Genetic screening in pediatric cardiomyopathy using microarray capture and high-throughput sequencing

Tam Le[1], Mehdi Keddache[2], Patrick Putnam[2], Stephanie Ware[3]

[1]University of Cincinnati College of Medicine, [2]Cincinnati Children's Hospital Medical Center Division of Human Genetics, [3]Cincinnati Children's Hospital Medical Center Division of Molecular Cardiovascular Biology

Cardiomyopathy is a clinically and genetically heterogeneous disease that affects the cardiomyocyte. Pediatric cardiomyopathy (PCM) is a genetic disease associated with significant morbidity and mortality. Treatment based on underlying etiology improves clinical outcome, however up to 70% of PCM patients are considered idiopathic. The ability to perform genetic analyses for precise diagnosis has been limited by the heterogeneity of genes and lack of prevalence data. Therefore, there is a need for more comprehensive and efficient genetic screening for cardiomyopathy, particularly in the pediatric population. To develop this technology, we performed a pilot study using microarray-based genomic selection and massively parallel sequencing. A custom array consisting of the coding exons of 110 genes known to cause PCM was developed and used to test genomic DNA from 3 patients. Targeted enrichment was performed via hybridization capture to the custom array (sequence capture) followed by high-throughput sequencing. In addition, PCR enrichment followed by high-throughput sequencing was performed on 30 of the genes allowing for direct comparison of the 2 methods. The sequence capture method and PCR enrichment method interrogated 1,163,795 and 381,466 base pairs of DNA, respectively. On average per patient, 1196 of 1228 exons (97.4%) had an average coverage depth of at least 20x from sequence capture, and 835 exons (68%) had every base pair with a coverage depth >20x. Between method heterozygous base pair agreement was 93% in coding regions, with sequence capture appearing to have more sensitive heterozygous detection rates. An average of 788 variants was found per patient. For the 30 genes targeted by both methods, >20x average depth was seen in 98.2% of exons in sequence capture and in 97.7% of amplicons from PCR. We conclude that sequence capture allows for efficient enrichment of target genes for large-scale sequencing and holds promise for clinical diagnostic applications. By eliminating the front-end development required for PCR optimization, both time and cost can be reduced therefore making comprehensive genetic tests possible for PCM and other genetically heterogeneous diseases. In addition to identifying causative mutations with this method, further investigation of variants will allow genotype-phenotype correlation and investigation of genetic modifiers.