The Blueprint Genetics Arrhythmia Panel is an efficient diagnostic tool for hereditary arrhythmia disorders. The Panel includes majority of reported ventricular arrhythmia-associated genes. These genes code ion channel proteins defective in long QT syndromes (LQTS), short QT syndromes (SQTS), Brugada syndrome (BrS), and catecholaminergic polymorphic ventricular tachycardia (CPVT). Our panel also involves diagnostics for arrhythmogenic right ventricular cardiomyopathy (ARVC). These diseases carry a strong risk for sudden cardiac death. The Arrhythmia Panel is a powerful diagnostic solution whenever other clinical assessments have failed to narrow reliably the exact diagnosis. In addition to patient diagnostics, the Arrhythmia Panel has potential in forensic autopsies in cases where reason for sudden cardiac death is investigated.

The Arrhythmia Panel provides a high quality read-out of all clinically relevant genes associated with channelopathies and ARVC. Our OS-Seq™ technology provides high coverage clinical grade sequencing and enables reliable diagnostics for patients with significantly lower costs and faster turnaround time (basic service TAT 21 days and express service TAT 7-10 days). The Arrhythmia Panel has undergone rigorous validation process during its evolution at Blueprint Genetics. Our unique sequencing technology combined with in-house built bioinformatics pipeline with channelopathy and ARVC mutation and knowledge database, together with our experienced team of geneticists and clinicians, forms the most efficient hereditary cardiovascular disease diagnostics service in the market. Our variant classification schemes and clinical interpretation processes have been developed and validated with thousands of patients with hereditary cardiovascular disease. Blueprint Genetics publically shares all classified variants identified in cardiomyopathy and channelopathy patients to improve future diagnostics (ClinVar; http://www.ncbi.nlm.nih.gov/clinvar/). Our mission is to improve the quality of diagnostics and management of hereditary arrhythmia disorder patients and their families.

Genes Covered by Panel

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<tr>
<td>AKAP9</td>
<td>CALM2</td>
<td>DSC2</td>
<td>KCND3</td>
<td>KCNJ2</td>
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<td>KCNE1</td>
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<td>DSG2</td>
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<td>CAV3</td>
<td>DSP</td>
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<td>KCNQ1</td>
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<td>SCN4B</td>
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<td>CTNNA3</td>
<td>GPD1L</td>
<td>KCNE3</td>
<td>LDB3</td>
<td>RANGRF</td>
<td>SCN5A</td>
<td>TRPM4</td>
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<td>CALM1</td>
<td>DPP6</td>
<td>JUP</td>
<td>KCNH2</td>
<td>LMNA</td>
<td>RYR2</td>
<td>SLMAP</td>
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The test covers 47 genes with evidence of association with hereditary arrhythmia disorders. All protein coding exons and exon-intron boundaries are covered. Sequencing is also targeted to other regions with reported pathogenic or likely pathogenic mutations.

Description of Test

Blueprint Genetics offers a comprehensive Arrhythmia Panel that covers the genes associated with hereditary arrhythmia disorders. The genes are carefully selected based on the existing scientific evidence, our experience and existing mutation databases.

Coverage

In our latest validation of the Arrhythmia Panel, median sequencing depth in the target region was 693x on a nucleotide level and 99.83% of the nucleotides had at least 15x coverage.

Analytical validity

Analytical validation is a continuous process at Blueprint Genetics. Our mission is to improve the quality of the sequencing
process and our standardized validation process follows each modification. In our latest validation the Arrhythmia Panel had sensitivity of 0.991 and specificity of 1.000 to detect single nucleotide polymorphisms. Our panel had also good performance to detect insertions and deletions. Sensitivity was 1.000 for both indels ranged 1-5 bp and 6-19 bp. This panel has not been validated to identify larger deletions, insertions or complex rearrangements.

Diagnostic yield

Blueprint Genetics provides genetic diagnostics for hundreds of hospitals and clinics around the world. The diagnostic yield varies substantially between hospitals and countries. Diagnostic yield (detecting a pathogenic or likely pathogenic mutation) with Arrhythmia Panel is currently 24.0% in our laboratory. Please review our variant interpretation strategy here.

Bioinformatics

We have developed a unique ventricular arrhythmia sequence analyzer and interpretation pipeline. In the core of our bioinformatics lies our in-house created and curated cardiomyopathy and channelopathy mutation database, which is a synthesis of over 2000 original publications on hereditary arrhythmia disorders and existing mutation databases. The analysis pipeline includes rigorous quality control steps to ensure validity and consistency of results, and incorporates gene variability data from thousands of publicly available human reference sequences in order to eliminate false positive findings and deliver data of the highest relevance. Reference databases currently used are 1000 Genomes (http://www.1000genomes.org), NHLBI GO Exome Sequencing Project (ESP; http://evs.gs.washington.edu/EVS), Exome Aggregation Consortium (ExAC; http://exac.broadinstitute.org). In addition, we apply the following \textit{in silico} variant prediction tools in our analyses: SIFT (http://sift.jcvi.org), Polyphen (http://genetics.bwh.harvard.edu/pph2/) and Mutation Taster (http://www.mutationtaster.org).

Through our online ordering and statement system, Nucleus, the customer can access specific details of the analysis of the patient. This includes coverage and quality specifications and other information on the analysis. This represents our mission to build fully transparent diagnostics where customer has easy access to the details of the analysis.

Clinical Interpretation

In addition to our cutting-edge proprietary sequencing technology and bioinformatics pipeline, BpG also provides the customers with the best-informed clinical statement on the market. Clinical interpretation requires fundamental clinical and genetic understanding on hereditary cardiovascular diseases. At Blueprint Genetics our geneticists and cardiologists, who together, evaluate the results from the sequence analysis pipeline in the context of phenotype information provided in the requisition form, prepare the clinical statement. Our goal is to provide clinically meaningful statements that are understandable for all medical professionals, even without training in genetics.

Variants reported in the statement are always classified using the Blueprint Genetics Classification Scheme modified from the ACMG guidelines (Richards et al. 2015), which has been developed by evaluating existing literature, databases and with thousands of clinical cases analyzed in our laboratory. Variant classification forms the corner stone of clinical interpretation and following patient management decisions. Our statement also provides allele frequencies in existing databases and \textit{in silico} predictions. We also provide PubMed IDs to the articles or submission numbers to public databases that have been used in the interpretation of the detected variants. In our conclusion, we summarize all the existing information and provide our rationale for the classification of the variant.

A final component of the analysis is the Sanger confirmation of variants classified as likely pathogenic or pathogenic. This does not only bring confidence to the results obtained by our NGS solution but establishes the mutation specific test for family members. Sanger sequencing is also used occasionally with other variants reported in the statement. In the case of variant of unknown significance (VUS) we do not recommend risk stratification based on the genetic finding. Furthermore, in the case of VUS we do not recommend use of genetic information in patient management or genetic counseling. For some cases Blueprint Genetics offers a special service to investigate the role of identified VUS.

We constantly follow the development on the field of hereditary cardiovascular diseases and adapt new relevant information and findings to our diagnostics. Relevant novel discoveries can be rapidly translated and adopted into our diagnostics without delay. These processes ensure that our diagnostic panels and clinical statements remain the most up-to-date and relevant on the market.

Sample Requirements

- 3ml of EDTA blood
- Purified DNA 10μg
About the Disorder

All the diseases included in the BpG Arrhythmia Panel manifest with similar symptoms such as palpitations, pre-syncope/syncope or sudden cardiac death leaving the differential diagnostic challenges entirely to cardiology investigations. Although clinical evaluation combined to rest-, stress- and Holter-ECG, and echocardiography is considered helpful in diagnostics, they rarely offer definitive diagnosis of specific arrhythmia disease. Effective and safe arrhythmia treatments have been challenging to develop as severe arrhythmias represent a heterogeneous group of diseases with diverse cellular mechanisms. The role of molecular genetic diagnostics is increasing and could dominate the diagnostics, prognostics, and treatment of hereditary arrhythmia diseases.

Until today, more than 2,200 channelopathy mutations have been characterized from >35 genes. These genes encode proteins constituting ion channel structures such as their subunits and other interacting proteins. Gene diagnostics of LQTS relies largely on sequencing KCNQ1, KCNH2 and SCN5A genes respective to LQTS 1-3 that covers more than 75% of all LQTS patients. More than 1,300 LQT mutations have been discovered only from 58 exons in these three genes. Rare LQT syndromes, namely LQTS 4-13, have alone only relative significance for differential diagnostics but as a group they constitute marked entity with >200 reported mutations in 10 genes. Moreover, Crotti et al. discovered novel genes related to long QTc when they described CALM1 and CALM2 mutations in infants with recurrent cardiac arrest and dramatically prolonged QTc. Mutations in any of the rarely affected genes may modify disease severity in any common LQTS. Importantly, detecting different LQTS gene mutations enable gene specific lifestyle modification including avoidance of certain arrhythmic triggers and have significance in therapy decisions (Cerrone & Priori 2011). Moreover, certain polymorphisms in NOS1AP, which is not a LQT gene, predict higher mortality in LQTS patients but also associate to increased QTc duration and acquired LQTS in healthy population (Tomás et al. 2010, Jamshidi et al. 2012). Multigenetic nature of LQTS has been recognized and holds prognostic value. Carriers of two or more mutations were detected in 10% of Japanese pediatric LQTS patients and they had longer QTc, higher risk for arrhythmias and death compared to patients with single mutation (Izumi et al. AHA 2012 abstract 16835). Similar findings have been also reported by Tester and coworkers (Tester et al. 2005). This is indicative that multigene panel may be imperative for comprehensive risk assessment and well-informed clinical decisions.

Until today, causative mutations have been characterized from 18 genes in BrS, of which SCN5A is the most important. Gene test is positive only in one fifth of the patients fulfilling BrS clinical criteria. Disease penetrance is not yet well defined and prognosis is variable between BrS cohorts, possible reflecting lack of easily interpretable diagnostic criteria. In near future, molecular genetics is likely to increase uniformity of this important clinical entity. Of all SCN5A mutation carriers 25% have typical BrS ECG finding and 80% will develop it during flecainide challenge, which is also predictive for sudden cardiac death in BrS (Kaplinger et al. 2010). Sequencing large RYR2 gene (105 exons) offers gene diagnosis in >65% of CPVT patients. CPVT is also caused by CASQ2, TRDN and CALM1 mutations and CPVT-like disease occur with KCNJ2 and ANK2 mutations. SQTS and diseases reminding it are linked to mutations in KCNHH2, KCNQ1, KCNJ2, CACNA1C, CACNA2D1 and CACNB2 genes. In addition to classic ion channels disorders, severe arrhythmias appear in cardiomyopathies, of which ARVC is important as it lacks a clear morphologic sign. Therefore, it constitutes a major differential diagnostic challenge from channelopathies.

Large sequencing panels are essential in clinical diagnostics of channelopathies as there is substantial overlap with genetics, as well as clinical features, of different forms of classic arrhythmia diseases. KCNJ2 gene is found in LQT7, SQTS, BrS and idiopathic ventricular tachycardia, and SCN5A associates to LQT3, BrS, atrioventricular block, sick sinus syndrome and dilated cardiomyopathy. Comparing to single gene PCR tests, targeted multigene sequencing strategies enable efficient diagnostics and assessment of multigenic disease modulators. Therefore, genetic testing is moving from inefficient single gene testing to large gene panels covering most of the genes associated with severe hereditary arrhythmia diseases.

The benefits of genetic diagnostics in arrhythmia diseases has reached the level where international guidelines are recommending ‘effective’ gene diagnostics for these patients (Ackerman et al. 2011). Effective means that testing should provide either diagnostic or prognostic information or influence therapy selection. These criteria are best achieved by covering majority of causative genes using targeted sequencing (Ashley et al. 2012). Genetic diagnostics is an important component of patient’s clinical evaluation and assessment of family members. It has been recommended that previous research laboratory-based genetic tests should be now repeated and confirmed by current genetic tests that are superior to the research-based genetic tests performed in the 1990s and early 2000s (Napolitano et al. 2005; Taggart et al. 2007; Ackerman et al. 2011). Genetic diagnostics is becoming one of the most definitive and powerful diagnostic and prognostic tools in the field of channelopathies. All patients who undergo genetic testing should receive pre-test and post-test genetic.
counseling so that they can understand the implications of testing.

References

Ackerman, M.J. et al. HRS/EHRA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies: this document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA). *Europace* 2011, 13(8), 1077–1109. Link.


