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.
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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 β-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].

Refresher corner

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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 haplo-insufficiency; 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 *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 channnelopathies. 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^{2+} lead to largely similar cardiac phenotypes.

Another example of complexity is the case of mutations in the γ2 subunit of the AMP-activated protein kinase gene (*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], phosphatidyl inositol 3-kinase–protein kinase B–glycogen synthase kinase 3β [PI3K-Akt-GSK3β]), 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, α-tropomyosin) 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^{2+} channel of the sarcoplasmic reticulum that is known as the ryanodine receptor (*RYR2*), and calsequestrin (*CASQ2*), the Ca^{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^{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 achievment of better understanding of corresponding functions, and for the development of new therapeutic strategies. ■

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