GGCX Splice-Site Mutations: Functional Investigation in a Chimeric Minigene System

Watzka M, Soos J, Czogalla K, Liphardt K, Oldenburg J

Institute of Experimental Haematology and Transfusion Medicine, University Clinics Bonn, Germany

Background

Vitamin K is an indispensable cofactor for the endoplasmatic enzyme gamma-carboxylase (GGCX), which catalyses the posttranslational modification of glutamate residues into gamma-carboxy glutamate residues (Gla). Gla residues are essential for the function of coagulation factor II, VII, IX, and X. Combined deficiency of these factors (VKCFD) is caused by defects in either GGCX or VKORC1 and represents a rare autosomal recessive disorder. In the case of GGCX, three splice site mutations have been identified in the last years, but functional analysis and proof of causality has not been performed until now.

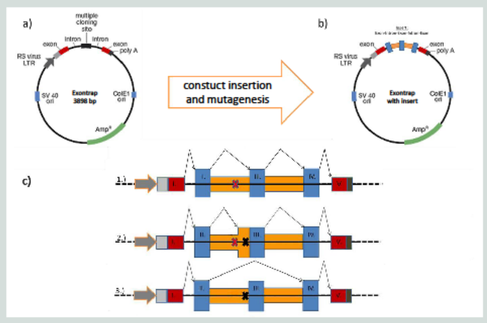
Methods

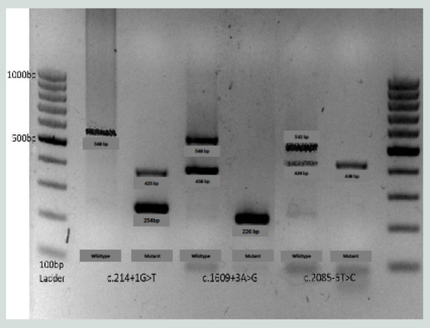
Three different GGCX splice site mutations and the corresponding wt sequences (c.214+1G>T, c.1609+3A>G, c.2085-5T>C) have been cloned into the ExonTrap-vector within their natural surroundings. The GGCX part of each vector consists of 3 flanking GGCX exons and 4 GGCX introns. Together with two insulin exons within the vector, these constructs form chimeric minigenes. After transfection into COS-cells, the expressed mRNA was isolated, reverse transcribed, and amplified. The resulting band pattern was analysed and each DNA fragment sequenced to identify exon-exon boundaries.

Results and Conclusion

In genetic analysis, roughly 10 % of mutations affect splice site consensus sequences. Although several databases allow splice site score calculation, these values do not necessarily reflect patients phenotype.

Therefore, we established an assay, useful for examination of splice site mutations. In all three mutations investigated, we observed complete loss of the wild type band and exon-skipping. In contrast to in-silico analysis, all mutations could be identified as causative for VKCFD1.

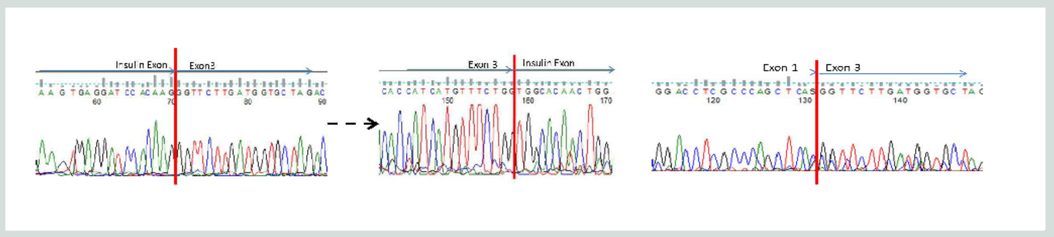




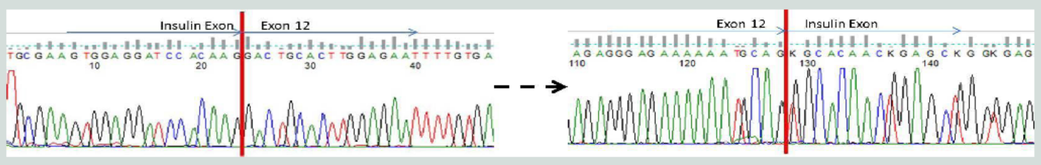
possible resulting splice variants:

1) wildtype
2) activation of a cryptic splice site
3) exon skipping

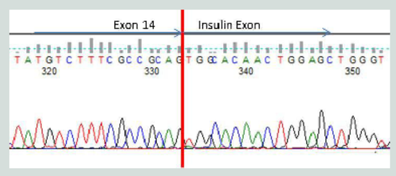
RT-PCR products of the correponding wt and mutant GGCX constructs



c.214+1G>T, skipping of exon 1 and 2 / skipping of exon 2



c.1609+3A>G, skipping of exon 10 and 11



c.2085-5T>C, skipping of exon 15