**Evaluation for Heritable Cardiovascular Disorders using Targeted Next-Generation Sequencing on Formalin-Fixed Paraffin Embedded Tissue**

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**BACKGROUND**

Heritable cardiovascular (CV) disorders are diverse and include channelopathies (CP), cardiomyopathies (CM) and connective tissue disorders (CTD). Because they are associated with catastrophic CV events, identification of those at-risk is paramount. While a strong family history can prompt screening, post-mortem genetic testing is often lacking. Furthermore, ideal specimens (i.e. whole blood (WB)) often are note retained at autopsy. Ubiquitous use of formalin-fixed paraffin embedded tissue (FFPET) in autopsy makes it an ideal source for interrogation; however its use in traditional sequencing is limited owing to genomic integrity. Targeted next generation sequencing (NGS) technology offers the ability to circumvent such limitations. The primary aim was to evaluate the efficacy of testing FFPET for heritable CV disorders using NGS.

**METHODS**

Paired FFPET (heart) and blood (WB or dried blood spot (DBS)) samples were obtained from 13 patients. 7 were random autopsy samples, 3 were autopsies with clinical phenotype of a heritable CV disorder but unknown genotype, and 3 were surgical samples from patients with genotype-confirmed hypertrophic cardiomyopathy (HCM) on blood. Extracted genomic DNA underwent Agilent SureSelect targeted capture of 101 genes associated with CP, CM, and CTD, followed by sequencing on the Illumina MiSeq. Quality metrics were compared. Variants were classified by consensus.

**RESULTS**

In quality comparisons of 10 cases using the CM discovery panel (63 genes), there were no significant differences between FFPET, WB and DBS in average percent mapped reads (60 vs 55 vs 60%), average depth of coverage (1259 vs 1425 vs 1490X), and Phred quality scores (all >30). Analysis of surgically derived FFPET from HCM patients, confirmed pathogenic mutations in *TPM1*, *MYH7*, and *MYBC3*, previously detected on WB. In the 3 autopsy cases with unknown genotype, testing on FFPET identified pathogenic mutations in *FBN1*, *RAF1*, and *MYBC3*, consistent with the clinical phenotype of Marfan syndrome, Noonan syndrome, and HCM, respectively. There was 100% concordance for all genotype calls, with no false positives or false negatives.

**CONCLUSIONS**

The results of this validation study show similar performance characteristics for NGS of DNA derived from FFPET (less than 15 years old), WB, and DBS, in the evaluation of inherited CV disorders. Such has important implications for molecular genetic testing when only FFPET is available. Additionally, interrogation of such tissues has important implications for extensive genotype-phenotype correlation in archival tissue, which is currently ongoing.