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Sequencing of Uncertain Significance

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In the initial reports that identified the long-QT syndrome (LQTS)-associated genes, mutation status was proven by a combination of linkage mapping, DNA sequencing, ion channel expression analysis and functional assessment.¹⁻³ Examination of both expression and function proved providential because various mutations affected ion channel expression levels, membrane trafficking and/or function.⁴ Specificity of disease association was confirmed by demonstrating absence of the identified mutations in the genotypes of a few hundred unaffected individuals. The comprehensive nature of this analysis soon proved impractical as the number of observed variants in LQTS genes increased. Functional investigations continued,^{5, 6} but not at the same rate as “disease-associated” variant reporting.^{7, 8} The typical method for assigning disease association status shifted from a more comprehensive analysis to a more limited assessment that generally focused on analyzing frequency of the questionable genetic variant in databases of gene sequenced, phenotypically normal individuals. It was believed that the rarity of LQTS made it unlikely that true positives would appear with any significant frequency in a collection of normals, so variants seen in these databases were deemed less likely to be disease-causing. The rationale for this approach rested in a study of nearly 50,000 infants in Italy using a combination of QTc measurement and genotyping that estimated the prevalence of LQTS to be approximately 1 in 2000.⁹ As technology improved, the number of “normal” sequences available for comparison increased to a current standard that routinely uses databases of a few thousand people.

In this issue of JCE, Kaltman et al.¹⁰ report that the current standard is inadequate. Kaltman used the gnomAD database containing 123,136 genotyped patients, and they looked for 1415 LQTS disease-associated variants. They found 347 of these variants in the database, of which 7 were present at an allele frequency greater than 1:1,000 and 65 were present at an allele frequency greater than 1:10,000. (Considering the reported disease prevalence, any single pathogenic variant should be found at a frequency far less than the overall LQTS population frequency of 1:2,000.) Kaltman looked more closely at the 65 identified variants and found that most lacked high quality data supporting a conclusion of pathogenicity. Only 9 of the 65 variants had published functional data, and only 5 had documented linkage with disease phenotype in family studies. Kaltman rightly pointed out that more data need to be required for assigning disease association status given the implications of this decision.

Of interest in Kaltman's report is the occurrence in the gnomAD database of several variants that had functional data supporting LQTS behavior. Kaltman's findings resemble those of Norton et al., who did a similar analysis for dilated cardiomyopathy.¹¹ Norton used the NHLBI exome sequencing project as their database of phenotypic normals. Like Kaltman, Norton found that a large percentage of reportedly causative variants in dilated cardiomyopathy were present in the normal population database. Where Kaltman and Norton differ slightly is their interpretations of this finding. Kaltman starts off their discussion by stating that "a significant number of [LQTS] variants ... designated as disease-causing or likely disease-causing are probably mislabeled" (although to be fair, they soften this by later stating that their data "suggest that many of the variants associated with LQTS ... are either bystanders, modifiers of disease, or associated with reduced penetrant forms of disease"). Norton considered but rejected the idea that the variants found in the general database were false-positives. Instead, they postulated that these variants were low penetrance or disease susceptibility altering. Norton backs up this assertion with a discussion of the functional alterations proven for 13 of the 31 variants found in the general database. The issue of low or variable penetrance is not a trivial one for LQTS as anyone who has taken care of family members with the same mutation but markedly different QTc values can attest.

How should the clinician with both a patient and gene sequencing data in front of him/her interpret these findings? It would be easy and certainly consistent with the current quality of data to at this point say "I have no idea!" but that would not be particularly helpful. I do think that both Norton and Kaltman make valid points. Norton has the advantage that a higher percentage of the variants identified in their study had functional data consistent with disease effect, so it is reasonable for them to conclude that these variants are not false-positives. Kaltman is right to be frustrated by the amount of data available for LQTS mutations. My interpretation of Kaltman's statement is that they are asking us to move back in the direction of the prior standard where the "disease-causing" label was reserved for variants with a combination of functional, linkage and allele frequency data. To that end, I am excited about plans to use high-throughput patch clamp analysis to provide the needed functional data for many of these ion channel sequence variants.¹² When those data become available, My interpretation of a patient's genotype results will be very different for a variant with proven alteration of ionic current levels or gating behaviors compared with one that does not have any observable effect on channel function (with the caveat that interactions between ion channel alpha subunits and various associated proteins within the environment of the cardiac myocyte could either positively or negatively affect these findings).

In situations where the functional data are inconsistent with the observed clinical phenotype, supportive data could be obtained from induced pluripotent stem cell-derived cardiac myocytes. Moretti et al. and Itzhaki et al. were first to describe use of these cells, when harvested from LQTS patients, to replicate the LQT phenotype on a cellular level.^{13,14} In situations where it is particularly important to make a diagnosis, these cells could be generated from the patients in question and gene manipulation could be used to study function of these cells with and without the genetic variant in question. This level of investigation would obviously be limited by the amount and complexity of work involved, but it could resolve questions of causality where it was necessary to do so.

In the meantime, while we wait for these more comprehensive analyses, what are we to do? I think we need to consider gene sequencing data to be supportive rather than definitive. Genotype needs to be interpreted in the context of the individual patient's phenotype and of the linkage to family members with either disease genotype or phenotype. The level of evidence for disease causation needs to be clearly communicated (by genotyping company to clinician, and by clinician to patient). When the data don't allow us to draw firm conclusions, we need to state what we do and do not know. This is likely to cause some uncomfortable conversations, but that is preferable and less likely to do harm than giving unreliable and potentially wrong information.

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