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REVIEW ARTICLE
Unraveling Monogenic Channelopathies and Their Implications for Complex Polygenic Disease

J. Jay Gargus

Department Physiology and Biophysics and Division of Human Genetics, Department of Pediatrics, University of California, Irvine

Ion channels are a large family of >400 related proteins representing >1% of our genetic endowment; however, ion-channel diseases reflect a relatively new category of inborn error. They were first recognized in 1989, with the discovery of cystic fibrosis transmembrane conductance regulator, and rapidly advanced as positional and functional studies converged in the dissection of components of the action potential of excitable tissues. Although it remains true that diseases of excitable tissue still most clearly illustrate this family of disease, ion-channel disorders now cover the gamut of medical disciplines, causing significant pathology in virtually every organ system, producing a surprising range of often unanticipated symptoms, and providing valuable targets for pharmacological intervention. Many of the features shared among the monogenic ion-channel diseases provide a general framework for formulating a foundation for considering their intrinsically promising role in polygenic disease. Since an increasingly important approach to the identification of genes underlying polygenic disease is to identify "functional candidates" within a critical region and to test their disease association, it becomes increasingly important to appreciate how these ion-channel mechanisms can be implicated in pathophysiology.

Introduction

Ion-channel diseases reflect a relatively new category of inborn error. They were first recognized in 1989, with the isolation of cystic fibrosis transmembrane conductance regulator (CFTR [MIM 602421]) (Riordan et al. 1989), then explosively advanced as positional and functional studies converged in the dissection of components of the action potential of excitable tissues. Although it remains true that diseases of excitable tissue (nerve, muscle, and heart) still most clearly illustrate this family of diseases, ion-channel disorders now cover the gamut of medical disciplines, causing significant pathology in virtually every organ system, producing a surprising range of often unanticipated symptoms, and providing valuable targets for pharmacological intervention. Although there have been a number of recent reviews on specific ion-channel diseases, the intent of this review is not to catalog new alleles or phenotypic variations for these syndromes. It is, rather, to provide a general framework for understanding monogenic channel diseases, highlighting major themes common to these disorders across organ systems and formulating a foundation for considering their intrinsically promising role in polygenic disease.

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Address for correspondence and reprints: Dr. J. Jay Gargus, 328 Sprague Hall, 839 Medical Sciences Court, University of California, Irvine, Irvine, CA 92697-4034. E-mail: jjgargus@uci.edu

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Ion Channels as Physiological Mechanisms

Ion channels are unique protein mechanisms that influence physiology in a fashion distinctly different from enzymes. Ion channels carry out no biochemical transformations. Product and substrate are inorganic ions and differ only in regard to the side of the membrane on which they reside. Their major function is the rapid conductive transport of ions diffusing down their electrochemical gradient, as if through a water-filled pore, and their major design features are their mechanism of gating (how they open and close this pathway) and their selectivity (which ions are allowed access to the pathway). Both of these features are now described in exquisite molecular detail for at least a few prototypes (Doyle et al. 1998; Morais-Cabral et al. 2001). However, the transported ion itself is rarely of any physiological consequence: it is the transmembrane currents and their contribution to controlling the cell membrane potential that primarily dictate physiology and create pathology. Since cells have but one membrane potential, it is the great integrator in signaling pathways and a natural substrate for summing subtle polygenic abnormalities. Also—unlike biochemical pathways, the overall flux of which is predominated by a single rate-limiting step—membrane potential is intrinsically a continuous variable fully reflecting subtle changes in the entire host of contributing channels. Al-

though a biochemical pathway may allow polygenic control points, this is largely idiosyncratic, whereas it is the default for membrane potential.

Ion channels are a large family of related proteins sharing features that have proven useful in identification of their genes in the genome. For example, the large superfamily of voltage-gated potassium channels shares a monomer structure of six transmembrane helices and a signature pore motif that forms the conductive pathway (Hille 2001). The functional channel is a tetramer, and the essence of potassium-channel function is manifest in the panoply of combinatorial heteromultimers formed as monomers from family members coassemble, which creates tremendous functional diversity. This diversity conveys to them the modulating functions in signal processing as they interact and "decide" the output. The voltage-gated sodium and calcium channels are the "doers" able to execute the result of signal processing, and, although they can be perceived to share the structure of the potassium channels, in their case, where a crisp uniformity of channel function is required, a fixed pseudotetramer structure is hardwired into the large endoduplicated genes that encode a pseudotetrameric functional monomer. In addition to the major, α-channel subunits that directly contribute to the conducting pore, there is also a host of auxiliary subunits that modify channel function. The human genome contains >400 channel genes (GeneCards) representing 1%–2% of our genetic endowment (Venter et al. 2001). The large number of channel genes does not reflect redundancy. Many channel genes are expressed in a remarkably tissue-selective fashion, whereas others, broadly expressed, predominate in the physiology of only a few tissues. Both of these features allow channels to produce tissue-selective disease and to offer highly selective targets for therapeutic intervention. Since their job is not really the movement of ions, but rather the shape of the electrical signal they produce, physiology requires the diverse vocabulary provided by multiple members of a gene family. It is becoming increasingly clear that this gene-family diversity is tremendously amplified again, not only through combinatorial monomer assembly, but additionally through alternative splicing, allowing one channel locus to produce multiple splice isoforms and, hence, multiple functionally distinct protein products. That nature requires a genetic investment in this huge array of subtly differing channels might lead one to expect that subtle changes matter. Hence, it becomes less surprising that pairs of diseases as apparently distinct as migraine and ataxia or paralysis and myotonia—or even with dominant versus recessive inheritance—are found to be allelic. Again, that subtly differing alleles produce a wide range of disparate phenotypes suggests the potential of this family of genes for contributing to polygenic disease.

Patch electrode electrophysiology has been critical to defining channel mechanisms, working hand-in-glove with in vitro expression studies of cloned wild-type and mutant channels (Noda et al. 1983; Hille 2001). Because of the magically tight (gigaohm) seal between the patch electrode and cell membrane, the technique has the sensitivity to monitor a single ion-channel molecule in real time during its millisecond dance between conformations: "closed," where it is nonconducting, and "open," where it passes on the order of 10^7 ions/sec, producing a current of 10^{-12} amps, a picoamp. For the large family of voltage-gated channels, this transition is induced by a change in the membrane potential. They next spontaneously enter an "inactive," nonconducting conformation that is distinctly different from the closed state, since while inactive, a channel cannot be opened. Only by restoring the resting membrane potential does the conformation become reset to the closed state that has the potential to open.

Ion Channels as Mechanisms of Disease

The cardinal feature of ion-channel disease of excitable tissues is a periodic disturbance of rhythmic function. In the heart, this is manifest as a potentially fatal arrhythmia; in skeletal muscle, as periodic alterations in contractility, ranging from paralysis (the inability to contract) to myotonia (the inability to relax); and, in the CNS, as a seizure, ataxia, or migraine (table 1). The remarkable finding is the absence of overt functional abnormality the vast majority of the time. In the extreme, this results in the tragic—but still very common—situation where no phenotype is obvious until the moment of death. Although many insights have been garnered as to how a variety of stresses serve to create the decompensation that allows these phenotypes to become manifest, the mechanisms that compensate, in the long run, for a constitutionally defective channel still largely remain to be defined. Ion-channel phenotypes in nonexcitable tissues are more diverse, altering signaling involved in endocrine secretion (Thomas et al. 1995), the function of cytosolic compartments (Kornak et al. 2001), and complex epithelial secretory and resorptive functions, which, in the kidney, produce secondary changes in systemic electrolyte balance and blood pressure (Hansson et al. 1995; Simon et al. 1996). Although cystic fibrosis is historically the first recognized genetic ion-channel disease, it remains one of the least understood. Therefore, neither it nor the other epithelial ion-channel diseases will be discussed further. They have, however, been recently reviewed (Lifton et al. 2001; Jentsch et al. 2002).

Didactically, it is most useful to begin with the cardiac phenotype of arrhythmia and the Long QT (LQT) syndromes, demonstrating how component parts of the membrane potential are dissected by the genetic lesions

Table 1

Channelopathy Phenotypes

Phenotype and Disease	Gene	Pathogenic Mutation[a]
Cardiac arrhythmia:		
LQT1	*KCNQ1*	Dominant negative, decrease I_K
LQT2	*KCNH2*	Dominant negative and LOF, decrease I_K
LQT3	*SCN5A*	GOF slow inactivate, increase I_{Na}
Brugada syndrome	*SCN5A*	GOF short inactivation
LQT5	*KCNE1*	Dominant alter modulation, decrease I_K
LQT6	*KCNE2*	Dominant alter modulation, decrease I_K
LQT7[b]	*KCNJ2*	Dominant negative, decrease I_K
JLN/LQT1[c]	*KCNQ1*	Recessive LOF, strong decrease I_K
JLN/LQT5[c]	*KCNE1*	Recessive LOF, strong decrease I_K
Muscle weakness:		
SCCMS	*CHRNA1*	GOF increase activation, increase I_{Na}
SCCMS	*CHRNB1*	GOF increase activation, increase I_{Na}
SCCMS	*CHRNE*	GOF increase activation, increase I_{Na}
Periodic paralysis:		
HOKPP[d]	*CACNA1S*	Missense, alter E/C coupling
HOKPP	*SCN4A*	GOF decrease inactivation, increase I_{Na}
HOKPP	*KCNE3*	Alter modulation, decrease I_K
HYPP[e]	*SCN4A*	GOF decrease inactivation, increase I_{Na}
HYPP[b]	*KCNJ2*	Dominant negative, decrease I_K
Myotonia:		
Paramyotonia	*SCN4A*	GOF decrease inactivation, increase I_{Na}
K-activated	*SCN4A*	GOF decrease inactivation, increase I_{Na}
Becker	*CLCN1*	Recessive LOF, strong decrease I_{Cl}
Thomsen	*CLCN1*	Dominant negative, decrease I_{Cl}
Malignant hyperthermia		
MHS1	*RYR1*	Missense, alter E/C coupling
MHS2	*SCN4A*	GOF decrease inactivation, increase I_{Na}
MHS5	*CACNA1S*	Missense, alter E/C coupling
Seizures:		
BFNC1	*KCNQ2*	Haploinsufficiency, decrease I_K
BFNC2	*KCNQ3*	Haploinsufficiency, decrease I_K
ADNFLE	*CHRNA4*	GOF increase activation, increase I_{Na}
ADNFLE	*CHRNB2*	GOF increase activation, increase I_{Na}
GEFS+	*SCN1B*	GOF decrease inactivation, increase I_{Na}
GEFS+	*SCN1A*	GOF decrease inactivation, increase I_{Na}
GEFS+	*SCN2A*	GOF decrease inactivation, increase I_{Na}
GEFS+	*GABRG2*	Dominant missense, decrease I_{Cl}
JME	*GABRA1*	Dominant missense, decrease I_{Cl}
JME	*CACNB4*	Dominant missense, I_{Ca}
SMEI	*SCN1A*	Haploinsufficiency, decrease I_{Na}
…[f]	*KCNA1*	Haploinsufficiency, decrease I_K
…[f]	*CACNA1A*	Dominant negative, decrease I_{Ca}
Ataxia		
…[f]	*CACNA1A*	Dominant negative, decrease I_{Ca}
SCA6	*CACNA1A*	Dominant CAG expansion, decrease I_{Ca}
EA2	*CACNA1A*	Haploinsufficiency, decrease I_{Ca}
EA1	*KCNA1*	Haploinsufficiency, decrease I_K
Migraine:		
FHM1	*CACNA1A*	Dominant missense, decrease I_{Ca}

[a] E/C = excitation/contraction; GOF = gain of function; LOF = loss of function; ion currents are designated as I_K, I_{Cl}, I_{Na}, and I_{Ca}.

[b] Phenotype includes Andersen syndrome.

[c] Phenotype includes deafness.

[d] Phenotype includes hypokalemia.

[e] Phenotype includes hyperkalemia.

[f] Single report; see text.

and how this creates a natural substrate for polygenic interactions. It further provides specific paradigms for how subunit interactions can be manifest; how loss-of-function and gain-of-function phenotypes occur; and how dominance can arise through either haploinsufficiency, gain-of-function, or in a "dominant-negative" fashion. The muscle and CNS phenotypes, although not lending themselves to as simple a "functional cartoon," are easily built by extrapolation from this substrate. They further illustrate the power of gene families in identifying candidate disease genes and reinforce the intrinsic polygenic nature of such phenotypes.

The Cardiac Phenotype: Arrhythmia

Details of the LQT syndrome and its clinical management have been recently reviewed (Towbin and Vatta 2001; Marban 2002). There are two major LQT syndromes, the Romano-Ward syndrome (RW [MIM 192500]), which has dominant inheritance and a phenotype limited to the heart, and the Jervell and Lange-Nielsen syndrome (JLN [MIM 220400]), which has recessive inheritance and an additional sensorineural hearing loss. LQT is a fatal arrhythmia syndrome that, as the name suggests, prolongs the QT interval measured on the electrocardiogram (EKG). This is the long interval of the cardiac cycle during which the ventricles repolarize; their depolarization is reflected by the QRS waves (fig. 1*a*). A design feature that is "engineered" into the ventricular action potential by the mix of ion channels in its membrane is a prolonged plateau phase of depolarization, a phase not present in the classic rapid action potential of nerve (fig. 1*b*). LQT syndrome is caused by mutations in these cardiac ion channel genes (fig. 2). The plateau phase is a time during which calcium enters myocytes to produce contraction (fig. 1*c, bottom*), but, as importantly, the plateau phase serves to hold sodium channels in the "inactive" nonexcitable state until the depolarizing wave has spread to all of the electrically coupled myocardium (fig. 1*c, top*). In this manner, it can be assured that all ventricular myocytes will repolarize and regain the ability to depolarize again in synchrony. If this mechanism fails and asynchrony is initiated, an endlessly looping futile depolarization wave spreads through any newly excitable domains of the tissue. Such asynchrony is lethal, since, unless the entire ventricular muscle depolarizes and contracts together, it does not pump blood but only fibrillates. As seen in figure 1, during the plateau phase, there is a struggle between the depolarizing sodium and calcium currents and the repolarizing potassium currents, with the potassium currents finally overcoming the depolarizing currents and restoring the resting membrane potential. Mutations that serve to enhance the sodium current or to reduce the potassium currents predictably prolong the plateau and are associated with LQT

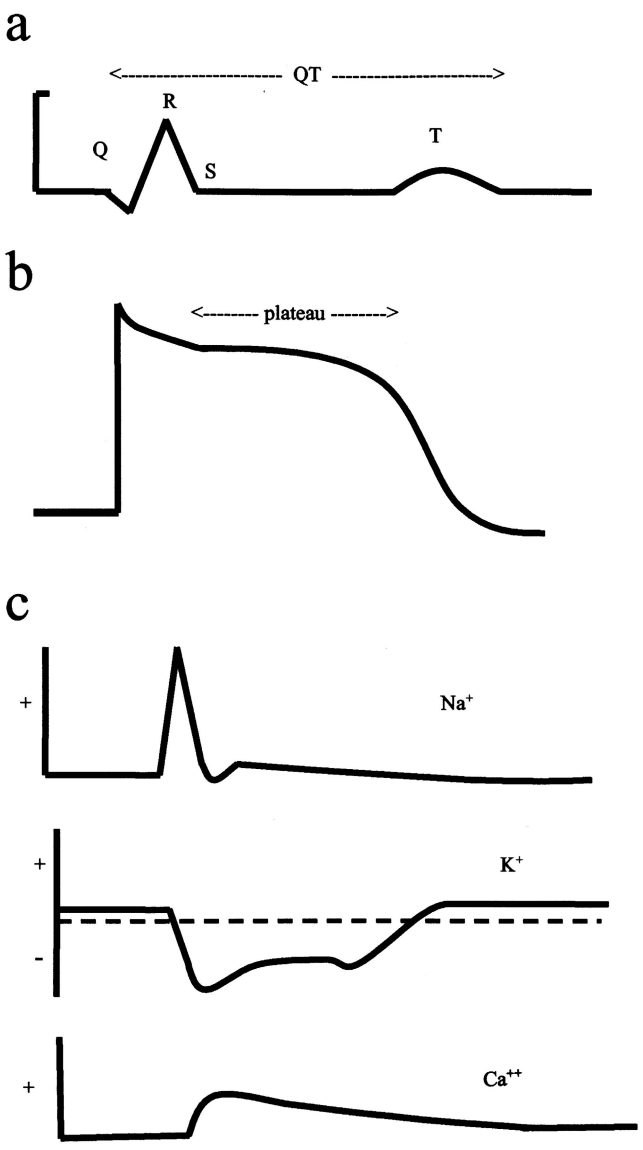

Figure 1 Time correlation of EKG waves, ventricular myocyte action potential, and the individual participating ion currents. *a,* EKG trace, with each component wave labeled above, and QT interval, from the start of the Q wave to the end of the T wave, indicated with brackets. LQT syndrome prolongs this interval. *b,* Ventricular cardiac myocyte action potential correlated in time with the EKG above. The plateau phase of prolonged depolarization is indicated. By convention, depolarization is an upward deflection from the baseline. *c,* Ion currents underlying myocyte action potential, correlated in time with the EKG and action potential. Individual traces are shown for a pure sodium, potassium, and calcium current, the sum of which produces the action potential. Sodium and calcium channels produce depolarizing currents, reflected as an upward deflection. Potassium channels produce a hyperpolarizing current, reflected as a downward deflection.

syndrome. This is a dangerous situation, since prolonging this struggle opens the opportunity for asynchrony and susceptibility to arrhythmia, as described above.

LQT mutations have now been found in six cardiac

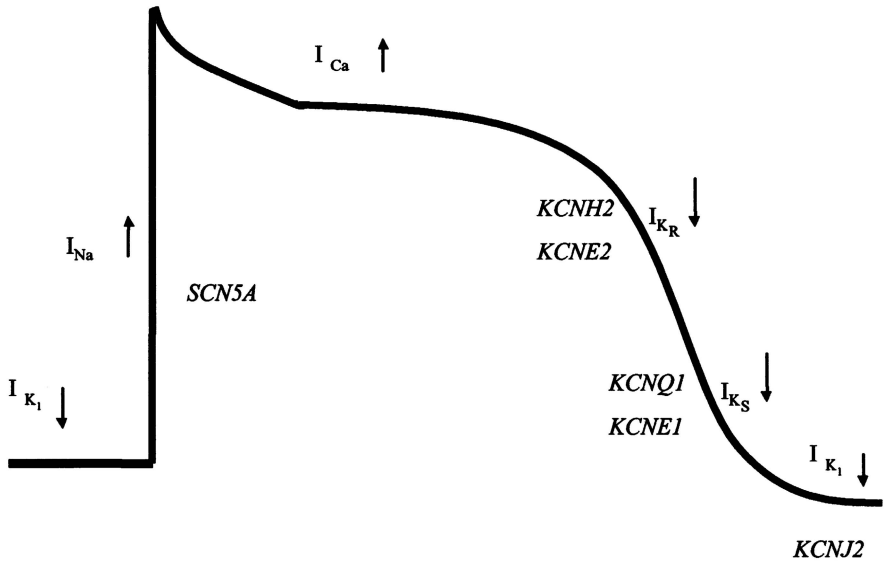

Figure 2 Ventricular cardiac myocyte action potential, showing the contribution of individual ion channels and gene products. The name of the current is placed adjacent to the time at which it predominates in the action potential. Upward arrows indicate a depolarizing current; downward arrows indicate a hyperpolarizing current. The name of the genes contributing the channel subunits are placed adjacent to the current name. Each gene named has dominant alleles that produce the RW LQT syndrome. The two genes forming the I_{Ks} channel also have recessive alleles producing the JLN LQT syndrome.

ion-channel genes, one encoding the cardiac sodium channel and five encoding the primary and auxiliary subunits of three different potassium channels (fig. 2). Although this provides a nearly complete dissection of the major channels involved during the plateau phase, one major actor, the cardiac calcium channel, encoded by *CACNA1C,* has yet to yield a pathogenic allele. It is unfortunate that nearly all of these mutations are rare, "private" mutations, so routine genotypic diagnosis of these disorders is still not practical, obviating postmortem diagnosis. On the other hand, a careful reading of an EKG QT interval, corrected for heart rate (QTc), can make the diagnosis (Marban 2002). Despite this, however, the disease is still not uncommonly diagnosed only after repeated deaths in a family, with a majority of patients being asymptomatic until death but having a family history positive for undiagnosed symptoms or deaths. There is a characteristic context of death observed in the syndrome that further illuminates the critical balancing act performed by multiple cardiac channels in normal physiology. The context is that of excess adrenergic outflow caused by high emotion or exertion. The reason that this is a risk is that heart rate increases dramatically in this "fight or flight" response, and, although we may think about this in terms of the β adrenergic receptor increasing the rate at which the heart depolarizes and contracts, it is clear that to depolarize more rapidly, it must also *repolarize* more rapidly. In fact, specific cAMP-dependent channel regulatory mechanisms assure this coordination, effectively shortening the QT as the heart rate rises. However, in a patient with an intrinsic defect that lengthens the QT, the adjustment cannot keep pace, and an arrhythmia ensues.

The Dominant LQT Genes

LQT1 (MIM 192500) was the first locus identified, positionally through linkage, that causes this syndrome (Keating et al. 1991). All of the other LQT genes were identified as functional candidates within a mapped interval. *LQT1* is located on chromosome 11p15.5, and, although the *Ras* oncogene was initially considered to be the candidate, it was ultimately demonstrated that mutations in a potassium-channel α subunit gene, *KCNQ1*, were causal (Wang et al. 1996). Over 30 different pathogenic alleles have been reported in this gene, and they are the most common cause of RW syndrome. They primarily confer a dominant-negative phenotype when studied in vitro (Wollnik et al. 1997). Defective subunits coassemble with wild-type copies, producing, through combinatorials, a supermajority of defective channel tetramers and, hence, reduced current. Naturally occurring truncated splice isoforms of this channel behave similarly (Demolombe et al. 1998), suggesting that perhaps these mutants are taking advantage of a native mechanism for channel regulation. When expressed in vitro, *KCNQ1* produces a current not recognizable in the heart; however, when expressed together with its β subunit, encoded by

KCNE1, they can be recognized to produce the I_{Ks} channel underlying the slow delayed rectifier K^+ current that participates in repolarization (fig. 2) (Barhanin et al. 1996).

LQT2 (MIM 152427) was the first LQT gene cloned, taking advantage of a candidate gene approach within the region of chromosome 7q35-36 where a second locus was identified in families not mapping to chromosome 11 (Curran et al. 1995). The gene, *KCNH2*, was a strong functional candidate on the basis of its homology to a fly gene with a proven ability to create a rhythm disorder phenotype, ether-a-go-go (Warmke and Ganetzky 1994). Over a dozen alleles have been reported, and, like *KCNQ1*, many confer a "dominant-negative" phenotype when expressed in vitro. However, other dominant alleles appear to be simple loss-of-function alleles, suggesting that the membrane current via this channel is so finely tuned that a haploinsufficiency mechanism is adequate to produce dominance (Sanguinetti et al. 1996). Also like *KCNQ1*, coexpression of *KCNH2* with its β subunit, *KCNE2*, produces a current that can be recognized in the heart, in this case I_{Kr}, the rapidly activating delayed rectifier K^+ current (fig. 2) (Abbott et al. 1999).

The genes underlying *LQT5* (MIM 176261) and *LQT6* (MIM 603796), two adjacent loci on chromosome 21q22.1, are precisely the two highly homologous β subunits, *KCNE1* and *KCNE2*, respectively, discussed above. Both are proteins with only a single transmembrane alpha helix, and both function only to modify the behavior of the α subunit with which they multimerize. Dominant pathogenic alleles are missense and effectively achieve dominance by altering channel gating to produce less current (Splawski et al. 1997*b*; Abbott et al. 1999).

LQT3 (MIM 603830) is caused by a qualitatively different mechanism from those producing decreased potassium current discussed above. The etiology of this disease is mutations in the *SCN5A* gene found at chromosome 3p21-24 (Wang et al. 1995). It encodes a cardiac-specific voltage-gated sodium channel that underlies the rapid depolarization phase that produces the QRS complex and ventricular contraction (fig. 1; fig. 2). Channels encoded by pathogenic alleles have delayed or decreased inactivation after opening, which leaves excess inward depolarizing current during the plateau phase, thus delaying the time at which potassium currents can bring about repolarization. They are thus dominant "gain-of-function" mutations (Bennett et al. 1995). A distinct set of alleles producing the *opposite* effect on inactivation, a rapid recovery from inactivation, produce a different dominant arrhythmia syndrome, Brugada syndrome (MIM 601144) (Chen et al. 1998). The myocardium of these individuals contains a mixed population of sodium channels that are no longer locked in synchrony by a common period of inactivation, forming an ideal substrate for arrhythmia. It is surprising that some alleles are capable of producing either sodium-channel phenotype in a family (Grant et al. 2002), demonstrating the delicate balancing act between excitation and inactivation carried out by the sodium channel and hinting at modifier genes that alter this balance. Both features point to the potential participation of ion-channel mechanisms in polygenic disorders.

Andersen syndrome (MIM 170390) is a dominant multisystem disorder that includes long QT but also includes extra-cardiac findings, such as periodic paralysis and, more surprising, dysmorphology (Sansone et al. 1997). Dominant-negative alleles of the potassium channel gene *KCNJ2* are responsible for this syndrome, sometimes referred to as *LQT7* (MIM 600681) (Tristani-Firouzi et al. 2002). The *KCNJ2* channel subunits multimerize to form the inwardly rectifying potassium channel that governs the resting membrane potential of the cardiac myocyte. Current through this channel participates at the very end of repolarization, and, hence, dominant-negative loss of its function prolongs the process. The extra-cardiac findings imply that it contributes in a significant way to membrane signaling in muscle and perhaps in developmental processes underlying the dysmorphology (Preisig-Muller et al. 2002).

The Recessive LQT Genes

JLN differs from RW syndrome in that inheritance is recessive, and, although the cardiac arrhythmia phenotype is intermittent just as in RW syndrome, there is a constitutive sensorineural hearing loss. It is surprising that the JLN alleles are strong loss-of-function or null alleles in the same genes that cause dominant *LQT1* and *LQT5*; they are *KCNQ1* (Splawski et al. 1997*a*) and *KCNE1* (Duggal et al. 1998), the two channel genes encoding the I_{Ks} channel (fig. 2). The channel is abundantly expressed in the inner ear, where it is involved in potassium-rich endolymph production. Only complete loss of the channel significantly disturbs this secretory process, demonstrating that one mechanism to generate diverse phenotypes with different alleles is to change function such that the critical threshold in different tissues is crossed by each. Although it is still quite unusual to find dominant and recessive alleles in the same gene, as will be discussed below, this is not uncommon in channel diseases. It is also of note that another inner ear *LQT1* homolog, encoded by *KCNQ4*, has alleles that produce only deafness without LQT (*DFNA2* [MIM 600101]) (Kubisch et al. 1999).

The Prospects of Polygenic Cardiac Ion Channel Syndromes

From the description above of the interactions between the multiple cardiac ion channels in maintaining and terminating the plateau phase of the cardiac action potential, one can easily imagine how minor functional variants in these same genes could sum with one another to produce a major alteration in the plateau phase and, hence, a polygenic LQT, arrhythmia, or sudden-death phenotype. Although initial studies suggested that LQT is a highly penetrant Mendelian phenotype readily diagnosed by EKG, it has more recently become clear that there are both "weak" and "subclinical" alleles of these genes and likely polygenic interactions. Additionally, there is clearly an environmental component to the disease, seen as a pharmacogenetic syndrome, that greatly broadens the scope of the diseases involving these cardiac ion-channel genes. These more subtle features of disease produced by the LQT genes became most apparent in studies on families initially considered to have a case of "sporadic" LQT (Priori et al. 1999). Whereas half of these probands had the predicted new dominant mutation in one of the LQT genes, the other half were found *not* to have new mutations but, rather, to have families segregating a weak pathogenic LQT allele. These families had many silent carriers with no EKG abnormality, in generations both older and younger than the proband, or they had carriers who only expressed the phenotype after taking a medication with potassium-channel–blocking activity. These silent carriers presumably lacked a phenotype, because they lacked other unidentified susceptibility alleles at other loci carried by the proband. The pharmacological induction of the phenotype in such an individual suggests that environmental or genetic liability can sum with that contributed by the weak LQT alleles (Yang et al. 2002). Since the drugs that unmask the phenotype produce known effects like those of LQT mutations (K^+ channel block), presumably so does the unmasking genetic liability. The pharmacogenetic LQT syndrome has become clinically important, since many common medications can induce this potentially lethal disorder. These include antihistamines (such as Seldane), antibiotics, and cisapride, as well as the more predictable antiarrhythmics (Towbin and Vatta 2001). Thus far, all implicated drugs share a common mechanism of action that involves the block of I_{Kr} potassium channels, and a number of cases now explicitly demonstrate that weak or subclinical alleles in the LQT genes, encoding both Na^+ and K^+ channels, are contributory to the pharmacogenetic disease (Sesti et al. 2000; Makita et al. 2002; Yang et al 2002).

A special consideration of pathogenic weak LQT alleles is raised by the contribution of these genes to sudden infant death syndrome (SIDS [MIM 272120]). In a landmark prospective study spanning an 18-year period, Schwartz and coworkers from nine large maternity hospitals performed EKGs on all healthy newborns on the 3rd or 4th day of life, studying a total of >34,000 neonates. They performed a 1-year follow-up evaluation and observed that the infants who died of SIDS had a longer QTc interval at birth than did survivors or infants dying of other causes, even though none of their families had a history positive for LQT (Schwartz et al. 1998). Further, fully half of the infants dying of SIDS (12 of 24) had a QTc at birth >2 SD above the mean for the cohort. In a neonate, this finding alone predicts a 41-fold increase in the odds of dying of SIDS (Schwartz et al. 1998). Such screening is controversial, however (Zupancic et al. 2000; Schwartz 2001; Spooner et al. 2001). Because SIDS is so rare, despite the finding's dramatic odds ratio, only 2% of the neonates found to have a long QT die of SIDS. Therefore, the societal costs associated with saving one of those lives through universal screening and treatment can be large. Studies are beginning to add a molecular dimension to these cases of neonates with a long QT and SIDS. In a few cases, a new mutation in one of the LQT genes, producing an allele previously seen in a family with the classical syndrome, can be identified (Schwartz et al. 2000, 2001). It remains to be determined whether weak inherited mutations or even polymorphisms in two or more of the LQT genes contribute to the rest.

The Skeletal Muscle Phenotypes: Paralysis, Myotonia, and Hyperthermia

For the purpose of this review, the key features that the skeletal muscle ion-channel genes and phenotypes reveal are, first, how essentially the same ion channels and even the same types of mutations seen in the LQT syndrome produce interpretable yet distinct phenotypes in this different tissue and, second, that a wide range of phenotypes can be produced with allelic mutations in ion-channel genes. Both aspects are likely to be critical in evaluating the role of these mechanisms in polygenic disease phenotypes. For this reason, only those aspects of these diseases will be covered here. There have, however, been recent comprehensive reviews of the skeletal muscle ion-channel disorders and their treatment (Jurkat-Rott et al. 2002). Most simply, there are two major skeletal muscle phenotypes produced by ion-channel disorders, paralysis and myotonia. The third, apparently quite different, phenotype, malignant hyperthermia, a potentially lethal pharmacogenetic syndrome producing an anesthesia-induced elevated body temperature, is surprisingly closely related.

The skeletal muscle action potential is in many ways similar to the initial portion of that in heart (fig. 1). It is a fast depolarization followed by a rapid repolarization, much like that in nerve. Instead of arising endogenously

within the tissue, as at the cardiac pacemaker in the sinoatrial node, the inciting muscle depolarization arrives via the synaptic release of acetylcholine from a motor nerve terminal at the neuromuscular junction. A ligand-gated cation channel, the heteropentameric nicotinic acetylcholine receptor (nAChR), brings about the initial depolarization. Dominant gain-of-function mutations in three of the five nAChR subunits, encoded by *CHRNA1,* *CHRNB1,* and *CHRNE,* produce slow-channel congenital myasthenic syndrome (SCCMS [MIM 601462]), characterized by muscle weakness (Engel et al. 1998). Acetylcholine dissociates slowly from these mutant receptors, leaving them persistently activated, depolarizing the membrane. In the physiological condition, the tiny receptor-mediated membrane depolarization opens a few sodium channels, further depolarizing the membrane, causing more sodium channels to open in a reinforcing cycle until all sodium channels have explosively opened, spreading a rapid depolarizing wave across the surface of the muscle. At this point, all of the sodium channels are rendered "inactive" and nonexcitable, beginning the process of repolarization, a process completed when other channels restore the resting potential and return the sodium channels to the "closed" state. The major subunit of these sodium channels is the muscle-specific isoform α_4, encoded by *SCN4A,* a relative of the gene involved in *LQT3*. Alleles in this gene produce a diverse array of muscle phenotypes; they will be discussed below.

As the muscle depolarization reaches the T-tubules–specialized membrane invaginations to facilitate activation of contraction throughout the large muscle fiber, it activates the voltage-gated calcium channel, which is similar in structure to the sodium channel discussed above. The major, α_1, subunit is pseudotetrameric and contains the ion-conducting pore. Missense alleles give rise to one type of the complicated phenotype hypokalemic periodic paralysis (HOKPP [MIM 170400]) (Ptacek et al. 1994), discussed below. Its function is modified by auxiliary β, γ, and α_2/δ subunits, the skeletal muscle isoforms being encoded by *CACNA1S,* *CACNB1,* *CACNG1,* and *CACNA2D1,* respectively (Jurkat-Rott et al. 2002). This channel is responsible for rapidly immersing the contractile proteins throughout the large muscle cell in elevated concentrations of ionic calcium to bring about concerted contraction. It does this in part by allowing the passage of extracellular calcium through its pore, down its electrochemical gradient into the cytoplasm. But, additionally, a large cytosolic loop in the α_1 subunit contacts a *different* calcium channel located in a closely opposed *different* membrane compartment, the calcium-rich sarcoplasmic reticulum (SR). Through this physical connection, the voltage-gated channel directly gates the SR calcium release channel, called the "ryanodine receptor" (encoded by *RYR1*), opening it and spilling the intracellular calcium stores into the cytoplasm (Tanabe et al. 1988). Missense alleles altering either the loop of the α_1 subunit (Monnier et al. 1997) or its contact domains on the ryanodine receptor (Quane et al. 1993) (*MHS5* [MIM 601887] and *MHS1* [MIM 145600], respectively) render this complex hypersensitive to general anesthetics, such as halothane, triggering massive calcium release, muscle activation, and malignant hyperthermia. In the physiological condition, after opening, both the sodium and calcium channels spontaneously enter the nonconducting "inactive" conformation, beginning the process of membrane repolarization, a process completed by potassium channels that hyperpolarize the membrane, resetting the sodium and calcium channels into the "closed" conformation. Mutations in one of these potassium channel subunits, encoded by *KCNE3,* a relative of the *LQT5* and *LQT6* genes, have recently been shown to be one of the causes of HOKPP (Abbott et al. 2001), rendering every member of this *KCNE* gene family the site of a pathogenic mutation. The sodium and calcium channels then are kept closed by the large stabilizing current of the chloride channels, encoded by *CLCN1,* and both dominant and recessive allelic mutations that inactivate this channel produce myotonia-repeated muscle contractions, since, if the stabilization fails, repetitive cycles of sodium channel activation occur. The recessive loss-of-function alleles produce Becker myotonia (MIM 255700), with a phenotype characterized by stiffness and paradoxically weak hypertrophied muscles; the dominant-negative alleles leave more residual current and produce Thomsen myotonia (MIM 160800), which has a similar but milder phenotype without weakness (Meyer-Kleine et al. 1995).

Recognizing how these ion-channel disorders are manifest in the heart and muscle should help us understand, and perhaps predict, additional ion-channel disorders in these and other tissues. Once mutations are found in one ion-channel family member, the other family members are suspicious characters (table 2). For example, additional sodium-channel α subunit genes *SCN1A* and *SCN2A* have recently been recognized as seizure loci, as have additional KCNQ potassium-channel subunit genes *KCNQ2* and *KCNQ3*. Further, one can gain insight into probable mechanisms of action of these mutations on the basis of those previously seen in other family members.

Another insight best illustrated by the skeletal muscle sodium-channel lesions is the superficial diversity of clinical syndromes that can be produced by very subtle "tweaks" in channel function: clinically, nothing looks more different from paralysis than myotonia, but, commonly, a given patient can move from one state to the other. If one were forced to consider that the diseases were allelic, one might guess that the former was caused

Table 2

Channelopathy Gene Families

Gene Family	Tissue	Disease Phenotype	Pathogenic Mutation
KCNE1	Heart/ear	LQT5/JLN	Dominant negative/recessive loss of function
KCNE2 (100%)	Heart	LQT6	Dominant negative
KCNE3	Muscle	HOKPP	Dominant negative
KCNQ1	Heart/ear	LQT1/JLN	Dominant negative/recessive loss of function
KCNQ2	CNS	BFNC1	Dominant loss of function
KCNQ3 (80%)	CNS	BFNC2	Dominant loss of function
KCNQ4	Ear	DFNA2	Dominant missense
KCNQ5	CNS	6q14	
RYR			
RYR1	Muscle	MHS1	Missense
RYR2 (66%)	Heart	Ventricular tachycardia	Missense
RYR3	CNS	15q14	
SCN1A	CNS	GEFS+/SMEI	Gain of function/loss of function
SCN2A	CNS	GEFS+	Gain of function
SCN3A	CNS	2q24	
SCN4A (40%)	Muscle	HYPP/HOKPP/MHS/etc.	Gain of function
SCN5A	Heart	LQT3/Brugada S	Gain of function
SCN6A and *SCN7A*	Neuronal	2q23	
SCN8A	Motor endplate	12q13	
SCN9A	Neuroendo	2q24	
SCN10A	Nerve/muscle	3p22	
SCN11A and *SCN12A*	Sensory neurons	3p24	
SCN1B	CNS	GEFS+	Gain of function
SCN2B	Neuronal	11q23	
CACNA1S	Muscle	HOKPP/MHS5	Missense
CACNA1A	CNS	FHM1/EA2/SCA6/seizures	Many types
CACNA1F	Retina	Night blindness	Hemizygous loss of function
CHRNA1	Muscle	SCCMS	Gain of function
CHRNA4	CNS	ADNFLE	Gain of function
CHRNB1	Muscle	SCCMS	Gain of function
CHRNB2	CNS	ADNFLE	Gain of function
CHRNE	Muscle	SCCMS	Gain of function

NOTE.—Gene families ranked according to the percent of family members with proven disease association. Percent of members with proven association given in parentheses. Chromosome location is given for members yet to be associated with disease. Only the known pathogenic members are listed for large families having only a few known pathogenic members.

by hypomorphs and the latter by hypermorphs. However, *all* of the muscle sodium-channel alleles appear to share with the cardiac sodium-channel *LQT3* alleles delayed incomplete inactivation as their mechanism of pathogenesis. The range of phenotypes such a lesion in this gene can produce include:

1. Hyperkalemic periodic paralysis (HYPP [MIM 170500]) (Ptacek et al. 1991), characterized by short, mild, frequent attacks of profound weakness, beginning in infancy, provoked by rest after exercise or stress and often with myotonia between attacks. During an attack, plasma potassium levels rise to pathological levels, likely via release from the muscle. HYPP is characteristically caused by missense mutations in the transmembrane spans, and some HYPP alleles can additionally produce malignant hyperthermia (*MSH2* [MIM 154275]) (Moslehi et al. 1998). This phenotype is also a component of Andersen syndrome, discussed above, affecting *KCNJ2* (Preisig-Muller et al. 2002).

2. HOKPP (Bulman et al. 1999), characterized by infrequent, long-lasting, profound, painless episodes of weakness, beginning in the 2nd decade, provoked by glucose intake or insulin release or upon awakening. During an attack, the plasma potassium can fall to dangerous levels, presumably driven into the muscles. As mentioned above, two other loci, *CACNA1S* and *KCNE3,* also have alleles that produce this syndrome.

3. Paramyotonia congenita (MIM 168300) (Ptacek et al. 1992), a cold-exacerbated myotonia.

4. Potassium-activated myotonia (MIM 603967 .0012) (McClatchey et al. 1992), an unusual form of myotonia.

The essential biophysical feature of the sodium channel that makes the broad range of phenotypes mentioned above interpretable is the fact that, although a depolarization will make it more probable that the voltage-sensitive gate of the closed channel will open, when

sustained, depolarization also leaves behind more of the channels in an "inactive" nonexcitable conformation. Because the sodium channel is the major mechanism for propagating action potentials in all types of neurons and muscles, this delicate balancing act has the potential to move these tissues from a state of hyperexcitability to a state of inexcitability. This feature also explains why one sees profound muscle weakness with the SCCMS congenital myasthenia-acetylcholine–receptor mutations. One might guess that the prolonged receptor activation the mutant alleles produce will give a hyperexcitable muscle; however, the phenotype seen is produced by the loss of sodium channels into the inactive state in the chronically depolarized tissue, rendering the muscle inexcitable and, therefore, weak. It is likely that this feature of the sodium channel will play an important role in understanding phenotypes in the CNS and other tissues as well.

The CNS Phenotypes: Seizures, Ataxia, and Migraine

Seizures, the major ion-channel phenotype in the CNS, and one serving as a springboard for considering the others, is in many ways similar to the arrhythmia phenotype in the hyperexcitable LQT heart. It is a periodic disorder in which the normal rhythmic electrical activity of the tissue is temporarily lost. In the heart, this may be a once-in-a-lifetime event. In the CNS, it can be a lifelong chronic condition, epilepsy. During a seizure, an abnormally synchronous discharge occurs that produces stereotyped alterations in behavior. Whereas the heart is typified by its extremely homogeneous set of responding cells, the CNS displays maximal tissue complexity, both in terms of the number of different cell types present and in the number of different ways they connect with and influence one another in stimulatory or inhibitory fashions. Physiologically, global synchrony in the CNS is actively prevented. Therefore, although one can visualize the very specific and individual contribution each channel type makes to components of an action potential perturbed in LQT or skeletal muscle syndromes, one can much less specifically interpret how or even where the channel dysfunction underlying seizures occurs. A simplification of predicted molecular pathology derived from the cardiac and muscle syndromes, however, is that K^+ and Cl^- channels, which physiologically stabilize excitable tissue, will have pathological lesions that diminish their current and that Na^+ and Ca^{++} channels, which physiologically excite the tissue, will have gain-of-function lesions (table 2).

Idiopathic epilepsy is a common polygenic disorder that affects ~1% of the population and accounts for ~40% of all epilepsy. It is overwhelmingly a genetic disease, with MZ twins >95% concordant for the phenotype (Stoffel and Jan 1998). However, most of these genes still remain to be identified, since only a handful of rare monogenic epilepsy syndromes are recognized. Obviously, since ion channels control electrical activity in the CNS, as in the heart and muscle, they are strong functional candidates for this disorder and, in fact, are the first and, in humans, nearly the only proven genetic causes of epilepsy. Predictably, success to date has come only for the rare monogenic syndromes, and the focus here will be on extending these findings to the polygenic CNS disorders. The monogenic disorders have, however, been covered in detail in recent reviews (Jentsch 2000; Lerche et al. 2001).

The KCNQ Channel Family

The first rare monogenic seizure syndrome for which the major etiological genes were identified was Benign familial neonatal convulsions (BFNC), a rare autosomal dominant disorder characterized by a brief period of seizures in the neonatal period, generally resolving in weeks, but 10% having persistent adult epilepsy. The BFNC loci were mapped to chromosomes 20 and 8 (Leppert et al. 1989; Lewis et al. 1993). The *BFNC1* gene (MIM 121200) was positionally localized within chromosome 20q13.3, taking advantage of a family with the syndrome and a microdeletion chromosome (Singh et al. 1998). The interrupted locus contained a promising candidate gene, *KCNQ2*, a member of the *LQT1* gene family that was predominantly expressed in neurons. Unlike the *LQT1* alleles that demonstrate dominant-negative interactions in vitro, this first *BFNC1* allele was a null, and most subsequent alleles are simply loss-of-function alleles (Jentsch 2000). The relevant neuronal currents mediated by this channel must be so critically tuned that pathogenic alleles can achieve dominance simply via haploinsufficiency. Heterozygous null mice display a milder inducible pharmacogenetic syndrome, showing no basal seizures but only an increased sensitivity to seizure-inducing drugs (Watanabe et al. 2000). Presumably, the strain's polygenic background raises their seizure threshold making them appear much like silent-carrier family members of the probands with "sporadic" LQT syndrome who have disease produced by weak alleles.

Since the *KCNQ* family had the demonstrated ability to produce disease, other family member genes were sought by homology. *KCNQ3* was thus identified, and mapping to chromosome 8q was a tempting candidate for the second BFNC locus (MIM 120201), a hypothesis proven by finding loss-of-function alleles segregating in a family (Charlier et al. 1998). Although both KCNQ subunits produced channels when expressed in vitro, neither subunit alone produced a recognizable current. However, KCNQ2 and KCNQ3 were subsequently recognized to heteromultimerize to form the "M current," a long-sought signature potassium current activated by

muscarinic acetylcholine receptors (mAChR) (Wang et al. 1998). Therefore, both their gene-family relationship and their functional subunit interaction help to explain the common phenotype that mutations in either gene produce. The final member of this *KCNQ* family, *KCNQ5,* mapping to chromosome 6q14 (MIM 607357) (Lerche et al. 2000), and the only member still to be associated with a disease, is expressed in neurons and can also interact with KCNQ3 to produce the M current, making it an extremely strong functional candidate disease gene (table 2).

The Nicotinic Acetylcholine Receptor

A locus adjacent to *KCNQ2* on chromosome 20q13 was the first ion-channel gene demonstrated to contribute pathogenic alleles to an epilepsy syndrome. This position caused some confusion, since, for a time, it was mistakenly thought to cause BFNC. The gene, *CHRNA4,* encodes the most abundant neuronal isoform of the major subunit, α_4, of the nAChR. Unlike the mAChR, discussed above, which couples to channel gating via G protein activation (a G-protein–coupled receptor, GPCR), the nAChR is *itself* a ligand-gated nonselective cation channel, its activation depolarizing the membrane. The heteropentameric neuronal receptor/channel is related to the nAChR in muscle that participates in the phenotype of SCCMS congenital myasthenia. In nerve, it is composed of two α and three β homologous subunits, each with four transmembrane α helices. The rare seizure syndrome, autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE [MIM 600513]), which produces a phenotype with brief clusters of frontal seizures that occur at night, was mapped to this region in a family and missense alleles identified (Steinlein et al. 1995). To date, three alleles have been recognized, all altering the channel pore region and receptor function but having no obvious common effect on in vitro channel behavior. More recently, mutations in the most abundant neuronal β subunit, β_2, were also recognized to cause this syndrome (MIM 605375) (Phillips et al. 2001), suggesting that the channel isoform relevant to this phenotype is an $\alpha_4\beta_2$ pentamer. These missense alleles in *CHRNB2,* located on chromosome 1q21, produced the common in vitro effect of receptor activation, suggesting that some depolarizing gain of function is likely the mechanism of dominant pathogenesis, like that found in the SCCMS muscle pathogenic homologs. The vulnerable cell in the CNS, the function of which is perturbed by these mutations, however, remains to be defined (Lerche et al. 2001).

Febrile Convulsions

Fever lowers the seizure threshold for everyone such that at extremely high temperatures seizures will universally occur (Morimoto et al. 1993). Because fever is also common, it is not surprising that febrile seizures are by far the most common polygenic seizure disorder, affecting 3% of children worldwide (Wallace et al. 1998). Although these susceptibility genes have yet to be defined, a rare Mendelian dominant seizure syndrome that includes febrile seizures promises to point them out. This febrile seizure syndrome additionally evolves to include a variety of afebrile seizures and is called "generalized epilepsy with febrile seizures plus" (GEFS+ [MIM 604233]). It reinforces the notion discussed above that the interplay of multiple ion-channel mechanisms in the generation of a common physiologically relevant action potential renders ion-channel diseases natural substrates for observing locus and allelic heterogeneity and, therefore, likely participants in polygenic disease. In addition to perhaps paving the way toward understanding the common polygenic disorder, this monogenic seizure disorder is perhaps most informative in illustrating the complexity in discerning CNS pathophysiology when the relevant cell type being perturbed is unknown.

The first locus responsible for GEFS+ was mapped to chromosome 19q13.1 in a large family, and a missense mutation was identified in an auxiliary subunit of the voltage-gated sodium channel. This subunit, the β_1 subunit encoded by the gene *SCN1B* (MIM 600235), has a single transmembrane helix and functions only to modify the activity of the large α subunit. Subsequently, mutations producing GEFS+ or closely related syndromes were identified in two different adjacent neuronal sodium-channel α subunit genes, *SCN1A* (MIM 182389) and *SCN2A* (MIM 182390), on chromosome 2q24 (Escayg et al. 2000*b*; Sugawara et al. 2001; Wallace et al. 2001*b*). Not until the α_1 subunit alleles were coexpressed with their auxiliary β subunits did a reproducible picture of defective channel inactivation and sustained sodium current appear (Lossin et al. 2002). This is a picture similar to that observed with the *LQT3* and HYPP and HOKPP alleles of the gene family members *SCN5A* and *SCN4A,* respectively. As in the heart, most simply, a persistent sodium-channel current can be viewed as a gain-of-function dominant lesion that favors a hyperexcitable state; however, the periodic paralysis lesions caution that inexcitability through the inactivation mechanism is also possible.

Recently, however, a rare, very severe dominant seizure syndrome that is initially associated with febrile seizures but progresses to a malignant seizure phenotype, called "severe myoclonic epilepsy of infancy" (SMEI [MIM 607208]), proved to be allelic with GEFS+. All of dozens of known alleles were shown to be new mutations in *SCN1A,* and their nature (e.g., frameshift, nonsense) suggests they are predominantly functional nulls (Claes et al. 2001; Ohmori et al. 2002; Sugawara et al. 2002). The physiological interpretation of these findings is

clear; haploinsufficiency for the major subunit of the sodium channel produces a dominant *loss*-of-function severe phenotype in a critically tuned tissue. This would imply that a 50% reduction in depolarizing current in the relevant cells, which must produce *less* activation of those cells, produces CNS activation and seizures. One would have to presume that the critical target cells of this lesion natively have inhibitory silencing activity in the CNS. This notion, however, makes it tricky to explain how dominant *gain*-of-function alleles also produce a seizure phenotype if they function in the same tissue. It might be that the two allelic lesions impact *different* critical tissues, similar to the recessive and dominant LQT alleles, in this case, tuned to be more sensitive to losses or gains, respectively, in sodium current. In this fashion, the null is sufficient to shut off the inhibitory cell type, producing global hyperexcitability, and the gain-of-function GEFS+ phenotype arises from intrinsic hyperexcitability in a different cell type.

The remaining GEFS+ locus recognized to date perhaps gives further clues as to the nature of the hypothetical silencing inhibitory critical cells hinted at by the SMEI alleles. This gene, *GABRG2* (MIM 137164), found within a cluster of GABA receptor genes on chromosome 5q31, encodes the γ_2 subunit of the inhibitory $GABA_A$ receptor (Baulac et al. 2001; Wallace et al. 2001*a*). Very recently, one of the other members in this receptor gene cluster—*GABRA1*, encoding the α_1 subunit of the receptor—was recognized to carry a missense mutation segregating in a family with juvenile myoclonus epilepsy (JME [MIM 606904]) (Cossette et al. 2002). It is not unreasonable to consider these two seizure syndromes together, since, of the 17 distinct $GABA_A$ subunits recognized, the α_1 and γ_2 (together with the β_2) subunits are a preferred combination and the most widely coexpressed in the brain (Rabow et al. 1995). The $GABA_A$ receptor, like the nAChR discussed above, is a pentameric ligand-gated channel, but it differs in that, instead of conducting a depolarizing cationic current, it conducts a stabilizing chloride current. This receptor is the major mechanism through which GABA, the principle *inhibitory* neurotransmitter in the brain, functions (Hevers and Luddens 1998). It is at this site that the benzodiazepine class of seizure medications act to potentiate GABA effects and seizure inducers, such as picrotoxin, act to inhibit GABA (Hevers and Luddens 1998). So, physiologically, the inhibitory, neuronal silencing activity of GABA participates in preventing seizures. The GEFS+ allele produces a decrease in GABA-induced chloride current in vitro (Baulac et al. 2001), as does the JME allele (Cossette et al. 2002). Therefore, like the seizure-inducing drugs, seizure-inducing mutations in both loci alter receptor/channel function to produce *less* GABA-induced stabilizing current. GABA primarily acts to prevent the spread, not the initiation, of a seizure discharge, so the cells on which it functions may be the same as those activated by the gain-of-function sodium-channel GEFS+ alleles, giving all the alleles that produce GEFS+ a common target and common effect on membrane potential. Perhaps the inhibitory cells silenced by haploinsufficiency in SMEI are those cells with the physiological role of releasing the GABA; perhaps they are inhibitory interneurons, again producing the same postsynaptic effect in the recipient cells as the GEFS+ alleles.

Other CNS Ion-Channel Phenotypes: Of Mice and Men

Although some human "seizure" genes have not recapitulated that phenotype in mice (for instance, mice homozygous for null alleles of *CHRN4A* and *CHRNB2,* the two loci thus far recognized with alleles causing ADNFLE in humans, have a phenotype that alters the pain response, not seizures [Marubio et al. 1999]), spontaneous mutations producing a seizure phenotype in mice have led to the recognition of an important new category of ion-channel seizure disorders in humans, those altering voltage-gated calcium-channel function (Meisler et al. 2001). More importantly, those loci have proven to be particularly informative for their ability to tie the various neurological phenotypes together in an interpretable fashion. As discussed above, the voltage-gated calcium channels are similar to the voltage-gated sodium channel, and the major pore-containing α_1 subunit that dictates the channel's subtype is pseudotetrameric, with a function modified by the auxiliary β, γ, and α_2/δ subunits. Alleles in all four different types of neuronal voltage-gated calcium-channel subunit genes produce a monogenic absence seizure phenotype in mice. The "lethargic," "stargazer," and "ducky" mice are recessive phenotypes produced by mutations altering neuronal calcium-channel auxiliary subunits, *CACNB4* encoding a β subunit (Burgess et al. 1997), *CACNG2* encoding a γ subunit (Letts et al. 1998), and *CACNA2D2* encoding a protein cleaved to produce the α_2 and δ subunit (Barclay et al. 2001), respectively. Of these three genes, a human phenotype has been reported only for mutations in the first. The human *CACNB4* gene maps to human chromosome 2q22-q23, and two dominant alleles, a truncation and missense allele, were found in families with a dominant seizure syndrome (MIM 601949) (Escayg et al. 2000*a*). The human *CACNG2* maps to chromosome 22q13.1 and the human *CACNA2D2* gene to 3p21.3, both remaining only promising seizure candidate genes in humans. The "tottering" and "leaner" mice are recessive phenotypes produced by alleles of *CACNA1A,* the gene encoding the major α_1 subunit of the neuronal P/Q-type calcium channel (Fletcher et al. 1996). The P/Q-type calcium channel plays a major role in calcium

entry underlying synaptic release of the major *excitatory* neurotransmitter, glutamate, and, although this process is decreased in the mutants, suggesting they are hypomorphic alleles, it is unclear how this produces a hyperexcitable seizure phenotype (Caddick et al. 1999). Perhaps the critical target cells are themselves inhibitory, as suggested above for SMEI. Although these mice have a seizure phenotype, this is *not* the major phenotype caused by mutations in this gene in humans. Human mutations in *CACNA1A,* which maps to chromosome 19p13, have been shown to cause three apparently distinct and different late-onset neurological disease phenotypes: episodic ataxia type 2 (*EA2* [MIM 108500]) (Ophoff et al. 1996), familial hemiplegic migraine type 1 (*FHM1* [MIM 141500]) (Ophoff et al. 1996), and spinocerebellar ataxia type 6 (*SCA6* [MIM 183086]) (Zhuchenko et al. 1997). The *EA2* alleles are predominantly truncations, with >22 known alleles, including frameshift and splice-site mutations, but there are also five missense alleles, some of which alter conserved pore residues producing complete loss of function (Van Den Maagdenberg et al. 2002). It is, therefore, presumably a haploinsufficiency dominant loss-of-function syndrome. FHM is caused by at least nine known missense alleles, producing no obvious uniform functional change in calcium current in vitro; therefore, the relevant functional change they share in common is presumably subtle (Kors et al. 2002). Finally, SCA6 is a progressive degenerative phenotype primarily caused by trinucleotide expansion alleles. The polymorphic CAG repeat encodes a C-terminal polyglutamine repeat of 5–20 residues in unaffected individuals. Pathogenic alleles encode 21–30 glutamines (Ishikawa et al. 1997; Zhuchenko et al. 1997). The only point mutant allele recognized as producing the syndrome is G293R, which changes a conserved pore residue (Yue et al. 1997). An intermediate-length CAG-repeat allele with only 20 repeats produced the milder phenotype of EA2 (Jodice et al. 1997).

Thus, the four allelic *CACNA1A* diseases in human and mouse are clearly closely related at the molecular level. The phenotypes also can blend one into the other; for instance, many individuals with FHM have ataxia, as do most of the murine seizure mutants (Ducros et al. 2001; Zwingman et al. 2001), and, recently, a dominant-negative truncation allele has been reported to produce a syndrome of progressive ataxia and seizures in a child (Jouvenceau et al. 2001). In humans, the mildest alleles appear to be the missense mutations that produce FHM1. On a phenotypic continuum, these appear to be hypomorphic, since the truncations, pore mutations, and intermediate repeat expansions produce EA2, apparently a haploinsufficiency syndrome. Expanding the polyglutamine repeat by only a few additional residues yields the potent long-repeat alleles producing the progressive SCA6 syndrome, which can also be produced by one specific missense pore mutation. Although the mechanism of action of all pathogenic polyglutamine expansion alleles is controversial, if the three human *CACNA1A* phenotypes are truly the continuum suggested by the EA2 intermediate-repeat phenotype, these repeat expansion alleles should be dominant negative to eliminate more of the P/Q current than haploinsufficiency and to keep the progression of allele potency parallel to the severity of the symptoms and pathology. Although all of the human alleles produce the phenotype in heterozygotes, the mouse seizure alleles, which are missense, like FHM1, and demonstrated to be hypomorphic, produce no phenotype in heterozygotes, including knockout null alleles (Jun et al. 1999); decreased synaptic glutamate neurotransmitter release and seizures are produced only in the homozygotes.

On the basis of only one clear human case (Jouvenceau et al. 2001), is it clear that the phenotypes of FHM, EA, and SCA reflect a pathophysiology similar to that of seizures, a phenotype only reproducibly seen in mice? That the seizure phenotype in humans and mice is caused by mutations in calcium-channel auxiliary subunits (Escayg et al. 2000*a*) reinforces this notion, but perhaps the best clue comes from the other known episodic ataxia locus, *EA1* (MIM 160120). EA1 is produced by dominant loss-of-function alleles of perhaps the most "famous" potassium channel, "shaker" (Browne et al. 1994), named after its mutant phenotype in fly, and the paradigmatic first K^+ channel cloned (Tempel et al. 1987). It is a voltage-gated potassium channel formally called *KCNA1.* Knockout null alleles in the mouse produce a recessive seizure disorder, with the heterozygotes lacking a phenotype just like the calcium-channel mutants but, like them, showing a reduced seizure threshold (Smart et al. 1998; Rho et al. 1999). Although the vast majority of patients with EA1 have only ataxia, a few individuals have been reported to have seizures also (Zuberi et al. 1999; Eunson et al. 2000). It remains to be determined if the mice "shaker" mutants, like the calcium-channel mutants, have decreased synaptic glutamate release; however, it is intriguing that the homozygous *KCNA1* nulls have enhanced pain sensitivity (Clark and Tempel 1998), like the *CHRN4A* and *CHRNB2* nulls that failed to emulate the human ADNFLE seizure phenotype, mentioned above.

The Prospects of Polygenic CNS Ion-Channel Syndromes

Although it is obvious to consider the CNS ion-channel genes that were found above to produce the four cardinal related monogenic phenotypes as candidates for carrying the mutations and polymorphisms that will produce the much more common polygenic forms of the seizure, ataxia, or migraine syndromes, there are two major

questions to consider for the future. First, do these cardinal syndromes reflect the full range of polygenic CNS ion-channel phenotypes? Second, if we assume that rare monogenic syndromes do not directly demonstrate all of the critical ion channel participants in polygenic disease, from what has been learned of the pathogenic alleles in the monogenic syndromes, what are reasonable features to expect them to possess that would warrant candidate status?

First, it is likely that polygenic ion-channel disorders produce a wider range of phenotypes than those already revealed by the monogenic disorders. The salient feature of the CNS ion-channel disorders, as well as those in the heart and muscle, was that they arose from a *periodic* disturbance in normal rhythmic activity. They were intrinsically episodic disorders, although many progressed to become constant. Many of the common complex polygenic neuropsychiatric disorders share this character. Further, several of the drugs used to treat the recognized channel phenotypes are additionally used to treat these disorders (e.g., valproate is used to treat both seizures and bipolar disease). In that field, an enormous amount of attention has already focused on the neurotransmitter/neuroreceptor genes as functional candidates. They seem obvious candidates, since the pharmacology used to treat or to phenocopy these disorders typically targets those mechanisms. On the other hand, ion channels are perhaps more compelling candidates (Gargus et al. 1998). The monogenic disorders discussed above illustrate how ion-channel and neuroreceptor/channel mutants produce a common, shared disease phenotype (e.g., GEFS+ alleles of sodium channel and $GABA_A$ receptor genes). Moreover, they illustrate that essentially the only reason nature "bothers" to activate a neuroreceptor is to trigger a response from the ion channels sharing its membrane. Further, the channels reflect a tissue selectivity not achievable by the much more ubiquitously utilized limited repertoire of receptors; that is the very reason drugs targeting them are so prone to side effects and, perhaps, the reason so many receptor classes have yet to be demonstrated to have any functionally altered pathogenic alleles.

An increasingly important approach to the identification of genes underlying polygenic disease is to identify "functional candidates" within broad chromosomal regions and to test disease association with polymorphic markers within these candidates. Ion-channel candidates would be suggested by the known physiology, pharmacology, and pathophysiology; their appropriate or extremely narrow tissue distribution; their membership in a demonstrably pathogenic gene family; and their contribution to a demonstrably vulnerable component of the action potential or a salient disease-associated motif (e.g., a polyglutamine repeat in a disease showing anticipation) (Chandy et al. 1998; Dror et al. 1999). The existence of alleles sharing features common to pathogenic alleles underlying the monogenic channelopathies, such as dominant-negative inhibition of expressed channel function or incomplete inactivation, would be particularly suggestive (Miller et al. 2001; Tomita et al., in press).

For the discovery of a polygenic disease-associated gene to have full impact in modern medicine, it will be necessary to understand its function and to find small molecules able to alter that function, such "rationally designed" novel pharmaceuticals being one of the major hopes for the postgenomic era. Although often positionally identified disease genes have been slow to yield in both regards, ion channels are highly amenable and proven targets. Further, as our current pharmacopeia is overwhelmingly predominated by molecules targeting receptors (>80%), channels represent *qualitatively* different novel targets. For example, discovering ion-channel participants in a disease, such as the monogenic seizure disorders caused by *KCNQ2* and *KCNQ3* mutations, suggests not only a novel type of target for therapy (e.g., suggesting use of acetazolamide, a drug proven effective in a wide range of ion-channel diseases), but also that these channels themselves can further serve as the guides to *finding* entirely novel pharmaceuticals. Because the function of channel mechanisms is so well understood, it is clear that the therapeutic goal will be either a channel blocker or a channel activator, and many drugs of both types are already in current use. In vitro expression assays of channel function can be used in high-throughput drug screening and in drug optimization (Shieh et al. 2000). An example of this kind of breakthrough is retigabine, a novel class of seizure medication proven to activate the KCNQ2/KCNQ3 "M current" channel that is suggested as a seizure drug target by the role of these genes in BFNC (Wickenden et al. 2000). Since the channel mechanisms perturbed in such disorders are likely to be critical participants in producing the relevant membrane potentials that lead to a disease vulnerability, the drugs targeting them should be useful for treating the disease even in those *not* having an intrinsic defect in the targeted mechanism. Another advantage of pharmacology targeted to channels is the expectation, discussed above, that a narrow range of tissues express the channel in a physiologically important context; therefore, drug specificity is achieved and unwanted side effects are minimized. Therefore, in this postgenomic world rich in potential targets, even should ion channels reflect only a minority of the relevant etiological candidates in polygenic CNS disease, they may well provide the most rapid access to novel therapeutics, warranting prioritization for early evaluation for that reason alone. However, the demonstrated ability of ion channels to cause the wide range of monogenic CNS phenotypes discussed above suggests

they will likely be important common participants in the polygenic CNS diseases as well.

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Electronic-Database Information

The URLs for data presented herein are as follows:

GeneCards, http://bioinfo.weizmann.ac.il/cards/index.html (for database of human genes, their products, and their involvement in diseases)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for CFTR, RW, JLN, *LQT1*, *LQT2*, *LQT5*, *LQT6*, *LQT3*, Brugada syndrome, Andersen syndrome, *LQT7*, *DFNA2*, SIDS, SCCMS, HOKPP, *MHS5*, *MHS1*, Becker myotonia, Thomsen myotonia, HYPP, *MSH2*, paramyotonia congenita, potassium activated myotonia, benign familial neonatal convulsions *BFNC1*, benign familial neonatal convulsions *BFNC2, KCNQ5,* ADNFLE type 1, ADNFLE type 3, GEFS+, *SCN1B, SCN1A, SCN2A,* SMEI, *GABRG2,* JME, *CACNB4,* episodic ataxia type 2, FHM1, SCA6, and episodic ataxia type 1)

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