When High Throughput Meets Mechanistic Studies
A State-of-the-Art Approach in Brugada Syndrome

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The greatest insights in science are obtained through integrative approaches that combine the best aspects emerging and traditional technologies. The study by Veerman et al that appears in this issue of Circulation Research thoughtfully integrates big data high throughput technology, small data molecular approaches, and electrophysiological methods to draw conclusions important both to the understanding of Brugada syndrome and normal ventricular function.

More than 2 decades ago, Josep and Pedro Brugada first described a syndrome of ST-segment elevation, right bundle branch block, a high incidence of polymorphic ventricular tachycardia and ventricular fibrillation, and increased risk of sudden cardiac death. Research dating back to 1991 has described that the induction of phase 2 reentry by sodium channel block which was thought to be the cause of life-threatening arrhythmias in this syndrome, and which later became known as Brugada syndrome. In 1998, Chen et al showed linkage of the Brugada syndrome with a mutation in the SCN5A gene encoding the α subunit of the cardiac sodium channel protein. Because of the dynamic and temporary nature of the Brugada ECG pattern, sodium channel blockers (eg, ajmaline, flecainide, or procainamide) have been administered to unmask type 1 ECG patterns.

Since the discovery of the SCN5A mutation, a total of 12 Brugada syndrome susceptibility genes have been identified (Brugada susceptibility [BrS] 1–12). Type 1 BrS or BrS1, caused by the loss-of-function mutation in the SCN5A-encoded subunit of the sodium channel, represents the most common genetic substrate for BrS accounting for ≤30% of the disorder. Furthermore, mutations of the L-type calcium channel α1, β2, and δ2 subunits encoded by CACNA1, CACNB2B, and CACNA2B are estimated to cause 10% to 15% of Brugada syndrome. Functional analyses have shown that mutations in BrS genes result in the reduction of the cardiac sodium ($I_{Na}^c$) current, increase in the transient outward potassium ($I_{to}$) current, or reduction in the pacemaker ($I_{KATP}$) current. A large mutational analysis by Crotti et al in 129 unrelated patients with possible or probable Brugada syndrome concluded that among all of the 12 BrS-susceptibility genes discovered to date, the SCN5-mediated BrS1 continues to be the most common genetic substrate, whereas the other 11 genes account for ≤5% of the investigated cases.

The initial discovery of the SCN5A mutation occurred in a stepwise approach from clinical observation to molecular analysis and subsequently to mechanistic studies in the laboratory. More recently, discoveries of BrS-susceptibility genes have been facilitated via genomic sequencing—one of many high throughput technologies. High throughput technologies generated much enthusiasm in the scientific community during their early phase; however, with increased utilization, these technologies have come under increasing criticism. The most common criticisms of high throughput technologies include overfitting and random association. Overfitting limits the broad applicability of the results because the resulting model draws conclusions that are relevant only to the specific sample set used in the study. Random association is a type of overfitting that can occur when the number of variables analyzed approaches or exceeds the sample size. Although these concerns are valid, they can be properly mitigated when the right methodology is used.

Statistical methods that have the ability to account for large number of participants, variables, and computations, such as the $q$ value—a statistical measure of significance that considers the false discovery rate in large data sets—limit the risk of random association. Another standard method used in classification studies and machine learning to reduce the risk for random associations and overfitting is to divide data into training and independent test sets. Commonly, two thirds of the data or patient population (also called training set) are used to identify a biomarker, which then is validated in an independent test set containing one third of the data.

Because the technical details underlying a given study are so complex, scientific journals carry an important ethical responsibility to verify that research teams apply the correct methods to mitigate the pitfalls of high throughput technologies. A major strength of high throughput technologies that facilitates the discovery of new biomarkers is their capacity to screen without bias for disease associations because they do not limit the investigation to a preexisting assumption or hypothesis. Furthermore, high throughput methods are unprecedented in terms of the tremendous amount of data and knowledge they can generate in a short period of time, often leading to novel hypotheses. Big data analyses frequently reveal important general trends in a population or phenotype that would be impossible to recognize with classical, low-throughput experimental methods, even though the latter can provide more mechanistic insight.

Ultimately, the question should not be which method is more valuable—high throughput or mechanistic studies—but how these approaches best complement one another in helping to answer important scientific questions. A state-of-the-art
example of this integrative approach is the work published by Veerman et al\textsuperscript{1} published in the current issue of *Circulation Research*. The project builds on a previous discovery by their team published in *Nature Genetics* in 2013. Through genomewide association studies, the group had identified 2 significant association signals in patients with Brugada syndrome at the SCN10A locus (rs10428132) and near the HEY2 gene (rs9388451). Replication in an independent cohort confirmed both signals and identified 1 additional signal in SCN5A. The cumulative effect of all 3 loci on disease susceptibility was very large \( (P \text{ trend}=6.1\times10^{-41}) \). Whereas SCN10A and SCN5A encode sodium channels, HEY2 (also called HESR2, HRT2, and CHF1) encodes a basic helix-loop-helix transcription repressor that is expressed in the cardiovascular system.

Given its newly discovered association with HEY2 and findings that HEY2 regulates cardiac electric activity on a transmural level, it was hypothesized that Brugada syndrome may originate from altered transcriptional programming during cardiac development. Veerman et al\textsuperscript{1} used transcriptomic studies in human hearts and electrophysiological studies in HEY2 heterozygous knockout mice (HEY2+/−) to better understand the mechanism of the association of 6q22.31 with Brugada syndrome. They made the fascinating discovery that HEY2 plays an important role in the normal electrophysiological gradient in the ventricle. Their data suggest that a genetic variation at 6q22.31 (rs9388451) is associated with Brugada syndrome through HEY2-dependent alteration of ion channel expression across the cardiac ventricular wall.

First, they performed expression quantitative trait locus effect analysis in human left ventricle. All 8 protein-coding genes within 1 megabase of rs9388451 were assessed for possible expression quantitative trait locus effects using gene expression data of human left ventricular tissue of 190 deceased donors (Genotype-Tissue Expression database). Among the 8 genes, only HEY2 expression showed indeed significant association with rs9388451, making it the most likely driver of the 6q22.31 genome wide association studies locus, where the Brugada syndrome risk allele is associated with increased expression of HEY2. Because HEY2 is a transcription factor, the group then hypothesized that association of the locus with BrS involves ion channel gene expression. Using gene expression data from the Genotype-Tissue Expression database of human left ventricular tissue, Veerman et al\textsuperscript{1} discovered that the strongest association of all 15,617 transcripts tested was with the transcript of KCNIP2, which was positively correlated with that of HEY2. Similar to HEY2, KCNIP2 shows a pronounced increasing expression gradient from subendocardium to subepicardium. KCNIP2 encodes potassium channel–interacting protein 2, the \( \beta \) subunit of the voltage-gated Kv4 channel underlying \( I_{\text{s}} \). These data suggest that HEY2 is a relevant transcription factor to control cardiac electric function in humans.

The group validated these findings through an elegant approach using gene expression analysis in HEY2+/− mice. Their results confirmed the HEY2-dependent effect on Kcnip2 expression levels rather than noncausal association and in addition showed the effect of HEY2 on the pore-forming subunit of the \( I_{\text{s}} \) channel, Kcnd2. Finally, action potential recordings and patch-clamp technique revealed that the transmural difference of \( \text{V}_{\text{m}} \) and action potential duration in myocytes from endo- to epicardium is diminished in Hey2+/− mice because of increase in epi in Hey2+/− compared with wild-type mice. The authors concluded that heterozygous loss of HEY2 affects both depolarization and repolarization specifically in the epi layer.

The findings by Veerman et al\textsuperscript{1} give mechanistic insight on how the lead SNP rs9388451 at chromosome 6q22.3 near the HEY2 gene is associated with HEY2 transcript abundance in the human ventricle providing strong evidence that HEY2 is the causal gene at this locus. Coexpression analysis identified KCNIP2, encoding a subunit of the \( I_{\text{s}} \) channel, as the most significantly correlated gene, therefore, suggesting HEY2 as a transcription factor affecting the control of cardiac electric function in humans. In this article, the authors have successfully shown that HEY2 plays a role in the normal electrophysiological gradient in the ventricle. The group has applied sophisticated methods, including patch clamping of mouse myocytes, to determine that genetic variation at 6q22.31 (rs9388451) is associated with Brugada through HEY2-dependent alteration of ion channel expression across the cardiac ventricular wall.

### Disclosures

None.

### References


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