Closer Look at Genetic Testing in Long-QT Syndrome
Will DNA Diagnostics Ever Be Enough?

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Increasingly affordable sequencing has led to an explosion of DNA diagnostics and has fueled the hope for a genetic revolution in medicine. The popular vision is of definitive prediction of lifetime risk for a broad range of conditions from the primary analysis of each individual’s genomic sequence. The resultant panel of sequence variants will define the specific traits to which an individual is susceptible, triggering the institution of preventive measures or targeted therapies. Efficacy and safety will be largely assured for each of the chosen interventions. However, with the potential exception of pharmacogenetic studies in which the molecular targets are known, robust genotype-phenotype correlations are remarkably sparse and, in most instances, simply inadequate for rigorous risk prediction. Although genetics has transformed our understanding of the biology of disease, on closer examination it is not immediately evident when the “genomic revolution” will be realized as currently conceived for many cardiovascular conditions.

Current Genetic Testing in Familial Long-QT Syndrome

Nothing tests the tools of clinical risk prediction quite like sudden death. The difficulties encountered in the clinical application of genetic data, even in inherited conditions such as the long-QT syndrome (LQTS), in which the transmitted risk of sudden death is several hundred-fold greater than that in the general population, highlight some of the hurdles that must be overcome if DNA diagnosis is ever to transform cardiovascular medicine. Genetic testing for LQTS has been commercially available for several years, but a critical analysis of the clinical utility of test results reveals a rather mixed picture (see the Table).

Genetic testing is commonly performed in those with a family history of LQTS and a known mutation, yet often the rationale is debatable. Individuals meeting the clinical criteria for LQTS in such families have an inherited repolarization disorder regardless of the results of DNA testing, and genetic data offer essentially no additional predictive information.

Given the high a priori probability of LQTS in this context, the magnitude of any additional risk attributable to a mutation is difficult to estimate without large studies in unrelated individuals. Because of the high rates of de novo mutation in disorders that affect reproductive efficiency, it is unlikely that such studies will ever be feasible. Even for close relatives with a definitive clinical picture and a shared mutation, the risk for sudden death varies widely. Nevertheless, the family history is consistently a better predictor of outcomes than a specific DNA sequence abnormality. Indeed, discordant phenotype and genotype for the “known mutation” in a large affected family is as likely to represent a false-positive genotype as it is a misdiagnosis or phenocopy.

Individuals whose clinical status is indeterminate in such a family may be identified as at risk through a “positive” genetic test. However, the a priori probability of LQTS in such an individual is 0.5 even before any clinical evaluation, so once again, the additional risk conferred by sequence information is difficult to quantify in the face of relevant ECG abnormalities. A negative genetic test in this setting (indeterminate clinical status within an affected family) is as likely to reduce certainty in the causal role of the known mutation as it is to eliminate all doubt and is often unable to reassure physician or patient. The management strategy in such ambiguous situations is determined by physician and patient attitudes about risk rather than any rigorous data on mutation-specific outcomes. Fortunately, many patients, particularly those from families with inherited sudden death syndromes, have a very nuanced concept of risk that is helpful in management discussions. Even in clinically unaffected members of LQTS families with a known mutation, genetic testing may present difficulties. A substantial proportion of mutation carriers have normal QT intervals on a single evaluation, and the long-term outcome is unknown in those who are genotype positive and phenotype negative. There are some data that the natural history may correlate with clinical phenotype even in those who are genotype positive, but this may vary from family to family, as well as with the resolution of phenotyping. A negative genetic test in a clinically normal member of a well-characterized family theoretically leads to elimination of the need for future screening for the individual and his or her offspring. This is perhaps the most robust use of genetic testing at present, although here also the interpretation is dependent on the resolution of the phenotyping and the data supporting pathogenicity of the purported mutation.

When there is a family history of LQTS but no known mutation, then genetic testing is of even lower utility. As a result of genetic heterogeneity (mutations in unknown genes) or technical failure, ≈25% of LQTS families do not have...
abnormalities on screening of the known genes.\textsuperscript{6,12} Definitive attribution of causality is difficult without the rigorous genetic evaluation of the entire kindred, usually in a research setting. Even if a sequence variant is identified, its pathogenicity may be difficult to establish. As a result, changes in the management of those who are clinically indeterminate or clinically normal are at present unlikely to be based on the results of genetic testing.

In summary, the predictive utility of genetic testing in familial LQTS is so dependent on the a priori likelihood of disease that there is often little or no incremental information accruing from the test results. Rigorous data supporting the benefits of genetic testing over a careful family history and clinical evaluation are lacking.\textsuperscript{4} The psychological impact and effects on quality of life of genetic testing in LQTS are rarely considered.\textsuperscript{13} If medical consumers are to pay for genetic testing, they will surely require that the results offer some added value.

### QT Prolongation and Genetic Testing in the General Population

The limitations of genetic testing in familial LQTS are only amplified in isolated patients in whom the estimates of pathogenicity for a given sequence variant are not supported by robust cosegregation data. Clinical and genetic definitions of disease are so inextricably interdependent in large Mendelian families that great care must be taken to avoid circular logic as we move to prediction in single patients. There are unique confounders in monogenic conditions that preclude the direct translation of data on genotype-phenotype correlation to unrelated individuals, including shared genetic or epigenetic modifiers, strong ascertainment biases, and more typical environmental or other confounders. In this issue of Circulation, Schwartz et al\textsuperscript{14} explore the prevalence of congenital LQTS and grapple with these very problems.

Evaluating \(>43\) 000 healthy subjects between 15 and 25 days of age, these investigators identified a reproducibly prolonged QTc (\(>470\) ms) in 29 individuals. An additional 28 neonates exhibited a QTc interval of 461 to 470 ms, and 2 of these had further QT prolongation on Holter recording. Twenty-eight children with a QTc \(>470\) ms were screened for mutations in 7 of the known QT genes. Sequence abnormalities in the \(\text{KCNQ1}\) and \(\text{KCNH2}\) genes were identified in 12 of these 28 individuals; the remaining 16 children had no evidence of any sequence abnormalities in the genes screened. Genetic evaluation of an additional 14 individuals with QTc intervals of 461 to 470 ms revealed 4 further mutations.

The inevitable dilemmas arising from the current state of genotype-phenotype correlation in LQTS are highlighted in the efforts to tease apart the clinical and molecular diagnoses. As the authors acknowledge, there is no gold standard for DNA diagnosis, and a significant proportion of LQTS cases are not explained by mutations in the known genes. Using a conservative genetic diagnosis, Schwartz et al generate prevalence estimates in the range of 1:1500 to 1:4350 individuals. These estimates are considerably greater than previously published studies have suggested, yet they do not include more than half of those with a congenital QTc abnormality. The lack of impact of DNA diagnosis is evident only when faced with the decision on whom to treat; all of those with a QTc \(>470\) ms were treated regardless of the genetic information.\textsuperscript{5,14}

The value of any test is limited unless it reclassifies the patients into a new prognostic or therapeutic category. The impressive cohort collected by Schwartz et al will eventually yield quantitative estimates of the risks associated with the full spectrum of congenital QTc. Ideally, information on the risks associated with specific sequence variants will also become available, but as noted earlier, adequately powered studies may not be feasible, and meaningful predictive algorithms are unlikely with high false-negative rates for molecular diagnosis. Nevertheless, without quantitative data on the independent predictive utility of genotype and phenotype, it will be difficult to validate genomic sequence as a diagnostic tool.

### Improving Interpretation of the Results of Genetic Testing

It is possible that current genotype-phenotype correlation is limited only by a lack of relevant empirical data.\textsuperscript{6} In a second article in this issue, Kapp and colleagues\textsuperscript{15} have addressed potential deficiencies in the data supporting LQTS gene testing: the background “noise” in gene sequencing efforts and inaccurate structural predictions of pathogenicity.

To estimate the background noise in genetic testing, Kapp et al screened a large number of controls (\(>1300\)) at the same stringency as a cohort of definitively affected LQTS cases. As they note, this stringency of evaluation has been undertaken for remarkably few genes, even among those available for commercial testing. Missense variants were identified in 47\% (184 of 388) of LQTS cases and in 6\% (81 of \(>1300\)) of normal unaffected controls. Unfortunately, it was not possible to screen the same controls for all genes. Nevertheless, this study offers a real-world assessment of the scale of the background noise in the 3 LQTS genes and suggests signif-
icant contamination of existing mutation databases with simple polymorphisms.\textsuperscript{15}

These investigators then proceed to tackle the imperfect relationship between genotype and phenotype. They generate estimated predictive values for different classes of structural variant based on the ratios of missense variants in cases and controls for each functional domain in the channel proteins. Because the predictive values are derived from the data set used to define the pathogenicity of these same variants, external validation will be critical to confirm added value.\textsuperscript{14,15} Most algorithms attempting to improve the fidelity of genotype-phenotype relationships have integrated data from crystal structures or from relevant functional assays for the protein with sequence conservation.\textsuperscript{16,17} Although this may be feasible for an enzyme, it is considerably more challenging to apply to a channel protein that participates in distinct multimeric complexes as it is processed, trafficked through the cell, and shuffled between membrane microdomains. Many mutations validated in heterologous systems have not yet been studied in combination with wild-type alleles, far less in full native context, and even the most radical mutations may generate an unstable protein without functional consequences.\textsuperscript{12} As a result, it is not surprising that even with large data sets and sophisticated bioinformatic algorithms, we are unable to assign a graded pathogenicity to each sequence variant.\textsuperscript{15}

**Refining Predictive Algorithms**

As Kapp et al highlight, our concepts of risk prediction imply quantitative comparisons with objective risk-to-benefit ratios. In several areas, ongoing work promises substantial improvements in our understanding of the causal chain between genotype and phenotype. Next-generation sequencing offers massive changes in the scale of genotyping that will allow the efficient analysis not only of the primary LQTS genes but also of all potential modifiers.\textsuperscript{18} However, the effect sizes of the common modifiers affecting traits such as QTc are small, and interactions are complex. Even complete genomic sequence, capturing all of the genetic contributions to repolarization, may offer little additional information in the face of epigenetic, environmental, and stochastic contributions to the final risk of arrhythmia.

Many of these unknowns, including gene-gene interaction and transmitted epigenetic contributions, are at least partly accessible in the family history, which at present is the major determinant of pretest probability for genetic testing. The development and dissemination of robust family history tools would offer substantial advantages in the management of inherited disease long before genomic sequence is feasible.\textsuperscript{19} Characterization of pleiotropy and penetrance, the identification of misdiagnoses or phenocopies, and the definition of clear denominators for any risk estimates would immediately refine current genetic testing. Obstacles to such improvements include the lack of genetically trained clinicians, difficulties incorporating family information into standard electronic medical record formats, and the constraints of patient confidentiality.\textsuperscript{19} Innovative informatic solutions are required to capture objective data from linked relatives in a HIPAA-compliant fashion to generate composite but anonymized family risk metrics. The necessity of family engagement in the management of lethal inherited diseases may be a template for future efforts to remodel the delivery of health care.

Ultimately, modeling the true complexity of genotype-phenotype relationships is likely to prove difficult unless we can empirically test the effects of specific genetic variants in a fully native biologic context. This may take the form of innovative reevaluation of current phenotyping tools. Currently, vectorcardiographic tools are in development to refine the classification of repolarization beyond simple duration of the QT interval. Detailed characterization of the QT response during the first few weeks of life, when physiological stressors may offer unique insights into repolarization, and is an example of the enriched phenotypes that may drive future advances in human biology. Ex vivo cellular phenotypes may offer access to multiple interacting pathways contributing to a given disease, even though the specific assay end points may seem removed from sudden death. For example, the assessment of LQTS mutations might incorporate data from phagocytic pathways in which KCNH2 and other channels have been implicated.\textsuperscript{20} To complement the human genome project, we must embark on systematic strategies to discover new phenotypes integrating all the genetic, epigenetic, and environmental influences on disease physiology: a human phenome project. Well-defined perturbations could be used to define the systems architecture of disease phenotypes, expanding the dynamic range of old and new phenotypes alike. In LQTS, we might challenge individuals with existing drugs or novel small molecules to assess repolarization reserve. If the envisioned genetic revolution is ever to be realized, genetic data must outperform existing disease classifiers. Ultimately, genetics may prove most useful in highlighting specific disease pathways that then can be systematically tested with provoked phenotypes.

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**Disclosures**

None.

**References**

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