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Assaying the Splicing Activity of Novel Human Disease Variants of U4atac snRNA

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Abstract

In eukaryotes, pre-messenger RNA (pre-mRNA) splicing is an essential process in gene expression. Splicing is carried out by a dynamic multi-megadalton RNA-protein complex known as the spliceosome. Sequential transesterification reactions catalyzed by the spliceosome convert pre-mRNA to mRNA by removing the intervening sequences (introns) and joining the coding sequences (exons) together. Small nuclear RNAs (snRNAs) are essential splicing factors. Biallelic mutations of the human *RNU4ATAC* gene, which codes for U4atac snRNA, have been identified in patients diagnosed with Microcephalic Osteodysplastic Primordial Dwarfism type I (MOPD I). MOPD I is an autosomal recessive disorder characterized by extreme intrauterine growth retardation, multiple organ abnormalities, and typically early death. The mutations that have been studied biochemically reduce U4atac snRNA function and impair minor class (U12-dependent) intron splicing. Four novel patient mutations, 37 G>A, 46 G>A, 48 G>A and 118 T>C, have recently been discovered. To evaluate the functional effects of these newly discovered mutations on U12-dependent splicing, we incorporated each of these mutations into a modified human *RNU4ATAC* gene construct by site directed mutagenesis. Following verification of the mutations by DNA sequencing, we prepared DNA for use in an *in vivo* splicing assay that is based on genetic suppression. These mutations are expected to affect the binding of proteins to U4atac snRNA that are important in formation of the catalytically active form of the spliceosome. We do not yet know how the consequent defective U12-dependent splicing affects gene expression and yields the MOPD I disease pathologies, but this study allows us to better understand the mechanistic basis of MOPD I and will serve as an important foundation for further studies and possible therapeutic intervention in the future.