Analytical validation and clinical performance of a next-generation sequencing panel for inherited cardiovascular diseases.

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Purpose

Inherited cardiovascular diseases (ICVD) are characterized by extreme clinical and genetic heterogeneity. Comprehensive molecular testing conducted by traditional Sanger sequencing is limited by high cost and low yield. Next-generation sequencing (NGS) allows the simultaneous study of multiple genes, thereby bypassing these limitations. We compared the analytical and clinical performance of a NGS library with the Sanger method for genetic diagnosis of ICVD.

Methods

We designed a NGS library of 126 genes associated with development of ICVD (cardiomyopathies, channelopathies, familial aortic diseases and familial dyslipidemia). We analyzed with an Illumina Genome Analyzer IIx 44 unrelated ICVD patients, 31 of them previously studied by the Sanger method. We compared the results obtained with both techniques.

Results

The average coverage with this NGS library was 137x, 88% of the bases had an acceptable depth of coverage (>15x). The sensitivity and specificity in areas of good depth of coverage was 100% (global sensitivity and specificity: 96.6% and 99.99% respectively). This technique allowed us to shed light on several complex ICVD cases. In 2 patients with hypertrophic cardiomyopathy (HCM), a mutation in genes related to Noonan/LEOPARD syndromes was identified (in 1 of these families a previously identified sarcomeric mutation did not explain the phenotypic variability among carriers). One additional patient, diagnosed with Costello syndrome, was found to have a R4F1 mutation previously associated with Noonan syndrome. A case of HCM with a history of syncope during febrile episodes harboured a SCNA mutation previously related to Brugada syndrome. In one case of sudden death attributed to right coronary hypoplasia and with a doubtful familial history of HCM, a frameshift mutation in PKP2 associated with arrhythmogenic cardiomyopathy was found. An unexpected sudden death of a young man, occurred at rest and without evidence of cardiac diseases in the family, was later clarified by a novel RyR2 mutation. In a family with dilated cardiomyopathy and apical hypertrobasculaition, a novel TTN truncation was identified. In a patient with clinical suspicion of Marfan syndrome a FBN1 truncation was found—the same mutation had not been identified by Sanger sequencing. All of the mutations that were found were confirmed by the Sanger sequencing method.

Conclusions

Our NGS library had 100% sensitivity and specificity in regions of good coverage. The possibility to analyze large numbers of genes in a single study allowed the molecular diagnosis of a significant number of cases. Sanger sequencing in areas of low coverage and for confirmation of mutations is required for the clinical application of NGS techniques.