Genetics and dilated cardiomyopathy: limitations of candidate gene strategies

See page 1872 for the article to which this Editorial refers

One of the most intellectually appealing aspects of human genetics is the potential to define the primary cause of a disease, without making a priori assumptions, using the techniques of positional cloning. In many Mendelian traits, including hypertrophic cardiomyopathy, it has been possible to identify the disease genes. Although there is substantial evidence of familiality, dilated cardiomyopathy has proven less amenable to genetic dissection than hypertrophic cardiomyopathy. While formal data on heritability are lacking, Mendelian inheritance with low penetrance has been demonstrated in many small dilated cardiomyopathy kindreds. Several genetic loci have been mapped but there are too few informative recombination events at each locus to guarantee positional cloning. These difficulties may be due to the subtlety of the phenotype, genetic and environmental modifiers or hidden features of the biology of dilated cardiomyopathy such as somatic mosaicism or embryonic lethality.

Intermediate phenotypes

What is known of the genetic basis for dilated cardiomyopathy is the result of studying pedigrees with intermediate phenotypes. In these diseases the dilated hypocontractile heart is only one feature of the phenotype and other clinical manifestations allow precise definition of affection status.

The first such phenotypes to be studied were Duchenne and Becker muscular dystrophies, allelic X-linked disorders, which frequently exhibit myocardial involvement resulting in dilated cardiomyopathy and are caused by mutations in the dystrophin gene. Specific dystrophin mutations may present as dilated cardiomyopathy without skeletal involvement. Usually such families can be discriminated by X-linked inheritance. Dystrophin is a major component of a large membrane-spanning glycoprotein complex, the dystrophin-associated glycoproteins. The function of this complex is unknown but it has been implicated in mechanical and signal transduction. Other members of the dystrophin-associated glycoprotein complex, in particular γ and β sarcolecgamins, also cause dilated cardiomyopathy in the context of rare recessive limb–girdle dystrophies.

The Emery–Dreifuss variant of muscular dystrophy is distinguished by prominent abnormalities of atrioventricular conduction and tendon contractures. X-linked Emery–Dreifuss is caused by mutations in the nuclear lamina protein emerin. Emerin’s function is obscure but it may be involved in the higher order structure and regulation of the nucleus. The role of the nuclear membrane in conduction disease-associated cardiomyopathy is highlighted by the discovery of mutations in the lamin A/C gene in both an autosomal dominant variant of Emery–Dreifuss and dilated cardiomyopathy with conduction system disease. Interestingly lamin A/C mutations have also been found in a rare form of partial lipodystrophy. That such diverse phenotypes should result from defects in a single gene is intriguing and may suggest tissue-specific lamin interactions or involvement in a fundamental process, such as maintenance of differentiation, in a common mesenchymal cell lineage.

Further insight will come from other muscular dystrophy loci and the systematic evaluation of disease genes in model systems.

Isolated dilated cardiomyopathy

In isolated dilated cardiomyopathy, as noted above, no disease genes have been cloned to date. Dilated cardiomyopathy has a more complex genetic epidemiology than hypertrophic cardiomyopathy resulting in a trend to apply less robust genetic methods including candidate-gene and association strategies. Candidate-gene studies are heavily dependent on prior assumptions and as they afford no reliable way to differentiate mutations from rare incidental polymorphisms are prone to false positives. The very situations in which such a strategy is used (i.e. small families with insufficient power for linkage) are those in which candidate-gene analyses are most susceptible to error. Human association studies are subject to population structure artefacts which larger study size and transmission disequilibrium testing only partly mitigate. The lack of definitive human genetic data focuses attention on knockout or transgenic ‘models’ that fortuitously reproduce some component of the phenotype. These models can be difficult to relate to human dilated cardiomyopathy as the mutations are usually nulls or involve non-physiological cardiac expression.

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Desmin and α-cardiac actin each were identified recently as putative disease genes for isolated dilated cardiomyopathy using candidate-gene strategies. Tesson et al. in this issue reassess the role of both these candidates in a European population with dilated cardiomyopathy, with rather predictable results[7].

**Desmin**

The intermediate filament desmin was initially implicated in the myofibrillar myopathies, a heterogeneous group of diseases characterized by focal myofibril dissolution and intracellular aggregates containing desmin. Recently, molecular genetic techniques have confirmed that over 50% of kindreds with autosomal dominant myofibrillar myopathy have mutations in the desmin gene[8,9]. The phenotype in these families is dominated by a distal skeletal myopathy. There is frequently distinctive cardiac involvement with progressive atrioventricular block and a restrictive cardiomyopathy. The published mutations are clustered in the carboxy-terminal end of the rod domains of desmin where it is presumed they disrupt intermediate filament assembly.

Using a candidate gene approach Li et al. identified a missense mutation in desmin in one of 44 probands with isolated dilated cardiomyopathy[10]. This substitution was inferred to be the causal genetic event in the proband and his family from its location at a highly conserved residue, its predicted effect on secondary structure and its absence from over 900 normal chromosomes. One must be circumspect when interpreting these data as the phenotype in the kindred is free from skeletal myopathy and restrictive cardiomyopathy, there are no histological data and there is no evidence of linkage. To strengthen the case for a causal role for desmin in dilated cardiomyopathy we might look for desmin mutations in other probands with dilated cardiomyopathy or generate animal models bearing the original mutation. Tesson et al. performed a sensitive screen for desmin mutations in a cohort of 41 familial probands and 22 sporadic European dilated cardiomyopathy patients and found none[7]. There are no directly relevant animal models, although a desmin null mouse resembles a restrictive rather than a dilated myopathic phenotype. It is difficult to escape the conclusion that although desmin mutations are a frequent cause of restrictive cardiomyopathy in the context of myofibrillar myopathy, they rarely, if ever, cause isolated dilated cardiomyopathy[7].

**α-Cardiac Actin**

The verdict on a causal role for α-cardiac actin mutations in dilated cardiomyopathy also must remain ‘not proven’. Olsen et al. screening an undisclosed number of dilated cardiomyopathy patients found two different missense mutations in the α-cardiac actin gene[11]. While these substitutions did occur in conserved residues and were not seen in a large control population, again there was no evidence of linkage and there are no functional data. Several groups have attempted to replicate these findings in dilated cardiomyopathy probands from a variety of populations[12,13]. The addition of 86 cases from the current study by Tesson et al.[7] brings to over 270 the total number screened and adds a European cohort to the Japanese and African populations already studied. No mutations have been identified in any of these individuals suggesting that α-cardiac actin mutations rarely, if ever, cause dilated cardiomyopathy[7,12,13].

To add to the uncertainty, mutations in the same domain of α-cardiac actin have also been described in a single family with hypertrophic cardiomyopathy where there is ventricular dilatation late in the course of disease[14]. In this instance screening hypertrophic cardiomyopathy probands did reveal additional mutations, albeit in two young children[15]. It will require more compelling data to define unequivocally the role of α-cardiac actin mutations in human cardiomyopathies.

**Resolution**

We can begin to resolve such uncertainties by relying on classic genetic methods. Rigorous quantitative genetic epidemiology will help to define the basis of the apparent ‘complexity’ of dilated cardiomyopathy, identify heritable components of the trait and offer insight into the underlying biology of dilated cardiomyopathy. Such studies extract information from extended kindreds defining the complete range of phenotypes with each mutation. This methodology also avoids circular genotype–phenotype arguments based on old definitions of dilated cardiomyopathy and will lay the groundwork for a new nosology. Clinicians must emphasize the role of whole families in both genetic investigation and in clinical management. For example, when a proband has dilated cardiomyopathy, if all the other affected members have hypertrophic cardiomyopathy, this has very different diagnostic and prognostic implications.

The successful positional cloning of the disease genes at the known loci, facilitated by the completion of the Human Genome Project, will shed light on the mechanisms of dilated cardiomyopathy. Candidate-gene and association approaches may be useful for hypothesis generation but care must be exercised
interpreting the results of ‘genetic’ data where there is little information on segregation, unestimated heterogeneity, uncertain clinical context and no functional information. Reproducible identification of mutations in well-characterized populations and compelling functional data will be required to strengthen the case for a specific gene. ‘Private’ mutations in single families with atypical phenotypes will be difficult to confirm and will remain of uncertain relevance.

The genes identified in human diseases can now be evaluated in highly tractable model systems, such as the mouse and the zebrafish, helping to confirm these genes as disease-causing and to dissect the pathways from mutation to phenotype. Genome-wide analyses of these pathways offer rigorous methods for identifying true candidate genes that can then be tested in human populations. This two-way interchange between human disease and model systems will eventually fulfil a major goal of genomic approaches in heart failure; the identification of all the genes important in dilated cardiomyopathy.

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References

Parallel realities of guidelines and practice

See page 1877 for the article to which this Editorial refers

There are at least three good reasons for reading the paper by Hobbs et al. in this issue:
(a) it is focused on an area of care which is increasingly important in cardiology and public health;
(b) it recalls and underlines one of the most neglected aspects of heart failure, namely the conditions of discontinuity between specialist and general care;
(c) it is an attempt to provide a comparative view of the cultural attitudes and perceptions in different medical settings and health care systems.

There are at least three directly opposing reasons to suggest that the results should be taken as a stimulus for looking ahead, more than as a set of hard information on which to concentrate technical, or policy, discussion:

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