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EDITORIAL COMMENT

JPH2 Mutant Gene Causes Familial Hypertrophic Cardiomyopathy

A Possible Model to Unravel the Subtlety of Calcium-Regulated Contractility*

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It is estimated there are 7,000 rare (frequency ≤1%) single-gene disorders, and 1 or more genes have been discovered for more than 4,000 of these disorders (1). In these inherited disorders, the gene alone is capable of inducing the phenotype, the penetrance is usually age-dependent, and when identified, usually provides important insight into the molecular pathway leading to the phenotype. Expression of the human mutant gene in the mouse has become a standard approach to determine causality, elucidate the pathophysiology, and possibly to unravel targets to direct the development of new therapies. As Goethe remarked, it is often much easier to understand nature and its ways when it wanders off its beaten path. We are fortunate in the cardiovascular community to have several single-gene disorders for which multiple genes have been identified, such as the familial cardiomyopathies and the long QT, Brugada, and Wolff-Parkinson-White syndromes (2). Discovery of the responsible genes, even without a cure, has consistently and immensely improved the medical management of these disorders. The gene provides a precise diagnosis, and through genetic screening of kindred, one can detect those having the gene and requiring long-term medical follow-up and treatment versus those without the gene who can be relieved of this burden. Many of these disorders are inherited as autosomal dominant, which means only 50% of the offspring will inherit the gene and be vulnerable to develop the phenotype. Genetic screening to identify the 50% who do not have the gene and do not need medical follow-up or procedures will be tremendously comforting to them and also cost-effective. In those disorders inherited as recessive, such as many metabolic disorders, meaning the individual must have 2 copies of the gene to develop the disease, knowing the gene enables more precise genetic counseling. Individuals with a single copy of a rare disorder can be counseled to avoid a partner who has a copy of the gene and be assured none of their offspring will get the disease. A good example of the latter is Tay-Sachs disease, the incidence of which with genetic counseling was reduced by 90% (3).

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In this issue of *JACC: Basic to Translational Science*, Quick et al. (4) report the discovery of a missense mutation with alanine substituting for serine at residue 405 in the cardiac specific junctophilin 2 gene (JPH2-A405S) in a patient with the phenotype of familial hypertrophic cardiomyopathy (FHCM). The *JPH2* gene is hotly pursued because one of its alleged functions is to modulate the cascade of calcium release in the sarcoplasm reticulum. The implication being that it would be a therapeutic target to pursue, not just for FHCM, but also for heart failure due to other causes. The mutation was identified in a single proband showing, on echocardiogram, basal interventricular septal hypertrophy with a septal thickness of 23 mm,

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subaortic outflow tract obstruction due to systolic anterior movement of the mitral valve leaflet into the outflow tract, and myocardial fibrosis detected by cardiac magnetic resonance imaging (CMR). This patient has no siblings and no family history, and no other family members were available for genotyping. Ten years ago, genes were mapped to their chromosomal location by linkage analysis, which required DNA analysis of least 8 to 10 affected individuals spanning at least 2 and preferably 3 generations. The chromosomal locus was identified by showing DNA markers in close physical proximity to the causal gene would segregate only with those affected by the disease. It would then be possible to clone and sequence candidate genes in the region of the mapped locus to identify the mutation and show the mutation was present only in family members with the phenotype. Today, the availability of accurate, rapid, and inexpensive DNA sequencing makes it possible to pursue mutations through sequencing of suspected candidate genes in individuals with inherited disorders even if only a single family member is affected.

This study by Quick et al. (4) exemplifies a common dilemma today in our pursuit of genetic causality. In small families where we cannot prove causality by showing the mutation is present in only affected individuals, other means must be adopted. The phenotype described in this proband is not a problem because it is fairly typical for FHCM. The dilemma was produced by the widespread availability of DNA sequencing that indicates mutations in the human genome are very common, of which most are neutral with only a few disease producing. The DNA sequence of the genome of all *Homo sapiens* is 99.5% identical (5). The 0.5% of 3 billion nucleotides would indicate that about 15 million nucleotides account for the features that make each individual unique. However, most of the 15 million nucleotides are rearrangements, insertions, or deletions of large chunks of DNA or vary in the number of copies, all are referred to as structural variants. Fortunately, more than 80% of the variants that account for the unique features of each individual, including predisposition to disease, are due to single nucleotide variants, often referred to as single nucleotide polymorphisms (SNPs). The number of SNPs in the human genome is constant at about 3.5 million. Most of the SNPs are rare (frequency ≤1%), and these are the ones responsible for the rare single-gene disorders such as FHCM. It is estimated each human genome has about 50 to 100 of these SNPs that are alleged to be associated with rare diseases. Thus, finding a mutation in a particular DNA sequence or gene does not necessarily establish causality, particularly if more than 1 mutation is present in the same sequence or gene.

The investigators in this study, recognizing this dilemma, pursued functional analysis of the alleged FHCM mutation. Experimental studies have indicated the absence of 2 copies of the *JPH2* gene leads to lethality in the developing embryo. The investigators were very clever and are to be highly commended in developing their mouse model for functional analysis. To prove the mutation *403S* causes FHCM, they could have overexpressed the human mutation in the mouse as we (6) and others (7) have done with previous mutations associated with FHCM. This would prove causality, but to pursue functional analysis, there could be criticism of overexpression and abnormal stoichiometry between the wild type and the mutant. To avoid this criticism, they did 2 things: first they did not force expression of the human mutation but rather created a mutation in the analogous cardiac-specific *JPH2* gene of the mouse genome located at residue 399 (A 399S) and used it to develop a transgenic mouse expressing its own mutated cardiac *JPH2* mutation. This transgenic mouse was crossed with a cardiac-specific *JPH2* knockdown mouse to generate knock-in mice. This was performed to maintain stoichiometry of JPH2 levels similar to those observed in nontransgenic mice. Western blot analysis of cardiac tissue confirmed that cardiac JPH2 levels were similar in the wild type as in the mutant mice, maintaining stoichiometric balance between the wild and mutant genes. Conventional echocardiographic analysis of the mouse showed no change in left ventricular outer diameter, no difference in ventricular wall thickness in systole or diastole, and no change in any structural features compared with that of the wild type. There was no change in contractility, and no significant change in ejection fraction. An echocardiographic parasternal long-axis view was performed that showed a decrease in end-diastolic left ventricular volume due to a 26% increased basal interventricular thickness over that observed in the wild type. This septal basal hypertrophy was confirmed by CMR. Functional analysis by CMR showed diastolic dysfunction. Histology analysis showed enlarged myocytes, myocyte disarray, and increased fibrosis in the region of the basal septum. The mouse model, whether it was transgenic or due to knock-in by mutations of the myosin heavy chain causing human FHCM, consistently shows minimal hypertrophy, myocyte disarray, and fibrosis. The phenotype shown by Quick et al. (4) in this mouse model is consistent with FHCM and proves causality of the mutation.

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Junctional complexes referred to as JPH2 are those molecules that connect the sarcolemma of the myocyte to the sarcoplasm reticulum. These junctions are necessary in all excitable tissues including the brain. The sarcolemma membrane of cardiac myocytes have multiple invaginations referred to as transverse tubules (T tubules) because they are perpendicular to the plane of the sarcolemma membrane. Located in these T tubules are the so-called L-type calcium channels through which calcium flows. In cardiac muscle, the amino terminal of JPH2 is embedded into the sarcolemma of the T tubules, and the carboxyl terminal of the molecule is embedded into the sarcoplasm reticulum. The JPH2 decreases the time required for calcium activation to induce the type II ryanodine receptors to release calcium into the calcium-induced calcium release receptors. The structure and sequence of JPH2 proteins are conserved across several species. Elucidation of the function of JPH2 is being pursued with vim and vigor on the basis that it would provide further insight into the regulation of cardiac contractility as well as hope it could serve as a target for the development of novel drugs for cardiac failure. Experimental studies show that lack of both copies of the gene that encode for JPH2 is lethal, and the phenotype shows poorly developed T tubules and impaired contraction. It appears that JPH2 is necessary for the formation of T tubules and also has other functions that relate to cardiac contractility.

Is the main function of JPH2 to ensure anatomic development of the T tubules, or is there an additional function of regulating calcium? This is in part why the investigators have taken so much effort to develop this mouse model. It is to minimize confounding factors so functional analysis may further elucidate the alleged role of JPH2 in calcium regulation and contractility. We can expect new information in the near future from the exploration of this murine model expressing a mutant *JPH2*.

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