Progress in understanding the genetic basis of inherited disease has been driven by successive waves of new technology for identifying human genetic variation. The first inroads were made in monogenic mendelian traits with family-based linkage studies. In retrospect, this era of relatively laborsious, and hence rate-limiting, genetic technology had a silver lining in that time was left for thorough clinical phenotyping in extended families. This provided the most robust evidence of causality: statistically rigorous evidence of cosegregation of a proposed mutation with disease. Once supported by demonstration of further alleles in other families or demonstration of newly arising de novo mutations, the gene would rightly be considered proven. Such disease genes have stood the test of time and now underpin clinically important genetic tests.

Variants were considered likely to be pathogenic if they were rare (typically absent in just a few hundred control subjects), if they affected conserved residues, or if they could be predicted or shown to have an impact on the protein structure or function. We now know that these criteria are weak and, even together, insufficient. The true extent of human genetic variant variation has been revealed by the advent of next-generation technology allowing deep resequencing at the population level. The extent of low-frequency and rare variation in individual genomes turns out to be massively greater than expected. A large majority of nonsynonymous coding sequence variation has an allele frequency of <1%. Even in a study as big as 5000 individuals, about half of the variants identified were unique to 1 individual. In other words, a low allele frequency is a necessary, but in no way sufficient, criterion for a plausible disease-causing mutation in a dominant trait. Evolutionary conservation adds information, but it is a low bar because a high proportion of amino acid residues are conserved and because an impact of the mutation on the protein does not necessarily predict a causal role in a specific disease. Thus, for example, we find that most human genes can tolerate single-copy loss-of-function alleles (truncations, deletions, etc). These silent alleles would clearly score as likely to be pathogenic in any in silico or experimental assay, underscoring that demonstration of a functional impact is not the same as demonstration of disease causality.

At the same time, we have gained a body of evidence on the role of common genetic variation, typically operating through influencing gene expression. Genome-wide association (GWA) studies have been directed toward common disease but could in principle address the phenotype of inherited diseases, in which variable penetrance and expressivity are the norm and are not accounted for by locus or allelic heterogeneity. That said, GWA studies have shown that effect sizes of common noncoding variants are typically extremely small (and conversely that the number of genes influencing a trait is very large) and that power to detect them is low. With the burden of correction for multiple testing inherent in the GWA study approach, studies of many thousands of individuals have been needed for most traits, and modifiers of a mendelian phenotype will likely be similar.

Hypertrophic cardiomyopathy (HCM) has long been a paradigm for cardiovascular genetics. This reflects its clinical importance but also its tractability, being common and strongly familial with relatively high penetrance. It is for these reasons that within the spectrum of inherited cardiac conditions, it is in HCM and the long-QT syndromes that clinical use has been greatest, contrasting, for example, dilated cardiomyopathy (which is genetically heterogeneous) or arrhythmogenic right ventricular cardiomyopathy (in which penetrance is sufficiently low that large extended pedigrees are rare and complex inheritance is more common). Nevertheless, there are substantial unresolved complexities even within the genetics of HCM, and it is time to review the impact of the new awareness of the extent of human genetic variation. Questions to consider include the following: First, are the disease genes listed as causing HCM all adequately proven? Second, how many of the individual alleles reported as disease causing are reliable? Third, what is the basis of the substantial proportion (eg, 40%–50%) of families, let alone isolated cases, in whom myofilament gene mutations are not detected? Finally, and as considered in the accompanying article by Wooten et al, can common genetic variants be implicated that address either the variable penetrance or the myofilament-negative component of the disease?

The sarcomeric genes identified as HCM disease genes through linkage studies are robust and are the mainstay of genetic testing in public and private-sector laboratories. It is not the case, however, that all reported sarcomere disease genes for HCM have linkage support; for example, α-myosin heavy chain (MYH6), cardiac troponin C, and titin, all of which are often listed in reviews and sometimes included in testing
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schedules, are unproven. A bigger problem arises with the genes that were implicated through candidate gene screening thereafter. We know now that sequencing in cases and testing just the variants identified in controls is an inadequate strategy; rather, sequencing should be performed in controls also to determine whether there is a true excess mutation load of non-synonymous variants in cases. Few of the putative nonsarcomeric HCM genes can be considered robust, with only CSRP3 (encoding MLP) being adequately supported by genome-wide significant linkage data. For other genes, including those for a number of z-disk components, the weight of evidence is insufficient: Many of the variants initially reported to be absent in controls are now found to be low-frequency normal population variants; functional data were too nonspecific; and causality was not demonstrated in a relevant animal model. Such genes should be considered unproven and caution is needed, certainly before any clinical application. For example, ANKRD1-encoding CARP has been proposed as both an HCM disease gene and a dilated cardiomyopathy disease gene, but of the 11 missense alleles on which these reports are based, all but 3 are seen multiple times in the Exome Variant Server (http://evs.gs.washington.edu/ EVS/).

Within the proven HCM disease genes (MYH7, MYBPC3, TNNT2, TNNI3, TPN3, MYL2, MYL3, ACTC1, and CSRP3), we now know that we need to be more careful about assigning causality to individual alleles. A concerning number of alleles reported as disease causing (eg, in the Human Gene Mutation Database) are found in the normal population at too high a frequency to be pathogenic. On closer inspection, however, these turn out to be variants that are reported in probands rather than families and that are, in fact, already treated as variants of uncertain significance by diagnostic testing laboratories. The proportion of variants of uncertain significance found varies gene by gene, being higher for missense alleles in MYPC3 but generally much less a problem for HCM than, for example, arrhythmogenic right ventricular cardiomyopathy. Inevitably, there is a spectrum of penetrance, but those alleles that are sufficiently penetrant to document linkage in an extended pedigree (even if demonstrated only once but then made available, eg, through ClinVar [http://www.ncbi.nlm.nih.gov/clinvar/]) have demonstrable use.

Available evidence suggests that myofilament-negative HCM will not be explained by novel, penetrant, autosomal-dominant disease genes. Some of the residual families will have related mendelian traits, and a particular benefit of systemic genetic testing is that it reveals low, but significant, percentages of distinct HCM phenocopies (such as the Fabry, PRKAG2, FHL1, mitochondrial mutations). However, clinical experience suggests that myofilament-negative HCM is less likely to show familial recurrence, and when it does, affected relatives usually cluster in nuclear families only. This would argue for nonmendelian genetic inheritance, for example, for convergence of 2 or more susceptibility variants and the impact of shared environment. A significant proportion of myofilament-negative HCM appears as isolated cases, and they may have no strong genetic component at all (which is why testing candidate genes in such cases is problematic).

Within both known and unknown HCM genes, a gradient of effect size can be anticipated, ranging from rare penetrant alleles sufficient to cause disease, to more common variants that increase the likelihood of a clinical phenotype but are not alone sufficient, all the way to phenotypically silent variants (presumably the large majority). There is already evidence that medium-effect-size, low-frequency variants exist in HCM. An excess of compound and double heterozygotes is observed in HCM, as in other inherited cardiac diseases, that far exceeds what predicted by chance, indicating that many of these variants not only would produce a phenotype that reaches clinical recognition but also would require a convergence of deleterious alleles to do so. One of the best-documented examples is the partial splicing defect in MYBPC3, identified as a relatively common variant in South Asian populations. The beauty of this finding is that the variant is common enough, and with a big enough effect, for a reliable odds ratio to be calculated and a clearly validated mechanism exists. The difficulty outside this particular situation will be the usual one in the common variant/common disease world, namely that assigning function on the basis of in silico or biological characterization is not currently feasible and instead rigorous statistical evidence of association is required.

The report by Wooten et al presents one of the first GWA studies in HCM, with a stated goal to find common variants that affect penetrance and expression in HCM (ie, modifiers). A variant is reported in a plausible and interesting candidate gene, FHOD3, as being associated with HCM in a discovery cohort and in an independent replication cohort. FHOD3 is known to be involved in actin filament formation and has been shown to be required for the formation of the sarcomere. The single-nucleotide polymorphism that initially showed association in the GWA study is a common, intronic, polymorphism (minor allele frequency in HapMap of 0.48). The study reports an odds ratio of about 2 for the presence of HCM in both cohorts. A single missense mutation within FHOD3, although in notably weak linkage disequilibrium (r²=0.287), was then tested as a candidate, generating stronger evidence for association. Although the goals of the study are important, interpretation of this finding may require caution. The study design simply looked for single-nucleotide polymorphisms that were more frequent in HCM cases than population controls, treating HCM as a dichotomous trait. Because the variants tested were common, they clearly cannot be causing HCM but rather would have to be modifiers of the HCM phenotype. A counterintuitive aspect of the findings, however, is that the associated variant did not have any detectable effect on any quantitative aspect of the HCM phenotype. There was no association with septal thickness, age at diagnosis, or likelihood of obstructive features; the effect was the same in patients with or without myofilament mutations. In other words, if the association is real, the variant doubles the likelihood of a diagnosis of HCM being made independently of the underlying genetic cause but not by changing cardiac morphology. Conceivably, the susceptibility variant could operate in this way by rendering the ECG more abnormal in HCM patients. Of course, an alternative interpretation, long familiar from the field of genetic association studies, is that this is a chance finding. The discovery GWA study is likely to be underpowered (just 174 cases), and the discovery P
value falls well short of genome-wide significance. The best statistical support is for the missense variant subsequently tested; however, this was not the product of systematic fine mapping but rather was a selected candidate variant. FHOD3 was shown to be upregulated in HCM tissue, but more conclusive would have been evidence of allelic imbalance (indicating an effect of the risk haplotype on FHOD3 transcription). Knockdown of Fhod3 in Drosophila showed reduced cardiac fractional shortening, concordant with the previously published work in mammalian cardiomyocytes, but demonstrating a specific effect of the 1151I variant would require demonstration that it fails to rescue the phenotype.11 Experiments of this type, no doubt reinforced by further opportunities to test the observed genetic association in larger studies, will determine whether this novel finding does indeed open up a new line of inquiry.

Where do we go from here? GWA studies that seek to define a role of common variants in HCM will have to be powered to detect small odds ratio and will do better to test for association with a quantitative phenotype. This, of course, is quite difficult in HCM because of the local and asymmetrical nature of the hypertrophy. Additionally, if a modifier variant had a differential effect according to the underlying primary pathogenesis, such studies would need to be conducted in homogenous genotyped populations. The efforts that have been made in single, very large families are in this regard attractive.12 In the meanwhile, it seems more plausible that progress in understanding the basis of myofilament-negative HCM will be made in family members, then a negative genetic screen, instead of being a null result as at present, becomes a usefully reassuring finding.

Disclosures
Dr Watkins receives royalties from patents held by Harvard Medical School for genetic testing in HCM.

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