Reclassification of splicing VUS in neurological disease genes via RNA-seq

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Clinical genetic testing for neurological disorders is becoming widely available. However, its clinical utility is diminished by a large number of variants of unknown significance (VUS) detected in patients. In particular, the interpretation of splicing VUS poses a significant challenge due to their unknown effects on splicing. In this study, we sought to determine whether RNA-seq analysis can effectively reduce the number of splicing VUS in genes associated with neurological disorders. VUS that might affect splicing were identified in patients who ordered neurology genetic testing (single gene, multi-gene panel, or exome). VUS detected in genes expressed in bone marrow were selected for RNA-seq analysis. Blood was collected from the patients and healthy controls. RNA extracted from blood was analyzed using massively-parallel RNA-seq of cloned RT-PCR products (CloneSeq). Based on splicing events detected in RNA-seq, 86% (19/22) of the variants changed classification from VUS: seven were reclassified to pathogenic/likely pathogenic variants, while twelve were likely benign variants. Those variants that became clinically actionable included: four alterations affecting guanine at the last nucleotide of an exon (*ANDP* c.201G>C, *ANKRD11* c.226G>A, *NF1* c.586G>A, and *TSC1* c.2041G>A), two small deletions in introns (*FMR1* c.104+3_104+6delAAGT and *WDR45* c.976+5_976+10delGTGGGA), and one single nucleotide substitution in an intron (*ATRX* c.4957-3A>G). Of the three variants that remained VUS after RNA-seq analysis, two were missense alterations that had no splicing impact, but may still affect protein function. The remaining VUS was the only variant that had ambiguous RNA evidence. In summary, RNA-seq analysis provided useful evidence for all but one VUS and resulted in reclassification of 86% of the variants that would have remained VUS otherwise. Although RNA-based analysis may be limited to genes expressed in the blood or other readily obtainable tissues, our data indicates that RNA-seq analysis can help clarify pathogenicity of many splicing VUS identified in genes associated with neurological disorders.