

Scientific Poster Session



Monday, October 20 | 5:15 – 6:15 pm EST

Tackling RNA-caused Diseases: A Focus on RNU4-2

Mutations in U-rich RNAs are increasingly recognized as drivers of neurodevelopmental disorders. Although their short length and structured nature presents challenges, ASO technology offers a unique therapeutic opportunity. This work focuses on a recently identified mutation in RNU4-2, highlighting its functional characterization and n-Lorem's therapeutic strategy to address its toxic effects.

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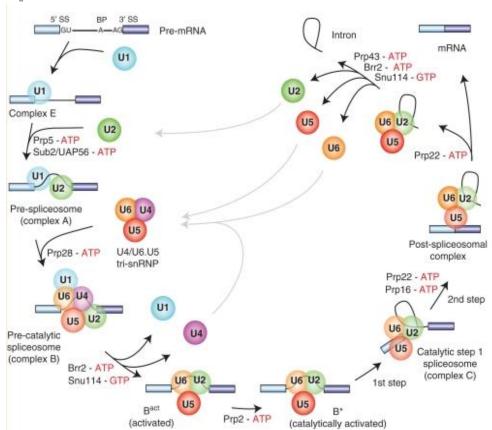
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Introduction: Splicing is a crucial process



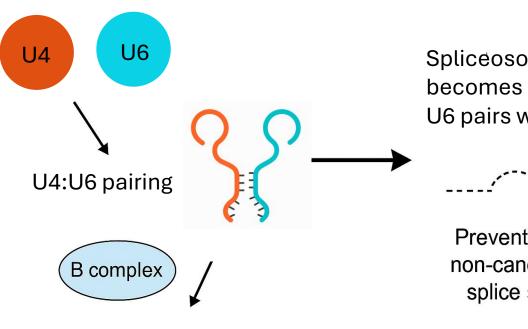


- Splicing is very important and very well-understood
- snRNAs are a subcategory of non-coding RNAs that are key components of the spliceosome.
- They are means of Quality Control as they ensure proper splicing
- The spliceosome assembly: Five snRNPs (U1, U2, U4, U5 and U6) mainly involved each containing a unique snRNA, and a total of approximately 100 additional proteins



RNU4-2 is a Tiny RNA with a Big Role





U4:U6 unwinding

U4 destabilizes

Spliceosome becomes active U6 pairs with U2

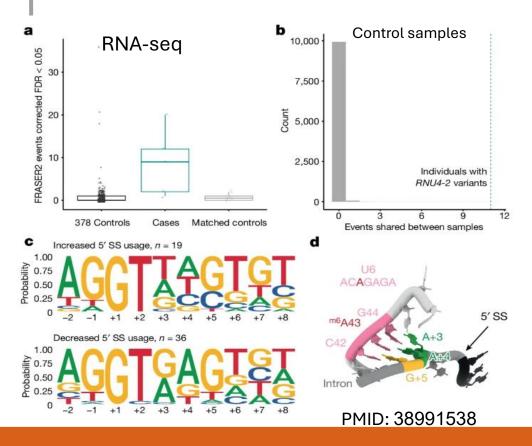


Prevention of non-canonical splice sites

- Assembly of the major spliceosome is initiated by binding of U1 and U2 snRNPs to the premRNA strand.
- The interaction between **U4 and U6 snRNAs is** crucial for stability of the complex and formation of active spliceosome
- Disassembly of the U4–U6 duplex + pairing with U2 snRNP at the 3' splice site = complete removal of the intron
- **Ensures spliceosome recognition fidelity**

RNU4-2 n.64_65insT Variant Disrupts the 5' Splice Site Usage



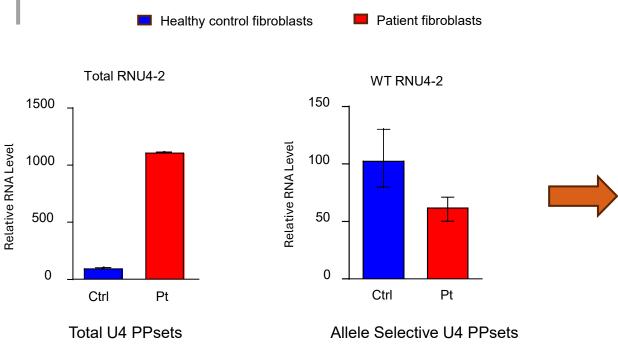


- Consistent with the importance of the critical region in 5' splice-site recognition, the most pronounced difference was observed for abnormal splicing events corresponding to increased use of unannotated 5' splice sites
- 5 of the genes implicated in the 12 shared events are in the Developmental Disorders Genotype-to-Phenotype (DDG2P) database21 and/or were associated with NDD in a previous large-scale analysis (NDUFV1, H2AC6, JMJD1C, MAP4K4 and SF1)
- We have a clear read-out

Indication that RNU4-2 mutation is a TGOF

Mutant RNU4-2 is 20 Times More Abundant Than Wild-type

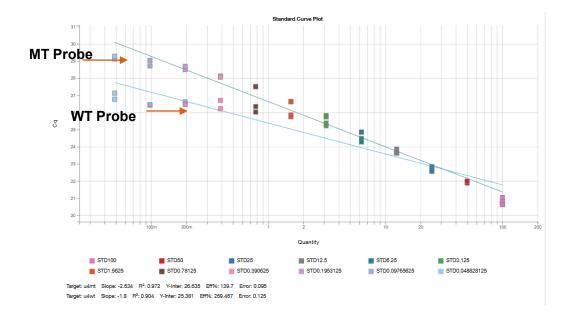




Total U4 PPsets Allele Selective U4 PPsets

Mutant RNU4-2 (MT-U4) is approximately 20 times more abundant

RT-qPCR using allele-specific probes showed significantly earlier Cq values for the mutant probe -> higher abundance



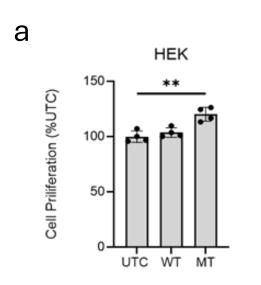
Indication that RNU4-2 mutation is a TGOF

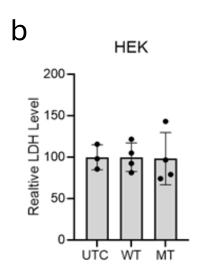
than WT, despite being transcribed from only one allele

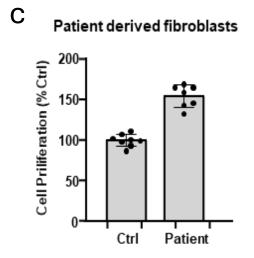


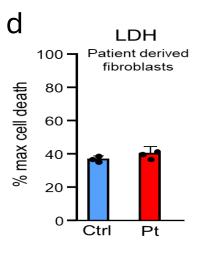


Overexpression of RNU4-2 n.64_65insT variant increased cell proliferation but didn't affect cell viability





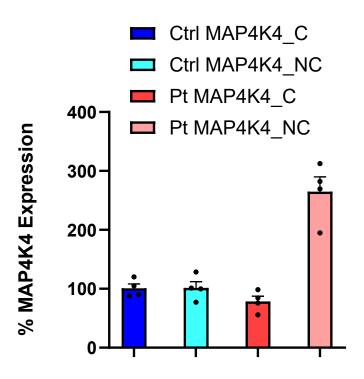


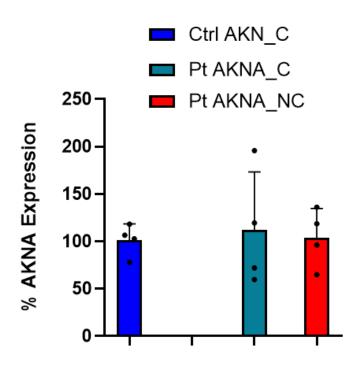




RNU4-2 n.64_65insT variant increased non-canonical NDD related transcripts









Allele-selective ASOs were designed and screened



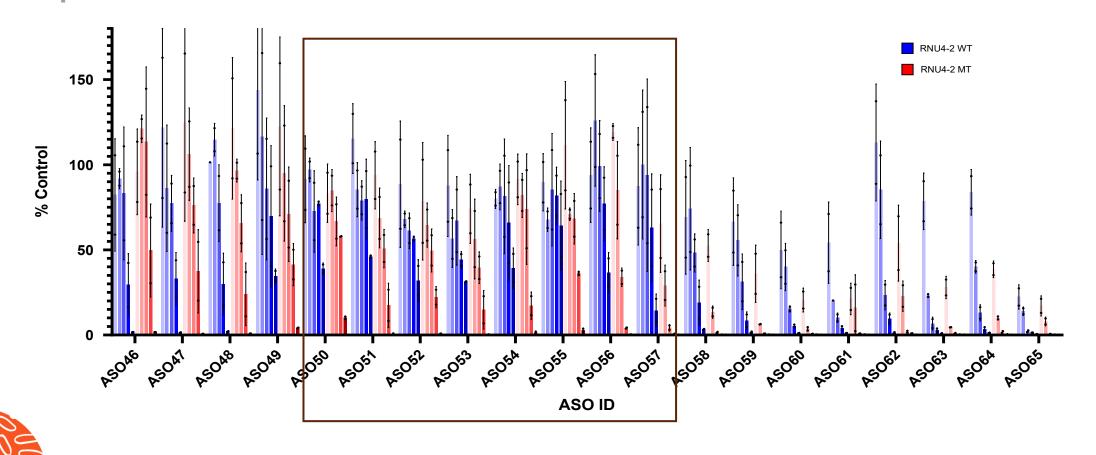
Mutation: n.64_65insT

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4-2									
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	AS019	AS039	AS059		AS079	ASO:	99	AS0119	
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AS015	5 AS	035	AS055	ASC	AS075		AS	0115	
AS014			AS054	ASO7	The second second	AS094		114	
AS013		ASO33 AS		AS073		AS093		13	
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AS010	AS030		AS050	AS070			AS0110 AS0109		
AS09	AS029			AS069		AS089			
AS08	AS028		048	AS068		AS088			
AS07	AS027	AS047		AS067		AS087			
AS06	AS026	AS046		AS066	AS08		AS0106		
AS05	AS025	AS045		AS065	AS085		AS0105		
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Allele-selective ASOs were designed and screened

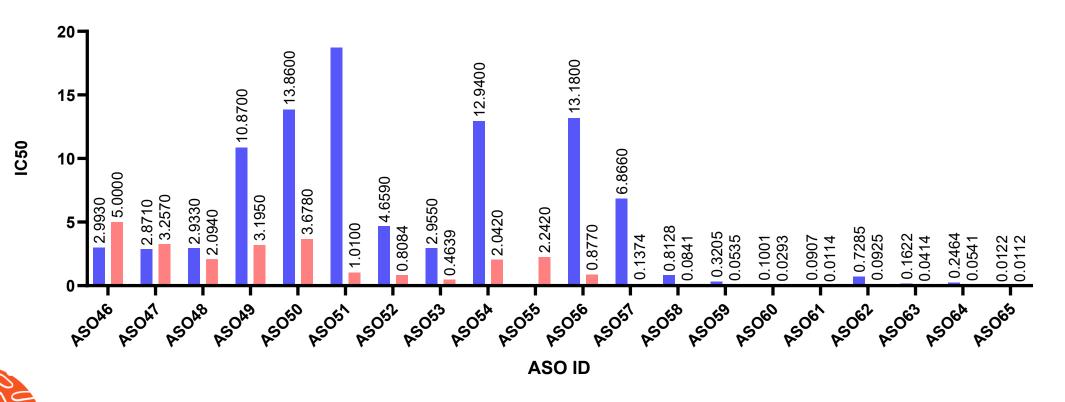






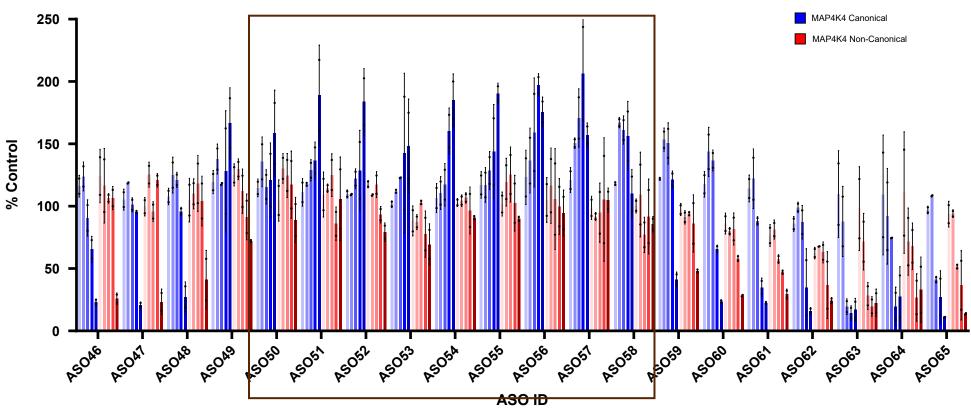


Several potent and selective ASOs





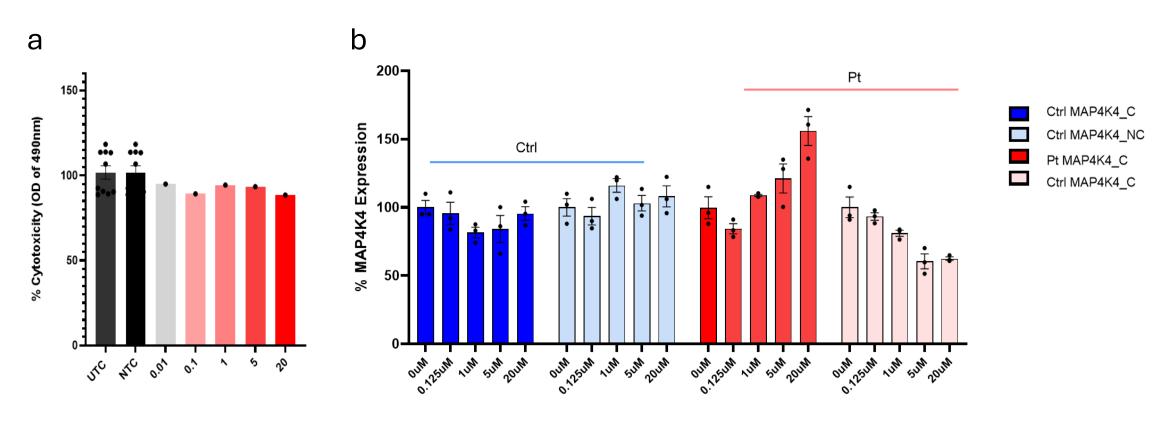
Allele selective ASOs selectively reduced non-canonical 5' splicing induced by RNU4-2 mutation





Allele-selective ASOs showed no cytotoxicity on cells









Conclusion

- RNU4-2 mutations disrupt 5' splice site fidelity and drive TGOF pathology
- A subset of lead ASOs shows strong allele-selective knockdown of mutant RNA and restoration of downstream splicing
- Lead ASOs don't show cytotoxicity on cell viability
- We developed highly promising allele-selective ASOs for the treatment of ReNU syndrome



Acknowledgement

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