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Disruption of Minor Intron Splicing by Disease-associated Mutations in U12 snRNA

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Disruption of Minor Intron Splicing by Disease-associated Mutations in U12 snRNA

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Abstract

In eukaryotic gene expression, the removal of introns from pre-mRNA is an essential function carried out by spliceosomes. Human cells have two distinct spliceosomes: U2-dependent and U12-dependent. U2-dependent spliceosomes, or major spliceosomes, remove over 99% of introns, whereas U-12 dependent spliceosomes remove less than 0.5% of introns. Mutations in spliceosome machinery are a notable cause of human disease. In particular, mutations to components unique to the minor spliceosome demonstrate that it plays a vital role in human development. Mutations in the gene encoding the minor spliceosomal small nuclear RNA (snRNA) U12, *RNU12*, are associated with two rare developmental disorders: 1) CDAGS syndrome (craniosynostosis and clavicular hypoplasia; delayed closure of the fontanelles, cranial defects, and, in some patients, deafness; anal anomalies; genitourinary malformations; and skin eruption) and 2) early onset cerebellar ataxia. Recent work identified rare biallelic variants in *RNU12* as the likely cause of CDAGS syndrome, while a single homozygous mutation was identified as the likely cause of early onset cerebellar ataxia. Mutations associated with these diseases are clustered in or near the stem-loop III of U12 snRNA, with three of the mutations located in the Sm protein binding site. Further investigation of the mutation associated with early onset cerebellar ataxia suggests that mutations in *RNU12* disrupt U12 snRNA function through the destabilization of the 3' stem-loop, which precedes overall destabilization of the U12 snRNA. Using our *in vivo* orthogonal splicing assay, we quantified the effects of pathogenic *RNU12* mutations on U12-dependent splicing. Splicing activity varies depending on the location of the mutation. Splicing was significantly reduced among three U12 variants located in the Sm protein binding site. Sm proteins are responsible for proper assembly of snRNPs (small nuclear ribonucleoproteins) prior to pre-mRNA splicing. Similarly, splicing activity was substantially impaired in the mutation located three nucleotides downstream of the U12 snRNA sequence. While the impact of this variant on the minor spliceosome complex is unclear, one possibility is that this mutation could affect 3' end processing of U12 snRNA. The effects of the two variants within stem-loop III of U12 snRNA differ. Whereas splicing activity was considerably diminished with the 86G>A mutation, activity in cells with the 84C>T mutation was near wild-type levels. It is presumed that these mutations affect the secondary structure of the snRNA due to the fact that they are located at the base of stem-loop III, farther away from the U12-65K protein binding site. Further work remains to fully define the mechanisms of splicing impairment that are the result of disease-associated mutations in U12 snRNA.

Introduction

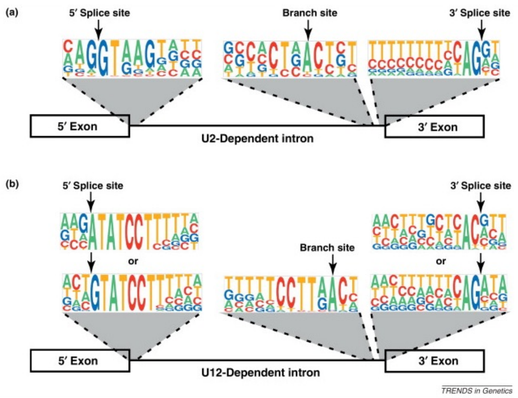

Figure 1. Splice-site consensus sequences for U2-dependent (a) and U12-dependent introns (b). Distinct splice-site and branch site sequences exist for each type of spliceosome. Graphical representations of the consensus sequences are shown within the boxes. The size of each letter represents the frequency of each base at each position over all introns and the bases are ordered by frequency from top to bottom. U2-dependent introns almost always begin with the dinucleotide GT and end with AG, whereas U12-dependent introns can have either AT and AC termini or GT and AG termini (Padgett, 2012).

Major and minor spliceosomes differ by:

- snRNP (small nuclear ribonucleoprotein) components
- Composition of introns in human genome
- Conservation of splice-site and recognition sequences

Mutations to minor spliceosome components are associated with:

- Microcephalic Osteodysplastic Primordial Dwarfism Type I (MOPD1)
- Roifman Syndrome (RS)
- Lowry Wood Syndrome (LWS)
- Myelodysplastic syndrome (MDS)
- Isolated Growth Hormone Deficiency (IGHD)

Introduction

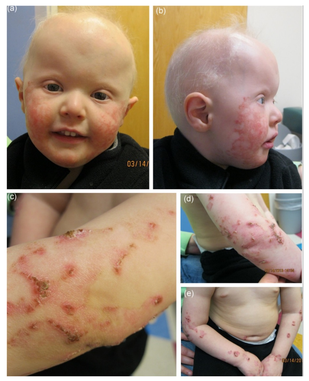

Figure 2. Affected individual with CDAGS syndrome. Patient shows characteristics of CDAGS syndrome, including craniosynostosis and skin eruptions throughout his body. He also had alopecia of the arms and legs and no eyebrows or eyelashes (Xing et al., 2021).

Clinical features of cerebellar ataxia include:

- abnormal development or degeneration of the cerebellum
- hypotonia at infancy
- delayed motor development
- abnormal gait
- speech and learning difficulties

Mutations in U12 snRNA cause

- CDAGS Syndrome
- Early Onset Cerebellar Ataxia (EOCA)

Characteristics of CDAGS syndrome:

- Craniosynostosis and clavicular hypoplasia
- Delayed closure of the fontanelles and cranial defects
- Anal anomalies
- Genitourinary malformations
- Skin eruption, including porokeratosis

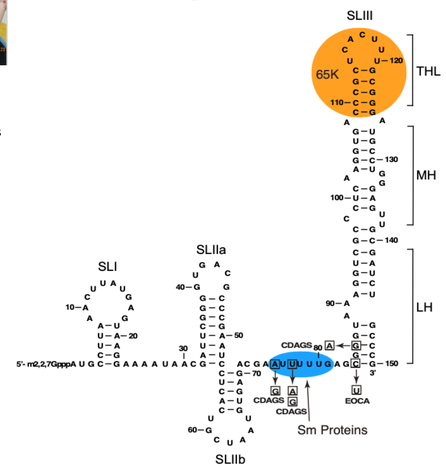

Figure 3. Mutations in U12 cause CDAGS Syndrome and Early Onset Cerebellar Ataxia. Schematic of U12 snRNA and its interactions with essential spliceosomal proteins. The nucleotides mutated in CDAGS syndrome and early onset cerebellar ataxia are boxed and labeled. The colored circles showing protein binding sites correlate with the colors on the graph in figure 5.

Results

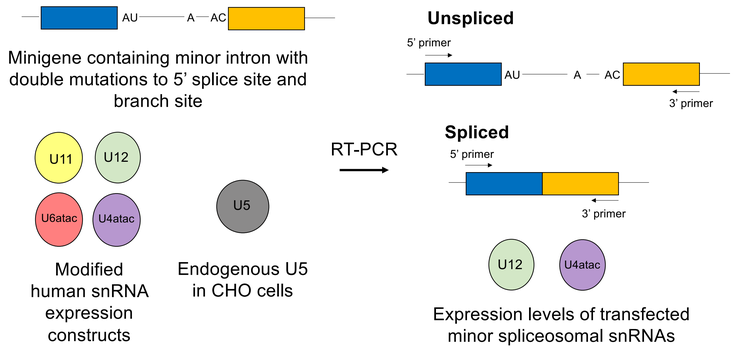

Figure 4. Representation of the orthogonal spliceosome that allows for genetic manipulation of the minor spliceosome. The left side displays the components that were transfected into Chinese hamster ovary cells. Complementary mutations exist within the mutated minigene and the modified human snRNA expression constructs in order to prevent the transfected minigene from being spliced by the endogenous spliceosome and the transfected snRNAs from splicing endogenous genes. The right side displays the unspliced and spliced minigenes, which are the predicted products of the orthogonal spliceosome assayed with RT-PCR. The levels of the transfected minor spliceosomal snRNAs can also be measured via RT-PCR.

Results

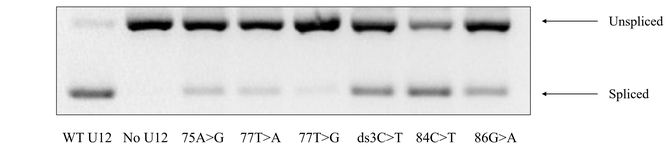

Figure 5. Agarose gel displays splicing activity resulting from orthogonal splicing assay. Variants show less splicing activity compared to wild-type.

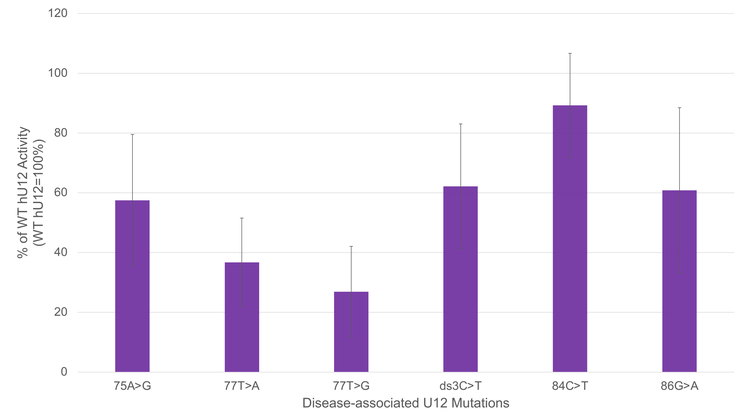

Figure 6. U12 mutations identified in patients disrupt U12-dependent splicing in vivo. Chinese hamster ovary cells were transfected with the components of the orthogonal splicing assay, including U12 that contained mutations found in patients with CDAGS and cerebellar ataxia. The cells were harvested and the RNA was extracted following a 48 hour incubation period. After cDNA synthesis, RNA splicing products were analyzed by agarose gel electrophoresis and quantified in comparison to wild-type. The average and standard deviation of four transfections were plotted.

Summary

- Most U12 snRNA mutations significantly reduce minor splicing
- Splicing activity varies based on the location of the mutation
  - Sm binding site mutations are the most severe
  - Mutations adjacent to stem-loop III are mild
  - Mutation downstream of U12 snRNA sequence has an effect between mild and severe
- Mutations in minor spliceosome components have severe effects on human development

References

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