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Working Group Report

Genetic and molecular basis of cardiac arrhythmias

Impact on clinical management

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Introduction

Clinical cardiologists who manage arrhythmias are increasingly faced with new complexities in management decisions. The once obscure science and jargon of medical genetics is assuming a much more prominent position in the mainstream medical literature, with seemingly weekly reports of new mutations to explain what once seemed very obscure diseases. This rapidly expanding knowledge base places the clinician — who usually trained when the concepts were not a major component of the medical school or fellowship training curricula — at a disadvantage in making day-to-day decisions with respect to managing common symptoms, such as unexplained syncope or heart failure. Even entertaining a diagnosis such as the congenital long QT syndrome or hypertrophic cardiomyopathy used to be a medical curiosity. Now, with increased public and physician awareness of these and even more esoteric conditions, the questions on patient management have become more common, and more complex. They include not only broad questions like 'How can I establish (or better yet, rule out) a diagnosis?' but also more specific issues such as 'Should this patient undergo genetic testing? Where? How? And how can I interpret the results?'

The first and second parts of this article attempt to answer these questions. They neither teach molecular genetics nor do they provide an exhaustive review of the current state-of-the-art of molecular and genetic cardiology relevant to arrhythmias. Rather, they try to put into a very practical perspective the ways in which ongoing progress in genetics may affect day-to-day clinical management.

The recognition that diversity in cardiac electrophysiology and indeed in many aspects of cardiac function can be attributed to variable expression of specific genes or variability in the function of their protein products has the potential to alter the way in which we think about normal and abnormal electrical heart function. The third part of the article reviews the potential for a genetic approach to understanding diversity in cardiac function, focusing in particular on ion channels and gap junction proteins as the central players in normal and abnormal electrophysiology. Moreover, integration of molecular function into a single cell and of single cells into cellular networks reveals a multitude of interactions eventually determining the generation and conduction of the cardiac action potential and, therefore, arrhythmogenesis.

This text is the outcome of a workshop convened by the Study Group on Molecular Basis of Arrhythmias of the Working Group on Arrhythmias of the European Society of Cardiology.

Key words: Sudden cardiac death, genetics, arrhythmia, molecular diagnosis, basic electrophysiology.

*See Appendix for details of Workshop on which this report is based.

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Inherited arrhythmogenic disorders

Long QT syndrome

The long QT syndrome is a familial disease[1,2] characterized by abnormally prolonged ventricular repolarization and a high risk of malignant ventricular tachyarrhythmias, occurring often, but not always, in the setting of high adrenergic activity, that is, physical or emotional stress. Two major clinical syndromes have been characterized based on the pattern of transmission of the disease: a more common autosomal dominant form with a pure cardiac phenotype (Romano–Ward[3]) and a rare autosomal recessive form characterized by the coexistence of cardiac abnormalities and congenital deafness (Jervell and Lange-Nielsen[4]).

Table 1 Mutations in families with familial dilated cardiomyopathy

Disease	Locus	Gene	Reference
XLCM	Xp.21.2	Dystropin	Muntoni *et al.*[70]
			Muntoni *et al.*[71]
			Milasin *et al.*[72]
			Ortiz-Lopez *et al.*[68]
Barth	Xq28	G.4.5	Bione *et al.*[69]
ADDCM	1q32	?	Durand *et al.*[73]
	2p31	?	Siu *et al.*[74]
	9q13-q21	?	Krajinovic *et al.*[75]
	10q21-q23	?	Bowles *et al.*[76]
	3p22-p25	?	Olsen *et al.*[78]
	15q14	Actin	Olsen *et al.*[79]
CDDC	1p1-1q1	?	Kass *et al.*[77]
FHCM	1q3	cTnT	Thierfelder *et al.*[39]
			Watkins *et al.*[54]
	3p	MELC	Poetter *et al.*[38]
	7q3	?	MacRae *et al.*[43]
	11p11.2	MyPBC	Bonne *et al.*[40]
			Watkins *et al.*[41]
	12q23-q24.3	MRLC	Poetter *et al.*[38]
	14q11-q12	BetaMHC	Geisterfer *et al.*[37]
	15q2	AlfaTM	Thierfelder *et al.*[39]
			Watkins *et al.*[54]
	19p13.2-q13.2	?	Kimura *et al.*[42]
FA.Fib	10q22-q24	?	Brugada *et al.*[89]
PFHB-I	19q13.2-q13.3	?	Brink *et al.*[90]
			De Meeus *et al.*[92]
LQTS (R–W)	3p21-p23	SCN5A	Wang *et al.*[9]
	4q25-q27	?	Schott *et al.*[8]
	7q35-q36	HERG	Curran *et al.*[12]
	11p15.5	KvLQT1	Wang *et al.*[5]
	21q22.1-p22	minK	Splawski *et al.*[15]
LQTS (JLN)	11p15.5	KvLQT1	Neyroud *et al.*[19]
			Splawski *et al.*[20]
	21q22.1-q22	minK	Schultze-Bahr *et al.*[16]
ARVD	1q42-q43	?	Rampazzo *et al.*[61]
	14q12-q22	?	Severini *et al.*[60]
	14q23-q24	?	Rampazzo *et al.*[59]
	2q32.1-q32.2	?	Rampazzo *et al.*[62]
NAXOS	17q21	?	Coonar *et al.*[58]
F-IVF	3p21-p23	SCN5A	Chen *et al.*[82]

XLDCM=X-linked dilated cardiomyopathy; ADDCM=autosomal dominant dilated cardiomyopathy; Barth=Barth syndrome; CDDCC=conduction defect and dilated cardiomyopathy; FHCM=familial hypertrophic cardiomyopathy; FA.Fib=familial atrial fibrillation; PFHB-I=progressive familial heart block type I; LQTS (R–W)=long QT syndrome Romano–Ward type; LQTS (JLN)=long QT syndrome Jervell and Lange-Nielsen type; ARVD=arrhythmogenic right ventricular cardiomyopathy; NAXOS=Naxos disease; F-IVF=familial idiopathic ventricular fibrillation.

Long QT syndrome genes

Five loci[5–8] have been associated with the Romano–Ward long QT syndrome and they are located on chromosomes 3, 4, 7, 11 and 21 (Table 1). As illustrated in Fig. 1, four long QT syndrome disease genes, each encoding an ion channel protein, have been identified: SCN5A, encoding the cardiac sodium channel (chromosome 3)[6,9–11]; HERG, encoding the I_{Kr} potassium channel protein (chromosome 7)[6,12]; KvLQT1, encoding the alpha subunit of the I_{Ks} potassium channel protein (chromosome 11)[5,13,14] and KCNE1, encoding minK, an ancillary subunit for the I_{Ks} channel complex (chromosome 21)[15,16]. The gene at the chromosome 4 locus (LQT4) has not been identified. Families linked to none of these five loci have been described, so there are other disease genes. The recognition that the long QT syndrome is actually a group of ion channel diseases with a similar phenotype has led to the new terminology for mutations: (i) LQT1 on KvLQT1, (ii) LQT2 on HERG, (iii) LQT3 on SCN5A, (iv) LQT5 on minK. Although the prevalence of each variant of the long QT syndrome has not been precisely defined, LQT1 is the most frequently encountered form, whereas LQT3 and LQT5 are rare.

Mutations in long QT syndrome genes

Most of the mutations identified to date in the long QT syndrome genes are missense mutations. These

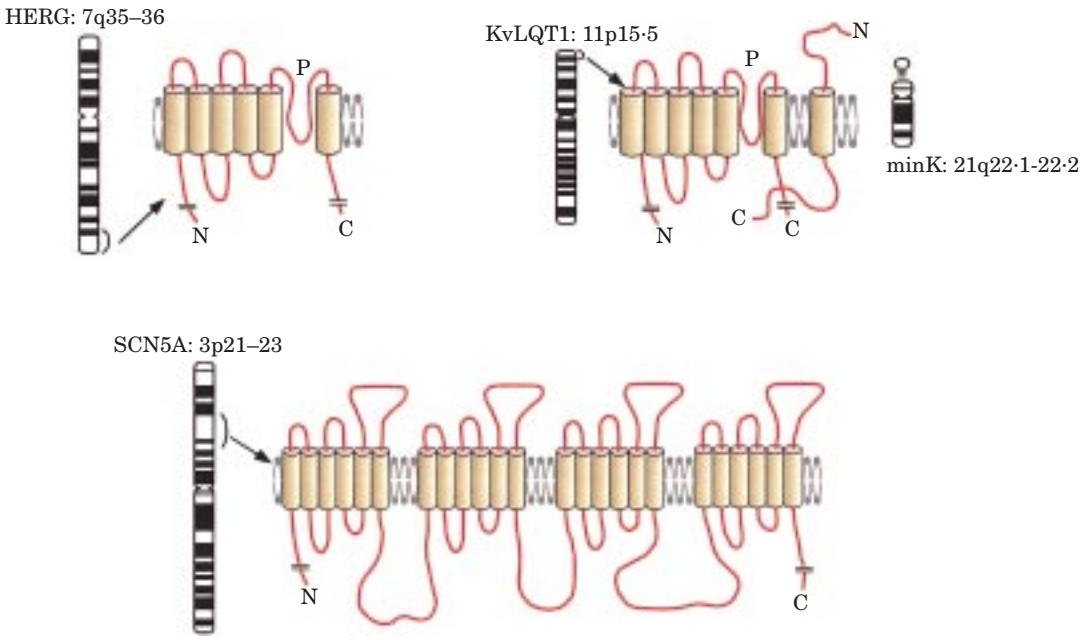

Figure 1 **LQTS genes Chromosomal locations of the genes and predicted topology of the ion channel proteins associated with the genetic variants of the LQTS.**

mutations are not confined to a single location but are found at various positions within each gene in different families. Thus, in most affected families, the long QT syndrome is due to a distinctive, or 'private' mutation. This remarkable genetic heterogeneity probably contributes to the variability in the clinical presentation.

A few mutational 'hot-spots' (such as specific positions within a gene mutated in multiple families) have been identified in KvLQT1[17] and HERG[18]. Unrelated kindreds worldwide with the same mutation can therefore be studied to test the logical hypothesis that they share common clinical or epidemiological features. Contrary to expectations, initial studies indicate that substantial phenotypic heterogeneity remains even with an identical long QT syndrome gene abnormality. This, in turn, suggests that variable expression of as-yet-unidentified 'modifier genes' contributes to the clinical manifestations of the disease.

The Jervell and Lange-Nielsen (autosomal recessive) variant of the long QT syndrome (in which affected subjects have especially long QT intervals) arises in individuals who inherit abnormal KvLQT1 or minK alleles from both parents. The abnormal allele can be the same (usually in consanguineous families)[19,20] or different ('compound heterozygosity')[16]. Thus, parents of subjects with Jervell and Lange-Nielsen carry long QT syndrome mutations, although most (but not all) are aysmptomatic. Recently, a family with apparent autosomal recessive long QT syndrome without deafness has also been identified[21]. These findings all suggest that 'gene dosage' determines the phenotype (two abnormal alleles appear worse than one), and also highlights the extraordinary variability in the long QT syndrome phenotype[22–25]. The location of the mutations within the gene (for example, close to the regions encoding specific structures such as the pore, the voltage sensor, the S1–S6 region, or the N or C-terminal portions) or the type of mutation (the nature of the amino acid substitution, missense mutation vs deletions or insertions) may also play a role.

Functional consequences of mutations

The channels carrying I_{Kr} and I_{Ks} are multimeric; that is, alleles from both parents are thought to contribute to the channel complexes. When mutations in KvLQT1, KCNE1 or HERG are expressed alone or with wild-type alleles in oocytes or in other cell lines, they exhibit 'loss of function', i.e. the total current carried by the defective channel complexes is reduced. Some of the mutations not only reduce current but also modify channel kinetics. Many HERG and KvLQT1 mutations have been identified as 'dominant negative' because when the mutant protein is co-expressed with the native protein[13,14,26,27] the resulting defect in current exceeds 50%. One explanation for this phenomenon is that incorporation of a single abnormal protein subunit into the tetrameric channel structure is sufficient to alter the overall behaviour of the current.

By contrast, mutations in the SCN5A channels cause a 'gain of function'[10,11]. These mutations produce a persistent late sodium current which is not present physiologically and which is due to defective inactivation. In all described mutations, the sodium current is increased because of late reopenings of the channels, while in the three amino-acid deletion (ΔKPQ) long lasting bursts of channel activity are also present. These mutations also differ in severity, with the ΔKPQ deletion being associated with a quantitatively larger increase in

late sodium inward current[11]. It is generally difficult to develop specific therapies for loss of function (for example, the K^+ channel defects described above). By contrast, the gain in abnormal function exhibited by mutant SCN5A gene products raises the possibility that a cure could be accomplished by pharmacological agents that inhibit the 'gained' function, i.e. block the late I_{Na}. Indeed, some data suggest that these currents are especially sensitive to block by mexiletine or lidocaine[10,11].

Genotype–phenotype correlations

The different time and voltage dependence of the ionic currents involved in the long QT syndrome may help explain some aspects of the variable phenotype and raise the possibility of gene-specific treatment. Indeed, available data on several hundred genotyped patients indicate the existence of gene-specific differences in the triggers for cardiac events[28]. Exercise-related events dominate the clinical picture in I_{Ks}-related long QT syndrome (LQT1)[28]. I_{Ks} is the predominant K^+ current in conditions of high sympathetic activity, particularly at shorter cycle lengths. Thus, reduced I_{Ks} will be predicted to lead to inadequate action potential shortening with adrenergic stress and thereby account for the high prevalence of arrhythmic events in these patients during exercise. By contrast, most LQT3 patients experience events during sleep or at rest; they are also able to markedly shorten their QT interval during exercise[29]. In these cases, it seems likely that the presence of normal K^+ currents produces normal action potential shortening during exercise; however, at rest, defective inactivation of I_{Na} will result in an increase in the plateau inward Na^+-current. This apparently 'nice' distinction between LQT1 and LQT3 is, however, complicated by the reality that LQT2 patients also tend to display events both at rest and during exercise, thus pointing to the persistent limitations in current understanding.

There is an emerging sense that gene-specific therapy may be feasible for some forms of long QT syndrome. This related both to pharmacological therapy as well as to advice regarding lifestyle. A disorder based on disturbed inactivation kinetics of the sodium channel (LQT3) seems likely to respond to a sodium channel blocker. Indeed, in LQT3 patients, the QT-interval seems to shorten more than in LQT1 and in LQT2 patients in response to mexiletine, but individual exceptions do exist[29] and significant shortening of QT intervals by sodium channel blockers has been reported in some LQT2 patients[30]. It is also possible that while mexiletine or similar drugs shorten QT in LQT3, beta-blockade might still be required to suppress arrhythmias. As the amplitude of I_{Kr} increases when extracellular potassium concentration is increased, attempts have been undertaken to increase K^+ levels in long QT syndrome patients. To date, the QT interval has been shown to shorten significantly in LQT2 patients[31], but neither LQT1 nor LQT3 patients have been tested with this approach. Because I_{Kr} function is normal in the latter subjects, elevating potassium to increase I_{Kr} should shorten QT in them as well. The putative role of I_{Ks} in cardiac physiology suggests an especially favourable effect of beta-blockade and the avoidance of vigorous increase in heart rate (i.e. competitive sports) in LQT1 and LQT5. These examples demonstrate that gene-specific therapy may be feasible in the long QT syndrome. However, it should be emphasized that long-term trials are not yet available, and that, at the present time, beta-blockers remain the first choice therapy.

Drug-induced long QT syndrome

It has long been postulated that drug-induced long QT syndrome might represent a genetically-mediated 'forme fruste' of the long QT syndrome[32]. Recent studies have identified relatively large numbers of individuals who carry 'silent' mutations on long QT syndrome genes[22–25]. Thus, these persons, whose long QT syndrome mutations by themselves produce an alteration in repolarizing currents that is insufficient to prolong the QT interval at rest, may be especially sensitive to any drugs that affects K^+ currents. The combination of even a modest degree of I_{Kr} blockade, induced by a variety of drugs used for multiple purposes[33] and the silent mutations could produce a major prolongation in action potential that triggers the onset of Torsades de pointes. Indeed, occasional patients with typical drug-induced long QT syndrome and underlying mutations on long QT syndrome genes have now been identified. However, the rarity of this phenomenon means that genetic testing in patients with drug-induced long QT syndrome is not yet warranted in the absence of other indications (for example, family history, long baseline QT)[34,35].

Familial hypertrophic cardiomyopathy

Hypertrophic cardiomyopathy[36] is transmitted as an autosomal dominant disease. Its clinical phenotype is characterized by unexplained and inappropriate clinical left and/or right ventricular hypertrophy, which may be severe 4 to 5 cm), mild or even absent. Characterization of the distribution of left ventricular hypertrophy is arbitrary, but by convention hypertrophy is considered to be either asymmetric septal hypertrophy, concentric or predominantly distal ventricular. Any pattern of hypertrophy, however, may be seen including hypertrophy confined to the posterior or free wall. Characteristic histological features included myocyte disarray surrounding areas of increased loose connective tissue.

Clinically, there is marked haemodynamic heterogeneity among patients with familial hypertrophic cardiomyopathy. Systolic function may be hyperdynamic (with or without obstruction), 'normal' or impaired (10–15%). Diastolic dysfunction is the usual physiological abnormality, although the precise abnormality of ventricular filling and compliance is extremely variable.

Familial hypertrophic cardiomyopathy-related arrhythmias occur both at the ventricular and at the atrial level. Importantly, sudden cardiac death in

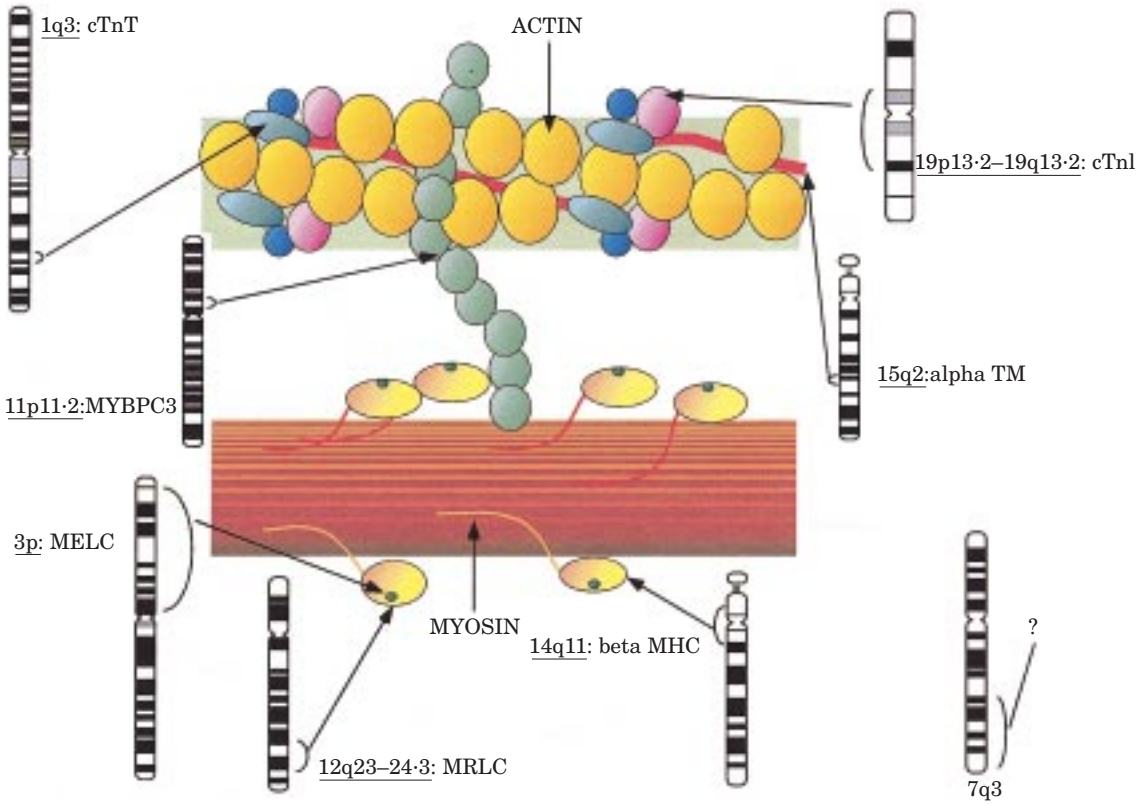

Figure 2 **FHCM genes Chromosomal locations of the genes and locations of the sarcomeric proteins associated with the genetic variants of FHCM.**

familial hypertrophic cardiomyopathy is not necessarily caused by ventricular arrhythmias. Atrial fibrillation in the presence of an accessory pathway, bradyarrhythmias and ischaemia may all lead to sudden death. In b-myosin heavy chain related patients (probably the majority), hypertrophy itself does not seem to be the main determinant of malignant ventricular arrhythmia. One caveat in interpreting electrophysiological changes in these settings is that a common secondary response to injury (such as pressure overload or coronary occlusion) is cardiac hypertrophy, which in diseased hearts produces further functional changes, notably in calcium handling. Thus, the extent to which any of the observed electrophysiological alterations are primary or secondary to the response to the disease process requires further study.

Familial hypertrophic cardiomyopathy genes

As illustrated in Fig. 2, there is considerable genetic heterogeneity in familial hypertrophic cardiomyopathy. Mutations in seven sarcomeric protein genes have been identified in families with familial hypertrophic cardiomyopathy (Table 1). These are: (1) beta-myosin heavy chain on chromosome 14[37]; (2) cardiac essential myosin light chain on chromosome 3[38]; (3) cardiac regulatory myosin light chain on chromosome 12[38]; (4) cardiac troponin T on chromosome 1[39]; (5) alpha tropomyosin on chromosome 15[39]; (6) cardiac myosin binding protein-C on chromosome 11[40,41]; and (7) cardiac troponin I on chromosome 19[42]. An additional locus has been identified on chromosome 7 in a large family with both familial hypertrophic cardiomyopathy and cardiac preexcitation (Wolff–Parkinson–White)[43].

The prevalence of the different gene abnormalities in familial hypertrophic cardiomyopathy is being delineated. To date, information on less than 100 genotyped families suggests that mutations in beta-myosin heavy chains and myosin binding protein C are more common than the others. In addition to this locus heterogeneity, there is, as in the long QT syndrome, marked allelic heterogeneity for all the recognized disease genes, and to date more than 85 different mutations have been reported (for reviews see[34,44,45]). The majority of mutations are missense mutations, although, for the cardiac myosin binding protein C gene, most of the mutations lead to an early stop codon, resulting in truncated mutant proteins[46]. Functional studies of mutant myosin indicates that sarcomeric contractile performance is depressed[47–49]. This in turn suggests that myocyte hypertrophy characteristic of familial hypertrophic cardiomyopathy reflects a compensatory response. The molecular (or other) determinants of myocyte disarray and myocardial fibrosis (interstitial and replacement) remain unclear. It may well be that these latter responses relate to the type of mutation (e.g. greater with troponin-related disease) and that sudden death and clinical arrhythmia are the clinical consequences of extensive disarray and fibrosis.

Genotype–phenotype correlations

Information on the genotype–phenotype relation in familial hypertrophic cardiomyopathy is still preliminary, as the published data on genotyped patients relates to only a few hundred individuals from centres that may reflect different referral biases. It is nevertheless clear that the phenotype varies not only with the type of mutation, but also within individuals bearing the same mutation. The 403 codon in beta-myosin is a hot spot for mutations; the arginine to glutamine mutation is associated with a poor prognosis, whereas the arginine to tryptophan mutation appears more benign[50–52]. Current practice suggests that if the ECG and two-dimensional echocardiogram are normal by age 25, then the patient can be safely reassured that he or she will not develop clinical familial hypertrophic cardiomyopathy. However, myosin binding protein C mutations appear to be associated with age-related penetrance during adult life[40,41,53]. Further information confirming the impression that adult onset of disease is an important feature seen with myosin binding protein C mutations would thus have a significant impact on management and counselling. The disease caused by troponin T mutations appears associated with mild or absent hypertrophy, a 20 to 25% incidence of non-penetrance and a high incidence of premature sudden death (possibly greater in young men although the numbers are small) which can occur even in the absence of significant clinical left ventricular hypertrophy[54–56].

Arrhythmogenic right ventricular dysplasia

Arrhythmogenic right ventricular dysplasia is a recently recognised familial cardiomyopathy[57]. The disease is characterized by fibro-fatty replacement of the right ventricular myocardium and life-threatening ventricular tachyarrhythmias originating from the right ventricle. Occasionally, the left ventricular myocardium is involved as well. Disease progression is associated with left ventricular involvement (50%), atrial dilatation and arrhythmias with embolic risk. Malignant ventricular arrhythmias are a common manifestation of the disease. Inducibility and reproducibility in the clinical electrophysiological laboratory is high, suggesting that re-entrant mechanisms related to the distinctive structural changes are likely. The disease appears especially common in North Eastern Italy (prevalence 1:1000) with an autosomal dominant inheritance (30%). An autosomal recessive variant of arrhythmogenic right ventricular dysplasia which is associated with a distinctive extra-cardiac phenotype (woolly hair and palmoplantar keratoderma) has been reported from the island of Naxos in Greece[58].

Molecular basis of arrhythmogenic right ventricular dysplasia

To date, four loci for autosomal dominant arrhythmogenic right ventricular dysplasia have been identified, two of which are in close proximity on chromosome 14 (14q23-q24 and 14q12- q22)[59,60]. A third locus was located on chromosome 1 (1q42-q43)[61], and the fourth on[62] chromosome 2 (2q32.1-q32.2) (Table 1). The autosomal recessive syndrome variant of arrhythmogenic right ventricular dysplasia has been linked to a locus on chromosome 17 (17q21), within the gene encoding a keratin, a reasonable candidate for the entity[58]. Further advances will facilitate recognition of the non-arrhythmic clinical presentations and the broader phenotype of arrhythmogenic right ventricular dysplasia/Naxos disease (Table 1).

Dilated cardiomyopathy

Dilated cardiomyopathy is a genetically heterogeneous and clinically heterogeneous disease[63], which can affect newborns, children, adolescents, adults and the elderly. The disease may be associated with other organ or muscle abnormalities or present as a pure disorder. Malignant life-threatening ventricular arrhythmia as well as atrial arrhythmia with a serious impact on cardiac function are frequently associated with the disorder. As in familial hypertrophic cardiomyopathy, sudden death in dilated cardiomyopathy may also be caused not only by ventricular arrhythmias but also by bradyarrythmias. Whenever spontaneous ventricular arrhythmia have been clinically documented, the inducibility and reproducibility of the arrhythmia in electrophysiological studies is usually low, favouring the possibility of a predominant role for non-reentrant mechanisms[64,65]. At least 30% of cases of dilated cardiomyopathy are inherited (that is, familial dilated cardiomyopathy, familial dilated cardiomyopathy) with a significant percentage of the remaining cases being acquired (that is, myocarditis, ischaemic heart disease etc.). Inherited dilated cardiomyopathy may have autosomal dominant, autosomal recessive, X-linked or mitochondrial transmission (Table 1).

Molecular basis of dilated cardiomyopathy

To date, genes for X-linked and autosomal dominant dilated cardiomyopathy have been mapped, demonstrating genetic heterogeneity[66]. The genes for two X-linked cardiomyopathies have been identified: the dystrophin gene which is also responsible for Duchenne and Becker muscular dystrophy[67,68], and G4.5 in Barth Syndrome (X-linked cardioskeletal myopathy with neutropenia, abnormal mitochondria and 3-methylglutaconic aciduria)[69]. Multiple mutations in both genes have also been reported[68–72].

Dystrophin is a large cytoskeletal protein which is found on the inner face of the sarcolemma and attaches at its N-terminal domain to F-actin in the matrix and to the dystrophin-associated glycoprotein complex (an oligomeric transmembrane protein) at its C-terminal domain. The protein encoded by the G4.5 gene is called 'tafazzin' but its function is unknown.

Genes for autosomal dominant dilated cardiomyopathy have been mapped to six different loci thus

far. 'Pure' dilated cardiomyopathy has been localized to 1q32, 2p31, 9q13, and 10q21-q23[73–76], while dilated cardiomyopathy with conduction defects has been mapped to 1p1-1q1[77] and 3p22-3p25[78]. Recently, mutations in cardiac actin[79] located on chromosome 15q14 have been identified, therefore so far actin is the only known gene for autosomal dominant dilated cardiomyopathy. Based on this finding, Olsen *et al.*[79] have now proposed that dilated cardiomyopathy results as a consequence of defective transmission of force in cardiac myocytes leading to heart failure.

Idiopathic ventricular fibrillation and the Brugada syndrome

Another interesting group of patients which has become a target for genetic studies is represented by individuals with so-called idiopathic ventricular fibrillation (that is, patients with a normal heart that experience cardiac arrest with documented ventricular fibrillation)[80]. A subgroup of these patients experience sudden death which may occur in families, apparently have no structural heart disease and have right precordial ST segment elevation, sometimes with right bundle branch block (Brugada syndrome[81]). These electrocardiographic characteristics may depend on exaggerated transmural differences in action potential configuration, especially in the right ventricular outflow tract. This could arise from dysfunction of a number of ion currents, such as I_{to}, L-type Ca^{2+} current ($I_{Ca(L)}$) and I_{Na}.

At least one variant of the Brugada syndrome is caused by defects in the sodium channel gene (SCN5A), i.e. the same gene implicated in LQT3[82]. In the Brugada syndrome, the mutations identified apparently lead to a loss of function while in LQT3 all cause a gain of function. Thus, the long QT and the Brugada syndromes appear to be separate allelic disorders.

Evidence that not all patients with the Brugada syndrome have defects on the cardiac sodium channel (Priori *et al.*, 1998 personal communication) suggest that, in analogy with the other inherited cardiac diseases, genetic heterogeneity is also present in Brugada syndrome.

Atrial fibrillation

Perhaps the commonest arrhythmia requiring intervention is atrial fibrillation. Data are now emerging from a number of laboratories on the potential molecular basis of electrophysiological changes observed in atria that have been fibrillating for hours to days and those that have been fibrillating for weeks to months[83–87]. They all share a marked shortening of refractoriness, probably reflecting decreased action potential duration early during atrial fibrillation. Available data suggest that a major mechanism is decreased inward current through L-type calcium channels and possibly sodium channels[88]. Later during the 'remodelling' that appears to accompany chronic atrial fibrillation, changes in expression and/or distribution of connexin proteins and/or other ion channel proteins, as well as changes in cellular ultrastructure, may play a role.

Inherited atrial fibrillation is considered uncommon and has been reported with autosomal dominant transmission. Recently, familial atrial fibrillation has been mapped to 10q22-q24 (a region of approximately 11 cM) in three families[89]. Expansion of the previously identified kindreds has allowed further refining of the map position and limitation of the gene critical region.

A fascinating issue concerning atrial fibrillation is its association with other disorders, such as dilated cardiomyopathy, familial hypertrophic cardiomyopathy and LQT4 and the possibility that a mutation in a gene responsible for one of these associated disorders could cause familial atrial fibrillation. For instance, is it simply circumstantial that a familial dilated cardiomyopathy locus[76] and the mapped atrial fibrillation locus are within the same relatively small region of 10q21-q24? Is there something different about the clinical course, and thus the causative gene responsible for LQT4[8] in which prolonged QTc appears to be associated with a high incidence of atrial fibrillation and slower heart rates than typically seen in the long QT syndrome? Could this be a different type of gene (i.e. not an ion channel) or a new channel disorder?

Progressive familial heart block

Two forms of progressive familial heart block[90] which differ in their ECG characteristics, have been reported. The first, progressive familial heart block-I, is defined on the ECG by evidence of bundle branch disease such as right bundle branch block, left anterior hemiblock, left posterior hemiblock, or complete heart block with broad QRS complexes. Progression of disease occurs with changes in the ECG, from a normal ECG to right bundle branch block to complete heart block. Typical manifestations of the disease are syncope, sudden death, or Stokes–Adams attacks. The second form of progressive familial heart block, known as progressive familial heart block-II, presents with complete heart block and narrow QRS complexes and is believed to occur due to atrioventricular nodal disease with atrioventricular block and an idionodal escape rhythm. Typically these patients present with sinus bradycardia and left posterior hemiblock, and develop syncope and Stokes–Adams attacks.

Genetically, progressive familial heart block-I is better studied than progressive familial heart block-II and appears to be inherited in an autosomal dominant fashion. Brink *et al.*[91] studied three South African families with progressive familial heart block-I, including one nine generation kindred, for linkage analysis. Using 86 family members (39 affected), linkage was identified on chromosome 19 at 19q13.2-q13.3 and the gene was localized to within 10 cM of the kallikrein

locus. Confirmation of this localization was subsequently reported by Bouvagnet *et al.* in a large Lebanese family[92]. Other candidate genes within the mapped region include Apolipoprotein C2 (ApoC2), creatine kinase MM isoform (CK-MM), myotonic dystrophy, troponin T and the histidine rich Ca^{2+} binding protein (a luminal sarcoplasmic reticulum protein). Myotonic dystrophy, CK-MM, and ApoC2 have been excluded as the causative genes.

Familial Wolff–Parkinson–White syndrome

Familial Wolff–Parkinson–White has been rarely reported but an inherited form of the syndrome associated with familial hypertrophic cardiomyopathy has been described and its locus mapped to chromosome 7q3[43]. It is unknown whether a single defect is responsible for both aspects of the syndrome or if two genes are located in close proximity (i.e. contiguous gene syndrome) and thus frequently co-segregate. In the latter case, familial Wolff–Parkinson–White could be caused by a single gene-defect on chromosome 7. However, other associations of familial hypertrophic cardiomyopathy and Wolff–Parkinson–White have also been identified. For example Kimura *et al.*[42] found mutations in the cardiac troponin I gene (on chromosome 19) in patients with familial hypertrophic cardiomyopathy and Wolff–Parkinson–White. Furthermore, some children with mitochondrial abnormalities and metabolic disease (Pompe disease) associated with familial hypertrophic cardiomyopathy also have been noted to have Wolff–Parkinson–White. Therefore, it currently appears that Wolff–Parkinson–White may have multiple different genetic aetiologies.

Molecular diagnosis of inherited arrhythmogenic disorders

Role of DNA screening in diagnosis of inherited arrhythmogenic diseases

The possibility of a genetic diagnosis means that genetic testing in routine clinical practice is also a possibility. Applications could include pre-clinical diagnosis and identification of patients who might benefit from prophylactic treatment for sudden death. For this approach to become a reality, however, several conditions must be met: the development of routine clinical DNA diagnostic testing facilities; a sufficiently large database to determine risk in relation to genotype, as well as the recognized heterogeneity in phenotype; an estimate of the efficacy of available treatments; and a consideration of the cost implications.

Molecular diagnosis has the potential to define with 100% sensitivity and 100% specificity the genetic status of any member of an affected family. However, for this potential to become fully expressed, it is necessary that all the genes and all the mutations within these genes causing a given disease be identified. This is not yet even close to reality for any of the inherited arrhythmogenic diseases discussed here. As a consequence, physicians still generally have to rely on clinical criteria to establish these diagnoses.

For some diseases, not even the specific affected gene(s) are known. In these cases (for example, familial atrial fibrillation, progressive familial atrioventricular block), the available genetic information is derived from linkage studies and provides only data on which chromosomal region the disease-gene is located. If this region is large, it may take years before the gene responsible for the disease is located. Thus, at this stage of knowledge, molecular screening for these entities is limited to research activities; it is not possible to consider genotype–phenotype correlations and, most importantly, the nature of the defect underlying the disease remains undefined. Linkage studies can, nonetheless, provide important information on whether only one gene is associated with the disease or if genetic heterogeneity exists (i.e. several genes accounting for a disease).

When a gene responsible for a disease is identified, it then becomes possible to search for specific mutations. The organization and the sequence of the disease genes are often not entirely known, and thus mutations are usually searched for only (at least initially) in portions of the gene. As a consequence, a positive finding (i.e. the identification of a mutation) is diagnostic, while a negative finding in a linked gene suggests that mutations may be present in an unexplored region of the gene (or that the linkage is incorrect). The diagnostic power of molecular screening is further limited (for all arrhythmogenic disorders discussed here) by the presence of genetic heterogeneity and the lack of identification of all of the genes responsible for the disease.

Implications of molecular diagnosis on patient management

When the genetic bases of familial hypertrophic cardiomyopathy and the long QT syndrome were elucidated, the hope of molecular biologists and clinicians alike was that it would become possible to reach, in a relatively short time, some important goals to establish genotype–phenotype correlations. In this respect, valuable information would be the ability to categorize mutations as 'mild' vs 'severe', in order to guide the therapeutic approach on the basis of the predicted risk. For the time being, this goal has not been achieved, and we are still far from being able to predict adverse or favourable prognoses based on the genetic defect.

A major goal in the long QT syndrome and familial hypertrophic cardiomyopathy remains, to have sufficient genotyped patients to understand the diagnostic, functional and prognostic implications of the different mutations. A problem in genetic testing in the long QT syndrome and familial hypertrophic

cardiomyopathy is that the disease-associated gene and specific mutations are still being identified. This research information is not yet widely implemented in commercial laboratories, and the resource demands for such an effort on a routine (or 'service') basis are generally beyond those available to the research laboratories engaged in the problem.

Genetic testing for the long QT syndrome

When should genetic testing be considered in dealing with long QT syndrome patients[93]. The cardiologist will confront three clinical scenarios:

The first situation is the patient who has a definite diagnosis based on established clinical diagnostic criteria. Here, genetic testing is not absolutely necessary because the cardiologist has most elements necessary to make a decision about initiation of therapy. However, genetic testing could be useful because, depending on the gene (and ultimately even the specific mutation) identified as responsible for the disease, modifications in management[29,94] may be suggested. Examples discussed above include the addition of mexiletine in LQT3 or lifestyle modifications such as limitation of strenuous or competitive exercise in LQT1. It should be pointed out, however, that in symptomatic patients with an established diagnosis of long QT syndrome, implementation of therapy with beta-blockers should not be delayed while waiting for genetic screening results.

A second scenario occurs when the diagnosis of long QT syndrome is only suspected or the patient has a borderline diagnosis based on clinical criteria. Under these circumstances, genetic testing could be very useful in establishing the diagnosis because identification of a mutated long QT syndrome gene would convert a suspected diagnosis to a certain one and would remove the cardiologist's hesitation in making therapeutic choices. However, failure to identify a mutation does not rule out the diagnosis (since only a minority of mutations have been identified to date). Although genetic testing in this situation is not yet widely available, techniques to automate screening for the hundreds of possible known mutations are now being developed and will probably be available in the next 5–10 years. A third scenario is an apparently asymptomatic relative of a patient with the long QT syndrome. Here, genetic testing can be especially useful if the disease-causing mutation has previously been identified in the proband. Otherwise the issues are the same as those for evaluating the 'borderline' long QT syndrome diagnosis.

Genetic testing for hypertrophic cardiomyopathy

Similar considerations apply in familial hypertrophic cardiomyopathy. Comprehensive screening of the disease-causing genes would, from the clinical perspective, be both inappropriate and impractical at this time. Specific clinical situations exist where DNA diagnosis is likely to have an important impact on management. For example, sudden death/resuscitated ventricular fibrillation in association with normal or near normal heart weight and/or mild morphological features in the young should lead to testing for mutations in the cardiac troponin T gene. Premature sudden death in association with obvious morphological features in the young have been associated with the Arg403Glu and Arg453Cys mutation in the beta-myosin heavy chain gene, and these mutations could be tested in this clinical context. Identification in the proband of troponin or myosin heavy chain mutations, which are associated with poor prognosis, would permit an early or even a pre-clinical diagnosis in family members with the potential for lifestyle modifications (avoidance of competitive exercise) and prophylactic treatment (amiodarone or implantable cardioverter defibrillator) to prevent sudden death.

Genetic testing for autosomal dominant dilated cardiomyopathy, arrhythmogenic right ventricular dysplasia, familial atrial fibrillation, progressive familial atrioventricular block

Until specific genes are discovered and characterized, molecular diagnosis should be considered a research tool only in large size families in which linkage analysis may be performed.

Genetic testing for dilated cardiomyopathy

In both X-linked dilated cardiomyopathy and Barth syndrome, definite diagnosis at the molecular level may be useful clinically since both are rapidly progressive and severe disorders. In the case of X-linked dilated cardiomyopathy where anticongestive and antiarrhythmic management initially, and cardiac transplantation shortly thereafter, is life-saving, determination of a mutation could help diagnose pre-symptomatic male gene-carriers. In Barth syndrome, therapeutic options are less clear-cut but a definitive diagnosis in family members and potentially in fetuses could be similarly useful.

The recent identification of mutations in the actin gene opens the opportunity to perform family screening for mutations; however, until the prevalence of actin-related autosomal dominant dilated cardiomyopathy is defined, the cost/benefit ratio of actin gene screening cannot be defined.

Genetic testing for Brugada syndrome

The identification of mutations in the cardiac sodium channel in families with Brugada syndrome opens the possibility to screen patients with the disease. The importance of identifying the defect obviously consists of the ability to identify the carriers before they become symptomatic. This is particularly important for a disease in which the first manifestation is often cardiac arrest. However, since not all patients with Brugada syndrome have mutations in the sodium channel (Priori *et al.*, personal communication), the cost/benefit ratio of mutation screening in the sodium channel gene cannot be defined until the prevalence of the genetic variant of the form associated to sodium channel defects is defined.

Ethical aspects of molecular screening

Important ethical aspects are involved when considering DNA screening in families affected by a congenital disease. Discussion with families about the information that could be provided by genetic testing is a most important first step. The experience of the team (which should include an appropriately trained genetic counsellor) in caring for patients with similar disorders is an important component for patient acceptance. One specific objective of counselling in arrhythmogenic disorders is to help the patient decide whether he/she should undergo genetic screening at all. These are the considerations involved when an individual is deciding whether or not to be tested:

(a) The patient should be given information on the (1) sample required; (2) use of the sample; (3) results of the test performed; (4) implications of these results for management of the patient and their family; (5) who will have access to the results.
(b) The patient should use this information to decide whether to give or withhold consent.
(c) There should be no coercion by anyone (healthcare team, family members, insurance companies).
(d) The consent form that patients sign should include statements that (1) all blood samples are coded to prevent identification; and (2) the results of screening will be communicated only to the patient and that no disclosure will be made to third parties (not even family members) without their written consent. Asymptomatic patients should have the option of providing samples (e.g. for family study) without being informed of the results.

Reimbursement and costs issues

Currently, DNA screening for arrythmogenic disorders is not considered a routine test, and therefore costs are not usually covered by insurance. Linkage to isolate the disease gene can be performed in large families. When small pedigrees or single patients, in whom linkage cannot be applied are studied, the only approach for DNA screening is the systematic search for known mutations in any disease-linked gene. As discussed above, clinical evaluation may help in selecting gene(s) to be screened first. Depending on the size of the gene and on the number of genes to be screened, costs may be substantial (>$1000 US per gene screened in each family). Currently, costs are covered almost exclusively by research funding of the laboratories involved in the field. The development of automated screens and identification of more mutations may change this in the near future.

Molecular basis of cardiac electrophysiology and arrhythmias

In the first two parts we have discussed monogenic arrhythmic disorders. These are determined or favoured by an inborn alteration and, for the most part, are characterized by a single alteration in one or more disease genes. This has allowed the use of 'paradigms'; namely, diseases such as the long QT syndrome in which it has been possible to trace specific mutations on ion channel genes to their electrophysiological consequences in the patient. Unfortunately for the practising cardiologist, these 'simple' diseases constitute only a small part of the clinical conditions associated with cardiac arrhythmias. The majority of cases affect patients in whom the arrhythmogenic substrate is complex. Indeed, the expression of the molecular systems responsible for normal and abnormal electrical activity vary significantly, depending on a variety of factors including age, regional factors (type of cells, myocardial perfusion), and underlying chronic diseases such as cardiac hypertrophy, myocardial infarction, and heart failure.

To approach this complex system of interacting molecular functions requires a somewhat different approach from that required to consider monogenic disease. Accordingly, in this section we discuss broader themes that are essential to understand the integration of gene expression, of ion channel function, and cell coupling in multicellular networks. This must be considered a first step toward the comprehension of more frequent and more complex arrhythmogenic conditions.

Diversity of gene expression in the heart

Understanding cell–cell variability in the cardiac action potential shape and the mechanisms underlying impulse propagation is the key to understanding normal and abnormal cardiac electrophysiology. Much of this variability can be attributed to variability in the characteristics of individual ion currents whose integrated behaviour determines the shape and duration of action potentials in individual cardiac cells, as well as to variability in cell–cell communications. Ion currents are now recognized to flow through specific pore-forming membrane proteins, termed ion channels. The first gene encoding an ion channel was cloned in 1984[95], and the succeeding decade and a half has seen the cloning of genes encoding most ion channels expressed in the heart, and in many other tissues[96–98]. Many of the proteins these genes encode share common structures and can be viewed as members of the same superfamily. For example, Fig. 3 shows the tremendous diversity of mammalian genes that make up the family of potassium channel genes. Since potassium channels are made up of four ion channel alpha subunit proteins, which are not necessarily identical, the potential for diversity in potassium currents is even greater than shown. This is further compounded by the identification of ancillary subunits (the products of different genes) that can assemble with potassium channel tetramers to modulate their function[99–101]. Figure 4 illustrates the major ion currents in the heart and the genes whose protein products are thought to form their structural basis. The dramatic increase in molecular genetic information

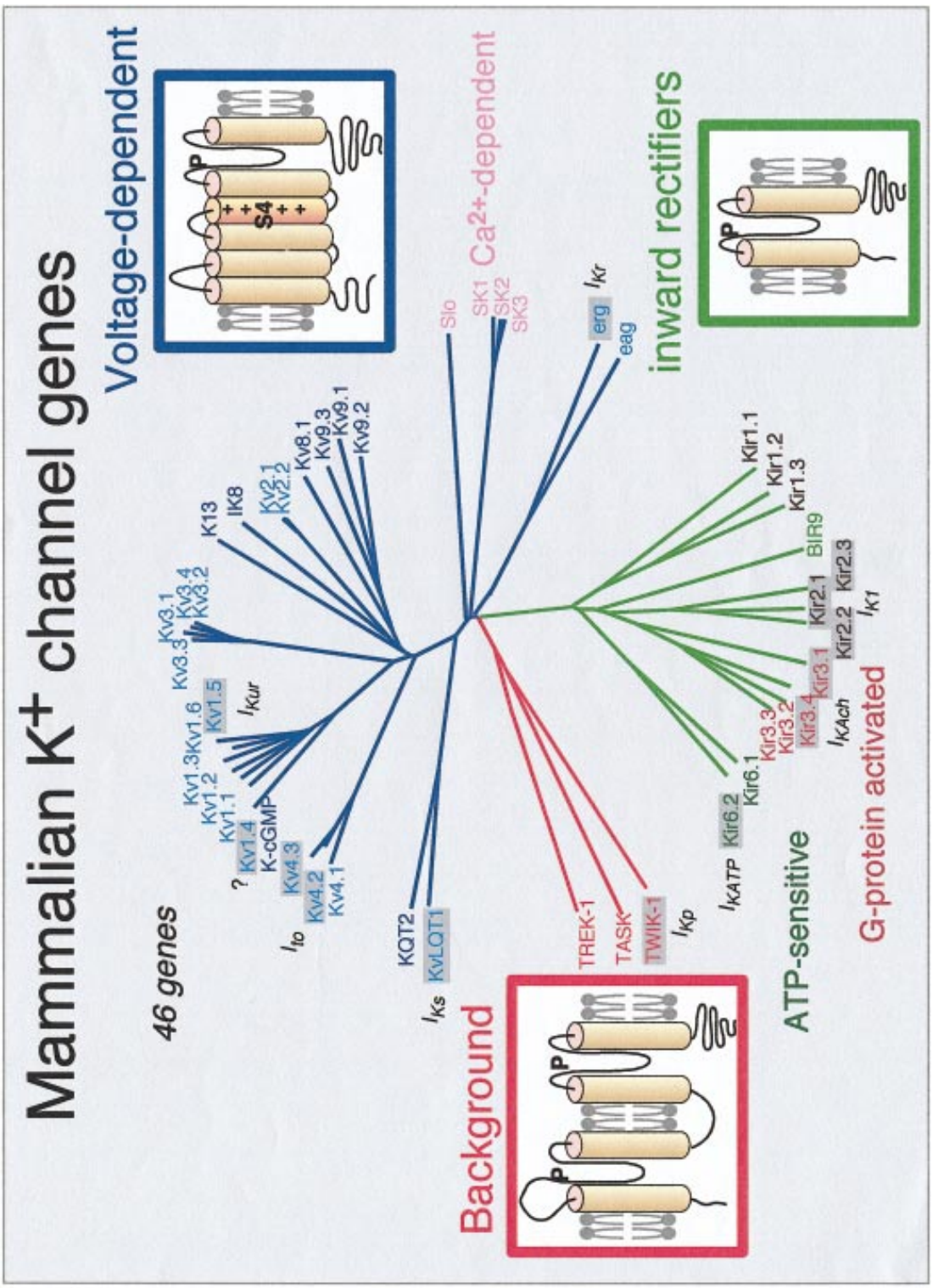

Figure 3 **Family tree of pore-forming K^+ channel subunits in mammals This dendrogram represents the degree of similarity between each gene, as determined by the ClustalW alignment programme. Sequences were extracted from the genbank database and correspond exclusively to rodent or human channels. The K^+ channel subunits are divided into three major groups according to their structural and functional properties as shown in the blue, green and red boxes. The major genes expressed in the heart are indicated in grey boxes, together with the probable current they underlie.**

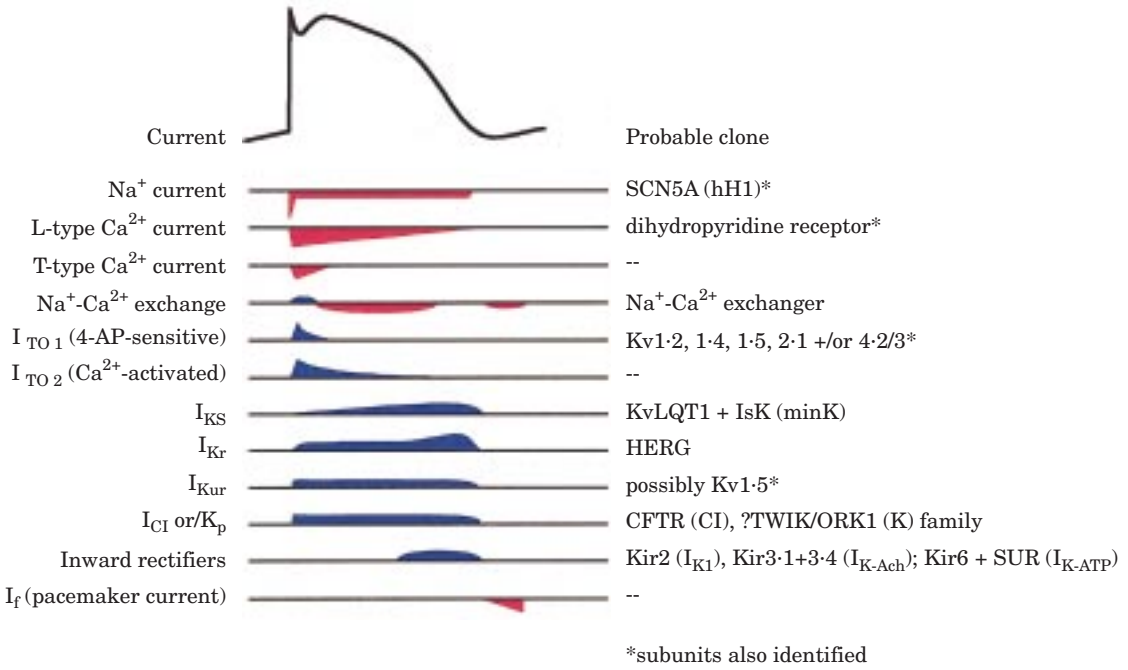

Figure 4 **Cardiac ionic currents and respective ion channel clones responsible for generation of the action potential** **Inward currents are drawn in red, outward currents in blue. The amplitudes are not to scale.**

underlying cardiac function is not confined to ion channels, but has extended to multiple other genes, including those controlling cell–cell communication (the connexins), the contractile apparatus, and cardiac development, to name a few. Cloning leads to important advances not only in our understanding of mechanisms of normal cardiac function but also as regards new insights into the mechanisms underlying common cardiac diseases and their therapy.

A common method of studying individual cardiac ion channel gene function is to express the gene of interest in non-cardiac (heterologous) study systems, such as mammalian cell lines or the eggs of the African clawed toad, *Xenopus laevis*. In some cases, expression of a single gene in such heterologous systems is sufficient to reproduce the physiological and pharmacological characteristics of a specific cardiac ion current; HERG expression to recapitulate I_{Kr} is an example[102] although coexpression of the minK subunit may increase I_{Kr} amplitude[103,104]. The systems have been especially valuable in delineating the functional consequences of ion channel gene mutations, although it should be recognized that mechanisms other than a simple dominant negative effect on channel gating (e.g. altered trafficking) may also play a role.

In other cases, faithful recapitulation of a specific cardiac ion current requires coexpression of more than one gene. Heterotetramers of Kv4.2 and Kv4.3 may determine the transient outward current in some species[105]. Other examples include coexpression of a structural gene and an ancillary subunit; one good example is the finding that coexpression of KvLQT1, a member of the potassium channel family shown in Fig. 3, with the minK gene is required to recapitulate I_{Ks}[13,14].

Another example is α-β_1 interaction during the development of an adult sodium current described below. In other cases, a gene product (e.g. Kv2.1) can be detected in the heart without a recognized counterpart among the known ion currents, and there remain ion currents (I_f) for which no corresponding gene has yet been identified. One very important observation is that ion channel genes are virtually never expressed exclusively in the heart. Thus mutations or blocking drugs may affect not only cardiac function but also functions in other organs. The best described example to date is the deafness displayed by patients with the recessive (Jervell–Lange–Nielsen) form of the long QT syndrome[4]. This arises because the two genes involved in the disease KvLQT1[19,20] and minK[16,106] are expressed not only in the heart but also in the inner ear, where together they control endolymph homeostasis[107]. It is not yet known whether parent carriers, who have mild, usually but not always asymptomatic mutations in KvLQT1[20] or minK[15] display subtle defects in hearing. This is an example of how molecular genetics may explain a diversity of symptoms through common mutations affecting function in multiple organs.

Development

One well-recognized form of variability in cardiac function is the stereotypical changes that are observed during development. Much of the information has been gathered in small rodents and may not be applicable directly

to humans, but it may be important because a common form of cardiac response to injury is regression to a fetal phenotype. Whether, for example, the electrophysiological changes associated with hypertrophy (for example, in patients with hypertension or heart failure) represent such a patterned response is an important consideration. Understanding the mechanisms underlying such a change in phenotype may be an important step in the prevention of arrhythmias in these common acquired disorders of cardiac function.

The embryo is the earliest stage at which ion currents have been recorded in heart tissue, on post-conception day 11 in mouse (normal gestation period 20·5 days). At this stage, the predominant inward current is L type calcium current (I $Ca_{(L)}$) and the predominant outward current is the rapidly activating component of the delayed rectifier, I_{Kr}[108,109]. Sodium current (I Na) appears later, and increases markedly just prior to birth[109]. There are important differences between sodium current recorded in neonatal animals and those recorded in adult animals[110,111]: I_{Na} in neonates is smaller, activates, inactivates, and recovers from inactivation more slowly than that in adult, and has a more positive voltage dependence of inactivation than that in adult. Some data suggest that this difference between neonatal and adult sodium current may reflect expression of a β_1 subunit and/or α-β_1 assembly to produce the 'mature' phenotype[112,113], and that this change may reflect the sympathetic innervation of the heart that occurs around the time of birth. This may be one example of a more general influence of sympathetic innervation as a modulator of cardiac electrophysiology[114]. It is likely that multiple mechanisms will be identified. The possibility that acquired diseases (for example, myocardial infarction) in which regional cardiac denervation may play a role is an obvious one that requires further study[115]. Another intriguing observation during development is the consistent embryotoxicity of specific I_{Kr} blockers such as dofetilide or almokalant in the 13–14 day post-natal rat[116]. Since I_{Kr} is the predominant (if not the sole) repolarizing current at this stage[108], it has been postulated that embryotoxicity is due to failure of cardiac repolarization, with death due to arrhythmias secondary to triggered activity (which have been demonstrated under these experimental conditions) or simply membrane depolarization. Whether similar considerations apply to humans has not been determined.

The pattern of expression of the connexins, which form the gap junction channels and belong to one gene family, also varies during development. Cx43 mRNA is detectable in mouse heart from day 9·5 post-conception. Initially it is expressed only in the ventricle but later it spreads throughout the whole heart. Two weeks after birth the message starts to diminish again to a steady level that is maintained during adulthood[117]. Cx40 is detected from 9·5 days post-conception. The message is initially confined to the atrium and left ventricle but during development it spreads throughout the whole heart[118]. After 14 days post-conception the Cx40 message starts to diminish in the ventricles from epicardium to endocardium until in the adult Cx40 is restricted to both atria and the proximal conduction system[117]. Cx45 mRNA is expressed from day 11 post-conception at a constant level throughout the whole heart until week 3 post-partum when it starts to decrease until in adulthood, only low-level expression in the proximal conduction system is detected[117].

Regional diversity in cardiac electrophysiology

While heterogeneity of cardiac electrophysiology is increasingly recognized as a contributor to cardiac arrhythmias, it should also be recognised that there is substantial heterogeneity in the electrophysiological properties of individual cells even under physiological conditions. A trivial example is the difference among the electrophysiological properties of sinoatrial node, atrium, atrioventricular node, conducting system, and ventricular myocardium. These differences presumably reflect variability in expression and/or function of the repertoire of ion channels whose integrated activity determine the distinctive action potentials in each of these regions. More recently, it is increasingly recognized that there is considerable potential for cell-to-cell variability in action potentials and gene expression within such specified regions. For example, a survey of atrial myocytes revealed a consistent I_{TO} in only about 60% of cells, a consistent I_K in approximately 15% of cells, and both currents in 30% of cells[119]. Studies of mRNA expression have also demonstrated striking cell–cell variability in expression of individual ion channel genes[120]. Two long QT syndrome genes, HERG and KvLQT1, were identified in a majority of cells in most regions. In contrast, minK was most abundant in the sinoatrial mode (in about one third of cells), but much less abundant in ventricular muscle cells (10–29%). This is consistent with a more recent report that, at least in the mouse, minK expression appears restricted largely to the conducting system[121]. Similarly, M cells, which, as described below, appear to play a role in the genesis of long QT-related arrhythmias, have distinctively long action potentials which markedly prolong at slow heart rates[122], a characteristic also seen in Purkinje cells[122,123]. One report suggests that this distinctive action potential behaviour is paralleled by a reduction in I_{Ks} (compared to endocardial and epicardial cells[125].

Electrophysiological studies have identified Kv4.2 and/or 4.3 (depending on these species) as the ion channel gene whose expression in heterologous systems results in a current most closely resembling human I_{TO}[126]. One of the important features of human I_{TO} is its usually rapid recovery from inactivation[127]. It was this observation that first raised the suggestion that Kv1.4, an initial leading candidate for I_{TO}, might not, in fact, encode this current, since Kv1.4 recovers very slowly from inactivation[128]. Interestingly, the human endocardium also displays a transient outward current but one which, unlike that recorded in epicardium, recovers from inactivation very slowly, and is therefore not regularly observed in endocardial action

potentials[127]. It therefore remains conceivable that while Kv4.x encodes epicardial I_{TO}, expression of other channels, including Kv1.4, may still contribute to the electrophysiological properties of cells in other regions.

The connexins are also expressed in a chamber-specific and tissue-specific fashion. In heart, connexins 40, 43 and 45 have been detected at the protein level[129]. The phosphoprotein Cx43 is the most abundant cardiac connexin. It forms gap junctional channels with a main conductance of about 45 pS between cardiac myocytes in all parts of the heart, with the possible exception of the sinoatrial and atrioventricular nodes. In the ventricle, Cx43 is more abundant in the intercalated disk than in the lateral cell borders, which partially explains anisotropic impulse conduction. In the atrium (with the exception of the crista terminalis) the difference between end-to-end and side-to-side connections is much less pronounced. Although Cx43 has been reported to be present in the sinoatrial node[130,131], in atrial cells it is probably intercalated between the pacemaker cells[132]. Rabbit sinoatrial node pacemaker cells are found coupled by high conduction (250 pS) channels formed by an unidentified connexin (Verheule *et al.*, 1998, personal communication). In atrial gap junctions, Cx40 is co-localized with Cx43[129]. Cx40 is also a phosphoprotein with a main conductance of 160 pS. Guerrero *et al.*[133] presented evidence that Cx40 and Cx43 contribute equally to impulse conduction in the atrium. In most species (including man) Cx40 is also found in the proximal conduction system. Cx45 forms channels with a conductance of about 20 pS which are very sensitive to transjunctional voltage, that is, even at small voltage differences between neighbouring cells, Cx45 channels close quickly. Cx45 has been reported to be abundantly present in all parts of the heart by some authors[134], but others find it only in part of the conduction system and in very limited amounts in the rest of the heart. The role for Cx45 in impulse conduction has not yet been established.

Integration of ion channel function into the cellular environment

While ionic channels and connexins participate in the generation of the cardiac action potential and in cell-to-cell communication, it is important to recognize that, experimentally, single channel data are mostly obtained in preparations that are removed from the cardiac cell environment (e.g. membrane patches, cloned channels in Xenopus oocytes). Figure 5 is a schematic diagram of a cardiac ventricular myocyte, demonstrating the complex physiological environment in which ion channels function to generate an action potential[135].The cellular environment is highly interactive and modulates the behaviour of the single channel through interactions with other channels, or with the ionic milieu of the cell. An example is illustrated in Fig. 6. In this scheme, the $I_{Ca(L)}$ induces Ca^{2+} release from the sarcoplasmic reticulum through the Ca^{2+}-induced-Ca^{2+} release process[136]. The released Ca^{2+} in the myoplasm, in turn, modulates several ionic currents (including $I_{Ca(L)}$ itself)[135]. In Fig. 6, the myoplasmic Ca^{2+} is shown to increase the conductance of I_{Ks}, to drive I_{NaCa} for the purpose of Ca^{2+} extrusion, and to participate in the inactivation of $I_{Ca(L)}$[137–139]. The effect on the action potential is complicated; increased I_{Ks} acts to shorten the action potential duration, as does reduced $I_{Ca(L)}$. I_{NaCa} is an inward current (when operating to extrude Ca^{2+} from the cell) and its augmentation acts to prolong action potential duration. The net effect depends on the quantitative balance of these processes. This balance can depend on the basal expression of these channels or of the proteins (such as kinases) that regulate their activity, diseases or other processes that modulate the expression, and other important factors, such as rate or adrenergic activity. Importantly, many of these latter processes may also be modulated by changes in intracellular calcium.

The example above serves to illustrate a most important point, namely that the current through an ion channel is determined by its intrinsic kinetic properties *and* its interaction with the cellular environment. Because of the highly interactive nature of the cell, altered function of a particular channel (e.g. due to modulation by calcium) will have an indirect influence on the currents through other channels. For example, the late current that underlies the LQT3 form of the long QT syndrome acts to depolarize the membrane during the plateau phase of the action potential. The increase in membrane potential, in turn, alters the magnitudes and time course of other plateau currents (for example $I_{Ca(L)}$,I_{Kr},I_{Ks}) which together, determine the action potential duration.

The concept that the action potential is determined by the interaction of various ionic currents is increasingly well-appreciated in the context of the long QT syndrome. The action potential plateau is maintained by a delicate balance between inward (depolarizing) and outward (repolarizing) currents. In the long QT syndrome, the action potential is prolonged by an increase in inward current (late I_{Na} in LQT3) or a decrease in outward current (I_{Ks} in LQT1, or I_{Kr} in LQT2). It should be emphasized that the effect on the action potential could be very different for the different mutations. For example, the generation of an early afterdepolarization at plateau potentials (phase 2 early afterdepolarization) involves recovery and reactivation of $I_{Ca(L)}$[137,138]. This can be achieved if the action potential plateau is sufficiently prolonged at a specific range of membrane potential. It is conceivable that such conditions are created by some mutations but not others.

Similarly, distinctions can occur in the rate dependence of action potential duration. Through the process of adaptation, action potentials shorten with increasing heart rate[142]. This phenomenon raises the possibility of depressing early afterdepolarizations with fast pacing in the long QT syndrome. Recently, it was found that LQT3 shows much greater shortening of QT interval with an increase in heart rate during exercise

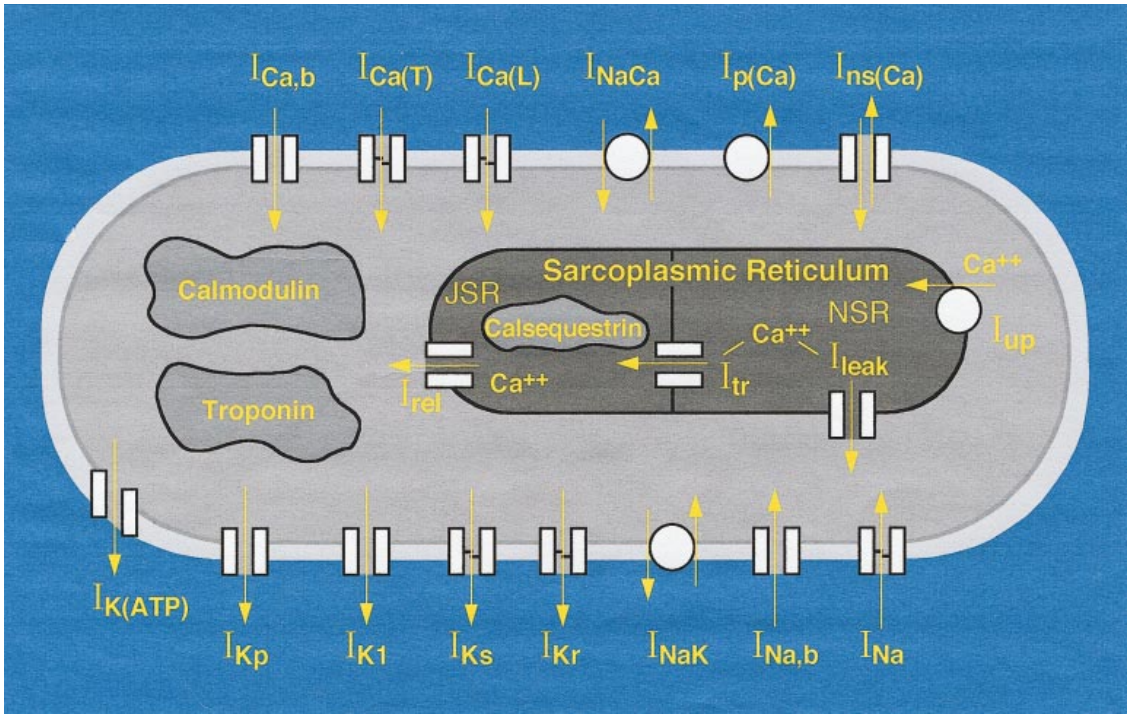

Figure 5 **Schematic diagram of a computer model used to simulate the electrical activity of a cardiac ventricular cell The cellular environment is highly interactive and modulates the behaviour of membrane ion channels. The integrated behaviour of the cell generates the action potential. I_{Na}=fast sodium current; $I_{Ca(L)}$=calcium current through L-type calcium channels; $I_{Ca(T)}$=calcium current through T type calcium channels; I_{Kr}=fast component of the delayed rectifier potassium current; I_{Ks}=slow component of the delayed rectifier potassium current; I_{K1}=inward rectifier potassium current; I_{Kp}=plateau potassium current; $I_{K(ATP)}$=ATP sensitive potassium current; I_{NaK}=sodium–potassium pump current; I_{NaCa}=sodium–calcium exchange current; $I_{p(Ca)}$=calcium pump in the sarcolemma; $I_{Na,b}$=sodium background current; $I_{Ca,b}$=calcium background current; $I_{ns(Ca)}$=nonspecific calcium-activated current; I_{up}=calcium uptake from the myoplasm to network sarcoplasmic reticulum (NSR); I_{rel}=calcium release from junctional sarcoplasmic reticulum (JSR); I_{leak}=calcium leakage from NSR to myosplasm; I_{tr}=calcium translocation from NSR to JSR. Calmodulin, troponin and calsequestrin are calcium buffers[132,137,161,162].**

than the other long QT syndrome types[29]. Assuming that the QT interval reflects the degree of action potential duration prolongation in the long QT syndrome, it is possible that fast pacing has a greater effect in LQT3 due to the specific involvement of I_{Na} in this syndrome. A possible explanation is that with fast pacing Na^+ accumulates in the cell, lowering the Na^+ gradient across the membrane and the associated electrochemical driving force for Na^+. Through this mechanism (or altered function of the electrogenic sodium–calcium exchanger), the magnitude of I_{Na} is reduced. The effect of such a reduction will be negligible during the rising phase of the action potential, when I_{Na} is so much larger than other currents. However, the plateau I_{Na} contribution through mutant channels is of a much smaller magnitude and could be significantly affected by such changes. As stated earlier, this current operates at a critical time, when the action potential is determined by a very delicate balance of small currents. It is conceivable, therefore, that such a small reduction of late I_{Na} during this phase could result in shortening of action potential duration at fast rates.

Ion channel function in multicellular networks

The first section of this part described the diversity and variability of ion channels involved in cardiac electrical excitation, and the second illustrated the level of complexity related to the integration of individual channels into whole cell function. Assembling single cells in the multicellular excitable tissue introduces further significant interactions between ion channel function and structural properties of the tissue. These interactions play an important role in depolarization, repolarization and arrhythmogenesis.

Interaction between cell-to-cell coupling and ion channel function

The simplest model used to explain cardiac electrical propagation was originally derived from conduction models developed for the nervous system. In this model, cardiac cells are merged into a syncytium-like conducting structure composed of a cell membrane carrying the

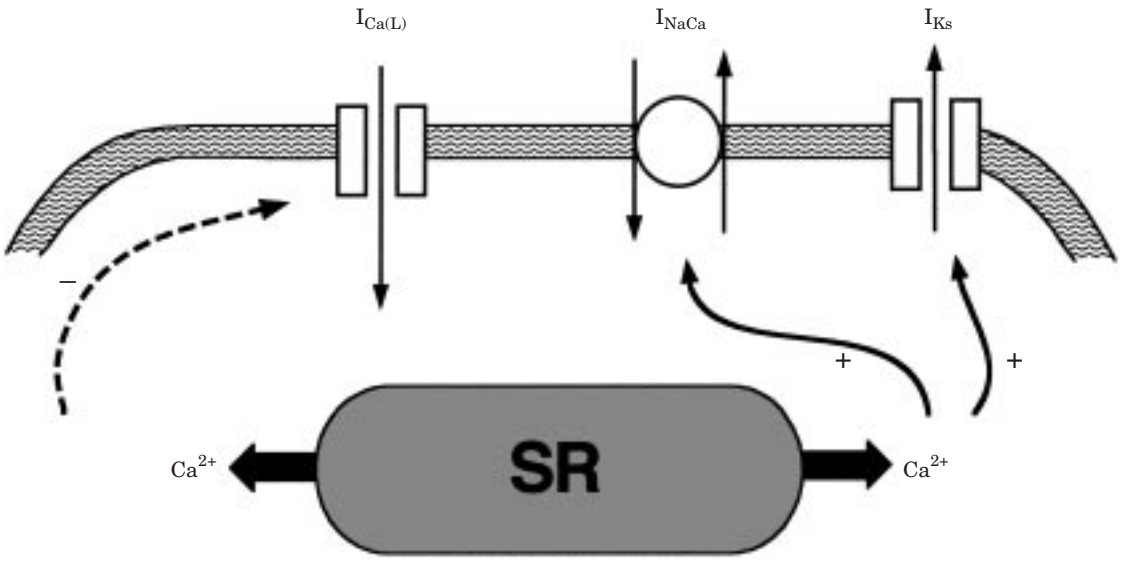

Figure 6 **Example of interactive processes in a single cell $I_{Ca(L)}$ triggers Ca^{2+} release from the SR. Ca^{2+}, in turn, activates I_{NaCa} and augments I_{Ks} (+ indicates a positive, enhancing effect). Ca^{2+} also acts to inactivate $I_{Ca(L)}$ in a 'negative feedback' type process (indicated by −). Ca^{2+} may have multiple other actions as indicated in the text.**

ion channels that generate the action potential, and a single continuous intracellular space (in this space, the electrical conductances of the cytoplasm and the gap junctional connexons are merged together). Many important insights into the electrical propagation process have been derived from this so-called 'continuous' model[143]. It has been successfully applied to describe global effects of inhibitors of Na^+ channels[141] of acute myocardial ischaemia[145] and hypoxia/anoxia[146] on conduction. However, in many instances, more complicated models are needed to describe the conduction process appropriately.

A first step in describing more closely the relation between electrical propagation and cardiac structure involved simulation of single cell chains with interconnections representing the gap junctions[147]. While the properties of such models are not markedly different from the 'continuous' model when electrical cell-to-cell coupling is normal, a distinctly different behaviour is unmasked once the electrical coupling of the cells diminishes. Interestingly this behaviour includes feedback interaction between cell-to-cell coupling and ion channel function. Partial closure of connexins (ischaemia, hypoxia[145,146]) or a decrease in expression (heart failure[148]) of connexins might affect conduction in several ways. First, conduction velocity decreases to a much greater extent than with inhibition of ion channels[149] and can reach a few centimetres per second or be even slower. Accordingly, re-entrant arrhythmias which occur in partially uncoupled tissue can form circulating excitation of very small dimensions (micro-reentry). By contrast, simulations suggest depressed I_{Na} alone cannot account for this phenomenon. Secondly, a change in cell-to-cell coupling feeds back on the way ionic channels in the membrane are activated and on the role of ionic channels in conduction (and most probably, repolarization). This is illustrated in Fig. 7 which depicts a row of simulated cells in a state of advanced cell-to-cell uncoupling. Due to the high degree of discontinuity which is introduced by the uncoupled cells, there is a long conduction delay between the cells. Since the action potential in the driver cell has to furnish local electrical circuit current to the driven cell, this current has to flow as long as the driven cell has not reached its threshold for depolarization, i.e. for activation of the Na^+ inward current. In the normal case of propagation, the conduction delay between two cells is very short. During this time the Na^+ channels of the driver cell are open and furnish sufficient electrical charge to excite the driven cell. With advanced degree cell-to-cell uncoupling and the concomitant delay, the inward Na^+ current will be inactivated before the driven (downstream) cell reaches threshold, and consequently, flow of Ca^{2+} inward current later during the action potential is necessary to assure propagation. Thus, the conduction process can change from being solely Na^+ current dependent, to being Na^+ and Ca^{2+}-inward current dependent, merely by a change in the degree of cell-to-cell coupling[149,150]. Feedback interactions between electrical coupling and ionic current flow are also expected to occur during the action potential plateau and during repolarization. Due to the small ion current density and the high membrane resistance during the action potential plateau[151], the electrical interactions between adjacent tissue regions are stronger and extend over longer distances during this phase than during rest or during the action potential upstroke. As a consequence, differences in intrinsic action potential durations among adjacent tissue regions will be relatively small during normal cell-to-cell coupling and unmasked during uncoupling. In such a way,

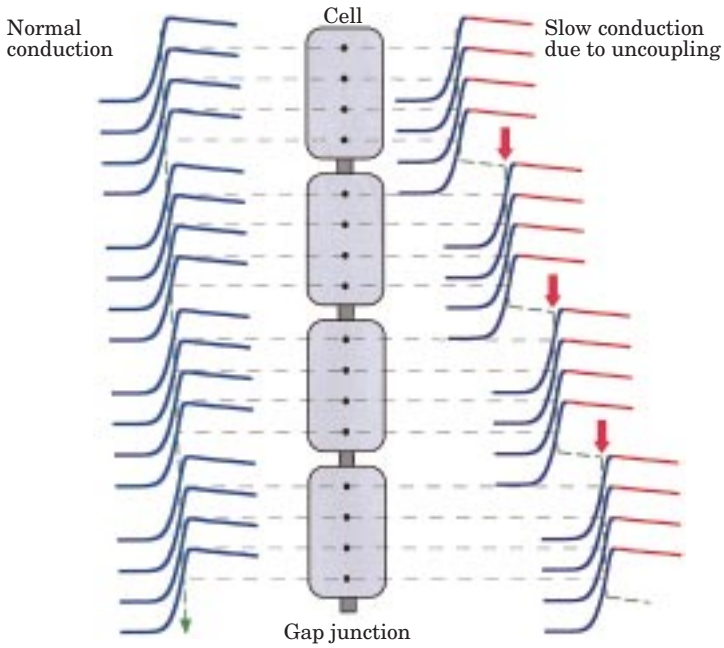

Figure 7 **Involvement of $I_{Ca(L)}$ and I_{Na} in propagation The middle panel illustrates schematically a column of 4 cells connected by gap junctions. Propagation during normal cell-to-cell coupling is depicted by the action potential upstrokes in the left row. Note that normal propagation is relatively continuous, i.e. major delays between the excitation of subsequent cells are absent. Propagation during advanced cell-to-cell uncoupling at a propagation velocity which amounts to 1/10 of normal velocity is shown in the left column. In this case propagation is highly discontinuous, i.e. the membrane sites in a single cell are activated almost simultaneously and a long conduction delay exists between subsequent cells. Due to this delay, the action potential upstroke of the driven cell occurs at a time when the driver cell is at its early plateau phase (red). Therefore $I_{Ca(L)}$ is necessary to support propagation.**

the reduction of electrotonic interaction by cell-to-cell uncoupling is predicted to unmask intrinsic heterogeneities in repolarization with both events serving to promote reentrant arrhythmias.

Interaction between tissue structure and ion channel function

As with the discrete pattern of gap junctions at the microscopic level, the macroscopic cardiac structure introduces obstacles and discontinuities for propagating electrical waves. Typical examples of discontinuities are branching fibres (Purkinje system, atrial trabecula) and/or connective tissue layers (remodelled ventricular tissue in infarcted and/or hypertrophic hearts, normal midmural layers of normal hearts[152], ventricular trabecula in ageing myocardium[153]). Such discontinuities have been shown to affect membrane channel function. In any situation where electrical waves propagate through anatomically *discontinuous* tissue and emerge from an isthmus[154], turn around the end of an obstacle[155], or emerge from a small fibre into a large tissue mass[156,157], the propagating wave becomes *curved*. In a curved wavefront, the mismatch between the excitatory local current produced by the excited cells upstream, and the electrical load of the non-excited cells ahead of the wavefront downstream affects both conduction velocity and the activation of ionic currents. At a convex wavefront, this mismatch will lead to a local conduction delay and to a localized increase of the amount of depolarizing inward current[156]. Conversely, at a concave wavefront (or, similarly, at a site of collision) conduction velocity will locally increase and the inward current will be locally reduced[158]. This dependence of ionic current activation on wavefront curvature is likely to be responsible for the larger effects of inhibitors which bind to open Na^+ channels at sites where the propagation wave is convex than at sites where it is linear[159]. The curvature of the wavefront will also play an important role in determining the ionic channels involved in impulse propagation. Thus, at divergence points, if the wavefront is markedly curved, large local conduction delays result. As with the case of advanced cell-to-cell uncoupling (see Fig. 7), the Ca^{2+} inward current then becomes essential for propagation, and application of Ca^{2+} entry blockers produces localized conduction block[160]. Interestingly, the interaction

between the macroscopic tissue architecture and excitation, leading to local divergence or convergence of wavefronts and changes in ion channel function, is further modulated by the degree of local gap junctional coupling[161].

Prospective

In the near future most genes responsible for inherited arrhythmogemic conditions will be identified and the genomic structure of disease-related genes will also be defined. This will produce results that are needed for successful management of patients and families. One major result will be the definition of the 'molecular epidemiology' of inherited arrhythmogenic conditions. Qualitative statements such as 'rare' or 'common' should be replaced by actual numbers defining the prevalence of each condition in the general population and the relative prevalence of each variant of a disease.

The availability of screening methods with sensitivity and specificity close to 100%, combined with complete clinical information prospectively collected, will define the penetrance of each disease and, within a disease, of each genetic variant. This will lead to guidelines for the management of asymptomatic gene carriers based on the predicted risk of becoming asymptomatic.

Given the very large number of mutations associated with arrhythmogenic disorders, it may be more realistic to study carefully the larger group of individuals with defects in the same gene rather than attempting to define genotype–phenotype correlations for each mutation. If distinctive features are identified that segregate patients with specific genetic variants of the same disease, gene-specific therapy may result.

The identification of the genetic or environmental factors that modulate the expression of these diseases and understanding the mechanisms whereby relatives with the identical mutation can have a radically different clinical history, will also be useful for patient management.

An intriguing aspect of investigating the molecular bases of inherited arrhythmogenic disorders lies in the hope that information provided by studies of relatively rare inherited conditions may help elucidate mechanisms for the acquired variants. The importance of the discovery of the gene for familial atrial fibrillation, for example, will be enhanced if it will lead to an understanding of the causes of the much more prevalent 'lone' (and even disease-associated) atrial fibrillation. Similarly, recent data suggest that the acquired long QT syndrome may develop in individuals carrying otherwise apparently 'mild' mutations in long QT syndrome-related genes. Polymorphisms of these genes could also predispose to other acquired arrhythmias.

Finally, the step from defective gene function to the clinically manifest arrhythmias involves further complexities related to the environment in which the abnormal proteins exert their function. Further study of the multiple interactions among ion channels, pumps and exchangers on one hand and the structure and connectivity of the cellular network on the other should improve our understanding of the mechanisms that determine the occurrence of the electrical disturbances that lead to lethal arrhythmias.

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Appendix

This paper summarizes the outcome of a workshop held at the European Heart House, Sophia Antipolis, France, 2–5 October 1997. The need for the workshop was proposed by Silvia G. Priori. It was organized by the Study Group on Molecular Basis of Arrhythmias of the Working Group on Arrhythmias of the European Society of Cardiology, and its funding was administered by the Working Group itself. The workshop was co-chaired by Silvia G. Priori and André G. Kléber. Participating in the workshop and coauthoring the paper were J. Barhanin, Institut de Pharmacologie Moleculaire et Cellulaire, Laboratoire de Genetique de la Neurotransmission CNRS, Valbonne, France; R. N. W. Hauer, University Hospital of Utrecht, Heart Lung Institute, Utrecht, The Netherlands; W. Haverkamp, Medizinische Klinik und Poliklinik, Innere Medizin C-Universitat Münster, Münster, Germany; H. J. Jongsma, University of Utrecht, Physiologic Laboratory, Utrecht, The Netherlands; A. G. Kléber, Department of Physiology, University of Bern, Bern, Switzerland; W. J. McKenna, Dept of Cardiological Sciences, St George's Hospital Med. School, London, U.K.; S. G. Priori, Molecular Cardiology and Electrophysiology, Fondazione S. Maugeri, IRCCS, Pavia, Italy; D. M. Roden, Division of Medicine and Pharmacology, Vanderbilt University Medical Center, Nashville, TN, U.S.A.; Y. Rudy, Case Western Research University, Department of Biomedical Engineering, Cleveland, OH, U.S.A.; K. Schwartz, UR 153 INSERM, Pavillon Rambuteau, Groupe Hopitalier Pitié-Salpétrière, Paris, France; P. J. Schwartz, Dipartimento di Cardiologia, Policlinico S. Matteo, IRCCS, Pavia, Italy; J. A. Towbin, Ped Molecular Cardiology, Baylor College of Medicine, Texas Children's Hospital, Houston, TX, U.S.A.; A. M. Wilde, Department of Clinical and Experimental Cardiology, Academic Medical Centre, Amsterdam, The Netherlands. The final preparation and organization of the manuscript were the responsibility of André G. Kléber, Silvia G. Priori, Dan M. Roden and Peter J. Schwartz.