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High-throughput Allele-Specific Expression Analysis Can Detect Allelic Imbalance in Clinical Patient Samples as a Proxy for Quantitative Measures of Gene Expression

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Abstract:
RNA analysis has recently emerged as a powerful tool for variant classification. However, most RNA analysis performed on clinical RNAseq data involves the detection of qualitatively different transcripts produced as a result of aberrant splicing. Identifying quantitative differences in the amount of transcript produced from an allele at the gene-level is a more difficult task and has historically relied upon quantitative real-time PCR (qRT-PCR) experiments. In this study we used the phASER tool on high-throughput clinical paired DNAseq and RNAseq samples to detect allelic differences in gene expression caused by *cis*-acting variants. phASER employs RNASeq reads to phase heterozygous variants (called from DNASeq data) relative to one another within a given gene. Allele-specific expression (ASE) analysis is then applied to evaluate the relative expression of the two alleles present at heterozygous loci to produce a single expression measurement for each haplotype. To validate this method in our clinical samples, we performed ASE analysis on samples from two patients heterozygous for a known pathogenic variant in the 5`UTR of the *MLH1* gene (*MLH1* c.-27C>A) that has been shown to result in reduced promoter activity. As predicted, this ASE analysis was able to detect significant ASE of *c.-27C>A* and a downstream benign polymorphism (*c.655A>G*) in both probands and not in controls. We then performed this analysis on two novel variants impacting the canonical donor splice sites of the noncoding first exons of *BRCA2* (*BRCA2* c.-40+2T>C) and *TP53* (*TP53 c.-29+1G>C*). We suspected these variants may cause ASE based on literature reports demonstrating ASE for other variants impacting the same canonical donor sites. We identified two patients heterozygous for the *BRCA2* variant and one for the *TP53* variant and were indeed able to show significant ASE for benign downstream heterozygous polymorphisms in the 5`UTR (*c.-52A>G, c.-26G>A*), exon 10 (*c.1114A>G*), exon 11 (*c.3396A>G*), and exon 14 (*c.7242A>G*) of *BRCA2*, and in exon 3 of *TP53* (*c.215C>G*). ASE was not detected in controls. Although preliminary, these data indicate that ASE analysis using phASER is capable of detecting RNA allelic imbalance associated with variants identified through clinical DNA samples. These results raise the tantalizing possibility that high-throughput ASE analysis could be used to identify patients with allele-specific expression of unknown origin. Future directions of this study include validating the method for additional variants and variant types and assessing potential candidate variants in a variety of genes using our in-house database of >300000 paired DNAseq and RNAseq samples.