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The Significance of Exon 17 Mutations Within FVIII Gene in Wasit City Patients with Hemophilia A

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Abstract: *Objective*: Hemophilia A is an X chromosome-linked disorder caused by different abnormalities in F8 gene, resulting in the absence of impaired molecule production of factor VIII (FVIII) in the plasma, an important protein in the intrinsic coagulation pathway. The hereditary testing of the F8 gene encoding FVIII is utilized for confirmation of HA diagnosis, which fundamentally diminished serious confusions of this disease and at last leading to longer duration of life. *Aims of study*: Exon 17 mutations in the FVIII gene were detected and analyzed in 10 HA Iraqi patients. *Patients and Methods*: This study included 10 Iraqi patient with hemophilia A and 5 healthy members as control. This work done in medicine & science college laboratories as well as AL Zahra Hospital. These patients' prior diagnoses were based on DNA testing and family history. *Results*: During the screening for exon 17 among the HA patients, results showed 6 (60%) from 10 patients had this mutations. Discussion: It has been shown that the severity of F8 gene mutations is also correlated with their types and locations. Our data feature and information emphasize the prominence of exon 17 for its association with HA patients' positive family ancestry, and we are continue to operate for other exons mutations. *Conclusions*: Our findings are advantageous for prenatal diagnosis, carrier detection, and HA diagnosis. Our research also shows that patients with HA suspicion should undergo F8 gene mutation screening because there is an association between mutations and severity in our case studies.

Keywords: Hemophilia A, Factor 8 Gene, Exon 17, Point Mutations, Frameshifts Mutations

1. Introduction

Deficiency in coagulation factor VIII encoded by F8 results in Hemophilia A is a common hereditary X-linked recessive bleeding disorder hemophilia A (HEMA) [1, 2]. HEMA occurs in 1:5.000–10.000 males with approximately one third of the cases being spontaneous mutations [3]. Almost four times as frequent as hemophilia B, hemophilia A affects over 80% of individuals, and around half of those who have it have the severe variant [4].

The only gene known to be related to hemophilia A is factor VIII (F8). F8 spans 186 kb of genomic DNA and is located at the terminal end of the long arm of the X-chromosome (Xq28). It has 26 exons, which together code for a polypeptide precursor with 2351 amino acids [5]. Three homologous A domains, two homologous C domains, and the unique B domain make up the mature FVIII protein. These domains are organized from the amino - terminal to the carboxyl-terminal end in the following order: A1-A2-B-A3-C1-C2. Due to the fact that each domain has unique binding sites for various elements of the coagulation cascade, the diverse domains are crucial to the way that FVIII functions [6, 7]. These binding areas may be impacted by genetic abnormalities, leading to HA. [8]. The telomeric end of the X-chromosome is home to the F8 gene (186 kb; 26 exons), which is more prone to methylation cytosine deamination mutations due to its high GC content. 2,537 mutations have been identified by the CDC Hemophilia A Mutation Project (CHAMP) [9]. Moreover this gene has been found that two hotspot inversions, known as intron 1 and intron 22 inversions, account for 40 to 50 percent of people The telomeric end of the X-chromosome is home to the F8 gene (186 kb; 26 exons), which is more prone to methylation cytosine deamination mutations due to its high GC content. 2,537 mutations have been identified by the CDC Hemophilia A Mutation Project (CHAMP) [10] in this gene.

Additionally, it has been found that two hotspot inversions, known as intron 1 and intron 22 inversions, account for 40 to 50 percent of patient suffering from severe hemophilia A [10-12] The massive size of the gene plus the fact that different mutations occur in patients who are not related due to the high frequency of de novo mutations. make it hard to analyze every relevant mutation in a patient [13, 14]. People from all racial and ethnic comunities are affected by hemophilia A [15, 4].

2. Materials and Methods

2.1. Samples Collection and DNA Extraction

After obtaining the informed consent from the patients, 5–10 ml of peripheral blood were collected in tubes containing EDTA. Genomic DNA was extracted from peripheral blood leukocytes by real-time PCR assay (Real-Q KIT Screening Kit; BioSewoom, Seoul, Korea) [16]. With informed consent, blood samples were taken from patients and their family members. Unlinked HA patients from Wasit province, Iraq, aged between 5 and 54 and 5 healthy members as control, who were evaluated from AL Zahra Hospital for DNA quality and quantity using UV-spectrophotometry (260 nm and 280 nm) or agarose gel electrophoresis, were included in the study.

2.2. Screening Test for Detection of Exon17 Mutations

The FVIII coding gene (F8exon)'s 17 mutations were screened using a real-time PCR assay (quantitative PCR, qPCR) and direct sequencing using a Multigene Optimax (Thermo Cycler) instrument. The F8 coding sequence was amplified with PCR and automatically sequenced. In order to avoid DNA-dimer formation, we redesigned the primers for the PCR reaction.

2.3. Thermocycling Program for Exon17

The polymerase chain reaction (PCR) products were electrophoresed on a 2% agarose gel and visualized under UV light. Direct sequencing for exon 17 with 298 base pairs was performed using NTI vector Pro, Clustal W method of MEGA4 Pro, NCBI/ BlAST program, Chromos Pro, and Mutation Surveyor, among other programs.

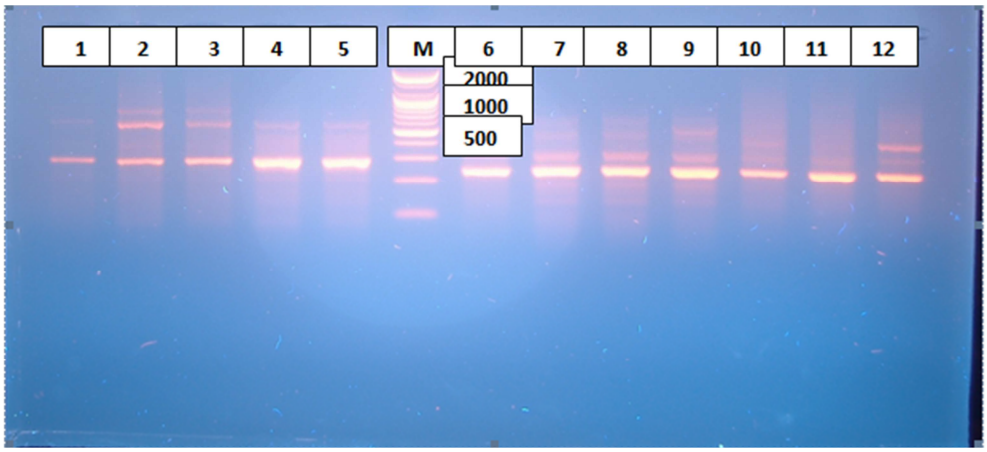

Figure 1. *PCR products of FVIII gene on 1% agarose gel at 70 voltages for one hour. Exon 17.*

Lane 1T, 2, 3, 4, 5P: Lane-M-standard molecular weights: Lane 6T, 6, 7, 8, 9, 10, 11, 12, P. Gel was stained with Ethidium bromide staining.
*p for patients; T for control

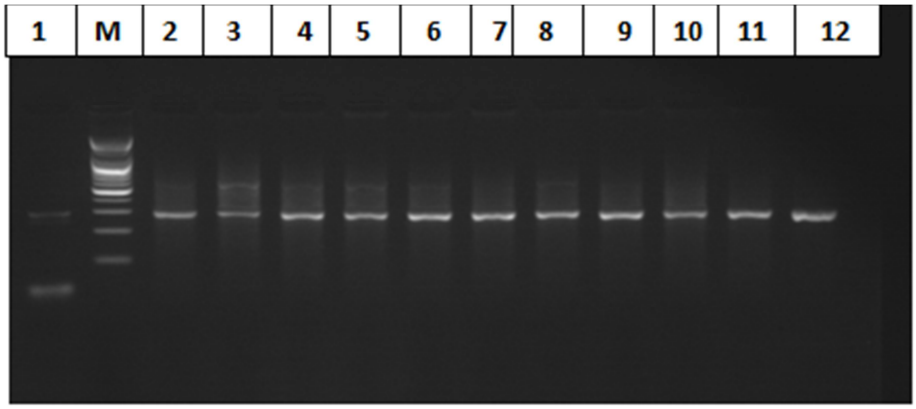

Figure 2. *PCR products of FVIII gene on 1% agarose gel at 70 voltages for one hour. Exon17.*

Lane-1T: Lane-M-standard molecular weights: Lane: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 P: Gel was stained with Ethidium bromide staining.
*p for patients; T for control

2.4. DNA Sequence Analysis

DNA sequence information is using to identify the genes and regulatory instructions that exist in the DNA molecule as well as changes in a gene that may be the origin of this disease. It can also represent the genetic information that is carried in a specific DNA segment, a whole genome, or a complex microbiome.

These results can be applied to genetic counseling in Alkut where consanguineous marriages are more prevalent. Hemophiliac patients group data & Laboratory diagnosis of these hemophilia A patients are depicted in (Table 1).

Table 1. *Hemophiliac patients group data.*

Patient sample no.	Mutation\Genome		Mutation type	Family history		Consanguinity state		severity		
1	4623delT		Framshift	positive		positive		Severe		
2	nill			positive		positive		moderate		
3	3211A>C		Point mutation	negative		negative		moderate		
4	nill		-	positive		negative		severe		
5	44712delG		Framshift	positive		negative		mild		
6	4511A>G		Point mutation	positive		positive		Severe		
7	3121G>A		Point mutation	negative		positive		moderate		
8	4155del T		Framshift	positive		positive		Severe		
9	nill		-	negative		negative		Moderate		
10	nill		-	negative		positive		severe		
	Yes	No		Positive	Negative	Positive	Negative	Severe	Moderate	Mild
Total	60	40		60%	40%	60%	40%	50%	40%	10%
	10									

3. Results

In our investigation, a novel frameshift deletion mutation (44712delG) in the F8 gene was found in the HA family of wasit patients. This study validated an existing mutation's pathogenicity, introduced a new mutation to the list of pathogenic variants in the F8 gene, and characterized the clinical symptoms and patient's blood clotting function. Direct mutation analysis or indirect linkage analysis has been performed on HA. Point mutations are the most prevalent kind of deficiency, possibly causing the disease. The second most frequent type of gene deficiency is deletions resulting in frameshifts [17-19]. Our understanding of the genetics mechanisms behind hemophilia in this area will improve with the identification of the causal mutations. The majority of the mutations found in our study were harmful, and these mutations were closely linked with severity of the phenotypes, especially for variations on the B-interfaces region's and their closest adjacent regions in the full-length molecule [13, 14]. Deleterious amino acid alterations result in this genetic disease. The study's testing for mutations reveals three point mutations in patients no. (3, 6, 7) with negative family history in patients no. (3, 7) and positive in patients no 6 and positive consanguinity in patients no. (6, 7) negative in patients no 3 as well as three Framshift deletion mutations in patients no. (1, 5, 8) appears in this study of the FVIII gene reveal with positive family history and positive consanguinity in patients no. (1, 8) and negative consanguinity in patients no 5 state as showed in figures 3, 4.

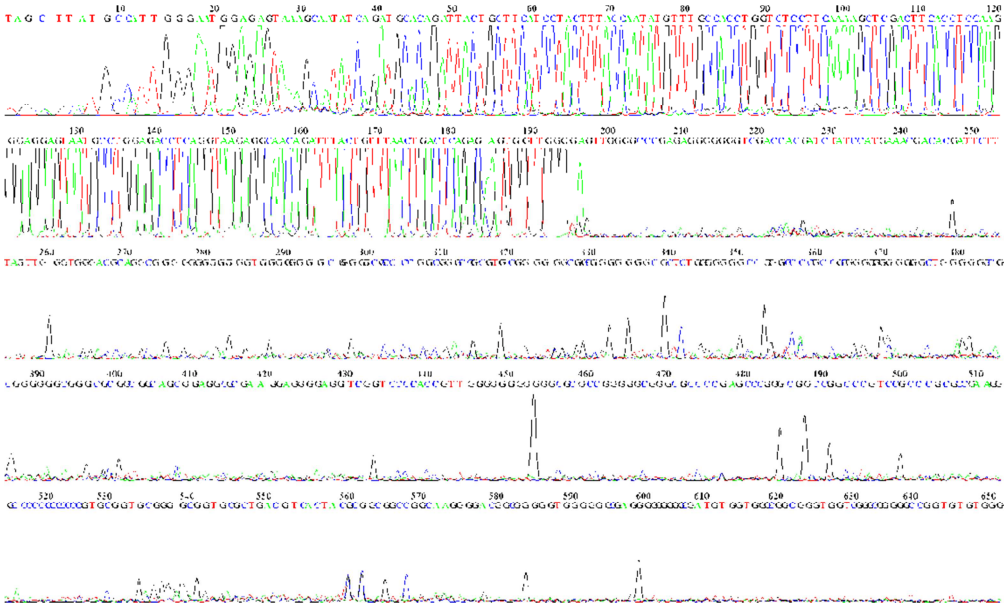

Figure 3. *DNA sequencing (forward) for patient no. 5 detect with exon17.*

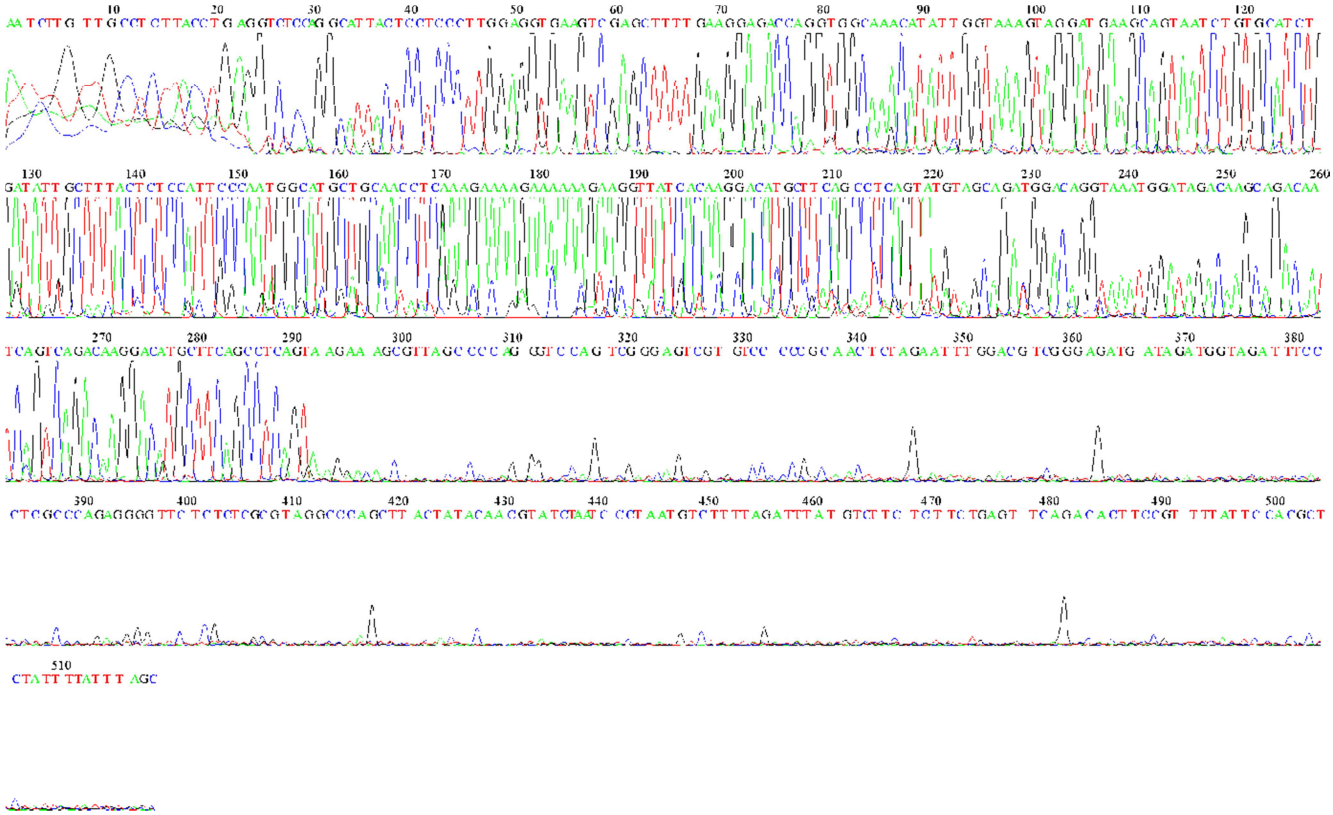

Figure 4. *DNA sequencing (reverse) for patient no. 5 detect with exon17.*

Patient no. 6 represent in this study detect with mutation in exon 17 of the FVIII gene reveal with positive family history and consanguinity state as reveal in figures 5, 6.

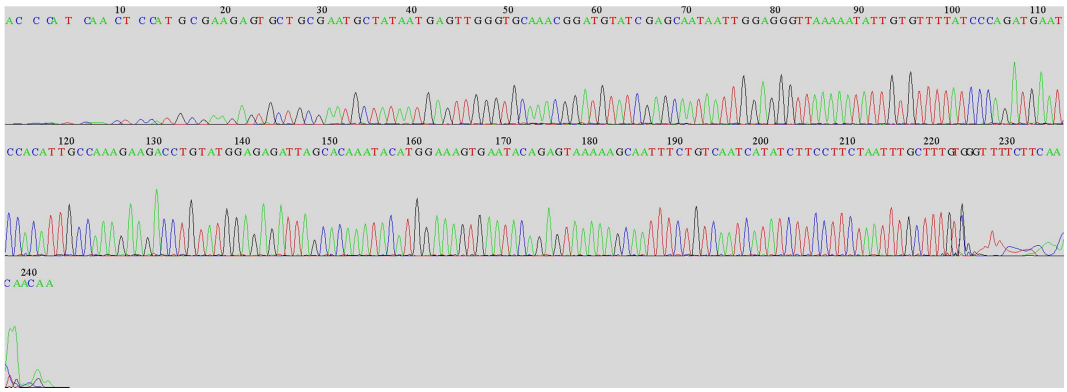

Figure 5. *DNA sequencing (forward) patient no. 6 detect with exon17.*

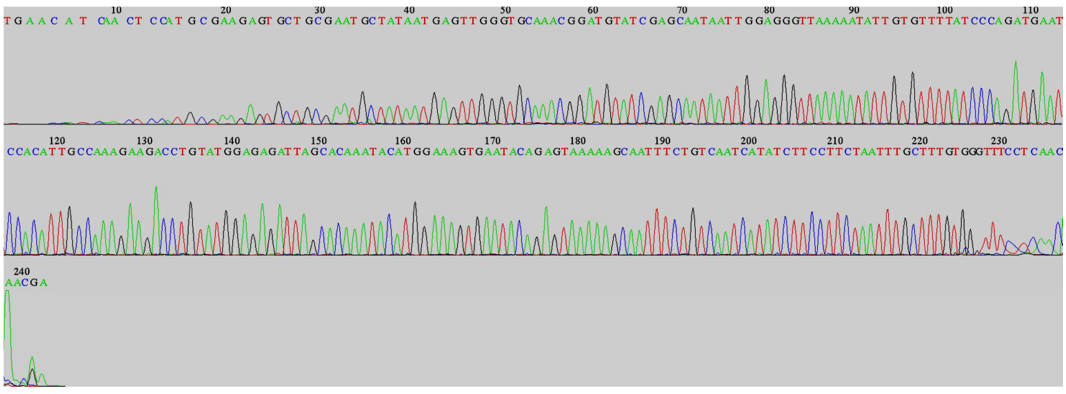

Figure 6. *DNA sequencing (reverse) for patient no. 6 detect with exon17.*

4. Discussion

The factor VIII gene's coding region has 70 CpG dinucleotides. As predicted, nearly all mutations in patients with mild to moderate hemophilia A were found in the current research; however, only 40% of mutations in patient with severe disease were identified. The coding area included the triggering mutation [20]. In this analysis, hemophilia A patients were screening for mutations in some coding regions and splice sites of the FVIII gene.

The identified mutation in the most often, is utilized to describe the patient's clinical phenotype, revealing a strong correlation between the genotype and HA phenotype. Patients with severe hemophilia A represent the majority of cases where frameshift mutations that result in an early stop codon have been described. For this, various frameshift mutations are recorded in the research [21].

Also in the case patient no 5, who bears from mild haemophilia A, in which this mutation shows a premature termination codon resulting in truncations in the B domain of the FVIII protein; so the clinical phenotype and genotype are incompatible [19]. In addition, we found three frame shift mutations in this analysis caused by by deletions.

The number of frameshift mutations observed in this study in 30 (40%) severe hemophilia A patients—was result by a lack of hypo-xanthine phosphoribosyl transferase [22]. A slight fluctuation was observed in factor VIII protein sequence at targeted mutated region, which was at the flexible region of factor VIII. The loop mutation in the flexible region of factor VIII domain A1 was found close to the -sheet 10. At elevated temperatures (300 K/60 °C), the flexibility was increased by the change of the flexible region. Due to the flexible region's reduced flexibility, the significant point mutation resulted in an increase in protein folding and compactness. The significant variation of flexible region would trigger an unfolding process and consequently denature the protein [23]. Frameshifts mutations causes disease mostly because it disrupts a critical protein function, as according variations in the folding free energy upon mutation [24, 25].

5. Conclusions

Early hemophilia diagnosis and treatment enhance the patient's quality of life by avoiding the appearance of problems at an early stage. Knowing a specific mutation is very important since it can help with genetic counseling and predicting the likelihood of developing anti-FVIII antibodies, the most serious hemophilia consequence. a current treatment. Considering that it is a large gene (7.2 kb of coding sequence, represented by 26 exons. Thorough identification of mutations in a certain genetic defect may reveal previously unknown or unappreciated regulatory components of the gene responsible for the disorder or may indicate the presence of other genes responsible for the phenotype of that specific disorder.

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References

[1] Mann, K. G. (1984) in Progress in Hemostasis and Thrombosis, ed. Staet, T. H. (Grune & Stratton, Philadelphia), Vol. 7, pp. 1-23.

[2] Sadler, J. E. & Davie, E. W. (1987) in The Molecular Basis of Blood Disease, eds. Stamatoyannopoulos, G., Nienhuis, A. J., Leder, P. & Majerus, P. W. (Saunders, Philadelphia), pp. 575-630.

[3] Bicocchi MP, Pasino M, Lanza T, Bottini F, Boeri E, Mori PG, Molinari AC, Rosano C, Acquila M. Analysis of 18 novel mutations in the factor VIII gene. British Journal of Haematology. 2003; 122: 810–7.

[4] Bolton-Maggs PH, Pasi KJ. Haemophilias A and B. Lancet. 2003; 361 (9371): 1801–1809.

[5] Husain N. Carrier analysis for hemophilia A: ideal versus acceptable. Expert Rev Mol Diagn 2009; 9 (3): 203–7.

[6] Bowen DJ. Haemophilia A and haemophilia B: molecular insights. Mol Pathol 2002; 55 (2): 127–44.

[7] Brower C, Thompson AR Hemophilia A. 2000 Sep 21 [updated 2008 Mar 25]. In: Pagon RA, Bird TC, Dolan CR, Stephens K, editors. GeneReviews [Internet]. Seattle (WA): University of Washington, Seattle; 1993-. Available from http://www. ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=gene&part=hemo-a.

[8] Keeney S, Mitchell M, Goodeve A. UK Haemophilia Center Doctors' Organization Haemophilia Genetics Laboratory Network. The molecular analysis of haemophilia A: a guideline from the UK haemophilia centre doctors' organization haemophilia genetics laboratory network. Haemophilia 2005; 11 (4): 387–97.

[9] Payne, A. B., Miller, C. H., Kelly, F. M., Michael Soucie, J., and Craig Hooper, W. (2013). The CDC hemophilia a mutation project (CHAMP) mutation list: a new online resource. *Hum. Mutat.* 34, E2382–E2391. doi: 1002/humu.22247.

[10] Andrikovics, H., Klein, I., Bors, A., Nemes, L., Marosi, A., Varadi, A., et al. (2003). Analysis of large structural changes of the factor VIII gene, involving intron 1 and 22, in severe hemophilia A. *Haematologica* 88, 778–784.

[11] Oldenburg, J., and El-Maarri, O. (2006). New insight into the molecular basis of hemophilia A. *Int. J. Hematol.* 83, 96–102. doi: 10.1532/ijh97.06012.

[12] Zimmermann, M. A., Oldenburg, J., Muller, C. R., and Rost, S. (2011). Unusual genomic rearrangements in introns 1 and 22 of the F8 gene. *Hamostaseologie* 31 (Suppl. 1), S69–S73.

[13] Higuchi, M., Wong, C., Kochhan, L., Olek, K., Aronis, S., Kasper, C. K., Kazazian, H. H., Jr., & Antonarakis, S. E. (1990) Genomics 6, 65-71.

[14] Wong, C., Dowling, C. D., Saiki, R. K., Higuchi, R. G., Erlich, H. A. & Kazazian, H. H., Jr. (1987) Nature (London) 330, pp. 384-386.

[15] Mannucci PM, Tuddenham EG. The hemophilias - from royal genes to gene therapy. *N Engl J Med.* 2001; 344 (23): 1773–1779.

[16] R K Saiki 1, D H Gelfand, S Stoffel, S J Scharf, R Higuchi, G T Horn, K B Mullis, H A Erlich. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science. 1988 Jan 29; 239 (4839): 487-91.

[17] Nataraj AJ, Olivos-Glander I, Kusukawa N, et al. Single-strand conformation polymorphism and heteroduplex analysis for gel-based mutation detection. *Electrophoresis.* 1999; 20 (6): 1177–85.

[18] Fodde R, Losekoot M. Mutation detection by denaturing gradient gel electrophoresis (DGGE) *Hum Mutat.* 1994; 3 (2): 83–94.

[19] Yee TT, Williams MD, Hill FG, Lee CA, Pasi KJ. Absence of inhibitors in previously untreated patients with severe haemophilia A after exposure to a single intermediate purity factor VIII product. Thromb Haemost. 1997; 78 (3): 1027–1029.

[20] Koeberl, D. D., Bottema, C. D. K., Ketterling, R. P., Bridge, P. J., Lillicrap, D. P. & Sommer, S. S. (1990) Am. J. Hum. Genet. 47, 202-217.

[21] Akkarapatumwong V, Intorasoot S, Oranwiroon S, Thano-Otarakul P, PungAmritt P, et al. (2000) Frameshift mutations with severe and moderate clinical phenotypes in Thai hemophilia A patients. Hum Mutat 16: 530-531.

[22] Rossiter, B. J. F., Edwards, A. & Caskey, C. T. (1991) in Molecular Genetic Approaches to Neuropsychiatric Disease, eds. Brosius, J. & Freneau, R. (Academic, New York), in press.

[23] Kamal MZ, Ahmad S, Yedavalli P, Rao NM. Stability curves of laboratory evolved thermostable mutants of a Bacillus subtilis lipase. *Biochim Biophys Acta.* 2010; 1804 (9): 1850–1856.

[24] Shen BW, Spiegel PC, Chang CH, Huh JW, Lee JS, Kim J, Kim YH. et al. The tertiary structure and domain organization of coagulation factor VIII. *Blood.* 2008; 111 (3): 1240–1247.

[25] Markoff A, Gerke V, Bogdanova N. Combined homology modelling and evolutionary significance evaluation of missense mutations in blood clotting factor VIII to highlight aspects of structure and function. *Haemophilia.* 2009; 15 (4): 932–941.