relief.

Hypokalemic periodic paralysis (HOK; MIM 170400) is a disease caused by mutations in the gene encoding the L-type (dihydropyridine-sensitive) calcium channel α1 subunit. HOK is an autosomal dominant disorder with complete penetrance in males and ∼50% penetrance in females. It is characterized by episodic attacks of weakness in association with decreased serum potassium levels. Similarities between HOKPP and hyperkalemic periodic paralysis led to the hypothesis that these disorders may be allelic. However, genetic linkage studies excluded SCN4A as a candidate gene for HOKPP (19,20). HOKPP was mapped to 1q31–q32 (21) and subsequently three different mutations within the α1 subunit of the dihydropyridine-sensitive calcium channel (CACNL1A3) were identified (22,23). This observation supported the view that HOK was not allelic to HyperPP (24,25). Recently, the CACNL1A3 gene was excluded in a French kindred with HOKPP, suggesting that HOKPP is a genetically heterogeneous disorder (26). Bulman et al. (27) also identified a family with HOKPP which appeared not to be linked to the calcium channel gene. They went on to identify a novel amino acid substitution in a highly invariant residue within the voltage-sensor of SCN4A, making the non-calcium channel variant of HOK the seventh disorder found to be due to mutations in the sodium channel gene. Whether or not HOKPP in the French kindred is linked to the sodium channel gene has yet to be determined.
EPISODIC ATAXIAS

Episodic ataxias are a clinically and genetically heterogeneous group of disorders (28) which are inherited as autosomal dominant traits. Patients experience episodes of ataxia which vary in severity and duration. They may also present with other signs of cerebellar dysfunction (29). Currently, two loci, EA-1 and EA-2, have been identified.

Episodic ataxia with myokymia (EA-1) (MIM 160120) was the first disorder in vertebrates which was shown to be due to a mutation in a potassium channel gene (30). In total, seven different missense mutations have been identified in KCNA1 (30–32). Myokymia is a fine rippling of distal musculature, due to spontaneous and repetitive discharges from peripheral nerves and occurs during and between attacks. Subclinical spontaneous motor unit discharges characteristic of myokymia can be identified by electromyography.

von Brederlow (33) and subsequently others (34–36) mapped the second EA locus to 19p13 (MIM#108500). The EA-2 locus fell within a previously defined 30 cM region (37) which was known to contain the gene for familial hemiplegic migraine (FMH) (MIM 141500). Two years later, mutations in the α1A subunit of the P/Q-type calcium channel gene, CACNL1A4, were found to be responsible for both FHM and EA-2 (38). In total seven different mutations were identified. All five of the mutations causing FHM were shown to be missense mutations resulting in amino acid substitutions (Fig. 2). In contrast, the two mutations causing EA-2 were shown to shift the translational reading frame, resulting in premature termination of translation. Both disorders are clinically very different; however, signs of clinical overlap have been noted. FHM is characterized by periodic, commonly unilateral, pulsatile headaches which may affect infants, children and adults and includes episodes of unilateral paralysis, which may outlast the headache. Some patients with FHM have been reported to have a cerebellar disturbance with dysarthria and horizontal nystagmus (39), as seen in patients with EA-2. FHM represents the only migraine gene which has been identified to date. The search is currently...
underway for at least one other FHM gene, as a number of families have been described which are not linked to 19p13 (40–42). In contrast, individuals with familial EA-2 experience discrete episodes of pancelebellar disturbances with dysarthria, titubations, dysmetric limb movements, severe truncal and gait ataxia and headaches, vertigo and nausea were reported in a subset of patients (28,43,44). The duration of attacks is usually variable, lasting from minutes to hours, although there are reports (45) of attacks lasting days. The attacks may be precipitated by emotional stress, exercise, alcohol ingestion, carbohydrate ingestion and onset of menses. Patients with EA-2 may have an associated migraine (non-hemiplegic) which presents after the onset of the ataxic symptoms. Interictal examination often reveals persistent nystagmus and residual mild cerebellar incoordination. Atrophy of the cerebellar vermis has been described in some families with EA-2 (46), but not in others (47–50). Like FMH and hyperkalemic periodic paralysis, the frequency and severity of attacks may decrease in middle to late life.

Shortly after the identification of the FHM/EA-2 gene defects, the trinucleotide repeat expansion disorder spinocerebellar ataxia type 6 (SCA6), was found to be due to a CAG expansion within CACNL1A4 (51). SCA6 (MIM 183086) is a mild but slowly progressive cerebellar ataxia of the limbs and gait. Patients present with dysarthria, nystagmus and mild vibratory and proprioceptive sensory loss (51). The disease progresses for 20–30 years resulting in the patient becoming wheelchair bound. Clinical onset begins in the forties with marked signs of cerebellar atrophy and cerebellar Purkinje cell loss, moderate loss of granule cells, loss of cells in the dentate nucleus and inferior olive (51). SCA6 is clinically distinct from either EA-2 or FHM and one could question the appropriateness of including the phenotype of a dynamic mutation as another example of phenotypic
heterogeneity, when the mechanism of the disease process may not have anything to do with an altered function of the channel per se, but rather a gain of function imparted on the channel by a contiguous stretch of polyglutamine. Currently, however, the mechanism resulting in neuronal cell death in SCA6 is not known, nor is it known if the expanded repeat impinges on the function of the calcium channel.

Mutations in the orthologous gene in mice were shown to be responsible for the naturally occurring and recessively inherited tottering (tg) and leaner (tg""lh"" mouse phenotypes (52). The distribution of these mutations is also presented in Figure 2. The tg mutation results in spike and wave discharges, mild ataxia and periodic convulsions. Spontaneous behavioral arrest is associated with synchronous, bilateral cortical polyspike discharges which are similar to those that occur in patients with absence epilepsy (53). Similar to EA-2, stress appears to trigger the episodic events. The leaner (tg""lh"" mutation, which is allelic to tg, causes profound chronic ataxia associated with Purkinje and granule cell loss within the anterior cerebellum. While spike and wave discharges in the leaner mouse have been detected, paroxysmal convulsions have not been observed. Both the tottering and leaner phenotypes are quite different from that of EA-2 and more complex than FMH. The recessively inherited lethargic (lh) mouse phenotype, which is associated with ataxia and seizures, was recently found to be due to a four base insertion within the Ca2+ channel β3 subunit (54). The neurological aspects of the phenotype are very similar to that of the tottering mouse (52), which may not be surprising given that the β3 and α1A subunits interact as part of a functional calcium channel.

Voltage-gated calcium channels regulate a number of biological functions, including the generation of action potentials in dendrites, allowing calcium entry into the cell thereby initiating neurotransmitter release and other intracellular regulatory processes, and play a pivotal role in the control of neuronal firing (55). Calcium channel types differ with respect to their voltage dependence, inactivation rate, ionic selectivity and pharmacology. The kinetics and the voltage dependence of inactivation define the specific channel subtype as L, N, P/Q, or R. Each channel is composed of five subunits α1, α2, β, γ and δ. The α1 subunit is able to form the structural channel and confer voltage sensitivity and may be encoded by one of at least six genes (A, B, C, D, E or S). The complex ramifications of mutations in any one subunit have not been fully appreciated, because the six α1 and four β subunits may form different combinations (56,57). In addition, the α1 subunit has been shown to undergo extensive alternative splicing (58,59). The P/Q-type calcium channels have been shown to bind directly to the G-protein γ6 complex (60) and mediate crosstalk between G proteins and protein kinase C (61). The distribution of the alternatively spliced products within the cerebellum and whether all of these products interact with G proteins is not known, suggesting that mutations within one of the subunits of the neural calcium channels may have a very complex effect on the cerebellum.

LONG QT SYNDROME

Of all the episodic disorders known to be caused by ion channels, long QT syndrome (LQTS) is the most tragic. LQTS derives its name from the patients’ electrocardiograph which shows a prolongation of the Q and T waves as a result of abnormalities of myocardial repolarization. It can cause ventricular arrhythmias, syncope and sudden death in often young and otherwise healthy individuals (62). LQTS is a genetically heterogeneous disorder in which four loci (LQT1–4) have been identified. The LQT loci were first mapped to 11p15.5 (LQT1; MIM 192500) (63,64), 7q35–36 (LQT2; MIM 152427) (65), 3p21–24 (LQT3; MIM 600163) (65) and 4q25–q27 (LQT4; MIM 600919) (66).

Using a candidate gene approach, mutations in an inwardly rectifying potassium channel gene, HERG (for human ether-a-go-go-related gene), were identified as the cause of LQT2 (67). Similarly, mutations in the cardiac sodium channel gene SCN5A were found to cause LQT3 (68). Using a positional cloning strategy, mutations in the voltage-gated potassium channel gene KVLQT1 were identified as the cause of LQT1 (69). In total, 10 different missense mutations and a 3 bp deletion were identified in 16 kindreds with LQT1. Additional missense mutations have since been reported (70). Mutations in the KVLQT1 gene have been estimated to be responsible for >50% of all LQTS (68). Defects in KVLQT1 have also been shown to be responsible for Jervell and Lange–Nielsen syndrome (MIM 220400), a recessively inherited disorder characterized by congenital bilateral deafness associated with LQTS (71) and, while formal proof has yet to be presented, KVLQT1 has also been implicated in playing a role in Beckwith–Wiedemann syndrome (MIM 130650) (72).

PAROXYSMAL DYSKINESIA

Familial paroxysmal dyskinesias (FPD) include choreoathetotic, dystonic and mixed forms and can be classified as either acquired or inherited (73). Both kinesiogenic (movement-induced) and non-kinesiogenic types have been noted (73–77). In addition, both autosomal dominant and autosomal recessive inheritance have been seen. In the summer of 1996, two groups independently mapped the gene for the non-kinesiogenic form of familial paroxysmal dyskinesia (FPDI) to a 10 cM region of chromosome 2q36 (78,79). Coincidently, the locus for another movement disorder, paroxysmal choreoathetosis with spasticity (CSE), was mapped to a cluster of potassium channel genes on chromosome 1p (80). Voltage-gated or ligand-gated ion channels and glutamate receptors are all potential candidate genes for these disorders.

Ion channel genes are probably expressed in every cell as they are required to perform a variety of functions, such as the maintenance of membrane electrical potential, cell signaling, secretion and absorption. Only a small proportion of ion channel genes which have been mapped are known to be responsible for heritable disorders. Mutations in genes encoding voltage-gated ion channels are known to be responsible for a number of episodic neurological and neuromuscular disorders and many of these mutations are providing insight into the physiological aspects of the wild-type channel.

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