Editorial

Discerning From the Good, the Bad, and the Ugly

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Catecholaminergic polymorphic ventricular tachycardia (CPVT) is one of the most malignant inherited arrhythmias, with onset of symptoms in childhood and a 70% risk of experiencing a cardiac event before the age of 20 years if untreated.[1] Presymptomatic diagnosis is challenging, because the resting ECG is normal, and a family history of adrenergic-related sudden cardiac death or syncope is only present in 30% of individuals.[1] The *hRyR2* gene, coding for the ryanodine receptor channel, has been linked to the autosomal dominant form of CPVT, and a mutation may be detected in ≈65% of probands.[2] A rare autosomal recessive form, linked to *CASQ2* homozygous mutations, has also been described.[2] Additional genes have been linked to the phenotype, but their prevalence is to date limited to sporadic cases.[2]

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Initially, it was suggested that *RyR2* mutations causing CPVT cluster in specific residues of the genes; however, reports of CPVT patients harboring mutations outside these clusters prompted the recommendation of screening the entire coding region of the gene.[2] Overall, the gene has a negative residual variation intolerance score (a gene-based score that ranks genes from intolerant to tolerant based on whether they have more or less common functional genetic variation),[3] hence suggesting that most variants are expected to be deleterious. The Heart Rhythm Society/European Heart Rhythm Association Expert Consensus[2] advises the use of genetic testing in everyone with a suspect clinical diagnosis of CPVT, based on the high yield of the test and on the malignancy of the disease that could manifest with sudden cardiac death as the first symptom. Recently, the American College of Medical Genetics and Genomics stated that variants on *hRyR2* detected as secondary findings in whole-exome screening (WES) studies are reportable information.[4]

Parallel to the growing awareness of the amount of human genetic variations not linked to an increased disease risk, few studies[5,6] have emphasized the concept of background noise in inherited arrhythmias, that is, the presence in ostensibly healthy individuals of variants considered potentially deleterious. The signal-to-noise ratio varies among conditions and ranges from 19:1 in the long-QT syndrome to 4:1 in arrhythmogenic cardiomyopathy,[2,5,6] based on the few studies available. It should be noted that these initial estimates come from data on patients and healthy controls collected by few groups. The current availability of large exome data from healthy controls, such as the Exome Variant Server and the Exome Aggregation Consortium[7] provides a large amount of novel information on human variation and has prompted investigators to reclassify some variants in light of their presence in these data sets.

Estimates on background noise in CPVT were missing, and only recently few studies started addressing the topic.[8] In a recent study published in this issue of the journal, Landstrom et al[9] investigated the incidence of *RyR2* and *CASQ2* variants in a total of 6517 subjects from 2 large exome databases (Baylor Miraca laboratories and Texas Children's Hospital); they then compared the findings to the Exome Aggregation Consortium database and to one subset of CPVT patients for which there is published data available.[10] The authors report that 8.8% of the individuals undergoing WES carried an *RyR2* or *CASQ2* variant as incidental finding, and 2.3% of these were classified as likely pathogenic. The majority of variants, as expected, were found in the *RyR2* gene, with only 15% of variants in the *CASQ2* gene. Although the data reported on *CASQ2* are interesting, it is not possible to draw substantial conclusions on the extent of background noise for this gene, because most of the changes were heterozygous, and the role of *CASQ2* in autosomal dominant CPVT is to date limited to sporadic reports. Similarly, the authors found a small subset of radical variants in the *RyR2* gene, whose interpretation remains challenging. Most CPVT-related mutations are missense substitutions, supposedly increasing the activity of the RyR2 channel. The physiological meaning of *RyR2* radical mutations and their potential effect is unclear, rendering the data provided by the authors difficult to interpret in the context of the CPVT phenotype.

One interesting piece of information emerging from the present study is the distribution of *RyR2* mutants along the gene topology in the 3 subsets of data. These results add novel and relevant information to aid the interpretation of functional effects of *RyR2* variations. Variants detected in the WES and Exome Aggregation Consortium data set were distributed along all the gene sequence, without any clustering pattern, in contrast with CPVT mutants. More than 60% of variants from the WES studies overlapped with those observed in healthy controls, with only 8% overlapping with CPVT cases. Even more relevant to the possibility of detecting concealed cases of CPVT, only 10% of the WES changes localized within one canonical hotspot. Overall, these data suggest that most RyR2 incidental findings should be considered of limited disease-causing potential.

An important limitation of this study is that the CPVT cohort was derived from the literature and from one source only, and it included only 33 out of 155 patients with a robust diagnosis and a 60% rate of RyR2 mutations. The rest of the

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cohort had only a suspicion of CPVT based on exertional syncope and ventricular ectopies during stress test, risking to adding to the complexity of interpretation of likely pathogenic changes. Because the CPVT data were collected from the literature, the authors should have considered including more than one cohort in the attempt to collect more genotype–phenotype information to apply to their comparison.

An important and novel element to be praised is the availability of clinical information in the Texas Children Hospital WES subset. One major weakness in the large healthy controls data sets resides in the lack of clinical and ECG information. Considering that most cases of inherited arrhythmogenic conditions, especially CPVT, will be undiagnosed before symptoms manifest, it is difficult to assess how healthy are the healthy controls in relation to one of these conditions. From this perspective, the effort of the authors to investigate how many patients undergoing WES could indeed have a pretest suspicion of CPVT adds substantial information. Only one patient underwent screening based on history of syncope and family history of sudden death, which indeed yielded to the discovery of a likely pathogenic mutation and appropriate clinical evaluation. All other individuals did not have a known clinical suspicion of CPVT, even when considering a potential link between RyR2 and idiopathic epilepsy. One could argue that this pediatric group could manifest CPVT symptoms later in life, but this is a weak argument considering that the authors properly focused their analysis on a condition with usual onset in childhood.

Altogether, the study of Landstrom et al[9] is a welcome addition to the scarce number of reports addressing the prevalence of background noise in CPVT and casts a strong warning to the use of genetic information derived as secondary findings in the absence of a pretest clinical suspicion. Although there is increasing evidence that even a gene with robust negative residual variation intolerance score could carry variants with limited pathogenic potential, the incidental detection of RyR2 variants should still warrant clinical assessment and the collection of family history. However, a growing body of data suggests that the presence of an *RyR2* VUS should never be considered per se sufficient to define the diagnosis of CPVT. In a wider perspective, this study provides an additional call for caution in the use of large Next Generation Sequencing genetic panels as a binary, diagnostic tool in the absence of thorough clinical assessment. The more genetic testing in cardiology evolves, the more its complexity is emerging, together with the evidence that its diagnostic application should be part of a complete clinical evaluation in centers with specific expertise in cardiovascular genetics.

Disclosures

None.

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