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EDITORIAL COMMENT

Importance of Clinical Analysis in the New Era of Molecular Genetic Screening*

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For the past 2 decades, a number of inherited cardiac arrhythmia syndromes have been shown to be linked to mutations in genes encoding cardiac ion channels or other membrane components. These include congenital and acquired long-QT syndrome (LQTS), Brugada syndrome (BrS), progressive cardiac conduction defect, catecholaminergic polymorphic ventricular tachycardia (CPVT), short-QT syndrome, early repolarization syndrome, and familial atrial fibrillation (AF) (1). In congenital LQTS, 13 genotypes have been identified in approximately 75% of subjects with clinically diagnosed congenital LQTS (1,2), and genotype-phenotype

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correlations have been investigated in detail. Thus, genetic testing is now a gold standard for diagnosing congenital LQTS, enabling risk stratification of cardiac events and better patient management (1). Mutations in the *RyR2* gene or *calsequestrin* gene can be identified in approximately 60% of typical patients with CPVT associated with bidirectional and/or multifocal ventricular tachycardia (1,2). However, the yield associated with disease-specific genetic testing is far short of 100%, even in congenital LQTS or CPVT. Moreover, causative mutations have been identified in a small number of patients with other inherited arrhythmia syndromes (1). The yield of disease-specific genetic testing is only 20% to 30% in BrS and is still unknown in progressive cardiac conduction defect, short-QT syndrome, early repolarization syndrome, and familial AF (1,2).

In BrS, the first mutation was identified in an alpha subunit of a sodium channel gene, *SCN5A*, in 1998 (3). Subsequently, genetic studies have identified 13 responsible genes on chromosomes 1, 3, 7, 10, 11, 12, 17, and 19 (1). Among 13 genotypes, more than 300 mutations have been identified in the major player, *SCN5A* (>75% of genotyped cases); however, a worldwide cohort reported that *SCN5A* accounts only for 11% to 28% of clinically diagnosed patients with BrS (4). Moreover, the majority of mutations were found in a single family or a small number of families. Therefore, a genotype-phenotype correlation is not available in most cases (1,5).

The relatively lower yield of disease-specific genetic testing except for congenital LQTS or CPVT is due mainly to the technology of genetic testing. Candidate gene analysis has long been used to identify a causative mutation in a gene, which is expected to relate to the pathophysiology of each inherited arrhythmia syndrome, such as cardiac ion channel genes. However, causative mutations do not always involve genes of ion channels or membrane components. Innovative advances in molecular genetic testing are overcoming this issue with the advent of more powerful molecular genetic screening tools, including genome-wide association study (GWAS) using gene array, as well as targeted, whole-exome and whole-genome next-generation sequencing techniques.

Several recent GWASs have disclosed significant association of numerous loci in some genes with electrocardiographic markers or arrhythmia syndromes. Arking et al. (6) first identified *NOS1AP (CAPON)*, a regulator of neuronal nitric oxide synthase, as a gene that is significantly associated with QT-interval variation in a general population derived from 3 cohorts (6). Subsequently, 2 groups conducted a meta-analysis of the GWAS and observed associations

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of single-nucleotide polymorphisms (SNPs) in several genes in addition to *NOS1AP* with QT interval, suggesting that these genes are candidate genes for LQTS or sudden cardiac death (7,8). Several GWASs also identified associations of SNPs in several genes, including *SCN10A*, with cardiac conduction parameters, such as QRS duration and PR interval (9–11). Regarding associations with cardiac arrhythmias, some SNPs in several genes, including *ZFHX3* and *KCNN3*, have been reported to be associated with AF (12–14). The association of a SNP in *CXADR* with ventricular fibrillation in acute myocardial infarction also has been reported (15). However, no responsible mutations have thus far been reported in these candidate genes in patients with clinically diagnosed inherited arrhythmia syndromes, such as congenital LQTS, familial AF, and familial conduction abnormalities.

Bezzina et al. (16) recently conducted a GWAS in 312 patients with BrS with type 1 electrocardiographic pattern and 1,115 controls. They detected 2 significant association signals at the *SCN10A* intronic locus (rs10428132) in chromosome 3p22 and near the *HEY2* gene (rs9388451) in chromosome 6q22 with BrS. *SCN10A*, which encodes the sodium channel isoform Nav1.8, was originally reported as highly expressed in cardiac neurons. Recent evidence indicates that *SCN10A* also is expressed in the working myocardium and the specialized conduction system, indicating a possible role for Nav1.8 in cardiac electrical function. *HEY2* is involved in patterning Nav1.5 (*SCN5A*) expression across the ventricular wall. In an experiment using *HEY2* knockout mouse, Bezzina et al. (16) suggested that loss of *HEY2* might affect the transmural expression gradient of sodium channel implicated in BrS.

In this issue of the *Journal*, Hu et al. (17) report on a clinical analysis and direct sequencing of *SCN10A* and all known BrS genes in 150 unrelated patients with BrS and 17 family members, as well as more than 200 ethnically matched healthy controls. They identified 17 *SCN10A* mutations in 25 of 150 patients with BrS (a yield of 16.7%). Twenty-three of the 25 (92.0%) displayed overlapping phenotypes, such as early repolarization syndrome and cardiac conduction defect. Patients with BrS with *SCN10A* mutations were more symptomatic and displayed significantly longer PR and QRS intervals than *SCN10A*-negative patients with BrS. Heterologous coexpression of *SCN10A* mutants (R14L and R1268Q) with wild-type *SCN5A* caused 79.4% and 84.4% reductions in sodium channel current, strongly implicating *SCN10A* as a major susceptibility gene for BrS. This study provides the first major step forward in more than 16 years in the identification of new BrS susceptibility genes, advancing the yield for detection of a genotype to more than 50%.

New molecular genetic screening technologies, such as GWAS and whole-exome and whole-genome next-generation sequencing, are promising tools for identifying new candidate genes responsible for inherited arrhythmia syndromes. However, no responsible mutations have been reported in the candidate genes identified by GWAS in patients with clinically diagnosed inherited arrhythmia syndromes. To the best of my knowledge, the *SCN10A* is the first gene to be suggested as a BrS susceptibility gene by both GWAS and direct sequencing techniques. Direct sequencing using the Sanger technique combined with a detailed clinical analysis, including genotype-phenotype correlation and functional expression studies, continue to play an important role in molecular genetic testing, even in the new era in which gene arrays and next-generation sequencing are available. The importance of a detailed clinical analysis including genotype-phenotype correlation as well as functional expression studies cannot be overemphasized. Even in GWAS and whole-genome or whole-exome studies, clinical misdiagnosis can contribute to confounding genetic noise. A detailed, precise clinical diagnosis is therefore a prerequisite for the identification of new potential candidate genes.

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