

# Analysis of F8 mRNA in haemophilia A patients with silent mutations or presumptive splice site mutations

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Mutation screenings in haemophilia A patients identify a huge variety of mutations in the factor VIII (F8) gene:

- about 40% missense mutations,
- 25–30% intron 22 inversions and
- less than 10% nonsense mutations, small or large deletions, insertions and splice site mutations, respectively.

Silent mutations do not result in amino acid substitutions and intronic variants are located outside the splice site consensus sequences. They cannot be easily classified as causative for haemophilia A. Further insight can be gained by mRNA analysis.

tation c.1752+5G>A caused a loss of exon 11. A small deletion in intron 13 (c.2114–8<sub>-</sub>25del18) resulted in the loss of the whole exon 14 in the F8 mRNA.

## Conclusion

Analysis of F8 mRNA from nine haemophilia A patients with silent mutations or pre-

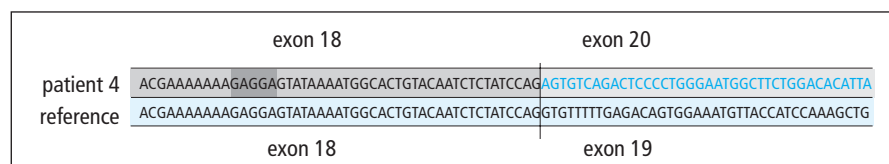
sumptive splice site mutations has revealed that three of the examined mutations cause an aberrant F8 mRNA due to loss of an existing or activation of a cryptic splice site.

This study also shows that examination of splice sites should not only rely on special prediction software but rather must be proved in mRNA analysis experiments.

## Methods and results

F8 mRNA was prepared for four haemophilia A patients with silent mutations and five patients with presumptive splice site mutations. Amplification of cDNA fragments was done using exonic primers located adjacent to the mutation-containing exons or introns. The obtained fragments were sequenced on an ABI automatic sequencer and compared to F8 wildtype cDNA. In this way, three of the nine mutations examined could be shown to have an effect on F8 mRNA splicing.

The silent mutation Tyr2017Tyr (c.6108C>T) resulted in partial loss of exon 19 in the F8 mRNA (► Fig. 1, ► Fig. 2). In addition, two presumptive splice site mutations have a similar effect. The point-mu-



**Fig. 1** Sequence analysis of patient 4 (with the silent mutation Tyr2017Tyr) compared to a reference sequence consisting of the end of exon 18 and the beginning of exon 19; the sequences of the reference and patient 4 are homologous until the end of exon 18, the subsequent grey region represents the beginning of exon 20. Therefore, exon 19 is missing in the cDNA of patient 4.

SpliceSiteFinder-like	[0-100]	74.7
MaxEntScan	[0-12]	7.0
NNSPLICE	[0-1]	0.9
GeneSplicer	[0-15]	2.9
Reference Sequence	6080 6090 6100 6115 6115+10 TACATGCTGGGATGAGCACACTTTTCTGGTGTATAGCAATAAGTGTAGCAATGTGGCA	
SpliceSiteFinder-like	[0-100]	84.0
MaxEntScan	[0-16]	85.6
NNSPLICE	[0-1]	
GeneSplicer	[0-15]	
Branch Points	[0-100]	
SpliceSiteFinder-like	[0-100]	74.7
MaxEntScan	[0-12]	7.0
NNSPLICE	[0-1]	0.9
GeneSplicer	[0-15]	2.4
Mutated Sequence	6080 6090 6100 6115 6115+10 TACATGCTGGGATGAGCACACTTTTCTGGTGTATAGCAATAAGTGTAGCAATGTGGCA	
SpliceSiteFinder-like	[0-100]	77.8
MaxEntScan	[0-16]	84.9
NNSPLICE	[0-1]	80.4
GeneSplicer	[0-15]	
Branch Points	[0-100]	

**Fig. 2** Splice site prediction by Alamut to simulate the effect of the c.6108C>T mutation (Tyr2017Tyr) on F8 mRNA splicing in patient 4; four different programmes indicate the probabilities for the use of the 5'- and 3'-splice sites. The wildtype sequence is shown at the top, the mutated sequence is below. Only GeneSplicer shows a minimal effect on 5'-splicing.

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