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Taking genes to heart

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Recently, there have been bewildering advances in our ability to read and interpret genetic information. These have come about through improvements in sequencing hardware, coupled with an increase in the density and assignment of single nucleotide polymorphisms (SNPs) and in the bioinformatic techniques needed to process and collate this information. This issue of Heart and Metabolism is dedicated to diseases of the heart that follow classical mendelian genetics, and therefore focuses on myopathies and ion channelopathies. In these diseases, candidate genes can be identified on the basis that they encode sarcomeric/contractile, or ion channel, proteins. Such candidate gene approaches to classical familial monogenic disorders are likely to be soon regarded as “old-fashioned” genetics, applicable largely to rare disorders with little global impact on disease burden. This situation contrasts with current trends in genetics that increasingly focus on more common multifactorial diseases in which several genes and environmental aspects interact to cause diseases such as diabetes, hypertension, and ischemic heart disease [1]. Dissecting the genetic components of such multifactorial diseases through association of genome-wide SNPs makes no a-priori assumptions regarding gene candidates, and is therefore likely to uncover novel regulatory pathways.

In a future issue of Heart and Metabolism, we hope to cover the more complex genetic contributions that occur in the common multifactorial diseases in which candidate gene approaches have often misled, and have therefore not resulted in reproducible findings. For this reason, this issue of Heart and Metabolism makes no mention of genome-wide association studies and the recent findings of SNPs that contribute to common cardiovascular disease [1]. Instead, we focus on old-fashioned genetics which, although implicated in the cause of relatively rare diseases, has a much greater impact on the affected family.

In the Main Clinical Article, Drs Deo and MacRae provide an authoritative overview of the use of genetic screening. The opening paragraph of their article provides an extremely concise précis of the main issues that confound genetic screening: penetrance, genetic and allelic heterogeneity, variable expressivity, and phenocopies. The paragraph is so well-written that it is worth reading a few times, as it provides the foundation for an extremely practical clinically orientated approach to screening that, from the outset, identifies the key limitations to widespread screening, namely genetic and allelic heterogeneity, which often lead to “private” mutations unique to a single family (discussed in the Case Report). Furthermore, because of variable expressivity, even within the affected family, the mutation often does not provide prognostic information. Despite presenting a superb summary of the generic problems in screening for mendelian disorders, Deo and MacRae offer a detailed review of the major phenotypes that may indicate an underlying inherited cardiac muscle disease – namely hypertrophic cardiomyopathy, dilated cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, and restrictive cardiomyopathy. For each of these diseases, Deo and MacRae give an elegant and pragmatic summary of the state of the art. Their final conclusions reinforce the use of screening within affected families to provide reassurance and to identify those individuals at risk in whom surveillance and therapy can be focused. These conclusions and other issues raised are further reinforced in the Case Report by Murphy and Gill in this issue of Heart and Metabolism and an earlier, high-profile Editorial [2].

The Basic Article by Dr Bezzina focuses on the genetic mutations that cause cardiomyopathy and channelopathies. Our current knowledge of the genes causing these diseases has been concisely tabulated within this review. Bezzina points out that cardiac hypertrophy can also be caused by non sarcomeric
protein mutations, copying the classic macroscopic phenotype of hypertrophic cardiomyopathy, but with a markedly different microscopic phenotype: lacking myocyte disarray but instead incorporating myocyte inclusions containing complex polysaccharides or lipid – the so-called lysosomal storage diseases. In addition to summarizing the mechanisms by which mutations in a particular gene give rise to cardiac muscle disease, Bezzina provides an extremely elegant and up-to-date summary of the cardiac channelopathies that cause arrhythmias in anatomically normal hearts. Once again, these channelopathies – namely, long- and short-QT syndromes, Brugada syndrome, conduction disease, sinus node dysfunction, and catecholaminergic polymorphic ventricular tachycardia – can be caused by a variety of mutations in different genes, not all of which in fact encode ion channels. Furthermore, in keeping with Deo and MacRae, Bezzina’s emphasis is on the use of screening within families. As she points out, the expressivity of a particular mutation within a family can vary enormously, and in extreme examples no disease is manifest in one carrier who dies in old age of non cardiac disease, whereas in another there can be heart failure or sudden death in childhood. Dissecting the cause for this wide range in expressivity/limited penetrance is likely to be a future area of research activity that will greatly enhance the current position of genetics in estimating prognosis and guiding therapy.

Although the mutations underlying some common genetic diseases such as sickle cell disease and cystic fibrosis have been known for decades, they have not yet led to targeted treatments. In this issue, Dr Sleeper and her colleagues outline therapies that are already licensed for treatment of the specific lysosomal acid hydrolase deficiencies affecting the heart: Fabry disease and Pompe disease. These are caused by the inability of the lysosome to degrade large complex lipids and sugars, leading to their accumulation in the myocardium, aorta, and valves. The products licensed currently are intravenous cell-permeable formulations of the deficient enzyme. However, Dr Sleeper and her colleagues have pioneered the use of gene therapy using vectors that result in long-term expression of the deficient protein. As beautifully illustrated in this article, these vectors can be tested in a naturally occurring canine model of lysosomal storage disease, in which they result in probably permanent transgene expression (at least 7 years) and, providing they are delivered early in life, they greatly attenuate the disease phenotype. As with other monogenic disorders, gene therapy holds great promise, provided a number of hurdles can be overcome.

This issue of *Heart and Metabolism* provides a comprehensive and contemporary description of monogenetic diseases of the heart. In such a fast-moving field, of one thing we can be certain: an update will be required very soon.

**REFERENCES**

Genetics of cardiomyopathy and channelopathy

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Conflicts of interest: None.

Abstract

During the past 20 years, we have witnessed a dramatic increase in our knowledge of the genetic basis of the cardiomyopathies and the primary electrical disorders (“ion channelopathies”). This review aims to provide an overview of the different genes linked to these disorders to date.

Keywords: Arrhythmia, cardiomyopathy, channelopathy, mutation, sudden cardiac death

Introduction

During the past 20 years, we have witnessed a dramatic increase in our knowledge of the genetic basis of cardiac disease. The greatest advancements have undoubtedly taken place in the understanding of the genetic basis of the cardiomyopathies and the primary electrical disorders, the latter commonly referred to as the “ion channelopathies”. Recognition of the genetic substrate in cardiomyopathy and channelopathy not only has provided clues to the underlying molecular mechanisms, but, importantly, has enabled the introduction of genetic diagnostic testing, providing new opportunities for patient management such as early, presymptomatic, identification of patients at risk of developing fatal arrhythmias. This review aims to provide an overview of the different genes linked to these disorders to date.

Cardiomyopathy

Cardiomyopathy is typically divided into several subtypes: hypertrophic cardiomyopathy, glycogen cardiomyopathy, dilated cardiomyopathy, restrictive cardiomyopathy, and arrhythmogenic right ventricular cardiomyopathy.

Hypertrophic cardiomyopathy

Hypertrophic cardiomyopathy (HCM) is a relatively common disorder with a prevalence of about 1: 500 [1]. It is recognized clinically by the presence of cardiac hypertrophy in the absence of an increased external load [2]. Echocardiographically, it is associated with preserved systolic, but impaired diastolic, function. Hypertrophy is asymmetric in about two-thirds of cases, with the septum being the predominant site of involvement. Significant left ventricular outflow tract obstruction is observed in approximately one-quarter of cases.

HCM is usually inherited as an autosomal dominant trait [3,4]. The first gene linked to HCM was reported in 1990 [5]. This was the gene encoding cardiac β-myosin heavy chain (MYH7), a component of the sarcomere, the contractile unit within the cardiomyocyte. The sarcomere is an immense protein complex that is organized into thick and thin filaments that, in
the presence of calcium and ATP, slide past each other, thereby generating contractile force (Figure 1). After the discovery of mutations in MYH7, mutations in another 10 sarcomeric genes were linked to HCM, leading to the proposition that HCM was a disease of the sarcomere (Table I). Most commonly affected are the MYH7 and the MYBPC3 genes, with the other genes accounting for far fewer cases. Mutations in sarcomeric genes account for about 60% of cases of HCM [6]. More recently, mutations have also been described in genes encoding sarcomere-associated proteins such as muscle LIM protein [7]. However, mutations in these genes appear to be rare.

**Glycogen cardiomyopathy**

Cardiac hypertrophy can also be triggered by defects in genes of metabolism [3]. Glycogen deposition is a shared feature of these metabolic cardiomyopathies. A number of features distinguish this form of cardiomyopathy from the sarcomere-related type. Histologic features associated with sarcomere-related hypertrophy (myocyte and myofiber disarray, myocyte hypertrophy, fibrosis) are notably absent in the glycogen cardiomyopathies, which, in contrast, contain myocyte vacuoles containing glycogen. Furthermore, patients with metabolic gene defects usually present with electrophysiological dysfunction.

Mutations in three lysosomal proteins produce such glycogen cardiomyopathy. Recessively inherited lysosomal acid α1,4-glucosidase (GAA) deficiency causes Pompe disease, X-linked lysosome-associated membrane protein (LAMP2) deficiency causes Danon disease, and X-linked lysosomal hydrolase α galactosidase A (GLA) deficiency causes Fabry disease; these three diseases are systemic disorders. Another gene associated with glycogen storage disease is PRKAG2, encoding the γ2 subunit of AMP-activated protein kinase (AMPK) [8]. AMPK functions as a metabolite-sensing protein kinase that is activated under

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Basic article
Genetics of cardiomyopathy and channelopathy

Dilated cardiomyopathy

Dilated cardiomyopathy (DCM) [9] is characterized by left ventricular chamber enlargement and systolic dysfunction, with normal or a modest increase in ventricular wall thickness. It has an estimated prevalence of 1:2500. Affected individuals gradually develop heart failure, often in association with life-threatening atrial or ventricular arrhythmias.

The disease is familial in about 35% of cases, pointing to an important role of genetics in disease pathogenesis [10]. In such cases, inheritance is most commonly autosomal dominant, but autosomal recessive, X-linked, or matrilinear (mitochondrial) inheritances also occur. DCM has been linked to 25 different chromosomal loci and genes. Known mutations affect proteins with a wide range of unrelated functions. Mutations have been identified in several components of the myocardial cytoskeleton, which, through a complex network of proteins, links the sarcomere to the sarcolemma and extracellular matrix and functions to transmit force generated during contraction (Figure 1). Cytoskeletal components found to be associated with DCM include cardiac muscle LIM protein (MLP), cypher/ZASP (LBD3), β-sarcoglycan (SGCD), desmoplakin (DSP), desmin (DES), dystrophin (DMD), telethonin (TCAP), and vinculin (VCL).

Mutations in the ubiquitously expressed nuclear envelope protein lamin A/C (LMNA) cause DCM associated with conduction disease [11]. A myriad of phenotypes, besides DCM, are associated with lamin A/C mutation, including Emery–Dreifuss muscular dystrophy type B1 and Charcot–Marie–Tooth disease. Several genes encoding sarcomeric proteins linked to HCM, including TTN, ACTC, TPM1, MYH7 and TNNT2, may also cause DCM. Other genes linked to DCM include genes encoding ion channel subunits. One of these is SCNS5A, which encodes the pore-forming subunit of the cardiac sodium channel. Conduction disease and atrial fibrillation are a common finding in patients with DCM harboring mutations in SCNS5A [12]. Another ion channel gene subunit linked to DCM is the sulfonylurea receptor 2A (SUR2A), which is the regulatory subunit of KATP channels in the heart. DCM is also caused by mutation in phospholamban (PLN), which has an essential role in calcium metabolism by modulating calcium-ATPase activity.

Restrictive cardiomyopathy

Restrictive cardiomyopathy is a rare disorder characterized by a normal or decreased volume of both ventricles, associated with bi-atrial enlargement, impaired ventricular filling with restrictive physiology, normal myocardial wall thickness, and normal or near-normal systolic function. A mutation has been described in cardiac troponin I (TNNTI3) in a family in which carriers exhibited restrictive or hypertrophic cardiomyopathy. Additional TNNTI3 mutations were also found in unrelated patients with restrictive cardiomyopathy [2,3].

Arrhythmogenic right ventricular cardiomyopathy

Arrhythmogenic right ventricular cardiomyopathy (ARVC) involves predominantly the right ventricle, with progressive loss of myocytes and fatty or fibrofatty tissue replacement – a process that appears to begin at the epicardium and gradually extends towards the subendocardium [13]. Left ventricular involvement is now commonly recognized. Significant advances in the understanding of the genetic basis of this disorder have been made over the past few years.

Mutations in five different desmosomal components (plakoglobin, desmoplakin, plakophilin-2, desmoglein-2, desmocollin-2) have been described, leading to the notion that ARVC is a disease of the desmosome. Desmosomes form specialized intercellular junctions that anchor intermediate filaments to the cytoplasmic membrane in adjoining cells, imparting mechanical strength. Mutations have also been described in two extradesmosomal genes. One of these is RYR2, encoding the cardiac ryanodine receptor. However, one could argue that the phenotype of these patients more closely resembles that of catecholaminergic polymorphic ventricular tachycardia (CPVT, see below), which also is caused by mutation in RYR2. The other extradesmosomal gene in which mutations have been described is TGF-β3, encoding transforming growth factor-β3. The contribution of mutations in this gene to the overall genetic profile of ARVC is not known with certainty.

Channelopathy

The cardiac action potential is mediated by the exceptionally well orchestrated activity of a diversity of ion channels. Cardiac ion channels are protein complexes in the membrane of cardiomyocytes which, via highly regulated opening and closing (gating), conduct a selective and rapid flow of ions through a central pore. Spatial heterogeneity of ion channel expression underlies the different action potential morphology of...
the different parts of the heart, which in turn ensures a coordinated contraction. The maintenance of normal cardiac rhythm is dependent on the correct movement of ions mediating the action potential in each cardiac compartment. Abnormalities in ion channel function can have disastrous consequences that manifest themselves as abnormalities of the electrocardiogram (ECG) and arrhythmias. These disorders of ion channels, commonly referred to as ‘cardiac channelopathies’, have been brought into focus in recent years, as mutations in genes coding for specific ion channels were shown to underlie specific forms of heritable arrhythmogenic disorders occurring in the structurally normal heart. These include long-QT syndrome (LQTS), short-QT syndrome, Brugada syndrome, conduction disease, sinus node dysfunction, and CPVT, discussed here.

**Long-QT syndrome**

LQTS, estimated to affect 1 in 5000 individuals, is a repolarization disorder identified by prolongation of the QT interval on the ECG [14]. It has long been recognized as a familial disorder, frequently presenting in childhood with syncopal episodes and potentially lethal torsades de pointes tachyarrhythmias, which occur in a significant proportion of untreated patients. Inheritance of the disease is either autosomal dominant or recessive. The autosomal recessive form (Jervell and Lange-Nielsen syndrome) is also associated with deafness.

Mutations in eight different genes encoding potassium (K$^+$), sodium (Na$^+$), or calcium (Ca$^{2+}$) ion channel subunits have been associated with the disorder (Table II). The pore-forming subunits of the slowly and rapidly activating repolarizing potassium currents (KCNQ1 and KCNH2 genes, respectively) are most often affected. Mutations affecting the potassium channel subunits (KCNQ1, KCNH2, KCNE1, KCNE2) prolong action potential repolarization – and, consequently, the QT interval on the ECG – by a net reduction in outward repolarizing K$^+$ current. Mutations in SCN5A, which encodes the pore-forming subunit of the sodium channel, lead to an increased inward Na$^+$ current during the action potential plateau, shifting the balance to prolonged repolarization. Mutations in SCN4B, an ancillary subunit of the sodium channel, have recently been reported in one family with LQTS [15].

Mutations associated with LQTS have also been described in genes encoding linker/adaptor proteins. These include the membrane adapter protein ankyrin-B (ANK2), caveolin 3 (CAV3 – a major component of caveoleas that constitute microdomains of the plasma-lemma [16]), A-kinase anchoring protein 9 (AKAP9) [17], and α-syntrophin (SNTA1), a member of the family of dystrophin-associated proteins containing several protein interaction motifs [18].

Jervell and Lange-Nielsen syndrome is caused by homozygosity (because of consanguineous parents) or compound heterozygosity for mutations in KCNQ1 or KCNE1. These genes are expressed in marginal cells of the stria vascularis, where they are believed to play a part in the homeostasis of K$^+$ in the endolymph, a K$^+$-rich fluid of the inner ear. This explains the deafness that is associated with this disorder.

Mutations in the KCNJ2 gene that encodes the inward rectifier K$^+$ channel Kir2.1 in both heart and striated muscle cause Andersen syndrome, a rare disorder that, besides mild prolongation of the QT interval, also exhibits extracardiac features, including skeletal muscle periodic paralysis and developmental problems. Another disorder manifesting with prolongation of the QT interval and extracardiac features is Timothy syndrome. This disorder combines, amongst other defects, severe prolongation of the QT-interval with syndactyly, autism, mental retardation, and facial dysmorphism. Considering the widespread expression of the CACNA1C gene and the importance of Ca$^{2+}$ as an intracellular signaling molecule, the widespread cellular and organ defects in this disorder are not unexpected.

**Short-QT syndrome**

The short-QT syndrome presents with a high rate of sudden death and exceptionally short QT intervals (QTc typically ≤300 ms). To date, only 30–40 patients have been described. In contrast to LQTS, in SQTS repolarization is hastened by an enhanced outward current during repolarization. Gain-of-function mutations in the KCNH2, KCNQ1, and KCNJ2 genes were identified in patients with the disorder (Table II). QT-interval shortening in the KCNJ2 subtype seems less severe than that for the other two subtypes.

**Brugada syndrome**

The Brugada syndrome is characterized by ST-segment elevation in the right precordial leads, with or without conduction abnormalities, and a significant risk of sudden cardiac death. The disorder is endemic in East and Southeast Asia, where it underlies the sudden unexpected death syndrome. Brugada syndrome is familial in about one-third of patients, in which case an autosomal dominant mode of inheritance is observed.

Genes involved in pathogenesis of Brugada syndrome encode the pore-forming and auxiliary subunits of the cardiac Na$^+$ channel encoded, respectively, by SCN5A and SCN1B [14,19] (Table II). The functional effects of Brugada syndrome on the sodium current are opposite to those found in LQTS. Loss-of-function mutations underlie...
Brugada syndrome and the frequently associated (mild) conduction disorders. Mutations in GPD1L, which encodes glycerol-3-phosphate dehydrogenase 1-like protein, also lead to Brugada syndrome by attenuation of the sodium current, an effect probably caused by interference with cell membrane expression of the channel [20]. Recently, loss-of-function mutations in two subunits of the cardiac Ca$^{2+}$ channel complex (CACNA1C and CACNB2) were associated with Brugada syndrome, in combination with somewhat shorter-than-normal QT intervals [21].

Cardiac conduction disease and sinus node dysfunction

Loss-of-function mutations in components of the cardiac Na$^{+}$ channel complex, namely SCN5A and SCN1B, also lead to cardiac conduction disease [19]. It is unknown why such mutations leading to loss of Na$^{+}$ channel function lead to conduction disease in some patients and Brugada syndrome (with conduction defects) in others. Mutations in SCN5A leading to loss of Na$^{+}$ channel function also cause a recessive form of sick sinus syndrome. Mutations in HCN4, which encodes the cardiac pacemaker channel, cause autosomal dominant sinus node dysfunction [14].

Catecholaminergic polymorphic ventricular tachycardia

Arrhythmias in the setting of CPVT are, typically, bidirectional and polymorphic ventricular tachycardia, exclusively triggered by adrenergic stimuli. The phenotype often presents in early childhood. In the majority of cases, CPVT displays an autosomal dominant mode of inheritance and is caused by mutations in the gene encoding the ryanodine receptor channel (RYR2; Table II). This is an intracellular Ca$^{2+}$-release channel on the sarcoplasmic reticulum that releases Ca$^{2+}$ in response to the entry of Ca$^{2+}$ through membrane Ca$^{2+}$ channels. A recessive form of CPVT is caused by homozygous mutation in the CASQ2 gene.
serves as the major Ca$^{2+}$ reservoir within the lumen of the sarcoplasmic reticulum. Symptoms are apparently more severe in CASQ2-related CPVT, including an earlier age of onset.

**Concluding remarks**

The identification of the mutation within a family affected by inherited cardiac disorders allows diagnosis in other family members independently of echocardiographic features, ECG features, or arrhythmic manifestations. This has led to the realization that inherited cardiac disorders exhibit variability in clinical expression [22,23]. As in the case of many other Mendelian disorders, reduced penetrance and variable expression are more the rule than the exception. Hence, not all carriers of mutations are clinically affected to the same degree by the disorders. Clinical expression is probably influenced by several factors, including age, sex, and environmental factors such as lifestyle, exercise, and blood pressure. Genetic modifiers are also expected to modulate disease penetrance and expression. Although some genetic modifiers are beginning to be uncovered [22,24], the nature of such modifiers remains, largely, unknown. The identification of genetic modifiers is regarded as the major next step in genetic studies of inherited cardiac disorders.

*See glossary for definition of this term.*

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Abstract

As the inherited basis of some cardiovascular diseases have begun to be uncovered, a practical understanding of genetics has become important for the cardiologist. The advent of new studies on the inherited contributions to common traits and the emergence of genetic testing for a broad range of cardiac and vascular conditions will only increase the need for fluency in genetics. In this article we have outlined the genetic concepts likely to be most useful to the practicing cardiologist, focusing the discussion around the inherited conditions most likely to be identified in general clinical cardiology.

Introduction

Genetic diseases lie along a continuum ranging from simple monogenic Mendelian disorders to complex traits, which arise from the interaction of a number of genetic and environmental factors. The concept of penetrance captures the distinction between genetic variants contributing to Mendelian disorders and complex disease traits. Penetrance for a genetic mutation is defined as the proportion of individuals carrying a particular genetic mutation who also demonstrate the disease phenotype. The mutations that lead to Mendelian disorders have high penetrances (approaching 100%), whereas for most variants contributing to complex disease the penetrance is quite low. The concept of genetic architecture describes the number of genes contributing to a disease trait, the number of variants per gene, and the magnitude of effect that each variant has on development of the trait. Although Mendelian disorders usually arise from inheritance of a single genetic mutation, many different individual genes may, when mutated, lead to a common disease phenotype (genetic heterogeneity). In addition, for any gene, many different mutations may also lead to the same disease phenotype (allelic heterogeneity). Both genetic and allelic heterogeneity introduce complexity when one goes about designing a genetic screening program for heart disease. Furthermore, although the penetrance of a disorder may be high, the exact manifestation of disease may vary from individual to individual, despite their inheriting the same mutation (variable expressivity). A final level of complexity arises from the fact that several distinct diseases may share a common “low-resolution” phenotype, but in fact have a different pathologic basis (termed phenocopies), with potentially different disease course and treatment.

Genetic screening

When should genetic screening be used? An example may help illustrate the approaches used for potentially heritable disorders. Consider an individual with a disease that does not appear to be arising from any...
known environmental cause – in genetic studies, this individual is called the proband. An initial step should be to establish whether the disease is familial, as this has relevance to pursuing a genetic diagnosis for the individual, and on managing risk within family members. In addressing familiarity, we must construct a careful family pedigree, asking about the health and manner of death of every relative. We need to be careful to distinguish two apparently similar situations with considerably different ramifications: one in which detailed pedigree information is available and no disease is apparent, and another in which there does not appear to be any other relative with the disorder but an inadequate family history is obtained. Only in the former case could we conclude that the disease is not familial, but either sporadic or attributable to environmental factors.

If the genetic architecture of the disease is such that there are a relatively small number of genes (low genetic heterogeneity) involved and there are causal genetic variants of moderate to high penetrance, genetic screening can be useful. Because many Mendelian disorders show significant allelic heterogeneity, screening for a single mutation tends to be unsuccessful, and sequencing of the gene is required to find likely causal variants. Several limitations exist with genetic testing of a single proband. Sequencing errors can occur, resulting in false positive and false negative results. Even with careful sequencing, a variant may be found in one of the candidate genes, but may not actually be causal for the disease. To establish a sequence variant as a potential mutation would require that it have the potential to have a deleterious effect. A mutation that is falsely assigned causality and used for genetic screening in family members would lead to both false reassurance and false alarm, as the inheritance of the variant would have no bearing on the likelihood of developing the disease.

How useful would the identification of a genetic variant be? Because of the bewildering genetic and allelic heterogeneity of most Mendelian disorders, the individualized prognostication and treatment that it was once hoped would follow genetic diagnoses have not materialized. We simply do not have enough prognostic information for individual mutations to provide mutation-specific predictions with any accuracy. As a result, the current utility of identifying a causal mutation in a proband is almost exclusively limited to facilitating screening of family members.

We can apply the above considerations to any disease with a heritable component. Below, we will address the approaches to hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), arrhythmogenic right ventricular cardiomyopathy (ARVC), and restrictive cardiomyopathy (RCM), highlighting how the known genetic architecture of the trait guides screening.

### Hypertrophic cardiomyopathy

The genetic architecture of HCM (Table I) makes it amenable to genetic diagnosis. HCM appears to be familial in a large proportion of cases and the pattern of inheritance is almost always autosomal dominant with high penetrance [1,2]. There are 12 known genes responsible for this disorder (not including several phenotypic mimics [3]), and mutations in one of eight sarcomeric genes explain approximately 60% of cases [4]. In the USA, there are various commercial tests available for genetic screening, at costs ranging from $500 to $3000, but the prevalence of HCM is too low to justify screening of the general population.

The allelic heterogeneity of HCM, which includes more than 400 causal mutations, makes individualization of treatment and prognosis on the basis of genetics implausible. It is unlikely that, for any mutation, adequate samples will ever be assembled for a reliable estimate of risk. Furthermore, the presence of incomplete penetrance and variable expressivity’ within families erodes confidence in the predictive utility of mutations. Attempts to prognosticate on the basis of genetic mutations have been difficult to replicate, and designations of mutations as ‘benign’ or ‘malignant’ are often the result of observational studies in small numbers of families [5]. It is, of course, expected that some mutations will have a more deleterious impact on protein function than

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<td>AICD</td>
<td>Autosomal recessive X-linked</td>
<td>Avoidance of exercise</td>
</tr>
</tbody>
</table>

ACEI, angiotensin-converting enzyme inhibitor; AICD, automatic implantable cardioverter-defibrillator.
Dilated cardiomyopathy

DCM is considerably more complex than HCM, in terms of both genetic architecture and known contributing environmental factors. Coronary artery disease, nutritional deficiency, viral infection, and toxins such as alcohol all can cause DCM. The prevalence of idiopathic DCM may be as high as 1:2500 adults [9], but the diagnosis requires an extensive work-up to exclude other causes, some of which may prove to be reversible [10].

More than 20 genes have been implicated in the pathogenesis of DCM, with autosomal dominant, recessive, and X-linked patterns of inheritance [11] (Table I). Penetrance is often low, and expressivity varies considerably. DCM can be syndromic, with other abnormalities such as the skeletal muscle dystrophies and retinal disease [12]. Given the fact that mutations in DCM are distributed widely over a large number of different potential causal genes, there is usually too low a likelihood of success to recommend genetic sequencing or genetic screening. Associated cardiac and non cardiac findings can help narrow the diagnosis. For example, in one small study, if atrioventricular block accompanied DCM, there was a mutation found in the lamin A/C gene in one-third of cases [13]. As with all cardiomyopathies, it is challenging to predict risk for particular mutations [14].

Although the complexity of DCM precludes genetic screening, clinical screening by ECG or echocardiography can often be very useful. An early diagnosis in asymptomatic family members of the proband allows the initiation of treatment with potentially disease-modifying agents such as angiotensin-converting enzyme inhibitors (ACEIs; see below). Individuals with mildly depressed systolic ejection fraction or mild left ventricular enlargement should be followed with more frequent screening. For every affected individual, care must be taken to exclude age-appropriate, potentially reversible causes, as these may contribute to disease even in the context of an inherited tendency [15].

Clinical guidelines recommend the use of ACEIs and β-blockers for all cases of DCM, independent of cause [16], and implantation of an implantable cardioverter-defibrillator in symptomatic individuals with severe left ventricular dysfunction. It is unclear if early initiation of treatment with ACEIs or β-blockers mitigates the disease course in individuals with mild echocardiographic abnormalities, or exclusively ECG abnormalities.

Arrhythmogenic right ventricular cardiomyopathy

ARVC is a genetically heterogeneous disorder, for which 12 genetic loci are currently identified [17] (Table I). Causal genes corresponding to eight of these loci have been found, with five encoding desmosomal proteins. The prevalence of ARVC is unknown, but has been estimated at 1:1000 to 1:5000 individuals [18]. ARVC is familial in nearly 50% of cases [19], and inheritance is usually autosomal dominant, with variable expressivity and incomplete penetrance.

The routine diagnostic work-up of a patient suspected to have ARVC includes ECG, Holter monitoring, signal-averaged ECG, echocardiography, and, potentially, cardiac magnetic resonance imaging [20]. If the clinical and family history and these
initial studies raise a high suspicion for ARVC, endomyocardial biopsy is often performed for confirmation, and an electrophysiological study may be performed to exclude benign right ventricular outflow tract tachycardia. The above diagnostic tests have been incorporated into criteria for the diagnosis of ARVC proposed by the Task Force of the Working Group on Myocardial and Pericardial Disease of the European Society of Cardiology and of the Scientific Council on Cardiomyopathies of the International Society and Federation of Cardiology [21].

A large percentage (up to 43%) of cases of ARVC can be explained by mutations in the plakophilin 2 (PKP2) gene [22,23], with more than 50 PKP2 mutations currently known [17]. The penetrance of ARVD appears to be low, possibly because of the insensitivity of the Task Force criteria [19,24]. Sequencing of the most commonly mutated genes may be useful in identifying family members of the proband who require long-term follow-up, especially because correct identification of affected individuals may be useful in prevention of sudden cardiac death. Testing for the four most common genes mutated in ARVC is available in the USA for a cost of $3000.

The relevance of genetic diagnosis to prognostication or individualization of treatment is limited by the fact that most mutations identified to date are rare, and “private” to individual families [25]. Given incomplete penetrance and variable expressivity within families, it is unclear to what extent we can extrapolate the risk of sudden death from one family with a given mutation to another, even if they share the same mutation. It is highly unlikely that we will be able to define a common risk profile for all mutations of a single gene [26–28].

If a genetic diagnosis cannot be made for the proband, then clinical screening of family members would occur, but those who appear to be “negative” for disease should continue to be screened at some regular interval [29]. The late appearance of ARVC in some individuals [30] requires that screening should continue throughout adult life.

Placement of an implantable cardioverter-defibrillator in patients with the diagnosis of ARVC remains an area of uncertainty [31]. As with HCM, attempts have been made to identify high-risk diagnostic features [32,33].

Restrictive cardiomyopathy

RCM demonstrates several rare hereditary variants, including familial idiopathic restrictive cardiomyopathy, and hereditary amyloidosis. Familial idiopathic RCM is extremely rare, with reports only from a small series of cases [34,35]. As yet, no gene has been identified. Furthermore, in some families with HCM, individual members can show a pattern of restrictive filling with little or no left ventricular hypertrophy [36,37]. In a systematic analysis of 1226 relatives of HCM probands, this “restrictive phenotype” of HCM was seen in 1.5% of individuals, and the diagnosis was accompanied by a high rate of dyspnea and mortality.

Hereditary amyloidosis represents a more common form of heritable RCM and typically involves a genetic defect in the transthyretin protein or apolipoprotein A-I protein, leading to misfolded proteins and infiltration of the myocardium with amyloid fibrils. RCM shows allelic heterogeneity, with multiple transthyretin mutations identified to date [38]. The pattern of inheritance is usually autosomal dominant.

Conclusions

General principles of the architecture of genetic disease can guide screening and diagnostic approaches for all the cardiomyopathies – and, in fact, for all inherited diseases. At present, the primary benefit of identifying a causal mutation in a proband is to facilitate screening in family members. A preclinical diagnosis achieved through screening programs can allow initiation of further monitoring programs for disease development, avoidance of high-risk behaviors, and potential implementation of disease-mitigating therapies. Although there is considerable incentive to offer genotype-based forecasting for patients, allelic and genetic heterogeneity and variable expressivity have rendered such individualization of care highly unlikely. Our ultimate desire for tailored prognostication and treatment is likely to be realized only when we can generate phenotypic profiles that can integrate individual genotypic and environmental information and yet be sufficiently common to allow accuracy in prediction and classification.

“See glossary for definition of these terms.”

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Imaging of myocardial receptors: applications in the evaluation of cardiac disease

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Conflicts of interest: None.

Abstract

Several positron labelled radiopharmaceuticals have been used to assess cardiac neurotransmission both at presynaptic and postsynaptic level. Some tracers compete with endogenous noradrenaline for the transport into the presynaptic nerve terminal mainly via the neuronal uptake-1 transport system. Several beta-blocking drugs have been labelled with 11C to act as radioligands for imaging the study of postsynaptic receptors by PET. The most extensively used is 11C-(S)-CGP 12177. This is a non-selective b-adrenoceptor antagonist with high affinity and low lipophilicity. Studies in patients have demonstrated diffuse down-regulation of b-adrenoceptor density in hypertrophic cardiomyopathy and in congestive heart cardiac failure, two disorders where there is evidence of elevated levels of sympathetic activation.

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Keywords: alpha adrenergic receptor, beta adrenergic receptor, cardiomyopathy, heart failure, PET

Positron emission tomography (PET) is commonly used to study cardiac metabolism by means of fluorine-18-labeled (18F) 2-fluoro-2-deoxyglucose (FDG). Left ventricular dysfunction is the endpoint of a progressive disorder that can be initiated by events damaging the myocytes or disrupting the ability to generate force, such as myocardial infarction, hemodynamic pressure, volume overload, or genetic cardiomyopathies. The common pathways, independent of the initiating event, are the compensatory mechanisms activated to preserve cardiac functional capacity. The most powerful compensatory mechanism is perhaps the activation of the sympathetic nervous system, resulting in an increase in adrenergic drive to the left ventricle.

Several positron-labeled radiopharmaceutical agents have been used to assess cardiac neurotransmission, at both presynaptic and postsynaptic levels. Four tracers have been used to study presynaptic sympathetic terminals: [18F]uorometaraminol [1,2], carbon-11-labeled ([11C]) hydroxyephedrine (HED) [3] and [11C]epinephrine [4]. These tracers compete with endogenous norepinephrine for transport into the presynaptic nerve terminal, mainly via the neuronal uptake-1 transport system (Figure 1). Once within the neuron, these compounds are metabolized and trapped, and hence serve as markers of sympathetic innervation.

To date, [11C]HED has been the tracer of choice in the clinical setting to study and quantify alterations in autonomic innervation. In the early stage after myocardial infarction, the area of reduced retention of [11C]HED was found to exceed the perfusion defect [5], indicating a greater sensitivity of sympathetic neurons to ischemia that was confirmed by the finding of reduced retention of [11C]HED in patients with advanced coronary artery disease but without a detectable myocardial infarction [6]. Denervation
of the heart has been demonstrated by decreased retention of \(^{11}C\)HED in patients after cardiac transplantation [7] or diabetes [8]. Using the same technique, it has been possible to demonstrate the occurrence of sympathetic re-innervation in the anteroseptal regions of the heart [9]. This has been correlated with recovery both of the sensation of angina pectoris and of contractile function in these patients [10,11]. As illustrated in Figure 2, both pre- and postsynaptic myocardial autonomic function can be assessed non invasively by combining different tracers [12,13], for example \(^{11}C\)HED and \(^{11}C\)(S)-CGP 1217.

Several \(\beta\)-blocker drugs have been labeled with carbon-11 to act as radioligands for imaging the status of postsynaptic receptors by PET [14]. Of these, the most extensively used is \(^{11}C\)(S)-CGP 12177. This is a non selective \(\beta\)-adrenoceptor antagonist that is particularly suited for PET studies because of its high affinity and low lipophilicity, thus enabling the functional receptor pool on the cell surface to be studied [15]. A graphical method has been developed for the quantification of \(\beta\)-adrenoceptor density (\(B_{\text{max}}, \text{pmol/g of myocardium}\)) from the PET data [16], providing parametric images of receptor concentration obtained without having to measure the input function.

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Figure 1. Diagram illustrating the \(^{11}C\)-labelled hydroxyephedrine (HED) transport model. NE, neuroeffector; U-1, uptake-1.

Figure 2. (a) Measurement of pre- and postsynaptic sympathetic innervation of the heart. Cardiac images in a patient suffering from hypertrophic obstructive cardiomyopathy. Left: Presynaptic sympathetic innervation measured with \(^{11}C\)-labeled hydroxyephedrine (\(^{11}C\)HED). Right: \(\beta\)-Adrenoceptor density measured with \(^{11}C\)-labeled (S)-CGP 12177 (\(^{11}C\)CGP). (b) Left: Catecholamine re-uptake in patients with hypertrophic cardiomyopathy (HCM) and control individuals, measured as volume of distribution (\(V_d, \text{mL/g}\)) of \(^{11}C\)HED (means are indicated by the horizontal lines). Right: Maximum number of available binding sites (\(B_{\text{max}}, \text{pmol/g}\)) for \(\beta\)-adrenoceptors in patients with HCM and control individuals, measured using \(^{11}C\)CGP (means are indicated by the horizontal lines). (Modified from [12], with permission.).
metabolites, and protein binding [17]. Studies in our institution [18] in a group of healthy individuals over a broad range of ages have yielded $B_{\text{max}}$ values of $8.4 \pm 2.0$ pmol/g of myocardium, a figure that is comparable to the values measured using in-vitro binding (Figure 2) [19].

Adrenergic neuroeffector axis abnormalities [20–22] are the hallmark of progressive myocardial dysfunction, and the salutary effects of $\beta$-blocking agents point to the detrimental effect of chronically increased $\beta$-adrenoceptor signaling. Both the norepinephrine uptake-1 mechanism and $\beta$-adrenoceptor density are reduced in the myocardium of patients with chronic left ventricular dysfunction, and have been demonstrated non invasively in hibernating myocardium [23] and in congestive cardiac failure [24].

The increased sympathetic activity to the heart in these patients is a generalized phenomenon that is likely to contribute to the remodeling process of the entire left ventricle. In keeping with this, downregulation of myocardial $\beta$-adrenoceptor density has been demonstrated in patients with coronary artery disease in the subacute phase of myocardial infarct, in the absence of symptoms or signs of congestive heart failure. Furthermore, the degree of myocardial $\beta$-adrenoceptor downregulation was directly related to the degree of left ventricular remodeling, 6 months after infarction [25]. As in ischemic cardiomyopathy, in dilated cardiomyopathy, $\beta$-adrenoceptor density is downregulated and is correlated to left ventricular ejection fraction and symptoms of heart failure (judged by New York Heart Association class) [26].

Adrenergic stimuli trigger life-threatening arrhythmias that are difficult to treat. Two disorders in which there is a broad range of evidence for chronically increased levels of sympathetic nervous system activation and diffuse downregulation of $\beta$-adrenoceptor density are hypertrophic cardiomyopathy (HCM) [12,27] and arrhythmogenic right ventricular cardiomyopathy [28]. To investigate further the relationship between left ventricular function and changes in neurocontrol of the heart in patients with HCM, we assessed [18] left ventricular function by echocardiography and myocardial $\beta$-adrenoceptor density by PET in a group of patients with HCM – one subgroup with, and another without, heart failure. Myocardial $\beta$-adrenoceptor density was directly proportional to ventricular function in patients with HCM, whether or not there were signs of heart failure.

Investigation of the $\alpha$-adrenergic receptors is the next step in the characterization of myocardial autonomic trafficking. $\alpha$-Adrenoceptor density has been found to alter as a result of myocardial ischemia [29], and the receptors have also been implicated in the development of ventricular hypertrophy [30,31]. Preclinical data are available for $\alpha_1$-adrenoceptors in rats [32,33] and large animals [34] (Figure 3); to date, only pilot studies have been performed in man [32].

In addition to studies of the sympathetic nervous system, the density and affinity constants of myocardial muscarinic receptors have been evaluated non invasively with PET and $[1^\text{C}]$methylquinuclidinyl benzilate (MQNB), a specific hydrophilic antagonist, both in experimental animals [35] and in man [36,37]. In patients with congestive heart failure resulting from idiopathic dilated cardiomyopathy, the mean receptor concentration ($B_{\text{max}}$) was significantly greater ($34.5 \pm 8.9$ pmol/g) than that in normal individuals ($25 \pm 7.7$ pmol/g), with no changes in affinity constants [37] – a clear indication that congestive heart failure is associated with an upregulation of myocardial muscarinic receptors paralleling the downregulation of $\beta$-adrenoceptors. A number of new ligands for different receptors in the heart are being developed and await further confirmation of their potential to provide a better insight into cardiac pathophysiology in the clinical arena. Preliminary studies in man have been carried out to image the distribution and uptake in the heart of two highly selective ligands for $\mu$ and $\delta$ opioid receptors.

Data obtained in experimental studies in dogs have shown the feasibility of the use of PET for non invasive quantification of dihydropyridine L-type Ca2+ channels with the antagonist 3-ethyl 5-methyl-(1)-2-[(2-(2-aminoethoxy)ethoxy)methyl]-4-(2,3-dichlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate (S12968) labeled with carbon-11 [39].

The technical development of new hybrid PET–computed tomography scanners with the possibility of dual respiratory and cardiac gating could, in the near future, allow the imaging of active plaques in the epicardial coronary arteries. To date, active, inflamed plaque imaging has been performed with [18F]FDG in the carotid, iliac, and femoral arteries, with good reproducibility [40]. [11C]PK11195 is a selective ligand for the peripheral benzodiazepine receptor (PBR) which is expressed in various tissue and organs, including macrophages [41]. Inflammation is characterized by macrophage infiltration [42] and can be detected by PBR binding. At present, tracers such as [11C]PK11195 are under study to monitor either systemic arterial therapies or local, plaque-based, therapy.

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Gene therapy for cardiovascular manifestations of lysosomal storage diseases

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Abstract

Cardiac disease causes morbidity in several lysosomal storage diseases, which are the result of deficient activity of lysosomal enzymes. Mucopolysaccharidosis (MPS) causes aortic and valvular disease, Pompe disease causes cardiac muscle weakness, and Fabry disease causes left ventricular hypertrophy. Enzyme replacement therapy involves intravenous injection of enzyme modified with mannose 6-phosphate, which can be taken up by cells, and is currently approved for some lysosomal storage diseases. Gene therapy can result in secretion of mannose 6-phosphate-modified enzyme into blood, from where it can, similarly, be taken up by cells. Gene therapy has been effective in animal models of lysosomal storage disease, and holds great promise.

Keywords: Animal models, gene therapy, lysosomal storage disease, mucopolysaccharidosis, valve disease

Introduction

More than 50 different forms of inherited lysosomal storage diseases are known to occur in humans; most result in high morbidity and mortality. The aggregate incidence is approximately 1 in 7000 live births [1]. Most lysosomal storage diseases are caused by loss of normal function of a specific lysosomal acid hydrolase, resulting in inability of the lysosomes to degrade large complex substrates that have been targeted for degradation after endocytosis or autophagy. Lysosomal accumulation of the substrate affects the architecture and function of cells, tissues, and organs; lysosomal storage diseases that affect the heart include mucopolysaccharidosis (MPS), Pompe disease, and Fabry disease. MPS results from deficiency in any of several enzymes involved in degradation of glycosaminoglycans, and results in mitral and aortic valve thickening and regurgitation, and aortic dilatation. Pompe disease is caused by deficiency of acid-α-glucosidase (AAG), and results in weakness of cardiac muscle because of the accumulation of glycogen [2]. Fabry disease is attributable to deficiency of α-galactosidase A and results in accumulation of globotriaosylceramide, a glycosphingolipid, and results in vascular disease, left ventricular hypertrophy, and cardiac conduction defects [3].

Enzyme replacement therapy has been developed recently for some types of MPS and for Pompe and Fabry diseases. It involves intravenous injection of...
mannose-6-phosphate-modified enzyme, which can diffuse to cells and be taken up via the mannose-6-phosphate receptor. The active enzyme can be delivered to the lysosome, where it catabolizes stored substrate. Other treatment alternatives include hematopoietic stem cell transplantation for MPS [4,5], and substrate reduction therapy, which is being tested for Fabry disease [3].

Therapeutic gene transfer holds great therapeutic potential, and has proven successful in several animal models of lysosomal storage disease. The most common form of gene therapy involves the transfer into an animal of a cDNA that encodes a functional protein, resulting in long-term expression of the protein that was deficient. As transduced cells in a single organ can secrete enzyme that enters blood or diffuses to adjacent cells, a small percentage of transduced cells can be therapeutic for the entire animal. In-vivo gene therapy involves systemic injection of vector into the circulation, or localized injection into a specific organ. Ex-vivo gene therapy is comprised of removal of cells (such as hematopoietic stem cells), modification in vitro, and infusion of the modified cells back into the animal.

Mucopolysaccharidosis

MPS is caused by deficiency of enzymes that degrade glycosaminoglycans, and results in thickening and elastin fiber fragmentation in the aorta and cardiac valves. This is associated with upregulation of elastin-degrading proteins such as matrix metalloproteinases and cathepsins [6], and the changes can result in valvular stenosis or insufficiency, or both. Intravenous injection of plasmid DNA or viral vectors has been found to result in high expression in the liver, and high concentrations of enzyme in the serum [7]. Neonatal administration of a retroviral vector was shown to reduce the cardiac and aortic manifestations in mice with MPS I, which are deficient in activity of α-L-iduronidase [8]. Good hepatic transgene expression

![Figure 1](image)

**Figure 1. Echocardiograms in dogs with mucopolysaccharidosis (MPS) VII.** Normal dogs, dogs with untreated MPS VII, or dogs with MPS VII treated with retroviral vector underwent echocardiography at the ages indicated above the panels. (a–e) Echocardiographic images of the mitral valve obtained in the long axis from the right parasternum. The cranial (anterior) mitral valve leaflets are indicated by filled white arrows, and the chordae tendinae are indicated by open arrows. Note the marked thickening of the valve in the untreated MPS VII dog compared with that in the normal and treated MPS VII dogs. (d–f) Subjective severity score for echocardiogram parameters. Mitral valve thickening (MVT) (d), mitral valve regurgitation (e), and aortic valve thickening (AVT) (f) were scored from 0 (normal) to 4 (severely abnormal) for six or seven dogs with untreated MPS VII and for four dogs with MPS VII treated with retroviral vector, at the indicated ages. Values in the groups at a given age were compared statistically using Student’s t-test (*P = 0.005–0.05; **P < 0.005). Untreated dogs with MPS VII do not survive beyond 2 years, so it was not possible to compare values in treated dogs with MPS VII with those in age-matched, untreated dogs at older ages. (g) Aortic diameter. The aortic diameter determined in the long axis was normalized to the body surface area (m²), and statistical comparisons were performed as in (d–f). LA, left atrium; LV, left ventricle.
has also been observed with adenoviral vectors [9], SV40 vectors, and a transposon-based plasmid [10], and in-vivo treatment with adeno-associated virus or lentiviral vectors has reduced storage of glycosaminoglycans in the heart, although functional analyses were not performed [11]. Ex vivo hematopoietic-stem cell-directed gene therapy improved left ventricular function in mice with MPS I [4].

Studies involving large animals are more likely to be predictive of results in humans, because their larger size requires efficient scaling and their longer lifespan allows late evaluation of efficacy and toxicity [11]. Retroviral vector gene therapy has yielded impressive results for dogs with MPS VII or MPS I. Dogs affected with MPS VII due to β-glucuronidase deficiency that were treated at 2–3 days of age using a retroviral vector achieved very high serum β-glucuronidase activity [12] that has been maintained stably for up to 7 years (M. Sleeper, M. Haskins, K. Ponder, unpublished data). There was a marked reduction in mitral and aortic valve thickening and insufficiency [12,13], which has been sustained at 7.5 years (Figure 1). In addition, dogs with untreated MPS VII exhibit aortic dilatation and elastin fragmentation, and neonatal gene therapy with a retroviral vector has been found to result in a marked reduction in both of these parameters [13] that was maintained in the long term (Figure 1, Figure 2). Similar experiments in dogs affected with MPS I using a retroviral vector containing the canine α-L-iduronidase cDNA also resulted in stable enzyme activity and normal aortic diameters with mild aortic valve thickening and aortic regurgitation at 1 year [14], and improvements have been maintained at 2 years, although moderate mitral regurgitation was present (M. Sleeper, M. Haskins, K. Ponder, unpublished data). Marked improvement in the heart was also noted in MPS I affected cats that were treated as newborns with intravenous injection of a retroviral vector expressing the deficient enzyme (M. Sleeper, M. Haskins, K. Ponder, unpublished data). The improvement lasted for at least 2 years, when transient immune suppression was given at the time of gene therapy [11].

Pompe disease

Pompe disease results in the accumulation of glyco- gen in cardiac and skeletal muscle, and is associated with reduced contractility, the causative mechanisms of which remain unclear. In mice with Pompe disease, intravenous administration of an adeno-associated virus vector expressing AAG resulted in efficient transduction of liver, high concentrations of AAG in blood, and reduced lysosomal storage [15]. Cardiac function and mass normalization were sustained with gene therapy for at least 1 year in this model [16]. Therapy was more effective in clearing cardiac glycogen storage in heart muscle of young AAG-knockout mice than in that of older counterparts [2,15]. These results resulted in stable enzyme activity and normal aortic diameters with mild aortic valve thickening and aortic regurgitation at 1 year [14], and improvements have been maintained at 2 years, although moderate mitral regurgitation was present (M. Sleeper, M. Haskins, K. Ponder, unpublished data). Marked improvement in the heart was also noted in MPS I affected cats that were treated as newborns with intravenous injection of a retroviral vector expressing the deficient enzyme (M. Sleeper, M. Haskins, K. Ponder, unpublished data). The improvement lasted for at least 2 years, when transient immune suppression was given at the time of gene therapy [11].

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Figure 2. Pathology of the aorta. Aortae were collected from dogs at the ages indicated above the panels and then stained for elastin with Verhoeff’s van Giesen stain, which results in a dark color in the fibers. All images have the intima (I) at the upper left, and the adventitia (A) in the lower right. Lysosomal storage is indicated by the short vertical arrow, and elastin fiber fragmentation is identified with the long horizontal arrow. Low-power images (original magnification x5) are shown in the top panels, in which the box indicates the region that is shown at high power (original magnification x20) in the lower panels.

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emphasize that screening of newborns to allow early diagnosis and treatment is an important initiative.

**Fabry disease**

Fabry disease results in kidney and heart disease, at least in part as a result of the accumulation of storage material in the vascular system. Intravenous administration of adeno-associated virus serotype 8 encoding for human a-galactosidase resulted in normalization of tissue (including cardiac) substrate storage concentrations [17], although the mildness of cardiac disease in this model made it difficult to determine if there was clinical improvement.

**Summary**

Systemic gene therapy with viral vectors has reduced the clinical manifestations of cardiovascular disease in MPS and in Pompe disease, and will probably have a therapeutic effect for Fabry disease. As early treatment was more beneficial in some studies, identification of patients with lysosomal storage disease by means of screening of newborns [18], which is currently being introduced in some states in the USA, will be important in the future. Studies are currently under way to define the risk of insertional mutagenesis and to modify vectors in order to reduce the associated carcinogenic risks. If these concerns can be addressed, it is possible that, in the near future, gene therapy will be used to treat patients with these diseases.

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Anti-ischemic cardioprotection with trimetazidine

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Abstract
Alterations in cardiac metabolism are present in ischemic heart disease and heart failure, suggesting an increased utilization of non carbohydrate substrates for energy production, with a reduction in the efficiency of myocardial oxygen consumption and ischemia-reperfusion damage. A direct approach to the manipulation of cardiac energy metabolism consists in modifying substrate utilization. Trimetazidine is a pharmacological agent shifting the energy substrate preference away from fatty acid metabolism and towards glucose metabolism. Recent studies suggest that trimetazidine has a positive influence on ischemia-reperfusion damage, endothelial function, and prognosis in patients with coronary artery disease.

Keywords: Endothelium, myocardial ischemia, nitric oxide, reperfusion damage, trimetazidine

The ischemia-reperfusion damage: from bench to bedside

It is commonly accepted that a rapid re-opening of an occluded vessel, by either mechanical means (coronary angioplasty or bypass surgery) or pharmacological means (thrombolytic or antiplatelet drugs), should be performed as soon as possible in patients with acute coronary syndromes. The opening of a coronary artery or the interruption of an ischemic period, such as in effort angina, exposes the entire heart to a complex cascade of events, so-called “reperfusion injury”, that influences the final amount of cellular damage, the endothelial function, the degree of mechanical dysfunction, and the short- and long-term prognosis. Reperfusion injury may affect various aspects of myocardial and endothelial function, with different and complex pathophysiological consequences [1]. The term encompasses several events, including microvascular damage, reperfusion arrhythmias, reversible myocardial mechanical dysfunction (stunning), and cell death (apoptosis or necrosis). Oxidative stress, intracellular calcium overload, neutrophil activation, metabolic alterations, and excessive intracellular osmotic load have all been proposed to explain the pathogenesis and consequences of inflammatory injury in ischemic reperfused myocardium.

Inflammatory and endothelial damage

The inflammatory process characterizes early and late reperfusion and is involved in tissue damage. Neutrophils feature prominently in the inflammatory component of post-ischemic injury. Ischemia-reperfusion prompts a release of oxygen free radicals, cytokines, and other proinflammatory mediators that activate
both the neutrophils and the coronary vascular endothelium [2]. Activation of these cells promotes the expression of adhesion molecules on both neutrophils and the endothelium; these recruit neutrophils on the endothelial surface and initiate a specific cascade of cell–cell interactions. This specific series of events is a prerequisite for the full expression of reperfusion injury, including endothelial dysfunction, microvascular collapse, impairment of blood flow ("no-reflow" phenomenon), myocardial infarction and apoptosis [3]. Endothelium-derived factors, such as nitric oxide and adenosine, exhibit a wide range of effects against neutrophil-mediated events and modulate the inflammatory response after reperfusion [3]. Alterations in endothelial function are pivotal in the development of reperfusion damage and the no-reflow phenomenon; here, the enhanced release or increased bioavailability of nitric oxide appears to be central. Besides its well-known vasodilatory effects, nitric oxide reduces microvascular dysfunction, platelet adhesion and aggregation, and leukocyte adherence or emigration [3,4].

**Apoptotic cell death**

Apoptosis is an energy-requiring physiological mechanism of cell death that regulates cell mass in many tissues; it is a genetically directed process that takes place in response to internal or external stimuli. Cardiomyocyte apoptosis has important pathophysiological consequences, contributing to functional abnormalities in the myocardium. It has been reported in a variety of cardiovascular diseases, including myocardial infarction or ischemia [5] and endstage heart failure [6]; a specific association with reperfusion injury has been suggested [7]. The cellular mechanisms underlying both ischemia-reperfusion injury and apoptosis may involve cellular calcium overload, left ventricle wall stresses, overproduction of oxygen-derived free radicals, cellular acidosis, inflammatory reaction, and microcirculatory dysfunction [7–9].

**Metabolic changes**

A metabolic protection of the ischemic myocardium appears to be a key factor in limiting reperfusion damage [9–11]. Major metabolic changes occurring during the early hours of myocardial infarction include increased secretion of catecholamines and production of circulating free fatty acids (FFAs). Under normal conditions, the myocardium depends on aerobic metabolism, with FFAs as the preferred energy source. During ischemia-reperfusion, FFA concentrations are greatly increased, and exert a toxic effect on the myocardium. This results in increased membrane damage, endothelial dysfunction, tissue inflammation, and decreased cardiac function. Decreasing plasma FFA concentrations and cardiac fatty acid oxidation, together with the stimulation of glucose and lactate uptake, might reduce these detrimental effects. This might be achieved by glucose–insulin–potassium (GIK) solutions at the time of reperfusion [12] and by inhibiting fatty acid oxidation with 3-ketoacyl coenzyme A thiolase inhibitors, such as trimetazidine [9].

### Anti-ischemic cardioprotection with trimetazidine

The anti-ischemic cardioprotection achieved with trimetazidine may involve several aspects, summarized in **Table I** and **Figure 1**.

### Trimetazidine limits accumulation of Na\(^{+}\) and Ca\(^{2+}\) and depresses intracellular acidosis

Dysregulation of intracellular Ca\(^{2+}\) homeostasis plays an important part in mediating myocardial injury. A marked increase in cytosolic free Ca\(^{2+}\) has been reported in ischemic myocardial injury, and the occurrence of intracellular Ca\(^{2+}\) overload has been suggested to lead to arrhythmias, contractile failure, and cell death. It has been proposed that trimetazidine has a key role in limiting the intracellular accumulation of protons that is responsible for cell acidosis during ischemia (**Figure 1**). Under conditions of acid load such as no-flow ischemia, trimetazidine acts in a dose- and time-dependent way in limiting the accumulation of Na\(^{+}\) and Ca\(^{2+}\) inside cardiac cells and depressing intracellular cell acidosis [13]. Trimetazidine also maintains intracellular adenosine triphosphate concentrations and increases plasma concentrations of adenosine, a nucleoside with protective

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**Table I. Anti-ischemic cardioprotection with trimetazidine: main effects.**

<table>
<thead>
<tr>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic efficiency: shifting of ATP production to glucose oxidation, a more energetically efficient pathway</td>
</tr>
<tr>
<td>Protection of endothelial function (increase in endothelial nitric synthase activity and nitric oxide availability; reduction in endothelin-1)</td>
</tr>
<tr>
<td>Modulation of the myocardial inflammatory reaction (reduction of neutrophil infiltration and activation)</td>
</tr>
<tr>
<td>Limitation of accumulation of Na(^{+}) and Ca(^{2+}) and intracellular acidosis</td>
</tr>
<tr>
<td>Reduction in necrotic and apoptotic cell death</td>
</tr>
<tr>
<td>Preservation of mitochondrial functions (reduction in mitochondrial permeabilization)</td>
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<tr>
<td>Protection against toxicity induced by oxygen free radicals</td>
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</tbody>
</table>
effects in myocardial ischemia [14]. In a recent randomized, double-blind, cross-over study comparing placebo and trimetazidine, Fragasso et al [15] assessed the effects of trimetazidine on the left ventricular phosphocreatine and adenosine triphosphate (PCr : ATP) ratio in patients with heart failure by means of in-vivo phosphorus-31 magnetic resonance spectroscopy. The mean cardiac PCr : ATP ratio was increased by 33% with trimetazidine, suggesting that the effects of trimetazidine are associated with the preservation of myocardial high-energy phosphate concentrations.

Trimetazidine reduces cell damage: necrosis and apoptosis

Various studies demonstrated that, in experimental conditions, trimetazidine reduces the myocardial damage caused by ischemia-reperfusion [9]. In patients with acute myocardial infarction, pretreatment with trimetazidine (40 mg orally about 15 min before thrombolysis, and then 20 mg every 8 h) can decrease the time to creatine kinase normalization, suggesting that trimetazidine reduces reperfusion damage and infarct size in patients with acute myocardial infarction who undergo thrombolysis, and affects remodeling after myocardial infarction [16]. Ruixing et al [17] reported that, in a rabbit model of ischemia-reperfusion, trimetazidine also reduced cardiomyocyte apoptosis and ischemia-reperfusion injury via antioxidant properties.

Trimetazidine preserves mitochondrial functions

Mitochondria are key factors in energy production in cells. Lipid oxidation is responsible for the production of much ATP resynthesis in the heart, but this process is less oxygen-efficient than glucose oxidation. During ischemia, lipid oxidation is suddenly blocked, but it is markedly increased during reperfusion, causing accumulation of potentially toxic metabolites (acyl coenzyme A) which may induce gene expression of cardiac-enriched uncoupling proteins and enter the mitochondrial fatty acid β-oxidation spiral. Reducing equivalents generated subsequently enter the mitochondrial respiratory chain complexes, resulting in the generation of a proton gradient across the inner mitochondrial membrane. Protons located within the intermitochondrial membrane space re-enter the mitochondrial matrix through ATP synthase, resulting in mitochondrial ATP synthesis. Mitochondrial ATP is exported to the cytosol by adenine nucleotide translocator, proposed to form part of the mitochondrial permeability transition pore. With increased β-adrenergic stimulation and mitochondrial fatty acid β-oxidation, concentrations of mitochondrial reactive oxygen species (ROS) may increase and activate uncoupling proteins or promote transition pore opening, or both. This in turn will result in proton leakage and dissipation of the membrane potential, thereby diminishing mitochondrial ATP production. Transition pore opening will also trigger programmed cell death (apoptosis). Acute or chronic administration of trimetazidine induces the partial inhibition of fatty acid β-oxidation (1) and determines the increase in glucose oxidation, energetically useful in ischemic heart. These metabolic changes could induce a reduction in ROS-induced cell damage (2), and in uncoupling proteins (3), with an inhibition of apoptosis (4). The final effect is a reduction in cellular damage and an improvement in cardiac function.

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carnitines, acyl coenzyme A, lysophospholipids). Trimetazidine inhibits the production of deleterious lipid metabolites and it can also reduce mitochondrial damage, inhibiting mitochondrial permeability transition-pore opening, and protects the heart from prolonged ischemia-reperfusion injury [18].

**Trimetazidine protects against toxicity induced by oxygen free radicals**

In mitochondria and endothelial or myocardial cells, trimetazidine reduces membrane damage induced by oxygen free radicals. This effect has also been reported in red cells after oral administration [19]. The drug acts as a potent antioxidant, and this mechanism of action could explain its cardioprotective role during ischemia and reperfusion, in which oxygen free radicals are generated and implicated in cardiac cell injury. Trimetazidine, at concentrations greater than 100 μmol/L, competed with cytochrome c in scavenging oxy radicals formed by the reaction catalyzed by the action of the xanthine oxidase enzyme upon xanthine. This scavenger effect was also observed when oxy radicals were generated by active human neutrophils [20].

**Trimetazidine protects endothelial function and modulates the myocardial inflammatory reaction**

Various studies have demonstrated that trimetazidine reduces inflammation and improves endothelial function in both acute conditions (ischemia-reperfusion damage, coronary angioplasty, thrombolysis) and chronic conditions (ischemic cardiomyopathy, stable angina). Trimetazidine maintains the integrity of cell membranes and of mitochondrial structure, and ensures the protection of myocardial cells that are at risk (Figure 1). Trimetazidine also protects postischemic hearts from neutrophil-mediated injury [21]. Tritto et al [22] reported that trimetazidine inhibited neutrophil activation in vitro and reduced cardiac oxygen radical production at reflow, independently of direct scavenger effects. Trimetazidine is also a useful drug in preventing inflammation after percutaneous transluminal coronary angioplasty (PTCA) [23,24]. Pre-procedural treatment with oral trimetazidine for 3 days significantly suppressed the increase in inflammatory markers before and shortly after PTCA [24]. Recent studies have analyzed the impact of trimetazidine on markers of inflammation in chronic ischemic heart disease. In patients with ischemic cardiomyopathy, we found [25] that plasma concentrations of C-reactive protein remained unchanged in the group of patients receiving trimetazidine, whereas a progressive increase in concentration was seen throughout the follow-up period (18 months) in the control group; this anti-inflammatory effect could be involved in the significant reduction in mortality and admissions to hospital observed in the same patients after 48 months of treatment [26]. In addition, a decrease in the serum concentrations of endothelin-1 has been reported in patients with diabetes receiving treatment with trimetazidine, both after short-term (2 weeks) and after long-term (6 months) treatment [27]. The mechanisms responsible for these favorable effects of trimetazidine on the inflammatory profile and endothelial function are poorly known. We have reported [28] that, in isolated rat heart subjected to ischemia and reperfusion, trimetazidine reduced ischemia-reperfusion damage and increased endothelial nitric oxide synthase mRNA and protein concentrations. In this way, trimetazidine exerts a significant, nitric-oxide-dependent, cardioprotection against ischemia-reperfusion injury and preserves the coronary endothelium. Preservation of the production of endothelial nitric oxide synthase and its bioavailability appears also to be a critical factor in the decrease in release of endothelin-1 and the preservation of endothelial function. Recently, Belardinelli et al [29] reported that trimetazidine improved endothelium-dependent relaxation in patients with ischemic cardiomyopathy. This effect was associated with antioxidant properties as measured by a reduction in plasma malondialdehyde and lipid hydroperoxide concentrations.

**Conclusions**

In the ischemic myocardium, metabolic antianginal treatment with trimetazidine induces a shift from utilization of FFAs towards utilization predominantly of glucose, increasing ATP generation per unit of oxygen consumption. This represents an innovative approach to the treatment of ischemic heart disease and to the reduction of ischemia-reperfusion damage. Trimetazidine offers unique positive effects and it is useful in improving ischemic cell metabolism and in reducing cell injury and endothelial dysfunction. All these effects could be clinically relevant, improving prognosis and quality of life in patients with coronary artery disease.

**REFERENCES**


Long-QT syndrome in a family with a KCNH2 mutation

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Conflicts of interest: None.

Abstract

Long-QT syndrome (LQTS) is an inherited ion channelopathy resulting in abnormal ventricular repolarization and abnormal prolongation of the QT interval on the electrocardiogram. Clinical features vary, from asymptomatic individuals to those with presyncope, life threatening ventricular arrhythmias and sudden cardiac death (SCD). This case report describes a family with a mutation of the KCNH2 gene expressed phenotypically as LQT2 syndrome. The variability in phenotypic expression, the importance of family and genetic screening, and the difficult dilemma of deciding which individuals with LQTS should receive an ICD are discussed.

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Keywords: Implantable cardioverter-defibrillator, KCNH2, long-QT syndrome, torsades de pointes

Case report

Ms X was diagnosed with epilepsy as a teenager and for years had recurrent blackouts diagnosed as seizures. In her 40s, the episodes became more frequent and the differential diagnosis of cardiac syncope was raised. An electrocardiogram (ECG) showed her QT interval corrected for heart rate (QTc) to be at the upper limit of normal. Holter monitoring was normal and she opted not to have an implantable loop recorder.

One year later, her older sister, Ms Y, suffered a cardiac arrest. Recurrent torsades de pointes ventricular tachycardia was documented and her ECG revealed abnormal prolongation of the QTc interval. She was diagnosed with long-QT syndrome (LQTS) and an implantable cardioverter-defibrillator (ICD) was implanted for secondary prevention. She was placed on prophylactic β-Blocker therapy.

Given the inherited nature of LQTS, ECG screening was performed in the family. The ECG of Ms X now showed a clearly prolonged QTc interval of 560ms (Figure 1a). She too was diagnosed with LQTS and only 1 week later presented with recurrent syncope and torsades de pointes ventricular tachycardia (Figure 1b). Like her sister, an ICD was implanted. The ECG of child A (a niece of Ms X) revealed a QTc interval at the upper limit of normal, but with clearly abnormal T-wave morphology (Figure 1c).

Family genetic screening for long-QT gene mutations revealed that several members were found to have a heterozygous mutation of the potassium channel subunit gene, KCNH2 (also known as the human ether-a-go-go-related gene [HERG]), on chromosome 7 (Figure 2). KCNH2 mutations are phenotypically expressed as LQT2 syndrome. The mutation, c.2306T>G (p.L769R), had not been reported previously. It was predicted to result in the non synonymous substitution of a conserved amino acid, indicating a high likelihood that it was a pathogenic variant.

Phenotypic expression within the family varied; with three sisters experiencing syncope and ventricular arrhythmia (Ms X, Ms Y, and Ms Z) while the children carrying the mutation were asymptomatic. Child A had reported infrequent dizzy spells characteristic of vasovagal episodes which improved with β-blockers.
Long-QT syndrome

Long-QT syndrome is a rare inherited genetic disorder caused by mutations in cardiac potassium or sodium ion channel genes [1–3]. Reductions in the effective repolarising currents of these defective ion channels lead to prolonged ventricular repolarisation and a susceptibility to ventricular arrhythmias and sudden cardiac death. The ECG QTc interval, representing activation and recovery duration of the ventricular myocardium, is abnormally prolonged.

Genetics

Most LQTS mutations have a heterogeneous autosomal dominant pattern of inheritance, with an estimated prevalence of around 1:10,000 of the population. Hundreds of mutations in eight genes (LQTS 1–8), have been identified to date [4]. LQTS 1–3 account for 95% of known mutations, with each syndrome exhibiting different genetic and clinical characteristics (Table I). Clinical presentation of long-QT syndrome can vary significantly due to the different genotypes and variable penetrance. Affected families tend to have their own novel or “private” mutations. Transmission is not strictly Mendelian with an excess of female carriers among the offspring of mutation carriers.

The LQT2 gene, KCNH2, is located on the long (q) arm of chromosome 7 and encodes the pore-forming subunit of the potassium channel. The mutation results in diminution of the repolarising rectifying potassium current (I_{Kr}) with abnormal ventricular repolarisation [5]. Over 200 mutations in the KCNH2 gene have been identified.

Clinical features and diagnosis

LQTS carriers may be identified because of symptoms, incidentally, or during family screening. Most mutation carriers remain asymptomatic throughout life, hence clinical disease is less common than the mutation rate. LQTS is identified by abnormal prolongation of the QT interval on the ECG. Normal upper limits of the QTc interval are <460 ms for women and <450 ms for men. However, highly variable disease expression means that such screening tests such as have a sensitivity less than 100%. Indeed, mutation carriers may have normal ECGs or dynamic QTc intervals as illustrated in the case of Ms X, whose QTc interval was initially normal. Causes of acquired prolongation of the QT interval should be excluded before a diagnosis of LQTS is made.

There is a spectrum of clinical manifestations in LQTS from presyncope to syncpe from ventricular arrhythmias and sudden cardiac death. The classic
arrhythmia is Torsade de pointes ventricular tachycardia. The mean age for first manifestation of the disease is 12 years, but there is a wide range, from the first year of life to as late as the fifth and sixth decades. Sudden death and syncope are uncommon in patients older than 40 years. Arrhythmias may be elicited by stress and emotion or may occur at rest or during sleep [6]. In LQT2 syndrome, sudden loud noises or startle such as an alarm clock, may trigger arrhythmic events. To assist diagnosis, clinical criteria such as the Schwartz scoring system have been developed to determine the probability of having LQTS, encompassing symptoms, family history, and the QTc interval amongst other features [7]. Additional ECG abnormalities such as abnormal T wave morphology are common. Wide-based T waves are most frequently seen in LQT1 whereas notched T waves are most commonly seen in LQT2. The diagnosis of LQTS may only be apparent after provocation tests such as treadmill testing which may yield an abnormal QTc response to exercise [8].

**Risk stratification**

The QTc interval is the strongest predictor of risk for cardiac events, with an interval exceeding 500 ms carrying a greater than 50% risk of an event before the age of 40 years. Other high-risk features include previous cardiac arrest, symptoms despite adherence to adequate β-blockade, symptoms before puberty, recurrent syncope believed to be caused by arrhythmias, male sex in LQT3 carriers irrespective of QT interval, or onset of syncope with noise or rest [9]. A family history of sudden cardiac death or the proband’s severity of symptoms do not appear to predict severity in other genetically affected family members. The location of the altered amino acids(s) within the ion channel may also affect prognosis, LQT2 carriers with mutations in the pore region are at increased risk for cardiac events compared with non-pore mutations [10].

**Genetic and family screening**

As an inheritable channelopathy, family screening in LQTS is clearly important. Identification of the typical diagnostic ECG and clinical features described above remains the main screening tool. Although LQTS is a clinical diagnosis, genetic testing for the more common types of LQTS has now become available, and can identify a mutation in 50–75% of probands in whom the diagnosis appears to be clinically certain. Genetic confirmation of LQTS is important both for risk stratification of the proband and for identifying mutation carriers within the family. Once identified, silent carriers of LQTS may be prophylactically treated with β-blockers and receive genetic counselling. A
negative guidance test does not, however, exclude the diagnosis, as non-coding variants or unidentified disease-associated genes will result in non-detection. False positive results are also possible since detection of a previously undescribed mutation does not establish a LQTS diagnosis and DNA variants of little significance are well recognised.

**Treatment**

Therapy for LQTS is directed toward reduction of the incidence of syncope and sudden death. The lack of randomized trials of treatment in LQTS reflects both the rarity of the disease and the heterogeneity of its clinical presentations. The data to guide management comes from large registries and referral centers with a bias toward patients with severe disease. Current therapeutic options involve the use of β-blockers and ICDs. Because of adrenergic triggering, affected individuals are also recommended to restrict their participation in athletic activities.

Prophylactic treatment with β-blockers has been shown to be effective in significantly reducing the risk of cardiac events and decreasing the death rate in LQTS [11]. β-Blockers have minimal effect on the QTc interval, but exert a protective effect by reducing the adrenergic stress that typically precipitates arrhythmias in LQTS. Long acting agents are recommended as first line treatment in all affected individuals, with efficacy assessed by the blunting of the exercise heart rate. They are effective in preventing cardiac events in approximately 70% of patients and thus do not provide absolute protection against fatal cardiac arrhythmias. Effectiveness in LQT3 syndrome is less clear than for LQT1 and LQT2.

Implantation of an ICD is indicated for LQTS carriers at high risk of sudden death. The pacemaker function is also used in those with pause-dependent or bradycardia-induced ventricular tachycardia. An important clinical dilemma has been deciding with certainty who should or should not have a defibrillator. The American College of Cardiology/American Heart Association/European Society of Cardiology have issued guidelines, which include the use of ICDs, for the management of LQTS [12]. Prophylactic β-blockade has a Class I indication for all individuals with abnormal prolongation of the QT interval, regardless of symptoms. ICDs have a Class I indication in secondary prevention for those surviving cardiac arrest, Class IIa for those with symptoms or syncope while taking β-blockers, and Class IIb for primary prevention in those with possible high-risk characteristics. Thus, ICD indications for secondary prevention in LQTS are clear but their use in primary prevention is more controversial and debated. One approach would be to implant an ICD for primary prevention in all patients with LQTS, given the risk of sudden cardiac death. In contrast, ICDs are typically implanted in young patients with LQTS, who will have the device for decades, with the significant risks of component failure or infection. Clinical judgment and risk assessment must prevail while outcomes of ICD use in primary prevention are pending.

**Conclusion**

Although a rare congenital disorder, LQTS can have devastating consequences, ranging from syncope to sudden cardiac death. β-Blockers and ICDs are the mainstay of treatment. A more detailed knowledge of the risks conferred by different genetic mutations, and long-term follow-up studies of patients receiving ICDs for primary prevention, will help further to inform decision making in the management of this syndrome.

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How do mutations cause disease?

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Abstract

The identification to date of most of the mutations responsible for monogenic disorders with familial inheritance has opened schematically two broad fields for cardiovascular research in the next decade or two: that of how a given mutation with a strong deleterious effect cause a disease, and that of how a number of genes, each with a weak implication in the disease phenotype, combine their effect between each other and with environmental factors to cause complex diseases. The first is between the hands of basic scientists using cellular and/or animal models. The second is between the hands of clinicians and geneticists for the identification of new susceptibility genes/mutations or the validation, in large cohorts of patients, of genes/mutations found in experimental models through the candidate gene approach. Between the two there is room also for assessing the important role of modifier genes and environmental factors in monogenic disorders. Altogether, patients, families, populations and animal models will continue for long to provide invaluable information for our understanding of how mutations cause diseases.

Keywords: Cardiomyopathies, channelopathies, genes, pathophysiology, environmental factors

Introduction

Mutations are irreversible hereditary alterations of the genetic material of living organisms that are responsible for – because they are hereditary – durable modifications of their phenotype. These modifications may or may not be deleterious to the functions and survival of the affected organisms. They need to be distinguished from polymorphisms, which are non pathological variations in the gene sequence that account for the different alleles of a gene in a given population.

The question to be addressed here implies a more or less direct link between a given mutation and its deleterious effect. More than a direct link, the question suggests that the mutation has a detrimental effect that is sufficiently strong to alter severely a physiological function, and/or the structure and function, of one or several organs, and lead ultimately to a disease. Such is the case in those monogenic disorders with Mendelian inheritance that are responsible for most familial cardiomyopathies or ion channelopathies.

Despite the strong effect of the mutation, it is nevertheless important to note that the complexity and length of the pathophysiological path from the mutation to the disease may differ significantly in different diseases. When Wang et al [1] described the first mutation in the \( SCN5A \) gene coding for the main subunit of the cardiac sodium channel, their finding was able to explain directly the long-QT syndrome (LQT3) observed in patients carrying the mutation. In contrast, when Seidman and colleagues [2] identified the first mutation in the \( \beta \)-myosin heavy chain gene (\( MYH7 \)) as a cause of familial hypertrophic cardiomyopathy (HCM), they were not able to explain how the mutation causes the disease, and the question remains under debate, as discussed below.

As will also be considered below, in autosomal dominant diseases – as in most familial cardiomyopathies and channelopathies – even a small mutation in one allele is sufficient to generate the pathologic
phenotype, whereas in autosomal recessive diseases, two mutations in the same gene – generally two different mutations on the two alleles – are necessary to generate the disease phenotype. In both cases, the genetic component of the disease is generally obvious simply by observing the members of the affected families, provided their phenotype has been assessed carefully.

Contrast is often drawn between the monogenic familial diseases and those common diseases that are also called “complex” or “multifactorial” because of the complexity of their pathophysiology resulting from the large number of factors involved. In these diseases, the phenotype is believed to result from the combined effects of mutations, functional polymorphisms, or both, in a number of genes, together with so-called environmental factors. In such diseases, the strength of each gene variant is considered to be weak and responsible for only a small part of the phenotype; it is only the combination of several such variants and their association with one or several environmental factors that is regarded as causative of the disease. This dichotomous distinction is erroneous, because what actually differs between diseases is the relative contribution of hereditary and environmental factors, which, at least from a theoretical point of view, can each vary from 0 to 100%. We will see below that, even in autosomal dominant diseases, environmental components may play an important role.

A role for “environment” having been recognized, the definition of what is an environmental factor also needs to be clarified.

Types of mutation, and their consequences

To answer, at least in outline, the question of “how mutations cause diseases”, the various types of mutations and their general consequences need to be described briefly.

Mutations comprise a broad set of gene alterations, ranging from the replacement of a single base pair by another, to small deletions or insertions, large deletions, duplications or inversions, triplet expansions, and other more complex and rare mechanisms that will not be considered here.

Mutations can affect any part of the genetic material – not only exons coding for proteins, but also the promoter and regulatory regions of the genes, and also introns. In the case of mutations affecting the coding regions of the genes, the replacement of a base pair by another does not alter the reading frame; accordingly, the resulting mRNA is entirely translated into a complete protein. Because of the degenerative nature of the genetic code, such a replacement does not change the amino acid sequence of the encoded protein if the resulting codon codes for the same amino acid as the “normal” (wild-type) one. If it does not, the replacement leads to missense or nonsense mutations. In the former case, a new amino acid replaces the “normal” one, and this may result in subtle to major changes in the function of the protein, depending on the place/role of the affected amino acid in the protein and the characteristics of the new amino acid. In the latter case, mRNA translation is stopped, resulting in truncated and often unstable proteins that are degraded by the ubiquitin–proteasome system. Such an outcome is also true in the case of small deletions or insertions of one or two base pairs (anything different from three or a multiple of three), which are called “frameshift mutations” because, by shifting the reading frame, they usually result in the occurrence, in the downstream mRNA sequence, of a premature stop codon, itself resulting in a truncated protein [3].

All the mutations that result in a partial or total loss of expression of the encoded protein, or in the synthesis of a protein that is partially or totally inactive, are classified as “loss-of-function” mutations. Therefore, the effect of loss-of-function mutations ranges from subtle alterations in the function of the protein, with no obvious change in its steady-state concentration, to more severe alterations in the structural, functional or biological activity of the protein, to the more or less complete loss of the protein encoded by the disease allele – a process called “haplo-insufficiency” or the “null allele” effect. This is the case, for instance, in familial hypercholesterolemia and with mutations in the gene coding for myosin binding protein C (MYBPC3), the second most common cause of familial HCM.

When a disease is caused by loss-of-function mutations, one usually finds a large number of different causal mutations that may affect gene transcription (mutations at the levels of the promoter and of the regulatory sequences of the gene), maturation of the primary transcript (for instance mutations affecting a splice site), or translation of the mRNA into protein (nonsense and frameshift mutations, which interrupt the translation of the mRNA into the final protein, which, consequently, becomes truncated). With respect to missense mutations affecting the coding regions of the genes (exons), they may also compromise the maturation and stability of the protein, but, in particular, they alter protein targeting within the cell, its assembly with other proteins into multimeric structures and eventual regulation by other proteins, and, finally, its function as, for example, an ion channel or enzyme, by altering its properties of activation or inactivation, or its interaction with substrate, respectively. This is the case for most of the mutations in the genes encoding sarcomeric proteins responsible for HCM, which lead to stable proteins that are fully incorporated into the sarcomere, where they play the dominant negative role of “poison peptide” [4].
The noticeable exceptions, here, are a number of mutations in the gene coding for the myosin binding protein C (MBPC) that are frameshift mutations leading to truncated, unstable proteins, resulting in haploinsufficiency; however, a number of these truncated proteins may also incorporate into the sarcomere and take on the role of poison peptide [3].

Interestingly, and very logically, a number of missense mutations are also able to modify the function of the protein in such a way that its function is not decreased but, on the contrary, is increased, the increased function being itself deleterious and responsible for the disease. This can be found in cases of familial HCM or in the \textit{SCN5A} gene mutation cited above [1].

As mentioned previously, the pathophysiological process leading from loss- or gain-of-function mutations to the disease is, in general, more difficult to decipher in familial cardiomyopathies than in channelopathies. For instance, although the situation is proving to be more complex than was originally anticipated [5], gain-of-function mutations in channels carrying depolarizing currents and loss-of-function mutations in channels carrying repolarizing currents both lead directly to prolongation of the QT interval, a feature visible on the surface ECG. In such cases, the pathophysiological link between the mutation and the disease is straightforward, with no complex molecular and cellular intermediates. In contrast, it is not clear how mutations in sarcomeric protein genes resulting in either decreased or increased myofibrillar sensitivity to Ca\textsuperscript{2+} lead to largely similar cardiac phenotypes.

Another example of complexity is the case of mutations in the \textit{\gamma}2 subunit of the AMP-activated protein kinase gene (\textit{PRKAG2}) responsible for glycogen storage cardiomyopathy, another form of HCM for which the mechanism remains under debate – not only with respect to the cardiomyopathy itself, but also with respect to the pre-excitation syndrome that is often observed at the level of the ECG [6]. Intriguingly, in familial HCM, despite the diversity of the initial functional defects conferred by the mutant proteins, they converge to induce the final phenotype of cardiac hypertrophy. This has led to the "common defect" hypothesis, according to which, regardless of the diversity of the initial defects caused by the mutations, a common impairment of cardiac myocyte mechanical function activates the classical intracellular signaling pathways (calcineurin-nuclear factors of activated T cells [NFAT], calmodulin-dependent protein kinase II [CaMKII], mitogen-activated protein kinase [MAPK], phosphatidylinositol 3-kinase–protein kinase B–glycogen synthase kinase 3\(\beta\) [PI3K-Akt-GSK3\(\beta\)], leading, in general, to maladaptive hypertrophy [7].

The mechanisms leading from mutations to dilated cardiomyopathies are even more obscure, all the more so because, besides mutations in genes coding for proteins of the cell cytoskeleton, mutations in genes coding for sarcomeric proteins that are generally implicated in HCM (actin, cardiac troponin T, \(\alpha\)-troponysin) are also involved. The hypothesis commonly advanced is that of a defect in force transmission throughout the myocardium that would lead the left ventricle to dilate as a compensatory mechanism for decreased left ventricular function. Interestingly, in a mouse model reproducing a type of human dilated cardiomyopathy, inactivation in the heart of the gene coding for the muscle LIM protein (MLP), leads to dilated cardiomyopathy as a result of a loss in the stretch sensor at the Z-line/costamere level and the resulting defect in hypertrophy signal transduction [8].

Environment

Finally, the environmental effects have to be considered briefly. From the point of view of a mutation, "environment" can be considered, very broadly, as everything but the mutation itself. Therefore, environmental factors can be another mutation in the same gene or in another gene, a functional polymorphism, a change in physiological conditions (eg, effort as opposed to rest, stress as opposed to quietness), or the occurrence of any pathological condition such as hypertension or diabetes. In fact, from observations made in large families among whom the same mutation yielded a different phenotype according to which affected family member was under consideration, it rapidly became clear that factors other than the mutation were involved in the final phenotype. This led to the first consideration of the possible existence of genetic modifiers – that is, one or several gene variants that exert an influence on the expressivity of the disease. This concept can be extended to the entire genetic background, and it has been well established, at least in mouse experimental models, that the same mutation has different effects depending on the strain in which it has been introduced (see [5] for examples).

Another aspect of the environment is the "way of life". Certain mutations need a specific context in order to exert their deleterious influence. This is the case, for instance, for mutations in the genes encoding the Ca\textsuperscript{2+} channel of the sarcoplasmic reticulum that is known as the ryanodine receptor (\textit{RYR2}), and calsequestrin (\textit{CASQ2}), the Ca\textsuperscript{2+}-storage protein within the sarcoplasmic reticulum, both responsible for catecholaminergic polymorphic ventricular tachycardia [9]. In essence, patients carrying these mutations have "no phenotype" at rest, and stress is a necessary condition in order that the mutation lead to the disease, most probably through a stress-induced increase in the accumulation of Ca\textsuperscript{2+} in the sarcoplasmic...
reticulum. Similarly, experimental data support the concept that the 1795insD mutation in the SCN5A gene is responsible, in the same mouse model or in patients, for coexisting LQT3 and Brugada syndromes, the expression of each depending on the heart rate [5].

Conclusion

Most of the mutations with a strong detrimental effect have now been identified and, fortunately for pathophysiologists, the extent of our understanding of how such mutations cause disease is far-reaching. In this context, pathophysiologists must certainly be grateful to geneticists for their continuing efforts to describe new mutations, because new mutations and the study of their relationship with the associated phenotype are, together with animal models, invaluable tools for our achievement of better understanding of corresponding functions, and for the development of new therapeutic strategies.

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To assess myocardial reperfusion in patients with acute anterior myocardial infarction treated by primary percutaneous coronary intervention (PCI), the relationship between the presence and severity of microvascular obstruction was studied, using cardiovascular magnetic resonance (CMR) and intracoronary Doppler flow measurement. CMR has been used to detect and quantify microvascular obstruction in patients after acute myocardial infarction, but has never been compared with coronary blood flow velocity patterns. Twenty-seven patients with first anterior ST-segment elevation myocardial infarction successfully treated with primary PCI were included. Coronary blood flow velocity was measured during re-catheterization 4–8 days after primary PCI. These measurements were related to microvascular obstruction determined by late gadolinium-enhanced CMR performed the day before re-catheterization. Early systolic retrograde flow was observed in none of eight patients without microvascular obstruction on late gadolinium-enhanced CMR, and in 10 of 19 patients (53%) with microvascular obstruction (P = 0.01). The extent of microvascular obstruction correlated with the diastolic: systolic velocity ratio (r = 0.44; P = 0.02), diastolic deceleration time (r = –0.61; P = 0.001), diastolic deceleration rate (r = 0.75; P < 0.0001), and coronary flow velocity reserve of the infarct-related artery (r = –0.44; P = 0.02). Furthermore, multivariate regression analyses, including extent of microvascular obstruction, infarct size, and transmural necrosis on late gadolinium-enhanced CMR, revealed that the extent of microvascular obstruction was the only independent factor related to early systolic retrograde flow and diastolic deceleration rate. It was concluded that the assessment of microvascular injury by late gadolinium-enhanced CMR corresponds well to evaluation by intracoronary Doppler flow measurements. By means of CMR, quantification of myocardial function, infarct size, and microvascular injury can be performed accurately with a single non-invasive technique in patients with acute myocardial infarction.

Commentary

The reperfusion of an ischemic area with oxygenated blood may cause severe and irreversible microvascular damage, resulting in adverse electrical, functional, and biochemical effects, together known as the ‘no-reflow’ phenomenon. It has been shown that the occurrence of no-reflow in humans after acute myocardial infarction is associated with less good prognosis and worse left ventricular remodeling. The no-reflow phenomenon can be detected invasively, at the time of primary PCI, by the Thrombolysis In Myocardial Infarction (TIMI) frame count or by intracoronary Doppler ultrasound. Coronary microvascular damage can also be assessed non-invasively by contrast echocardiography, nuclear medicine, and cardiovascular magnetic resonance. Both the invasive and the non-invasive approaches apply the term no-reflow, referring to the same pathophysiological event, namely microvascular obstruction, but they look at this event at different times, with different modalities, and different sensitivities.

Coronary magnetic resonance appears to be the most efficient and sensitive method of detecting microvascular damage, even when compared with the invasive approaches. However, the no-reflow phenomenon is an extremely dynamic process. Investigations taken at different times may give conflicting indications, independent of the investigational technique, as a consequence of the dynamic nature of the event or of treatments received by the patient, or both. It should also be considered that the no-reflow phenomenon varies widely in severity from patient to patient, most often with progressive worsening in the first 48 h, but sometimes improving with time. Moreover, studying the no-reflow with cardiac magnetic resonance is strongly affected by the technique chosen. Given the rapid diffusion into the normal interstitial space and in the area of microvascular damage, the distribution of gadolinium-based contrast changes in size over time. Thus the magnitude of the damaged area is affected by the timing of acquisition of the image after injection of the contrast. Other sequences, including gradient-echo with or without an inversion-recovery preparatory pulse, may have better spatial resolution. First-pass perfusion can present defects resulting from previous infarctions, or other flow abnormalities. Adjustments of the T1 time may also affect the size of the damaged myocardium.
The lack of a gold standard to define the area of microvascular damage is a major limitation to the development of strategies that focus more on myocardial perfusion than on vessel recanalization. An accurate assessment of the area at risk for reperfusion damage (no-reflow) would open an opportunity to test potential therapies.

Mario Marzilli

**Malonyl coenzyme A decarboxylase regulates lipid and glucose metabolism in human skeletal muscle**


Malonyl coenzyme A (CoA) decarboxylase (MCD) is a key enzyme responsible for malonyl CoA turnover and functions in the control of the balance between lipid and glucose metabolism. Gene silencing based on RNA interference (small interfering RNA [siRNA]) was used to determine the direct role of MCD in the metabolic responses in primary human skeletal muscle, silencing MCD gene expression in cultured human myotubes from healthy volunteers (seven men and seven women) with no known metabolic disorders. Thereafter, lipid and glucose metabolism and signal transduction were determined under basal and insulin-stimulated conditions. RNA interference-based silencing of MCD expression (75% reduction) increased malonyl CoA concentrations 2-fold and shifted substrate utilization from lipid to glucose oxidation. RNA interference-based depletion of MCD reduced basal oxidation of palmitate. In parallel with this reduction, palmitate uptake was decreased under basal (40%) and insulin-stimulated (49%) conditions, compared with that in myotubes transfected with a scrambled sequence. Furthermore, MCD silencing increased basal and insulin-mediated glucose oxidation 1.4- and 2.6-fold, respectively, compared with that in myotubes transfected with a scrambled sequence. In addition, glucose transport and cell-surface glucose transporter-4 content were increased. In contrast, the action of insulin on insulin receptor substrate-1 tyrosine phosphorylation, tyrosine-associated phosphatidyl inositol 3-kinase activity, Akt, and glycogen synthetase kinase phosphorylation was unaltered in myotubes transfected with siRNA against MCD compared with those transfected with a scrambled sequence. These results provide evidence that MCD silencing suppresses lipid uptake and enhances glucose uptake in primary human myotubes. It was concluded the expression of MCD has a key reciprocal role in the balance between lipid and glucose metabolism.

**Commentary**

Exposure of muscle (skeletal muscle or heart) to high concentrations of fatty acids is associated with a decrease in the ability of insulin to stimulate glucose uptake and metabolism (ie, insulin resistance). The accumulation of cytoplasmic fatty acid intermediates such as diacylglycerol, triacylglycerol, and ceramides may contribute to an impairment of insulin signaling in the muscle. Therapeutic strategies that decrease the exposure of muscle to fatty acids (ie, that decrease blood fatty acid concentrations) can help improve the sensitivity of muscle to insulin. However, the role of mitochondrial fatty acid oxidation in mediating skeletal muscle insulin resistance is controversial. Inhibiting fatty acid oxidation has the potential to increase muscle uptake of glucose, glycolysis, and glucose oxidation (by the Randle Cycle effect), thereby having an insulin-sensitizing effect. However, it has also been proposed that stimulating fatty acid oxidation can have an insulin-sensitizing effect, secondary to decreasing the cytoplasmic concentration of lipids that can inhibit insulin signaling (such as diacylglycerols).

An important regulator of fatty acid oxidation in muscle is malonyl CoA, which is a potent inhibitor of mitochondrial fatty acid uptake. Malonyl CoA concentrations, in turn, are regulated by malonyl CoA decarboxylase (MCD), which degrades malonyl CoA. As a result, inhibition of MCD should increase malonyl CoA concentrations and inhibit fatty acid oxidation. The study by Bouzarki K et al examined what effect inhibition of MCD in human skeletal muscle cells has on insulin sensitivity. Using an siRNA approach in human skeletal muscle cells, the authors showed that decreasing MCD activity results in an increase in malonyl CoA concentrations and a decrease in rates of fatty acid oxidation. Accompanying the decrease in fatty acid oxidation was an increase in insulin-stimulated glucose transport and glucose oxidation. Inhibition of fatty acid oxidation was also associated with an increase in insulin-stimulated glucose transporter-4 present on the surface of the muscle cells. This is a very important finding, because it suggests that inhibition of fatty acid oxidation (as opposed to stimulation of fatty acid oxidation) has an insulin-sensitizing effect in human skeletal muscle. It also suggests that fatty acid oxidation inhibitors have potential therapeutic benefit in the treatment of insulin resistance and diabetes.

Gary D. Lopaschuk
Allelic heterogeneity

If a number of different mutations occur in the same gene and produce a disorder, that gene is said to manifest allelic heterogeneity. This term is often used when a number of different alleles cause similar or different phenotypes in the human population (i.e., sickle cell anemia can be caused by various mutations in the alpha-globin gene).

Desmosomal components

Desmosomal components are the protein constituents that comprise the desmosome, a symmetrical, disc-shaped, cadherin-based intercellular junction that also links intracellularly to the intermediate filament cytoskeleton. Desmosomes function to provide mechanical stability as well as signal transduction pathways between cells including epithelia and cardiac muscle. The Ca$^{2+}$-dependent cadherins, desmoglein and desmocollin interact via their N-terminal regions, providing the sites for intercellular contact between desmosomes on adjacent/neighbouring cells. The cytoplasmic domains of desmoglein and desmocollin bind to intracellular proteins including plakoglobin and desmoplakin. Plakoglobin may serve as a molecular linker, as it also interacts with the N-terminal domain of desmoplakin, the C-terminal domain of which interacts with intermediate filaments of the cytoskeleton. Plakophilin proteins interact with the desmosomal components described. Recent research interest has focused on specific protein isoforms of the desmosome including desmoglein-2, desmocollin-2, and plakophilin-2, as mutations in the genes encoding these proteins underlie arrhythmogenic right ventricular cardiomyopathy.

Mutations in plakophilin-2 appear to be the most prevalent.

Penetrance

Penetrance is the percentage of individuals with a specific genotype that exhibit the associated phenotype. As an example, if 40% of all individuals who possess the “hazel eye” allele actually have hazel eyes, then the “hazel eye” allele has a 40% penetrance.

Phenocopy

A phenocopy is an individual whose phenotype (biochemical or physical characteristics) under a specific environmental condition is identical to that of another individual where the phenotype is determined by genotype (allelic composition).

Variable expressivity

Variable expressivity takes places when a phenotype is expressed at various degrees amongst individuals who have the same genotype. For example, individuals who possess the same allele for the gene involved in a quantitative trait like height might have a large variance (i.e., one individual might be 6 foot 9, while the other is 6 foot 4). This can make it difficult to predict a phenotype solely based on genotype alone. Age and environmental factors are some modifiers that can often influence the expression of a phenotype that is subject to variable expressivity.