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Research Article

MOLECULAR SIMULATION OF STRUCTURAL CHANGES AND MEMBRANE-BINDING CAPABILITY OF MUTANT I22I FVIII

Atanasov V.[1*], S. Hristova[2, 3], A. M. Zhivkov[3]

[1]Institute of Organic Chemistry with Centre of Phytochemistry, BAS, G. Bonchev str. bl. 9, Sofia 1113, Bulgaria

[2]National Specialized Hospital for Active Treatment of Haematological Diseases, Plovdivsko pole Str. 6, Sofia 1756, Bulgaria

[3]Rostislaw Kaishew Institute of Physical Chemistry, Bulgarian Academy of Sciences (BAS), Acad. G. Bonchev Str., bl. 11, Sofia 1113, Bulgaria

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Summary: Nearly half of severe Haemophilia A cases in humans are caused by F8 intron 22 inversion (Inv22).The F8 gene is located on the X chromosome (Xq28) and encodes FVIII, a large plasma glycoprotein that functions in the blood coagulation cascade. In the blood, it circulates mainly in a stable noncovalent complex with von Willebrand factor (VWF). Activation by thrombin leads to dissociation of VWF, interaction with factor IXa and forming an active membrane-binding tenase (Xase) complex. The activated FVIII (FVIIIa) includes five domains (A1, A2, A3, C1 and C2), but only C2 and C1 play essential role in the platelets membrane incorporation. The inv 22 F8 leads to a deletion of C2 domain and shortening and changing in the C1 primary structure. We modeled the 3D structure of the C1 * mutant domain of the I22I FVIII* from primary structure encoded by exon 23 in inv22 F8 gene. Superposition of 3D structure of mutant C1* fragment and C1C2 domains demonstrates a dramatic change in the tertiary structure. Through macromolecular docking, the interaction of the mutant fragment with a model anionic lipid membrane has been investigated. The results clearly show that I22I FVIIIa can not be incorporated into the anionic lipid membrane and to form an active membrane-binding Xase complex and it is proteolytically degraded as a defective protein. For this reason, the plasma levels of FVIIIa are critically low, which is typical for the severe form of haemophilia A.

Keywords: Computer simulations; Haemophilia type A; Inversion 22 in F8 gene; Mutant coagulation factor VIII; Protein docking to lipid membrane.

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*Corresponding author: ventseslav.atanasov@gmail.com

INTRODUCTION

Factor VIII (FVIII) is synthesized mainly in the liver as a precursor molecule (single chain protein) build by 2332 amino acid residues and signal peptide of 19 amino acids (AA), and undergoes various posttranslational modifications. FVIII consists of three type domains – A, B, C and three short binding fragments (A1-ar1-A2-ar2-B-ar3-A3-C1-C2). The mature protein is a hetorodimer and contains two chains – light chain (80 kDa) build by A1, A2 and B domains and heavy chain (200 kDa) – includes C1 and C2 domains (Shen et al., 2008). FVIII circulates as an inactive clotting cofactor in noncovalent complex with von Willebrand Factor (vWF), which stabilizes it. Binding to vWf prevents FVIII from premature interaction with the phospholipid membrane. FVIII is activated by proteolytic cleavage catalyzed by thrombin and Factor IXa. The The conversion of procofactor FVIII to an active FVIIIa is associated with cleavages in the heavy chain at Arg372 (A1-A2 junction) and at Arg740 (A2-B junction), and the light chain is cleaved near its NH_2 terminus at Arg1689. The cleavage at Arg740 leads to dissociation of the B domain from the rest of the molecule. The remaining subunits form a non-covalently linked heterotrimer, which consist of the A1 subunit (1–372), the A2 subunit (373–740), and a light chain containing the A3, C1, and C2 domains (1690–2332). Cleavage in the light chain at Arg 1689 and removal of the acidic region near its terminus drives the dissociation of vWF allowing FVIIIa to bind effectively to phospholipid surface. C1 and C2 domains are responsible for incorporation of the activated factor VIIIa in the platelets membrane. The binding of activated FVIIIa is accomplished by both hydrophobic and electrostatic interactions between positively charged groups in protein macromolecule (Lys, Arg) and negatively charged membrane surface (Wakabayashi and Fay, 2013). Between activated FVIIIa and FIXa is formed membrane bound tenase (Xase) complex with the participation of Ca^{2+} and presence of cell membrane rich in phosphatidylserine like the membrane of the activated platelets. Interaction between FVIIIa and FIXa is of a vital importance for the function and the activity of the tenase complex (Saenko et al., 1999). The role of the phospholipid membrane is to increase the local concentration of both components of the complex and possibly to provide their optimal mutual orientation.

Haemophilia A is an X-linked bleeding disorder, which is characterized by qualitative and quantitative FVIII deficiency resulting from various mutations in the F8 gene and is manifested by a disruption of blood clotting ability. Three clinical forms have been distinguished by the factor VIII levels: severe (<1%), moderate (2-5%) and mild (5-30%). Approximately 40% - 50% of patients with severe form of Haemophilia A are affected by the gene inversion with break point within intron 22 (Inv22), which develops as a consequence of homologous recombination between int22h1 – region and one of the extrageneous copies of homologous sequences (int22h2 and int22h3), localized close to the telomere of the long arm of X chromosome (Lakich et al., 1993; Naylor et al.,

1993). These telomeric sequences are in opposite orientation compared to its homologous sequence in intron 22. In a process of homologous recombination the orientation of introns from 1 to 22 is inverted. Inv22 leads to deletion of the C2 domain, shortening of the C1 domain and changing its primary structure.

Since C1 and C2 domains play a key role in interaction between activated FVIIIa with phospholipid double layer, occurrence of a mutation in the coding gene may has influence upon protein – lipid interaction. The aim of the present study is to show how the change in the structure of FVIIIa in consequence of a break point in Inv22 of F8 gene has an effect on binding of this coagulation factor to phospholipids membrane.

MATERIALS AND METHODS

An inverse PCR (I-PCR) was optimized and introduced as a diagnostic method for Inv22 screening, involving 3 steps: (1.1) BclI restriction; (1.2) Self-ligation of restriction fragments, providing BclI rings; (1.3) Multiplex PCR using 3 primer pairs. The visualization of the resulting PCR products was performed on a UV-transilluminator after a standard agarose gel electrophoresis. 30 patients with clinically verified severe form of Haemophilia A were tested for Inv22 by I-PCR (Atanasov et al., 2017).

The 3D-structure of the mutant factor VIII* was characterized in detail by the following servers: (2.1) PEP-FOLD 2.0 for prediction of 3D-conformaion of the mutant C1*-domain; (Thévenet et al. 2012). A SuperPose Version 1.0 was used for comparison of the 3D-structures of the mutant C1*-fragment with C1 and C2 domains of the functionally active protein VIIIa (Maiti et al. 2004); The macromolecule docking of the mutant C1* fragment to a model anion lipid membrane employing Membrane Builder was based upon the program CHARMM (Lee et al., 2018).

RESULTS AND DISCUSSION

Figure 1 presents the frequency of Inv22 in Bulgaria as judged from 30 patients with clinically proved severe form of Haemophilia A in comparison with the same frequency in Mexico and USA (Johnsen et al., 2017), (Luna-Zaizar et al., 2018). The data show lower frequency of this mutation for Bulgaria probably due to territory specific differences and to some extent to our smaller excerption.

F8 contains all 9030 bases found in *F8* exons 1 to 26 and encodes the full-length FVIII protein. The F8 encodes a large precursor protein of 2,351 amino acids. At the *N*-terminal, the precursor has a signal peptide of 19 amino acids. FVIII circulates in plasma bound to VWF as a heterogeneous mixture of heterodimers – heavy-chain fragments (A1a1/A2a2/B) and light chain (a3A3/C1/C2). Thrombin activated FVIIIa leads to lacking the B domain and dissociation of VWF, thus allowing the association of FVIIIa to the phospholipid membrane (Gilbert and Arena 1996).

Homologous recombination between *int22h1* and *int22h3* incompletely inverts *F8*. The transcription of I22-inverted *F8* lead to direct synthesis of *F8*-exonic-sequence–containing polyadenylated transcript *F8I22I*. *F8I22I* contains the

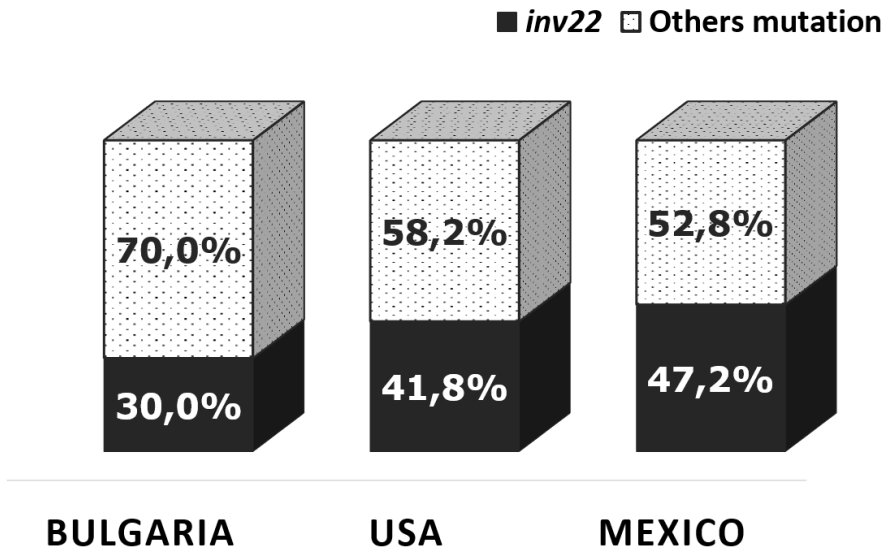

Figure 1. Frequency of F8 intron 22 inversion.

6756 bases found in *F8* exons 1 to 22 and, together with the 48 additional DCS bases in exon 23_C, encodes the $FVIII_{I22I}$ protein, which contains 2159 translated amino acid residues.

The synthesis of a mutant inv22FVIII* protein is proven in the latter five years. The question about its secretion is not fully clarified. Pandey and coworkers suggest that the mutant protein is not secreted, but entrapped in the cytoplasm of the endothelial

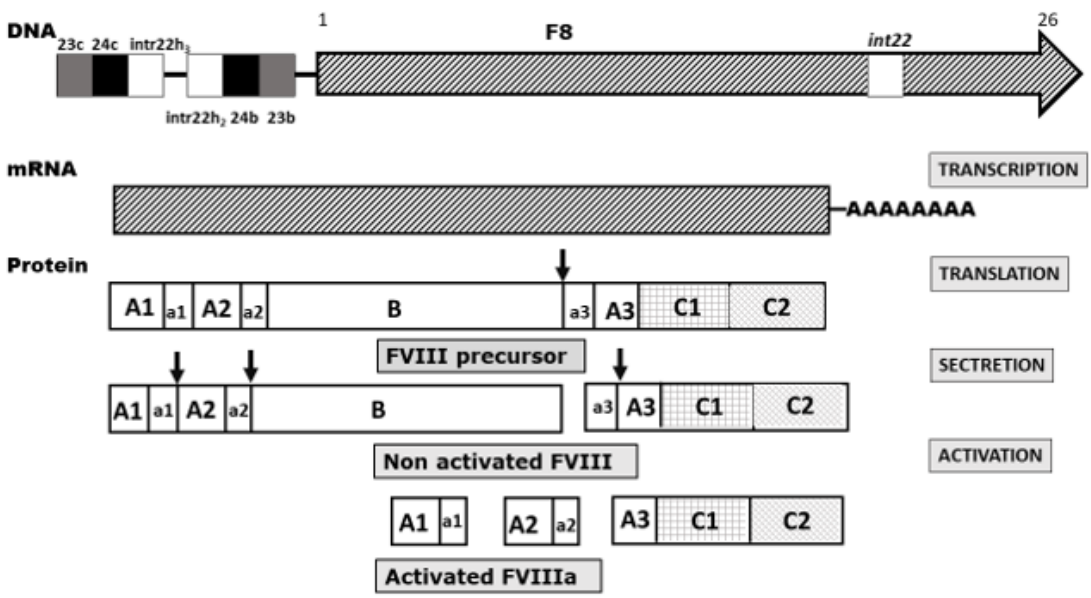

Figure 2A. Processing of functional F8 gene to active FVIIIa protein.

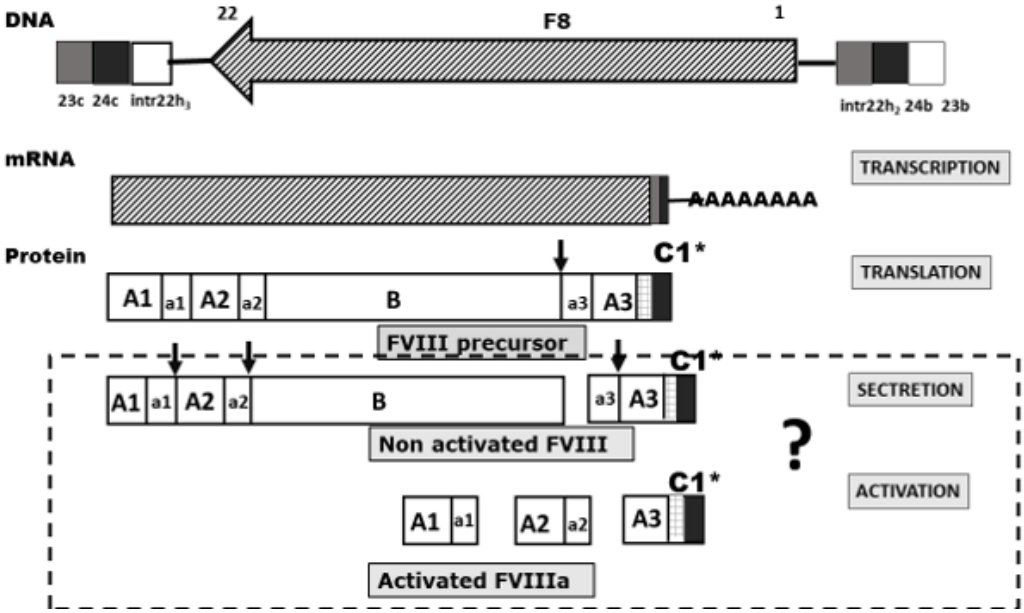

Figure 2B. Processing of intron-22-inverted F8 gene to mutant (no active) inv22FVIII* protein.

liver cells and degraded intracellularly (Pandey et al., 2013). But the authors don't explain why the mutant protein is not secreted as long as this mutation neither affects the signal sequence in the beginning of polypeptide chain, nor the sites responsible for chaperons binding, the latter assisting its secretion. Nevertheless, if the mutated protein is secreted and circulates in the blood, the question is whether it is able to incorporate in the platelets membrane and to form an active Xase complex.

The active form of coagulation factor VIII, FVIIIa is a trimer consisting of A1 (amino acids 1–372), A2 (amino acids 373–740) and linked A3 — C1–C2 (amino acids 1690–2332) domains. On Fig. 3 (left) is shown the tertiary structure of the functional active FVIIIa and the mutant protein 22FVIII*, originates as a result of Inv22 in the coding F8-gene. It is clear this mutation leads to loss of a whole C2 domain and the most of the C1 domain, which shortens with 44 AA. Only 4 AA and one small fragment of 16 AA remain unaffected with significantly changed primary structure (C1*).

In Fig. 4 is presented in outlines the change in the primary structure of the mutant 16 AA peptide fragment from Factor 22-VIII*. The number of hydrophobic amino acid residues decreases from 11 to 5 and increases the contribution of hydrophilic (non-charged, positively and negatively charged) aminoacid residues.

We used PEP-FOLD 2.0 to predict the tertiary structure from the primary structure of 16 aminoacid peptide from the mutant protein. For three dimensional comparison of the structures of C1 and C1* domains of functional and mutant protein, we used the SuperPose Version 1.0 software (Fig. 5A). C1 and C2 domains are of

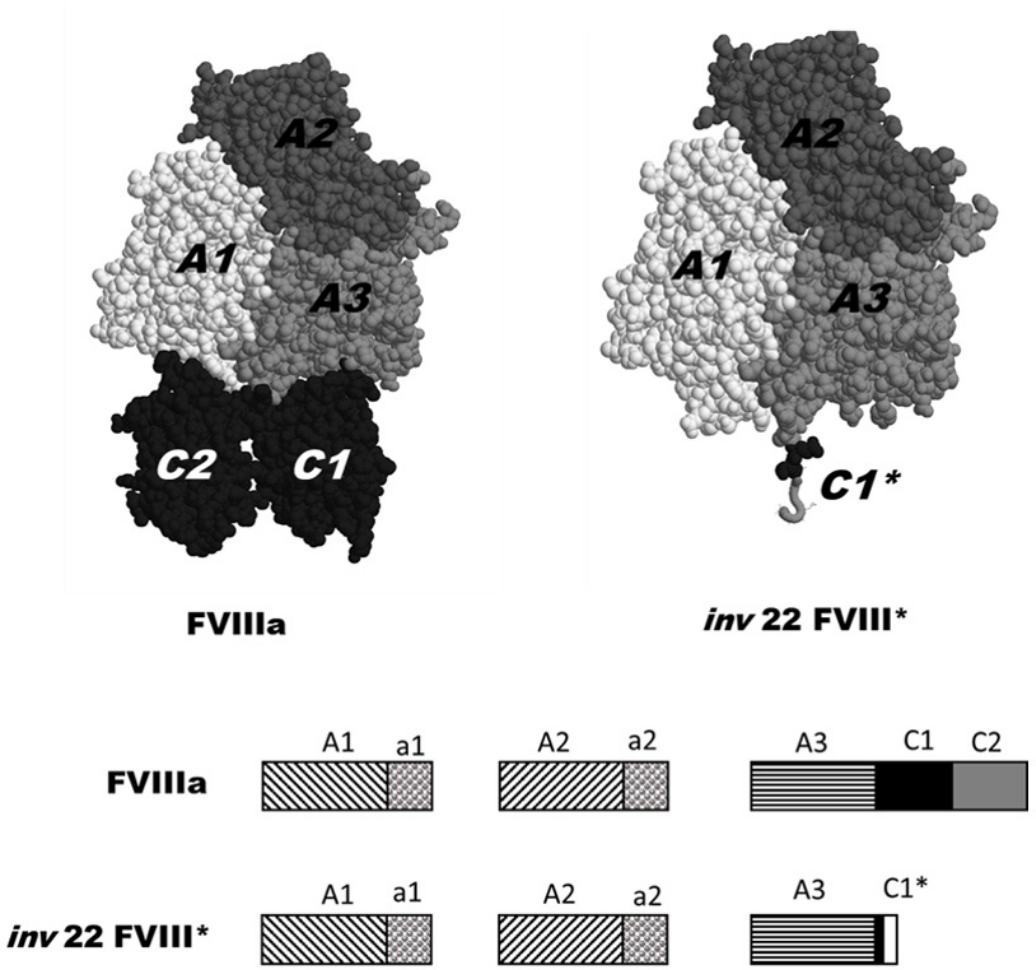

Figure 3. Structure of activated FVIIIa and mutant inv22FVIII*.

key role for interaction of the activated FVIIIa with platelets membrane. This binding is accomplished by both hydrophobic and electrostatic interactions. Membrane binding regions have been identified in the C2 domain and include Leu2251-Leu2252, Met2199-Phe2200, Lys2227, and Trp2313-His2315. C1 has a supporting role in protein-lipid interaction. More recent mutagenesis studies identified phospholipid – binding sites in C1 (Lys2092-Phe2093 , Gln2042-Tyr2043, and Arg2159 (Walter et al. 2013).

The inversion 22 in the F8 gene causes a drastic alteration in the primary and 3D-structure of the coagulation factor VIII*. This mutation leads to a loss of many key AA residues for interaction of the activated FVIIIa with the platelets membrane. Thus, C2 domain is not synthesized. Significant changes occur in the structure of the mutant C1* domain of 22-VIII* factor – the polypeptide chain is substantially shortened and in the remaining small segment of 20 aminoacids, the ratio of hydrophilic and hydrophobic residues are altered. The hydrophobicity of the mutated region in the C1* fragment is

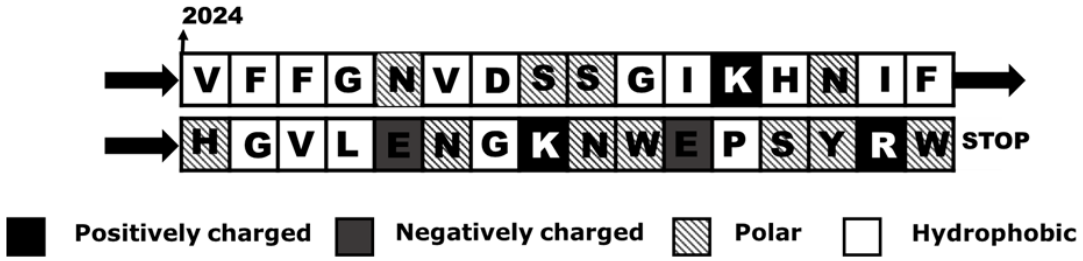

Figure 4. Amino acid sequence of 16 AA peptide fragment from C1 and C2 domains, coded by exon 23.

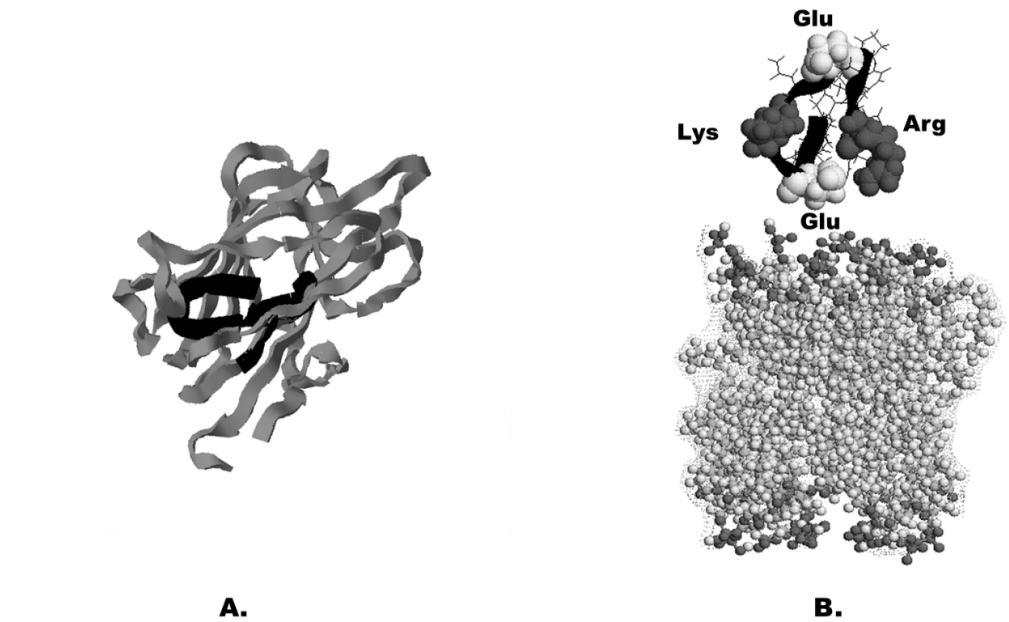

Figure 5. A. 3D superposition of C1 and C1* domens. B. Macromolecular docking of C1* domain to model anion lipid membrane.

decreased more than 60% in comparison with the similar region in the C1 domain of the functional activated FVIIIa.

Protein-lipid docking (Fig. 5B) was performed in order to check if remaining fragment C1* domain (altered in primary structure) can be incorporated in the platelet membrane through its C-terminal 16 aminoacid peptide. The macromolecular simulation indicates that the mutant C1* do not incorporate in model anionic lipid membrane as expected from the changes in the primary and tertiary structure. The results from the macromolecular analysis are fully consistent with experimental data for binding of FVIIIa and their mutant forms to phospholipids liposomes with different chemical composition (Wakabayashi and Fay 2013).

CONCLUSION

The absence of every aminoacid residues has a key role for protein – lipid interaction, thus decreasing the C1* domain hydrophobicity more than 60% and the structural changes in the protein macromolecule as a consequence of Inv22 in the F8-gene make impossible the mutant FVIII* to incorporate in the platelet membrane. Incorporation of this coagulation factor into the phospholipid membrane is of key role for building of an active complex with FIXa. Our results clearly show that I22I FVIIIa can not be incorporated into the platelets membrane and to form an active membrane-binding Xase complex and it is proteolytically degraded as a defective protein. For this reason, plasma levels of FVIIIa are critically low, which is typical for the severe form of haemophilia A.

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