Altering the Splicing of the *F8* mRNA Transcript as a Future Treatment for Haemophilia

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Key Points:

- Antisense oligonucleotides were successfully used to alter the splicing of the *F8* mRNA transcript in HuH-7 cells
- Splice variant products were confirmed to code for an in-frame, but truncated FVIII protein

Introduction:

- Haemophilia A (HA) is an X-linked inherited disorder (1:5000 male births) that results in FVIII coagulation factor deficiency
- Patients have a bleeding phenotype that is treated with exogenous FVIII concentrates
- 10% develop inhibitors against exogenous concentrates → increased disease burden
- 10% of patients have mutations mapping to the B domain of FVIII which is encoded by exon 14 of the *F8* mRNA transcript; almost all express the severe phenotype

Aim:

To demonstrate that antisense oligonucleotides (AOs) can skip exon 14 and bypass mutations associated with the B domain of FVIII

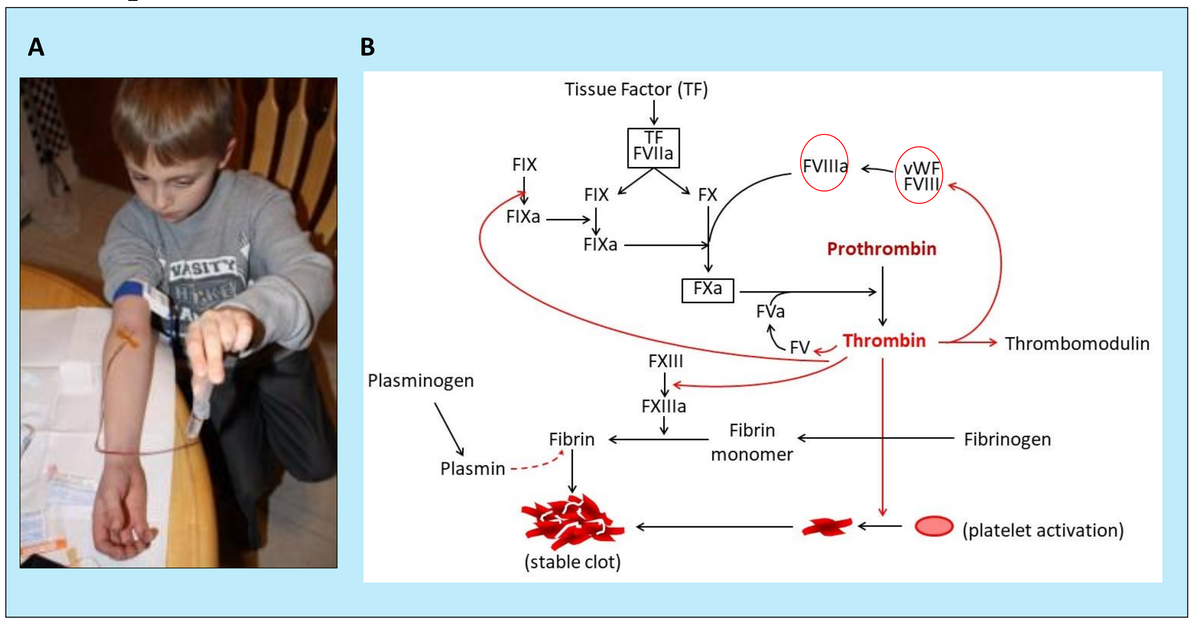

Figure 1. A) **A haemophilic child administering a prophylactic dosage of FVIII concentrates. *B)* A schematic diagram of the coagulation cascade. The FVIII in its active and inactive forms is highlighted.**

Method:

- HuH-7 cells were transfected with AOs designed to sterically inhibit splice site recognition of exon 14 in the FVIII pre-mRNA (*Figure 2.*)
- RNA was extracted and converted to cDNA for gene analysing
- A PCR targeting exon 14 and the flanking exons 13 and 15 was used to identify and isolate truncated products
- Truncated products were sequenced using the MinION Nanopore platform to determine if the modified sequence retained an in-frame codon usage

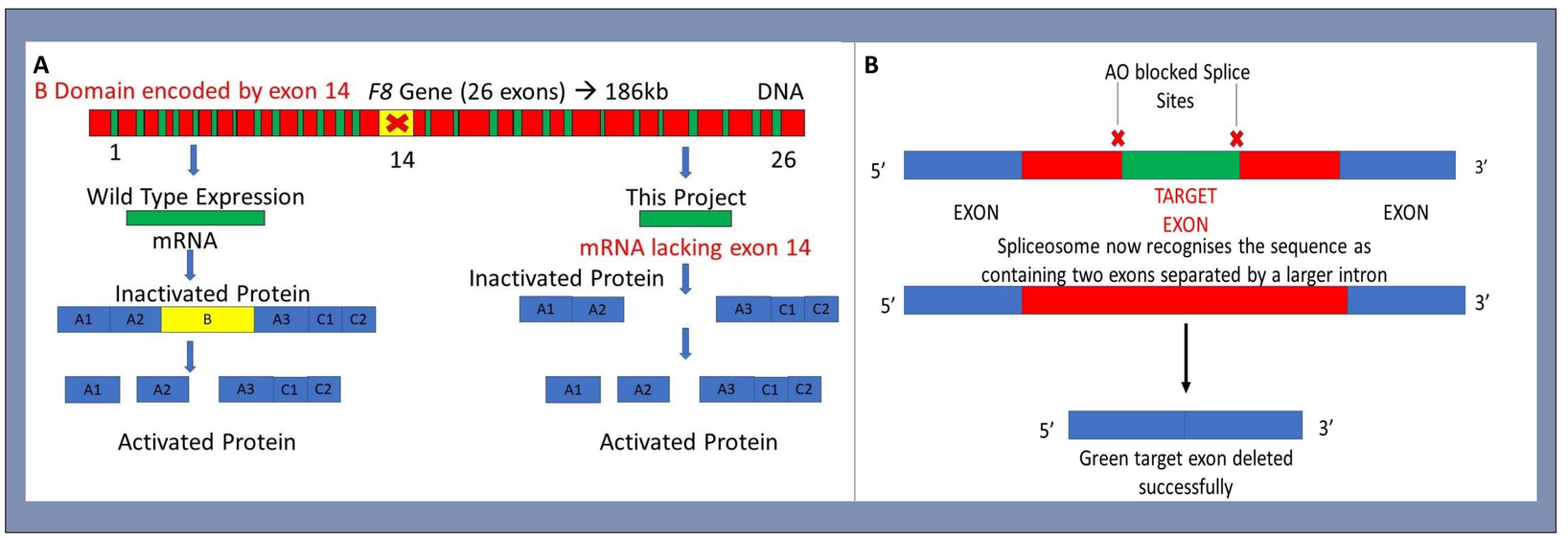

Figure 2. A) **Schematic of wildtype expression of *F8* and the alternative *F8* expression desired in this project using AO induced exon skipping. *B)* Using AOs to sterically inhibit splice site recognition by the spliceosome, consequently resulting in the deletion of the desired exon.**

Results:

- HuH-7 cells were successfully transfected with a range of AOs
- PCR and sequencing confirmed that one set of AOs induced a partial deletion of exon 14, whilst retaining in frame codon usage (*Figure 3.*)
- Three of the glycosylation sites encoded by exon 14 were determined to be preserved (*Figure 4.*)

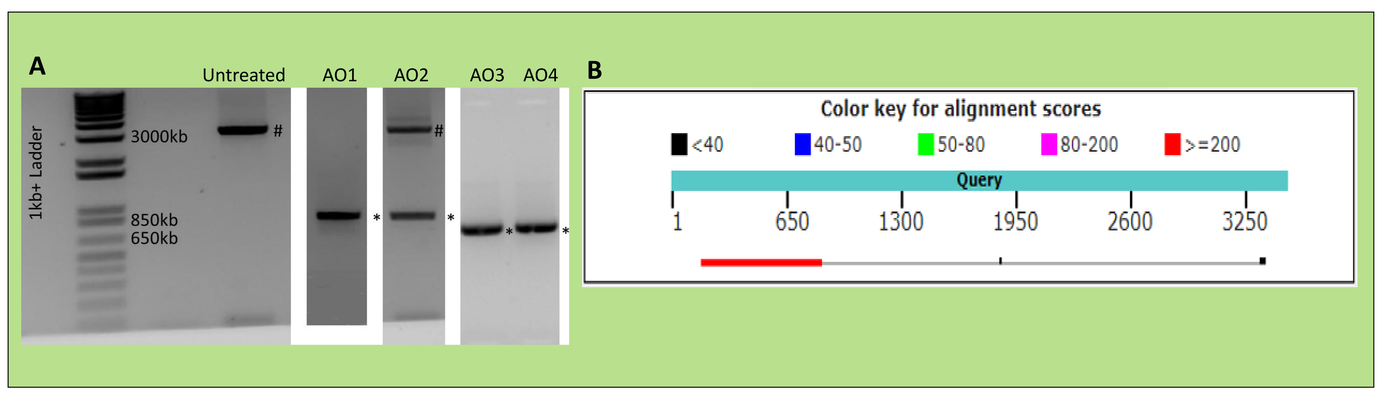

Figure 3. A) **Gel images showing isolation of unaltered exon 14 (#) and truncated products (*). *B)* An alignment of the truncated exon 14 products against the full length unaltered product. A large deletion of the 3' end of the exon is shown.**

Discussion:

- First demonstration of AO-induced skipping for a coagulation factor
- Skipping of exon 14 encoding the large B domain of the FVIII molecule is feasible using AO approaches
- The B domain is not required for FIXa cofactor function
- Deleting the FVIII B domain may be used in the future to bypass HA causing mutations
- This may increase endogenous FVIII levels, consequently replacing the need for exogenous concentrates for a subset of HA patients
- This may confer the benefits of: reduced frequency of treatment, reduced cost and reduced risk of inhibitor development

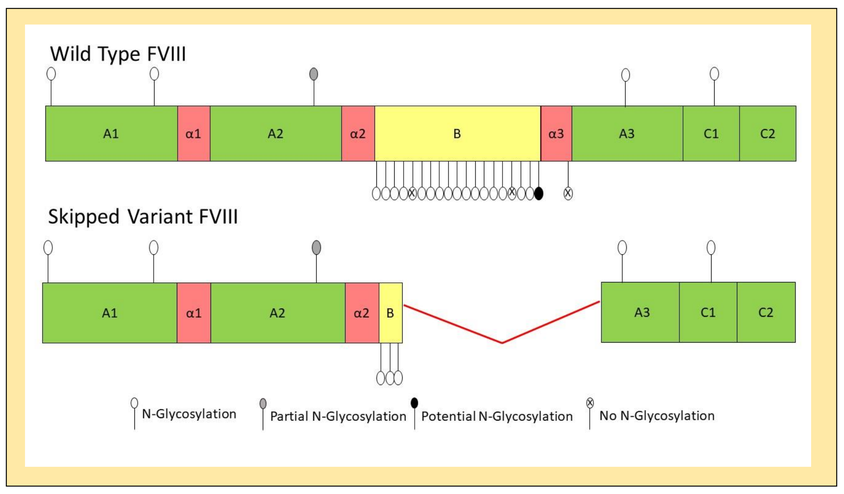

Figure 4. **A diagram comparing the full length wild-type FVIII and the predicted product from the altered splicing of the *F8* mRNA transcript demonstrated in this study.**

Research supported with grant funding from the Australian Haemophilia Centre Directors Organisation (AHCDO) John Lloyd Clinical Excellence Fund

Presented on 29/10/17 by Michael Menezes Email: m.menezes@wacth.org