TEN QUESTIONS/ALLIED PROFESSIONALS

Genetic testing for cardiac channelopathies: ten questions regarding clinical considerations for heart rhythm allied professionals

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What are cardiac channelopathies?

The study of cardiac channelopathies represents a relatively new discipline among heart rhythm specialists and allied professionals. In 1995, the discipline of cardiac channelopathies commenced with the discovery that defective cardiac channels were at the heart of congenital long QT syndrome (LQTS). Besides LQTS, the channelopathies include short QT syndrome, Brugada syndrome (BrS), Andersen Tawil syndrome (ATS), catecholaminergic polymorphic ventricular tachycardia (CPVT), congenital sick sinus syndrome, and some cases of autopsy-negative sudden unexplained death during infancy, childhood, adolescence, and beyond. The fundamental pathogenic mechanisms responsible for these disorders have been elucidated at least in part, and marked genetic and clinical heterogeneity is a common theme.

Is genetic testing available for these cardiac channelopathies?

Over the past decade, genetic testing for these cardiac channelopathies has been performed in select research laboratories throughout the world. Such genetic testing has been conducted principally for the purpose of discovery and genotype-phenotype correlations (to advance the science). In some cases, research participants have been the direct beneficiaries of the testing with results provided usually 1–2 years after submission of a blood sample. Recently, in May 2004, the first Clinical Laboratory Improvement Amendments (CLIA)-approved, commercial genetic test to detect cardiac channel mutations was released by Genaissance Pharmaceuticals (New Haven, CT). Their diagnostic test, FAMILION™, provides either comprehensive mutational analysis of the five cardiac channel genes implicated in LQTS or a *SCN5A*-targeted test for the only gene presently implicated in BrS. Genetic testing for ATS and CPVT remains a research lab–based test.

Who should undergo genetic testing for cardiac channelopathies?

All patients or family members for whom a clinical diagnosis of a channelopathy is suspected should seek genetic testing from either the commercially available test or from research laboratories depending on the suspected diagnosis. From a clinical test perspective, any patient and his/her first-degree relatives with a suspected clinical diagnosis of LQTS should be offered clinical genetic testing. LQTS clinical genetic testing should also be considered for patients with unexplained, exertional syncope or drug-induced QT prolongation/torsade de pointes who do not meet full diagnostic criteria for LQTS. Patients suspected of having BrS could undergo clinical genetic testing as long as it is recognized that the yield from the currently available test is approximately 20%.

What are the benefits of genetic testing for these cardiac conditions?

The genetic test can (1) elucidate the precise molecular basis in cases of a strongly suspected channelopathy, (2) establish a definitive molecular diagnosis when the clinical probability is intermediate such as in "borderline" LQTS, (3) confirm or exclude the presence of a disease-causing mutation in asymptomatic family members, and (4) help tailor treatment recommendations and management of a patient's specific channelopathy by characterization of the particular genotype.

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What are the current limitations in genetic testing for arrhythmia syndromes?

Clinical genetic testing is only available at the present time for LQTS and BrS. In the case of LQTS, approximately 25% of families with a strong clinical probability of LQTS will have a negative genetic test result. Therefore, it is critical to recognize that a negative test result *cannot* fully exclude the diagnosis as a stand-alone test. However, in cases where the clinical index of suspicion is intermediate at best, a negative test result may be used as another piece of objective evidence that has failed to establish the diagnosis. The BrS genetic test is only for the single gene, *SCN5A,* that has been associated with the syndrome. However, SCN5A BrS-causing mutations are found in approximately 20% of families satisfying the clinical diagnosis of BrS. While KCNJ2 mutations account for over half of ATS and RyR2 mutations account for over half of CPVT, these genetic tests are still conducted in research laboratories and clinical decisions cannot wait on the possibility of learning the genetic test result sometime in the distant future.

One of the other concerns with respect to genetic testing for cardiac channelopathies is the cost of genetic testing. Because of the extensive amount of DNA (12,000 base pairs in the case of the LQTS genetic test) that must be analyzed, the current comprehensive LQTS genetic test costs approximately $5400. Once a pathogenic LQTS/BrS-causing mutation is identified in the index case, the family-specific confirmatory test costs $900. Although the cost of LQTS/BrS genetic testing is consistent with extrapolations from breast cancer genetic testing and is less expensive than other diagnostic (electrophysiologic studies) or treatment (radiofrequency ablation, internal cardioverter defibrillator) modalities done routinely in heart rhythm services, this fee-for-service cardiac channelopathy genetic test represents a change from the free LQTS genetic test that has been offered as part of Institutional Review Board–approved research protocols in a few research laboratories over the past decade. This same transition has occurred already with cystic fibrosis genetic testing and *BRCA1/BRCA2* breast cancer genetic testing. As with these examples, it is expected that cardiac channelopathy genetic testing similarly will be reimbursed by the majority of insurance providers. In fact, our first LQTS case submitted for testing received prior approval for 100% coverage of the commercial LQTS genetic test.

What type of biological material can be used for genetic testing?

In general, for either the research-based or clinical genetic tests, 5–15 cc (1–3 tsp) of blood obtained from venipuncture placed in EDTA-containing tubes ("purple top") is requested as the source of genomic DNA for genetic testing. DNA isolated from a buccal (mouth cheek) swab can also suffice, particularly for confirmatory testing of family members. However, such sampling may not yield a large enough amount of DNA for comprehensive mutational analysis. The clinically available LQTS/BrS genetic test is currently set up for analysis from blood-derived DNA rather than buccal swab sampling. Umbilical cord blood can be obtained at the time of birth for newborn screening in cases where a molecular diagnosis of a channelopathy has been established in one of the infant's parents. Recently, we reported the first prenatal genetic test of LQTS derived from cultured amniocytes after amniocentesis. In cases of autopsy-negative sudden unexplained death, a cardiac channel molecular autopsy can be performed on EDTA-blood if isolated. Alternatively, genomic DNA can be extracted from a frozen piece of tissue. The tissue requested is typically left ventricle myocardium, although any organ (liver, spleen, thymus) with a high nucleus:cytoplasm ratio will suffice. Both research-based and clinical genetic testing will require that signed and dated informed consent accompany the samples to be tested.

What are the most commonly used methods to identify gene mutations?

The identification of gene mutations typically involves the polymerase chain reaction technique used to amplify or create many copies of a specific region of DNA sequence (amplicon) within the gene of interest. This is often followed by the use of some intermediate mutation detection platform such as single-stranded conformational polymorphism (SSCP) or denaturing high-performance liquid chromatography (dHPLC). These techniques are used to inform the investigator of the presence or absence of a DNA sequence change in the samples examined. However, direct DNA sequencing must be used to determine the underlying DNA change. A review of the resulting sequence chromatogram and the published normal DNA and amino acid sequence for the gene of interest will allow for the interpretation of whether the underlying DNA change is protein altering and potentially pathogenic or a nonpathogenic normal variant. In this approach, only samples believed to contain a DNA change are further analyzed by DNA sequencing. In some cases, the use of an intermediate mutation detection platform such as SSCP or dHPLC is bypassed for direct DNA sequencing of all samples interrogated. Typically, research laboratories quote research subjects 6–24 months as the time frame in which they can expect to learn of a positive result.

Importantly, the cardiac channelopathies require this comprehensive approach that is not predicated *a priori* on a specific set of mutations. This stems from the fact that many families affected by a channelopathy have their own private mutation not to be found in another unrelated family. Until saturation of all possible disease-causing mutations is achieved, conversion to a diagnostic gene chip annotated with specific mutations will not be possible and a sequencing-based approach as described above will be required.

The clinically available genetic test to detect cardiac channel abnormalities is a high throughput, automated, direct DNA sequencing–based assay. In the comprehensive mutational analysis for the five cardiac channel genes implicated in LQTS, the genetic test involves analysis of approximately 75 amplicons and over 12 kb of genetic material. In addition, each amplicon is interrogated with four-fold redundancy to maximize diagnostic accuracy associated with this comprehensive surveillance. Rather than reporting results to the research participant after a long period of time as with research testing efforts, results (both positive and negative) from the clinical genetic test are reported to the ordering physician within approximately 4 weeks.

How should the results of genetic testing be interpreted?

The patient and family suspected of having a cardiac channelopathy should be evaluated and managed by a heart rhythm specialist with particular expertise in this discipline. Because of issues associated with incomplete penetrance and variable expressivity, the results of the genetic test must be interpreted carefully and incorporated into the overall diagnostic evaluation for these disorders. The assignment of a specific variant as a true pathogenic disease-causing mutation will require careful scrutiny. If the cardiologist or cardiac channelologist lacks expertise with respect to the pathogenetic basis of these disorders, adding a geneticist to the patient's team may be worthwhile. However, given the myriad of genotype-phenotype considerations specific to these disorders, it seems reasonable to expect that the cardiologist/channelologist should be the primary physician responsible for directing the patient's care.

Should a genetic counselor be involved?

It may be beneficial to have an appropriately trained genetic counselor as part of the team to be involved in the communication process with the patient concerning the implications of genetic testing and genetic test results. A family history involving at least three generations should be taken at the onset of clinical evaluation of the patient and used as an evaluation for referral for further genetic testing and counseling.

What are the ethical issues involved?

Genetic information should be considered private and personal information with the potential for mishandling. Disclosure of confidential information to third parties, such as insurance companies or employers can have consequences to the patient. Patients should be well informed on the implications of genetic testing and in no way should be coerced into providing a sample for analysis. Full disclosure should be given as to the intent of either the research or clinical genetic test, the results of the analysis, and who will have access to the results. Legislation is advancing to protect patients from genetic discrimination.