Variants of Uncertain Significance and "Missing Pathogenicity"

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In this issue of the *Journal of the American Heart Association* (*JAHA*), Pottinger and colleagues[1] report their analysis of variants in 59 medically actionable genes identified in 900 racially and ethnically diverse adult participants of the NUgene biobank who underwent whole-genome sequencing. Accompanying clinical information was obtained via linkages to electronic medical records. Two hundred ten (23%) individuals were selected on the basis of 4 medical conditions of interest, with the remaining 77% individuals selected only for race/ethnicity. Data evaluation was focused on the 30 cardiac genes within the American College of Medical Genetics and Genomics 59-gene set,[2] with a particular emphasis on rare nonsynonymous variants that were listed in ClinVar.[3] Nineteen (2%) individuals carried variants that had been annotated as pathogenic or likely pathogenic. Although subclinical abnormalities were evident in some cases, none of these individuals had been diagnosed with the relevant cardiac disorders. Variants of uncertain significance (VUS) were present in 108 (28%) of 385 individuals who had echocardiographic data. Serial evaluation for periods up to 14 years revealed that VUS carriers had relatively greater increments in left ventricular end-diastolic and end-systolic diameters over time when compared with noncarriers. These results highlight the challenges of genetic variant interpretation and the potential clinical impact of incidental findings in biobank participants.

Since the discovery of the first cardiomyopathy gene mutation 3 decades ago,[4] criteria for predicting variant pathogenicity have progressively evolved. In the early days of genetic testing, variants were deemed likely deleterious if they were located in a highly conserved amino acid residue in a good candidate gene and were absent from 100 or more healthy control subjects.[5] Variant novelty remained an important factor as genetic testing of patient cohorts moved from single gene mutation screening to parallel analyses of multiple genes using next generation sequencing methods. The availability of human sequence databases, such as the Exome Sequencing Project and Exome Aggregation Consortium, provided disturbing new perspectives and recognition that many genetic variants previously considered to be disease-causing were also present in the general population.[6,7] In fact, the presence of such variants in the general population was considered to be compelling evidence that they were *not* disease-causing. A number of studies have now shown that predicted-deleterious variants in many genes are common and tolerated.[8,9] Given that the prevalence of predicted-deleterious variants in the general population far exceeds disease prevalence, it is increasingly apparent that current strategies for identifying bona fide disease-causing variants have their limitations. In an attempt to standardize genetic test results, the American College of Medical Genetics and Genomics devised a classification method in which variants were allocated into 1 of 5 grades (pathogenic, likely pathogenic, uncertain significance, likely benign, and benign) according to a weighted matrix that included variant characteristics, population frequency, family segregation, and functional data.[10] This stringent approach errs on the side of caution and reduces the potential for false-positive mutation calls, but also results in more VUS. With the introduction of multigene panels, exome sequencing, and whole-genome sequencing, the numbers of variants identified per person has increased progressively and the problem of VUS interpretation has magnified. Genetic testing is generally undertaken in affected patients who have a high a priori likelihood of a genetically mediated cardiac disorder. When genetic variants arise as incidental findings in population sequencing studies, any links with disease may be more tenuous and the prognostic significance of variants becomes more difficult to predict.

Recent genetics-first approaches have attempted to address this issue by looking at variant-related phenotypes in biobank participants. As an example, truncating *TTN*

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variants are thought to be the most common genetic cause of dilated cardiomyopathy.[11,12] However, although *TTN*tv are present in 10% to 20% of dilated cardiomyopathy cases, they are also seen in up to 3% of control subjects. Schafer and colleagues[12] evaluated cardiac magnetic resonance imaging data of 1409 individuals who self-reported an absence of cardiovascular disease and found that *TTN*tv carriers had significant differences in left ventricular size and shape when compared with noncarriers. Similarly, in a study of >71 000 individuals from the Geisinger MyCode Community Health Initiative and the Penn Medicine Biobank, Haggerty and colleagues[13] found that *TTN*tv carriers without known dilated cardiomyopathy had relatively lower left ventricular ejection fraction and a higher incidence of heart failure and atrial fibrillation than noncarriers. These observations indicate that the presence of *TTN*tv alone can affect cardiac size and function and suggest that additional factors may be involved in determining whether or not dilated cardiomyopathy is manifest. Age, sex, and ethnicity have already been shown to affect the penetrance of *TTN*tv,[11–13] but the role of each individual's unique combinations of genetic and environmental risk factors is relatively unexplored. Some of these factors might accelerate disease onset in *TTN*tv carriers while others may be protective.

In the study by Pottinger and colleagues,[1] pathogenic or likely pathogenic variants were found in a small proportion (2%) of biobank subjects, and the authors focused on the 385 individuals in whom VUS were evaluated. VUS were found in 24 (28.6%) of the 94 individuals known to have cardiomyopathy and in 84 (28.9%) of 291 individuals without cardiomyopathy. The significant overall association between VUS and longitudinal changes in cardiac chamber dimensions was found to be driven primarily by the 24 VUS carriers who had pre-existing cardiomyopathy diagnoses. Variants in some but not all of the genes in which these VUS were located would be anticipated to promote ventricular cavity dilation and the question arises as to whether the VUS were actually responsible for the cardiac changes seen. Additional targeted genetic testing of 102 cardiomyopathy-associated genes revealed an independent pathogenic variant in only 1 of these 24 VUS carriers; however, a comprehensive search for other potential genetic or environmental mediators in each individual is needed. Taken together, these data do suggest that at least some of the VUS identified in affected patients are likely to contribute to myocardial dysfunction and hence have been erroneously classified. It is important to note that temporal changes in cardiac function were not seen in VUS carriers without a previous cardiomyopathy diagnosis or for novel/rare variants that were absent from ClinVar.

These results point to a pressing need for more accurate ways to identify the subset of VUS that are truly deleterious. Ideally, a pathogenic variant would show strong co-segregation with affection status in a large family, relevant protein-altering effects supported by functional data, and recapitulation of disease in an animal model. Unfortunately, this is unrealistic for most variants, which tend to be novel or seen in small families. Moreover, while in silico predictions can be useful, extensive functional evaluation of every suspicious variant is currently impractical. Comprehensive cataloguing of genetic variants, development of high throughput methods for functional genomics, genotype-phenotype correlations, and longitudinal prospective clinical studies are priorities for future research.[14]

VUS identified by clinical genetic testing are often returned to patients but are not used for medical decision-making or predictive cascade testing in families.[10] On the other hand, whether VUS results found in population screening studies should be returned to clinicians and/or participants has been debated.[15–17] Routine disclosure of VUS in this setting raises a number of ethical issues with respect to sample identification, prior consent by participants to be recontacted, and preparedness of participants to receive results. It is also unclear whether the onus of responsibility for providing genetic results, genetics counseling, clinical screening, and periodic reassessment of variant pathogenicity would lie with the research team or with clinical services that are often underresourced. Systematic disclosure and follow-up cardiac investigations in all VUS carriers, many of whom might remain asymptomatic throughout life, is unlikely to be cost-effective for the community as a whole, and the potential for psychosocial harm could be considerable.

Pottinger and colleagues[1] now suggest that judicious disclosure of VUS to biobank participants may be warranted, particularly for individuals who have overt evidence of myocardial dysfunction. Knowing that a VUS was present would not alter the medical management of affected individuals who would already be flagged as needing ongoing clinical surveillance. However, the ability to upgrade a VUS to pathogenic or likely pathogenic status (or dismiss as benign) could have an immediate clinical impact both for patients and their families. The yield of cardiac genetic testing has not increased substantially over the past decade and remains less than 50% for many disorders.[18] We posit that VUS may hold the key to much of this "missing pathogenicity." Solving the problem of VUS interpretation is a rapidly emerging clinical imperative as genetic testing becomes more available and affordable and the futuristic vision of whole-genome sequence information in personal medical records looms closer.

Disclosures

None.

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