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Hearing the Noise: The Challenges of Human Genome Variation in Genetic Testing

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Variability of human genome

The completion of the Human Genome Project was a landmark achievement that revealed the reference DNA sequence for our own genome.[1] Almost immediately it became clear that there was no single 'reference' DNA sequence as even the approximately half-dozen human DNA samples used by the Human Genome Project contained tens of thousands of variations. As clinical genetic testing becomes more mainstream and various projects underway perform full DNA genome sequencing in hundreds of individuals, the extent of this genetic variation is increasingly being appreciated. It is widely recognized that most of this variation is probably not relevant for determining health or risk of disease and collectively has been referred to as 'genetic noise'. As in much of biology, separation of the 'signal' from the 'noise' can be challenging and as molecular genetic sequencing expands in use and in the amount of DNA that can be sequenced in a single assay, problems in distinguishing a diagnostic genetic change from background genetic variation will remain a difficult task for researchers and clinicians. Newer DNA sequencing technology can now complete the sequencing of an entire human genome several times over in a matter of days, orders of magnitude faster than the nearly thirteen years required for the initial first-pass done by the Human Genome Project consortium.[2] This technology, which will shortly be widely used in clinical genetic testing, will undoubtedly add to the difficulty of distinguishing signal from noise.

In this issue, Kapplinger and collaborators eloquently illustrate the breadth of genetic variation (the 'noise') in an important and lift-threatening genetic disease, arrhythmogenic right ventricular cardiomyopathy (ARVC).[3] Various authors had previously studied ARVC families to identify genes and screen patient cohorts to determine the contribution of various ARVC genes to the overall population of ARVC families. Although the screening of control populations for discovered disease-causing mutations is fairly standard in genetic studies, comprehensive DNA sequencing of controls to measure background genetic variation usually is not undertaken. This report and a modest number of others provide compelling

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data that this evaluation in control populations should become standard for many future genetic studies.

Kapplinger studied 175 individuals from the Netherlands and the United States with a confirmed diagnosis of ARVC. In this group of subjects mutations were found in 58% of cases after sequencing five ARVC genes (*PKP2, DSP, DSG2, DSC2* and *TMEM43*). Although these mutations were not labeled by the authors as being definitely 'pathogenic', the evaluation criteria used would have led to these mutations being classified as 'probably' or 'presumptively' 'pathogenic' by clinical laboratories and, more importantly, by many clinicians reading the laboratory reports. Interestingly, using the same evaluation criteria for mutations, 16% of 427 healthy controls without ARVC also had mutations, illustrating the level of genetic 'noise' and an overall frequency well beyond that predicted assuming the low prevalence of ARVC. In controls the majority of detected mutations were missense mutations arguing that many of this class of mutations were likely benign. When the authors turned to mutations predicted to cause more substantial consequences to the predicted ARVC protein structures (so-called 'radical' mutations) the prevalence of ARVC mutations dropped to 0.5% in controls yet remained as high as 43% in probands. The criteria for 'radical mutation' included: in-frame and frame-shift insertion and deletions, splice junction and nonsense mutations providing some guidance in how to interpret the likelihood of a given variant contributing to disease risk. However, as missense mutations may also cause disease the interpretation of a novel ARVC gene missense mutation likely requires more than just reading the mutation report from the laboratory. The investigators found that in individuals with confirmed ARVC missense mutations were grouped in "hot spots" in DSP and DSG2 in region of protein binding domains, and that mutations in affected patients occurred in highly conserved residues across species, whereas controls' missense mutations localized in highly variable residues. The complexity involved in interpretation of these mutations argues that, although ARVC clinical testing is accessible to many patients and cardiologists, significant skill maybe required to properly interpret mutation test results and helping to separate pathogenic genetic mutations from background noise.

A previous paper in long QT syndrome from Kapa et al. investigated the pathogenicity of LQT genes (SCN5A, KCNQ1 and KCNH2).[4] They screened a large cohort (388) of definitive long QT syndrome cases and also studied over 1300 normal controls. In that case, the "background noise" of mutations was again significant although at 6% less than the current report in ARVC. One explanation for the differences in background noise level is that the total number of patients and controls sequenced in long QT syndrome is substantially greater than in ARVC; thus we may expect that as more individuals are sequences for the ARVC genes that some of the background mutation noise may be reclassified as rare genetic variation reducing the ARVC mutation noise level somewhat.

An important element missing in the study of Kapplinger et al. was the genetic evaluation of the patients' families.[3] An analysis of cosegregation of a mutation within the family can be critical to help assess the causal role of a putative mutation. Unfortunately, this is difficult in research studies where ascertainment of large families is not always possible. In clinical circumstances, efforts to evaluate, recruit, and test multiple patients in a given family is even less likely to be completed. In some circumstances, investigators have taken additional steps

to assess mutation pathogenicity using *in vitro* cellular or *in vivo* animal assays but this approach is difficult to do when large numbers of mutations are identified and not possible in clinical situations when working with clinical laboratories.

Conclusions

Genetic testing continues to evolve, and in doing so reveals it to be an imperfect tool and one that requires careful interpretation before and after testing is done. Criteria for pathogenic mutations are not convincingly settled upon and are liable to undergo some changes as more knowledge is gained. Indeed, the pace of clinical testing seems at times to have moved faster and without circumspect consideration than perhaps research efforts would dictate. Stringent criteria ("radical" mutations, or missense mutations located in highly conserved and functionally important domains) and, whenever possible, cosegregation analysis can be used when applicable to help with genetic test interpretation. Furthermore, as underlined by Kapplinger et al.[3] and other authors[5–7] genetic tests must be integrated in the context of an expert clinical evaluation, together with a good family history and accurate clinical information, as for any other diagnostic test. Until the specificity of these types of molecular genetic tests is robust and understood, the clinical application of such tests is probably still better performed in referral centers with expertise in cardiovascular genetics.[5]

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References

1. Kraft P, Hunter DJ. Genetic Risk Prediction — Are We There Yet? N Engl J Med 2009;360:1701–1703. [PubMed: 19369656]
2. Baker M. Next-generation sequencing: adjusting to data overload. Nature Methods 2010;7:495 –499.
3. Kapplinger JD, Landstrom AP, Salisbury BA, Callis TE, Pollevick GD, Tester DJ, Cox MGPJ, Bhuiyan Z, Bikker H, Wiesfeld ACP, Hauer RNW, van Tintelen JP, Jongbloed JDH, Calkins H, Judge DP, Wilde AAM, Ackerman MJ. Distinguishing Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia-Associated Mutations from Background Genetic Noise. J Amer Coll Cardiol [query: current issue]
4. Kapa S, Tester DJ, Salisbury BA, Harris-Kerr C, Pungliya MS, Alders M, Wilde AA, Ackerman MJ. Genetic testing for long-QT syndrome: distinguishing pathogenic mutations from benign variants. Circulation 2009;120:1752–60. [PubMed: 19841300]
5. MacRae CA. Closer Look at Genetic Testing in Long-QT Syndrome: Will DNA Diagnostic Ever Be Enough? Circulation 2009;120;1745–1748. [PubMed: 19841296]
6. Hershberger RE, Lindenfeld J, Mestroni L, Seidman CE, Taylor MR, Tobwin JR. Genetic Evaluation of Cardiomyopathy: A HFSA Comprehensive Heart Failure Practice Guideline. Journal of Cardiac Failure 2009;15:83 –97. [PubMed: 19254666]
7. GeneTests: Medical Genetics Information Resource (database online). Copyright, University of Washington, Seattle. 1993-2010. Available at http://www.genetests.org. Accessed [accessed on December 19, 2010]