



confirmed by in vitro transcription and RNA structures of the variants predicted by SHAPE-MaP sequencing and analysis.

This system was tested in the human Factor VIII (F8) gene, mutations of which result in Hemophilia A. Prior work by theis group identified F8 as a gene with the highest number of variants per exon in the human population as well as the total number of putative splicing-sensitive point variants of those genes analyzed. A total of 97 known HA-causing variants across 11 exons were tested by generating heterologous splicing reporters for each exon (plus 100-250 bp of intronic sequence.) Variants associated with HA caused exon skipping in four exons, particularly in exon 16 where 6 pathogenic variants reduced inclusion of exon 16 in the spliced product. SHAPE-MaP-seq analysis indicated that one of the variants (exon-16<sup>c.5543A>G</sup>) forms a three-way junction structure that occludes the 3' splice site of exon 16, resulting in the aberrant splicing.

ASO's that targeted this structure, termed TWJ-3-15 (Three-Way Junction at the 3'-end of Intron 15) were designed and shown to significantly increase exon 16 inclusion relative to the controls. Importantly, ASO's targeting upstream and downstream regions of the splice site could also increase inclusion of exon 16. These are likely to perturb the influence of inhibitory elements found in the flanking introns. Multiple ASO's designed to target regions upstream and downstream of the splice site performed *better* than those that directly targeted the TWJ-3-15 structure itself, particularly when used in combination.

## APPLICATIONS

- ▶ Platform technology for the discovery of exonic splicing mutations
- ▶ Potential for personalized antisense treatment
- ▶ Antisense oligonucleotides that promote proper splicing despite targeting regions upstream and downstream of the splice site
- ▶ Antisense oligonucleotides that promote proper splicing *better* than antisense oligonucleotides designed to directly target the splice site, particularly in combination.
- ▶ Antisense oligonucleotides useful in the treatment of genetic diseases such as hemophilia A

## ADVANTAGES

- ▶ Technology opens up a wider variety of potential targets for ASO treatments - effectively any disease causing mutation in an exon that does not significantly affect protein function is a potential ASO target.
- ▶ Workflow can be used to identify combinations of ASO's relatively far upstream or downstream of mutation.
- ▶ Powerful combinations of ASO's that can target Hemophilia A caused by F8 mutations

## RELATED MATERIALS

- ▶ [An intronic RNA element modulates Factor VIII exon-16 splicing](#) - 01/11/2024

RELATED TECHNOLOGIES

► [Functional Rna Elements As Targets For Amelioration Of Aberrant Pre-Mrna Splicing](#)

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