In silico models for genotype/phenotype correlations of *F9* gene mutations causing severe Hemophilia B

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Objectives

In silico modeling of the amino acid substitutions caused by mutations in *F9* gene suggests the structural and functional effects on the mature FIX protein. In this work we intended to apply informatics tools to describe the genotype/phenotype possible correlations of selected *F9* gene mutations at exons 2 and 3 causing severe hemophilia B and their effect on the structure and function of the propeptide and Gla domains of FIX protein respectively.

Methods

Severe phenotype-associated mutations at exons 2 and 3 of *F9* gene were selected from the current Haemophilia B Mutation Database (http://www.factorix.org/). The PolyPhen program was used to perform multiple sequence alignments using the structural information of FIX protein (Polymorphism Phenotyping, http://genetics.bwh.harvard.edu/pph2/). The theoretical three-dimensional (3D) structure analysis of *F9* missense mutations was performed by the platform of the Swiss Model Workspace (http: //swissmodel.expasy.org/workspace/).

Table 1. *In sillico* analysis with PolyPhen2

Domain	Position	AA change	Damage probability
Propeptide	30 (-16)	Val>Ile	0.998
Propeptide	46 (-1)	Arg>Ser	1
Gla	58 (12)	Gly>Glu	1
Gla	84 (38)	Thr>Arg	1

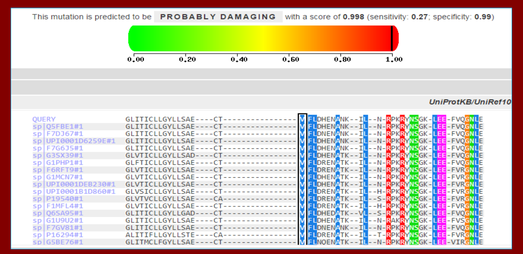

Fig. 1 multiple-sequences-alignment of *F9* gen mutations by PolyPhen program (update: uniprotkb 14-Dec-2011).

Results

The PolyPhen program based on multiple-sequences-alignment showed that amino acids in positions V30 (Fig. 1), R46 (propeptide) and G58, Y84 (Gla domain) are all highly conserved residues with a probability of deleterious effect (higher than 0.998) when they are replaced (Table 1).

Based on the Swiss Model Workspace program theoretical three-dimensional structure of the wild Gla domain (Fig. A) was constructed, in addition to the selected mutations in exon 3 that generate changes in Gly58>Glu (Fig. B) and Thr84>Arg (Fig. C). These changes result in alterations in the orientation of side chains of amino acids (Fig. B1) and affects network of hydrogen bonds (Fig. C1) relative to the wild-type protein (Fig. A1).

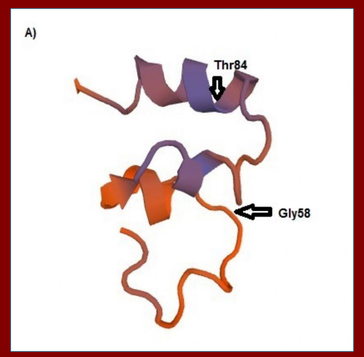

Fig. A) Theoretical three-dimensional structure of the wild FIX Gla domain.

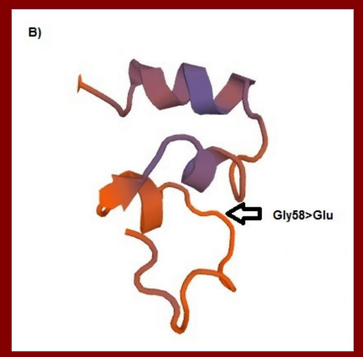

Fig. B) theoretical three-dimensional structure of the mutation Gly58>Glu FIX Gla domain.

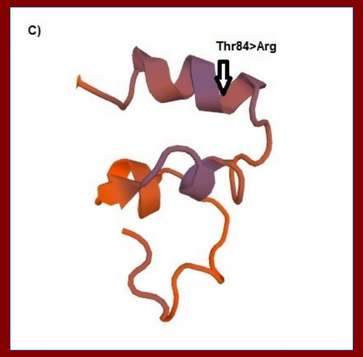

Fig. C) theoretical three-dimensional structure of the mutation Thr84>Arg FIX Gla domain.

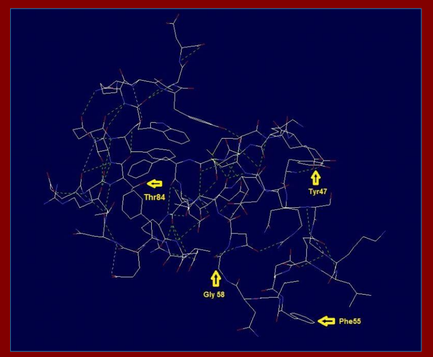

Fig. A1. Network of hydrogen bonds of the wild FIX Gla domain.

Fig. B1. Network of hydrogen bonds of the Gly58>Glu FIX Gla domain.

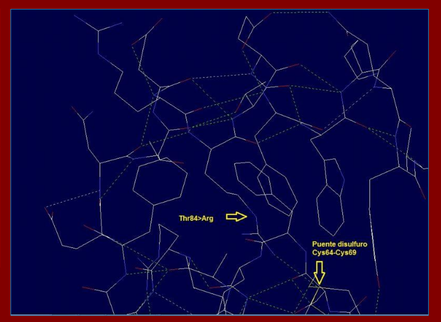

Fig. C1. Network of hydrogen bonds of the Thr84>Arg FIX Gla domain.

Conclusions

Selected mutations in the propeptide and Gla domains affect highly conserved amino acids and have an important functional effect on the carboxylation of glutamic acid residues, disrupting the interaction of mature FIX with platelets. The inclusion of the FIX propeptide in the established informatics models (not currently available), would have a strong impact on the structure / function of the FIX protein analysis.

References

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2. Adzhubei I, Jordan DM, Sunyaev SR. (2013). Predicting functional effect of human missense mutations using PolyPhen-2. Curr Protoc Hum Genet, Chapter 7: Unit7.20.

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