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EDITORIAL COMMENT

Drug-Induced Long QT Syndrome and Exome Sequencing

Chinese Shadows Link Past and Future*

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Seldom in medical history has a single clinical entity created greater havoc among a diversity of interests and individuals than drug-induced long QT syndrome (diLQTS) (1). Drug companies have witnessed powerful molecules, developed at extraordinary costs, that were suddenly withdrawn from the market after only a few cases of sudden cardiac death or life-threatening arrhythmias caused by therapeutic doses of very promising drugs. Regulatory agencies were confused. Individuals have experienced cardiac arrest while being treated for diseases as benign as hay fever (2–4). Physicians and scientists have been puzzled by this phenomenon for almost 100 years (5).

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The prototype of diLQTS has unquestionably been quinidine syncope (6,7). It was indeed the attention generated by quinidine syncope, largely related to the fact that this was the only antiarrhythmic drug effective in the management of atrial fibrillation (8), that prompted the identification of 2 key features: prolongation of the QT interval and torsades de pointes ventricular tachycardia. Both features could have immediately suggested a link with congenital long QT syndrome (cLQTS), but it was only in 1982 that Schwartz and Moss (9), anticipating the molecular era, posited for the first time that "quinidine therapy may exacerbate an underlying repolarization abnormality, possibly a subclinical forme fruste of idiopathic LQTS with incomplete expression". By the elegant use of the French language, they more simply meant LQTS patients with a normal or borderline QT interval. It took 20 years to go from hypothesis to proof of concept.

The identification of the first major genes for LQTS took place in 1995 to 1996 and has had a major impact on the approach and management of channelopathies in general and of LQTS in particular (10), thus opening the field for the attempts to uncover the genetic basis of the relationship between drug-induced syncope, QT interval prolongation, and cLQTS. The first such evidence was obtained when an elderly woman with cardiac arrest due to documented ventricular fibrillation associated with treatment with cisapride, a well-known cardiac delayed rectifier potassium current (I_{Kr}) blocker, was found to have the *KCNQ1*-Y315C mutation (11). Her baseline corrected QT interval was normal (430 to 437 ms) but was prolonged on cisapride to 530 to 590 ms, just before and after ventricular fibrillation; it normalized completely after cisapride withdrawal, and we demonstrated that the mutant protein produces a major loss in the cardiac slow delayed potassium rectifier current (I_{Ks}) current (11). This single case provided the first evidence that "silent" genetic defects may create a vulnerable substrate that, in the presence of appropriate triggers such as any drug that blocks I_{Kr}, may precipitate life-threatening arrhythmias and raised the intriguing possibility that some of the individuals at risk of diLQTS might in the future be identified by a still-unforeseen genetic approach. It also supported the very important concept of repolarization reserve, cleverly proposed by Roden (12) and the "2-hit" hypothesis implying the possibility of a genetic impairment in I_{Ks} increasing the risk of diLQTS on administration of an I_{Kr} blocker (13).

What followed was a dramatic acceleration in the search for the genetic link between drugs and diLQTS. Several groups contributed in a major way, among them, those led by Dan Roden (14,15), undoubtedly the investigator who has contributed more than anyone else to this specific topic, by Kääb et al. (16), and by Horie's group (17,18). This leads us to this issue of the *Journal*, in which Weeke et al. (19) used whole exome sequencing (WES) to detect genes contributing to diLQTS.

Weeke et al. studied 65 patients and 2 sets of control subjects: 148 drug-exposed control subjects and a reference group of 515 nonphenotyped individuals from the Exome Sequencing Project (ESP), with the focus on European-American ancestry. Given the small sample size and the consequent small power, they cleverly used different sets of analysis, progressively less stringent. They started by checking the number of amino acid coding (AAC) variants (missense, nonsynonymous, or frameshift), and they used it to identify the Bonferroni-adjusted p value ($p < 6.39 \times 10^{-7}$). By the Fisher exact test and exact logistic regression, no single AAC variant reached that genome-wide significance threshold. They then moved from the number of AAC variants to the number of genes containing those variants to test for gene-level association, and the Bonferroni-adjusted p value was accordingly increased to $p < 3.5 \times 10^{-6}$. Unidirectional (variable threshold) and bidirectional (sequence kernel association test) rare variant

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aggregate approaches were used, but, once more, not a single gene carrying AAC variants reached the threshold for significance. This negative result was interpreted as due to the likelihood that the study was underpowered for a genome-wide approach, and, accordingly, they applied a third analysis looking only at those genes with the strongest associations identified by variable threshold and sequence kernel association test. Empirically, a $p < 0.001$ was considered to select the "top" genes, and then the association was retested comparing the same group of patients with the 515 individuals from the ESP. For the validation, the investigators had to reduce the p value from 0.001 to 0.05, clearly to avoid losing the significance on the *KCNE1* gene already established (16). This approach is acceptable but is significantly limited by the absence of an independent population of cases and controls, which represents the gold standard for validation studies.

This staircase analysis led to the identification of 2 genes important for diLQTS: *KCNE1,* already known for its association with diLQTS (16,18), and *ACN9,* a gene whose association with diLQTS needs to be elucidated and supported by further studies. Finally, they limited their analysis to 20 genes with a strong level of evidence of association with congenital arrhythmia syndromes, and they looked at genes enriched with rare variants among diLQTS patients versus drug-exposed control subjects and versus ESP control subjects. Importantly, patients with diLQTS had a 2-fold enrichment of rare AAC variants in potassium channels compared with drug-exposed and ESP control subjects; this greater enrichment remained present after exclusion of *KCNE1*.

The study by Weeke et al. (19) represents a good example of how the WES technique can be used successfully, not only for the identification of new disease-causing genes, but also for the identification of rare variants modifying the susceptibility to complex traits.

After the first report in 2009 of selective WES in 12 humans (20), and the subsequent evidence that this method could successfully identify a gene responsible for a rare Mendelian disorder (21), >150 articles were published about gene discovery by WES in various Mendelian disorders (22). Three main approaches have been used so far to identify disease-associated genes through WES: family-based studies, case-parent trio studies, and unrelated individual studies.

In family-based studies, a number of affected individuals within a family are sequenced, and the shared mutations are analyzed and prioritized according to the supposed way of inheritance (i.e., autosomal dominant, autosomal recessive, or X linked). Examples are the identification of a *GATAD1* mutation as the cause of a form of autosomal recessive cardiomyopathy (23) and the segregation of 2 private mutations in 2 functionally related genes (*GUCY1A3* and *CCT7*) in an extended myocardial infarction family (24).

Case-parent trio studies are used to identify mutations responsible for very severe disorders not expected to be inherited from the parents. Both the parents and the proband are fully exome-sequenced, and the de novo novel variants identified become the main focus of the analysis. An example is the identification of mutations in calmodulin genes responsible for an extremely malignant form of cLQTS (25). Zaidi et al. (26) used a similar approach, but with a case-control analysis, and observed a marked excess of protein-altering de novo mutations in histone-modifying genes in patients with congenital heart diseases.

WES studies in unrelated individuals, as in the study by Weeke et al. (19), are more challenging but can be applied to a variety of questions. For example, Ng et al. (27), with only 10 unrelated subjects affected by the Kabuki syndrome in the discovery cohort and 43 affected individuals in the replication cohort, were able to identify *MML2* as the major gene responsible for this rare multiple malformation disorder. These successful studies based on a few unrelated individuals are possible mainly in monogenic disorders with minimal genetic heterogeneity. The same case-control approach proved successful also for the identification of genetic pathways/variants enriched in complex traits (28,29).

In line with these studies on complex traits and with the emerging shift from a common disease–common variant hypothesis to a rare variant–common disease/complex trait hypothesis (30), Weeke et al. (19) used a case-control approach to identify genes or gene pathways favoring the risk of diLQTS. The validity of this approach appears to be confirmed by the identification of *KCNE1* rare variants, as factors favoring the risk of diLQTS, as already demonstrated by Kääb et al. (16). The modest overlapping of the 2 studies does not detract from the finding.

The data of Weeke et al. (19) complement well those of Behr et al. (31) showing a failure of a genome-wide association study approach to identify common variants conferring a high risk of diLQTS, and suggest that rare variants indeed play a prominent role. The small sample size of the study by Behr et al. (31) does not allow excluding that the interaction of multiple variants with a modest individual effect might also contribute to this complex and uncommon phenotype.

The study by Weeke et al. (19), as it often happens with those led by Roden, may open interesting windows on the future. The WES technique, born as a very expensive tool aimed at the identification of new disease-causing genes mainly in Mendelian disorders, has progressively shown potential for going well beyond. It is becoming progressively less expensive with a constant improvement in its gene-coverage capability, possibly overcoming also the initial limitation in the identification of copy number variants (32). One could thus envisage that WES might become useful for the combined study of disease-causing and modifier factors and could replace existing genetic screening programs in clinical practice—a step toward the dream of a single test providing complete genetic information for a fully comprehensive tailored medicine. Things are actually moving very rapidly now. The U.S. Food and Drug

Administration has just granted marketing authorization for the first high-throughput genome sequencer (Illumina's MiSeqDx), which will allow the development and use of innumerable new genome-based tests. As Collins and Hamburg (33) have rightly stated, "this marketing authorization…represents a significant step forward in the ability to generate genomic information that will ultimately improve patient care."

The speed of the progress in the genetics of cardiac arrhythmias has been mind-boggling and, not infrequently, disconcertingly exciting. Still, it is a refreshing and soothing thought, at least for some of us, to realize that the directions for the future sometimes can be pointed out by the long shadows of the past.

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