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| **Alternative Splicing as a future treatment for Haemophilia A** |
| Aim:  Targeted skipping of F8 exon14 from pre-mRNA splicing using 2’Omethyl and/or phosphorodiamidate antisense oligoribonucleotides (AOs) to induce a truncated but functional F8 protein.  Method:  AOs specific to predicted F8 exon14 splice site elements on the pre-mRNA were introduced in HuH7 cells. Following 24h transfection, total RNA was extracted, converted into cDNA and PCR-assessed using primers spanning exon13&14 and14&15 boundaries. Truncated PCR products were assessed for inframe F8 coding. Factor VIII protein was analysed by immunoblotting with anti-F8 antibodies.  Results:  We have successfully induced exon skipping as determined by RT-PCR amplification of F8 fragments from AO treated cells. Different AOs induced different F8 pre-mRNA splicing in HuH7 cells. A wild-type product (3Kb) was present in all treatments, and excision events were observed in AO1-4, and 6 but not AO5. Of the excision products, 2 main F8 products were observed, 150bp and 800bp in size. Sanger sequencing confirmed 2 distinct truncated F8 gene transcripts with different disruptions to the reading frame. One had the intended B domain coding exon14 completed deleted. An alternative transcript partially excised exon14 sequences, but retains sequences towards the 3’end including the furin cleavage site.  Conclusion:  Gene skipping of mutant regions of the F8 B domain at the pre-mRNA stage is a potential novel treatment strategy for Haemophilia A. This study successfully excised exon14 of the F8 gene to generate a B domain deleted, as well as a partially deleted B domain F8 protein. |