Detecting Mutations Linked with Hemophilia A and B in Duhok Province

Eman Bunyan Ibrahim[1], Rana Adil Hanoon[2]

[1] Universty of Duhok , College of science /Iraq
[2]Universty of Duhok , College of health science
rana.hanon@uod.ac, +9647506071749

https://doi.org/10.15218/crewh.2024.08

Abstract

Abstract: Background & Objectives: This study aimed to identify mutations associated with hemophilia A and B in patients from Duhok Province, Iraq. The study sought to characterize genetic mutations in FVIII and FIX genes and their impact on disease manifestation.

Materials and Methods: Seventy-five hemophiliac patients (62 with hemophilia A, 13 with hemophilia B) and 75 control subjects were included. DNA extraction, PCR amplification of specific gene regions, and sequencing were performed.

Results: Several novel mutations were identified, particularly in intron 22 of the FVIII gene and exon 4 of the FIX gene. No mutations were found in FVIII intron 1. Amino acid changes resulting from these mutations were observed.

Conclusion: The research reveals novel mutations associated with hemophilia A and B in the Duhok region, contributing to a better understanding of the genetic basis of these conditions.

Keywords: FVIII Intron 22; FVIII Intron 1; FIX genes; Sequencing.

Introduction

Hemophilia is an inherited blood clotting disorder characterized by a deficiency in clotting factors, specifically factor VIII (FVIII) in hemophilia A and factor IX (FIX) in hemophilia B[1]. This leads to prolonged bleeding rather than faster clotting. Symptoms include internal bleeding, particularly in joints and muscles, with bleeding in the brain being a severe complication. Diagnosis involves assessing family history, physical exams, and blood tests to determine severity and rule out other causes[2].

Identifying genetic mutations is crucial for predicting inhibitor formation and detecting carriers, especially in females. In severe hemophilia A cases, intron 22 inversions and intron 1 mutations in the F8 gene are common. Initial screening involves searching for these mutations,

followed by comprehensive genetic sequencing if necessary. For moderate and mild cases, complete mutation analysis of F8 gene is essential. In hemophilia B, sequence analysis of the F9 gene is conducted on exons, intron-exon boundaries, and the promoter region, with fewer recurring genetic variations compared to hemophilia A[3].

One challenging aspect of this study may be the comprehensive genetic sequencing required to detect mutations in individuals with hemophilia, particularly in cases where common genetic defects are less prevalent. Another challenge could be obtaining a representative sample size from Duhok province for molecular screenings, ensuring inclusivity and accuracy in identifying genetic variations associated with hemophilia A and B in the region. Additionally, interpreting the significance of identified mutations and correlating them with clinical outcomes may pose a challenge, especially considering the diverse genetic backgrounds and potential environmental factors influencing the manifestation of hemophilia.

Objectives of the study:

1. To identify prevalent genetic variations associated with hemophilia A and B in Duhok province, focusing on factor VIII and factor IX.
2. To conduct molecular screenings to detect specific mutations linked to hemophilia A and B, including intron 22 inversions and intron 1 mutations in hemophilia A, and sequence analysis of the F9 gene in hemophilia B.

Materials and methods

This research was conducted at the Scientific Research Center within the College of Science, University of Dohuk during the period from July 2018 to February 2019. The study involved individuals diagnosed with hemophilia A (62 patients) and hemophilia B (13 patients), comprising cases with varying severity levels, including mild, moderate, and severe, who had abstained from receiving FVIII or FIX concentrate or any blood products for the past fifteen days. The age range of participants in both groups spanned from 1 year to 46 years. Patients were selected from the JIN Pediatric Hematology-Oncology Center in Duhok, and a questionnaire form was completed for each individual.

1- **Genomic DNA extraction:** Genomic DNA extraction utilized the Wizard DNA Purification Kit, with purity and concentration determined via Spectro-nanometer. Agarose gel electrophoresis involved preparing 1% and 2% gels, casting them, and loading DNA

samples with bromophenol blue dye and DNA ladder. Electrophoresis ran at 45 volts initially, increasing to 68 volts for 30 minutes. Gels were then soaked in ethidium bromide and viewed under a UV transilluminator for imaging. DNA concentration and purity were estimated using the Spectro-nanometer, with purity assessed as the OD 260/280 nm ratio[4].

2- **Primers**

Lyophilized primers were supplied by Macrogen Company. They were dissolved in a free DNase/ RNase deionized distilled water to give a final concentration of 100 pmol/µl as stock solution according to the manufacturer instructions. Twenty microliters of stock solution diluted in 80 µl of a free DNase / RNase deionized distilled water to obtain 20pmol/µl concentration primer as work solution.

A- Specific primers for FVIII (intron 22, intron1) and FIX genes

Eight primer sets were depended to detect FVIII intron 22,FVIII intron1, and FIX genes, as illustrated in Tables 1,2, and 3. All PCR primers were obtained from the literature and checked for specificity through NCBI primer BLAST[5,6].

Table (1): Primers sequence for hemophilia A(*F8* Gene) int22[5].

No.	Primers Name	Primer sequence of (5'→3')		Annealing Temp.	PCR Product size
1	Primer2 int 22	F	GCAACACTGCAGTCATGGTC	66ºC	723 bp.
		R	TTCAGTGAGCCAGGTGGAGC		
2	Primer3 int 22	F	GGC CGT CAG GTA CTC AAT AA	57 ºC	801 bp.
		R	GTTGCCAGGGACTATCGGGA		
3	Primer4 int 22	F	CCTATAAGCAGTCACTTGCC	66 ºC	1662 bp.
		R	TGGTACTGCCATCGTGATCG		

Table (2): Primers sequence for hemophilia A(*F8*Gene) int1[6].

No.	Primer	Sequence 5'→3'	Annealing Temp.
1	HA-9F	GTTGTTGGGAATGGTTACGG	65
2	HA-9cR	CTAGCTTGAGCTCCCTGTGG	65

3	Int1h-2F	GGCAGGGATCTTGTTGGTAA	65
4	Int1h-2R	TGGGTGATATAAGCTGCTGAGCTA	65

Table (3): primers Sequence of (*F9* Gene) used in this study[5].

Region	Primers sequences	Annealing	Band size
Exon 4	F 5-GGCTTCCAGGTCAGTAGTTTTGC-3 R 5TTTTCCAGTTTCAACTTGTTTCAGAGG-3	60 ºC	308 bp.
Exon 5	F 5-AAATGATGCTGTTACTGTCT-3 R 5-GTTTGTTAAAATGCTGAAGT-3	56 ºC	229 bp.
Exon 7	F 5-CAAATGTATTATGCAGTAAGAG-3 R 5-TGTACCAATCATATTAAAGAGC-3	56 ºC	218 bp.

B- **Amplification programs of hemophilia genes**

Significant programming of Thermo cycler (Applied Biosystem, Singapore) was accomplished after several trials for annealing steps. Suitable annealing temperature was selected for optimum amplification of specific sequences of FVIII and FIX genes, as are listed in Tables 4,5, and 6.

Table (4): FVIII genes amplification program (intron22).

Steps	Temperature (ºC)	Time	No. of Cycles
Initial incubation	94	5 min	1
Denaturation	94	30 sec	30
Annealing	54-66 depends on gene amplified	45 sec	
Extension	72	30 sec—90 sec depends on size amplified fragment	
Final extension	72	5 min	1
Hold	4	-	-

*annealing temp primer 3 (57), primer 2, 4 (66). *extension time. primer3 (45), primer 2,4 (90).

Table (5): FVIII genes amplification program (intron 1).

Steps	Temperature (ºC)	Time	No. of Cycles
Initial incubation	95	5min	1
Denaturation	94	1min	30
Annealing	65	1min	
Extension	72	2min	
Final extension	72	5min	1
Hold	4	_	_

Table (6): FIX genes amplification program.

Steps	Temperature (ºC)	Time	No. of Cycles
Initial denaturation	94	5min	1
Denaturation	94	30sec	30
Annealing	54-66 depends on gene amplified	30sec	
Extension	72	30sec	
Final extension	72	5min	1
Hold	4	-	-

*annealing temp. exon 7 (60), primer4,5 (56).

3- **PCR amplification components**

Amplification mixtures are detailed in the following Table (7).

Table (7): Amplification mixture and mixing amounts

Component	Component of one sample(µl)
PCR premix (Promega) include: *Taq* DNA polymerase, dNTPs, MgCl2 and reaction buffer (PH 8.5)	12.5

Forward primer	2, (20pmol)
Reverse primer	2, (20pmol)
DNA template	2, (50ng/ml)
Nuclease free water (DD.W)	6.5
Total volume	25

Analysis of *F8* Intron 1 Inversion

Sixty-two patients HA were screened, four primers (9F, 9CR, 2F, and 2R) used for detecting intron 1 mutation, multiplex PCR is performed for each genomic DNA sample. Those primers patterns have been used to test each of: Int1h-2F + Int1h-2R, 9F + 9cR, Int1h-2R + 9F, Int1h-2F + 9cR. Primer's final concentration was 10 pmol/µl (Table 8)[7].

Table (8): interpretation outcomes for *F8* Intron 1 Inversion

primer mix	Primer pair	Product	Genotype
(Int1h-2)	Int1h-2F + Int1h-2R +9F	~ 1kb	Normal
		~1.5 kb	Inversion
(Int1h-1)	9F + 9cR + Int1h-2F	~ 1.5 kb	Normal
		~1kb	Inversion

Analysis of PCR Products

PCR products (5 µl) were analyzed by electrophoresis, but the running conditions were manipulated to be 75 minutes on 80 volts and the gel concentration was 2%. Moreover, PCR products were photographed by gel documentary system version 3.0 (ATTO, Japan), and the amplicon size was further confirmed by CS analyzer software.

Sequencing of the PCR product

Sequencing was done to detect and identify the type of mutation in the gene of interest. Sixty-seven PCR products (49 patients' samples and 18 control samples) A, B were analyzed by sequencing the amplified target genes of int22 of hemophilia A and exon 4,5,and7 in hemophilia B. Sequencing was performed at Macrogen company, South Korea. Each successfully amplified fragment was sequenced using the same primers utilized for amplification and the Big Dye

Terminator V3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA).All obtained sequences were cleaned and trimmed using Geneious Prime software to confirm identity.

The sequences *Homo sapiens* coagulation factor VIII (*F8*), RefSeqGene on chromosome X (accession no. NG_011403.1) obtained from National Center for Biotechnology Information (NCBI)gene bank, were adopted as references for alignment searching for hemophilia A

The DNA sequences were aligned with the reference sequence of the *F9* gene *Homo sapiens* coagulation factor IX (*F9*), the Ref Seq gene on chromosome X (accession no. NG 007994.1) which is available in the NCBI. Database used as a reference for the alignment search for haemophilia B.

Statistical analysis

All data are analyzed by the SPSS (IBM Corporation, New York, NY, USA) statistical package (Version 25.0). Chi-square test for independence has been used to find the P-value.

Molecular analysis

DNA concentration obtained in the present work ranged between 100-500 ng/ml. The final concentration was adjusted to 50 ng/ µl which was applied in all PCR amplifications throughout the study.

Detection of intron 1 of hemophilia A

The results of this study included screening of Sixty-two patients, for detection of Intron 1 inversion, using 9F, 9cR, 2F, and 2R primers and by multiplex PCR. First amplification mixture was formulated to find mutation in 1 Int1h-1sequenceand the result was an amplification band of 1908 bp, while the second amplification components were assigned to identify mutation in Int1h-2 represented by 1191bp amplification band (Figure 1and 2).

In this study, a total of sixty-two patients were screened to detect Intron 1 inversion. The screening process involved the utilization of four primers: 9F, 9cR, 2F, and 2R, in conjunction with multiplex PCR. The first amplification mixture was specifically designed to identify mutations in the 1 Int1h-1 sequence. This amplification yielded a band of 1908 bp, indicating the presence of the mutation. Conversely, the second amplification components were tailored to detect mutations in Int1h-2, which was represented by an amplification band of 1191 bp (refer to Figure 4-1 and 4-2).

Figure (1): Amplicons of factor VIII gene of intron 1 inversion. Multiplex amplification of primers mixture of (Int1h-1) 9F + 9CR + Int1h-2F=1908bp. Lanes 1-14 represent non mutant individuals, L=DNA ladder = 100bp. Electrophoresis of PCR products run on 2% agarose.

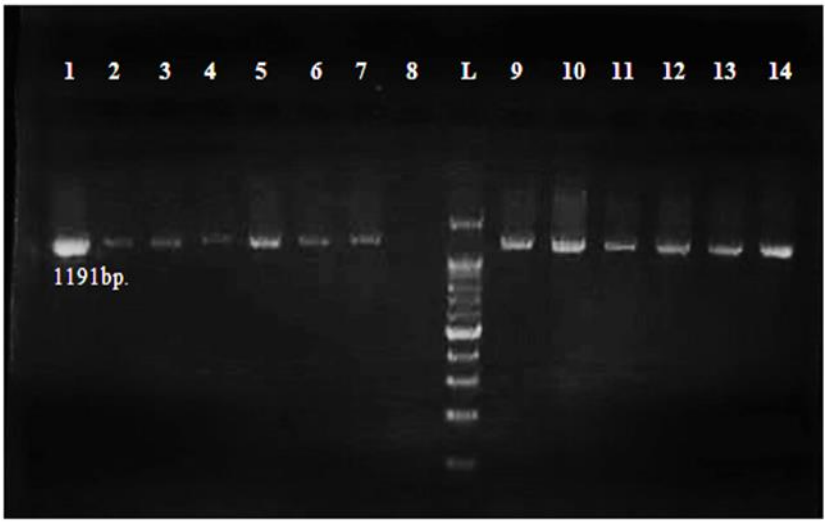


Figure (2): Amplicons of factor VIII gene of intron 1 inversion amplification products((Int1h-2) Int1h-2F + Int1h-2R +9F=1191bp Lanes 1-14 represent normal individual, L=DNA ladder = 100bp.PCR products run on 2 % agarose.

Detection of intron 22 of Hemophilia A

The intron 22 inversion mutation was detected by performing PCR using genomic DNA with three primers that differentiate between the normal DNA and patient DNA. This study supports the idea that overlapping PCR provides information about IVS-22 in 24 hours and is less complicated as compared to southern blotting technique, which takes about one week. The current research showed that after neglecting intron 1, all hemophilia A caused by spontaneous homologous recombination resulted in the inversion of intron 22 or other region of the *F8*gene. In this study, the middle region of Int22h-1selected. Three primers (2,3 and 4) are used to amplify this region for control (62 controls) and patients (62 patients), respectively. Wild-type and inversion templates are used for the PCR, a successful amplification was obtained for both cases, as shown in figures 3, 4, 5, respectively.

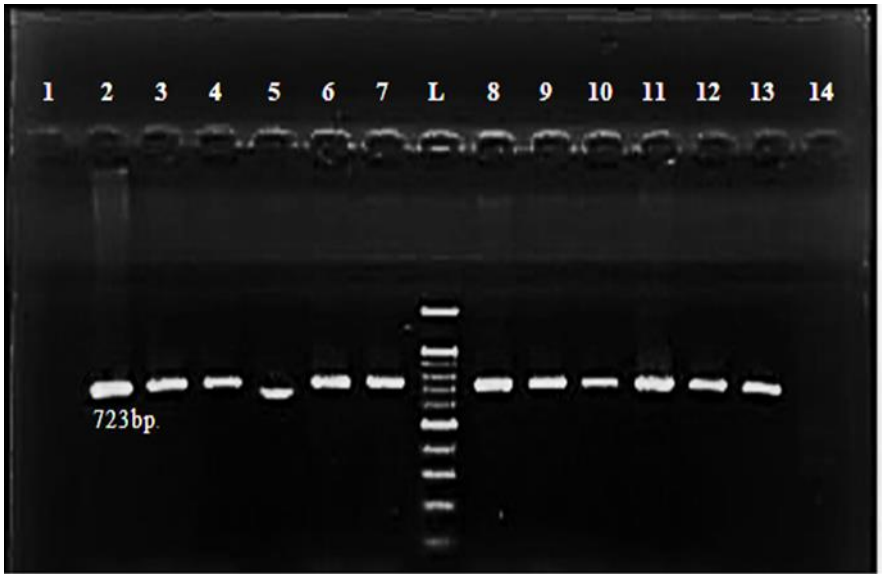


Figure (3): Amplification product of intron 22 of FVIII gene by primer 2, Lines 2_13with 723bp. L is a DNA ladder(100bp). PCR products run on 2 % agarose.

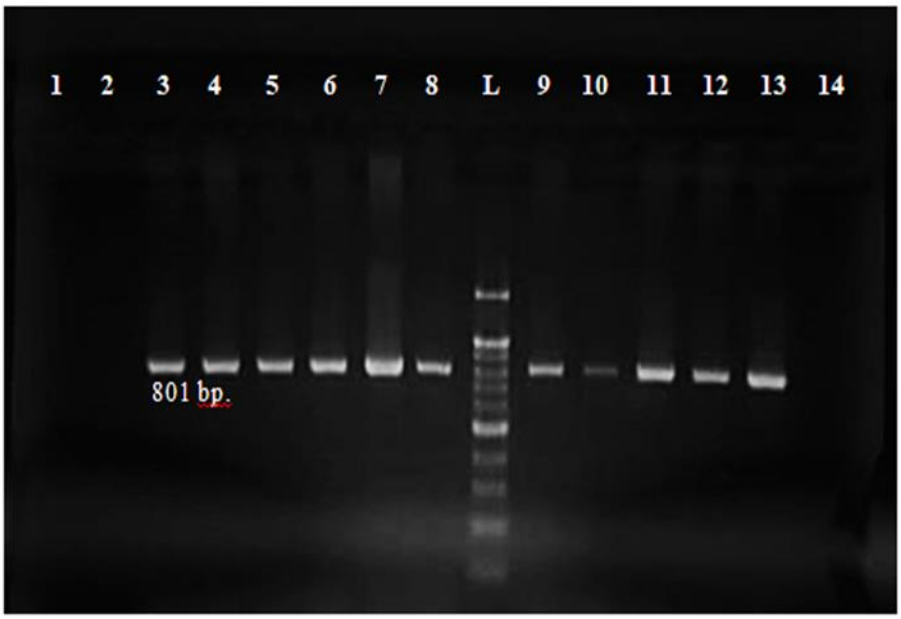


Figure (4): Amplification product of intron 22 of FVIII gene by primer 3 , Lines 3_13 with801 bp. L is a DNA ladder(100bp).PCR products run on 2 % agarose.

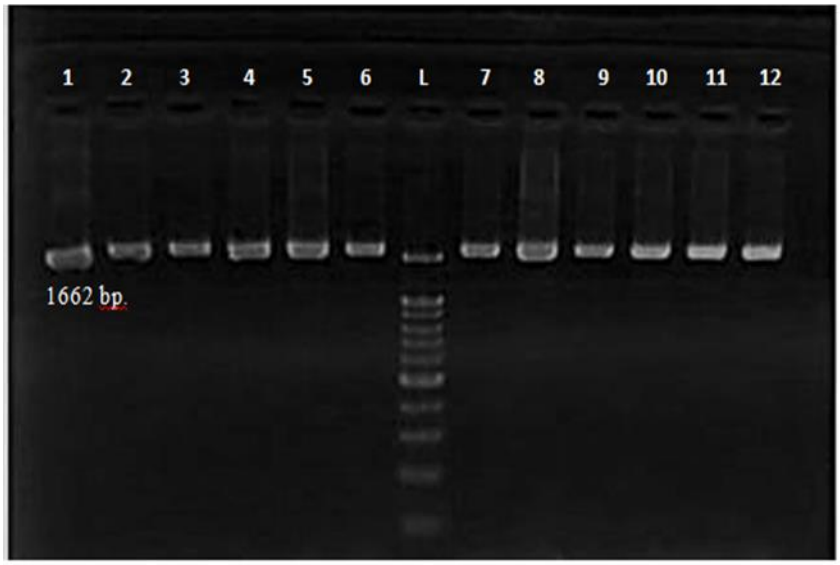


Figure (5): Amplification product of intron 22 of FVIII gene by primer 4 , Lines1_12 with 1662 bp. L is a DNA ladder(100bp).PCR products run on 2 % agarose.

Sequencing analysis of intron 22 FVIII gene

In this study, data on FVIII mutation for the sequencing analysis are confirmed from samples 36 (13 samples for primer 2, 11 samples for primer 3, and 12 samples for primer 4) with severe, moderate, and mild HA. A genetic abnormality reported from the intron 22 inversion in some patients, although no mutations have been identified in other patient studies, it is possible because that the mutation is outside the studied area (Tables 9).

Table (9): Detection of a gene mutation for hemophilia A patients and control.

Mutation Site	Ref-Seq. Gene		Target mutation		Type of mutations	Mutation Effect	Repeated	Clinical signs (phenotypic appearance)	
	Wild	Amino acid	Mutation	Amino acid				Disease nomenclature	Disease feature
Primer 2 (p2)									
g.142472	**CGG**	Arg	**CAG**	Gln	Substitution	Missense	11/13	Hemophilia A (F8)	8moderate 2sever 1mild
g.142302	**CAG**	Gln	**CAC**	His	Substitution	Missense	1/13	Hemophilia A (F8)	Moderate
g.142015,	**TGC**	Cys	**GGC**	Gly	Substitution	missense	3/13	Hemophilia A (F8)	2moderate 1 severe
g.142533,	**CAG**	Gln	**CAC**	His	Substitution	Missense	1/13	Hemophilia A (F8)	Moderate
g.10-p2-control									
Primer 3 (p3)									
g. 143222	**ACC**	Thr	**ACT**	Thr	Substitution	Silent mutation	2/11	Hemophilia A (F8)	2moderate
g.142717	CAA	Gln	CTA	Leu	Substitution	Missense mutation	1/11	Hemophilia A (F8)	Moderate

g.10-p3-control									
Primer 4(p4)									
g.143276	**GGC**	Gly	**GAC**	Asp	Substitution	Missense	12/12	Hemophilia A (F8)	7moderate 3sever 2mild
10-P4-control									

Table 9 shows the detected gene mutation that caused hemophilia A by the use of three primers. Four novel mutations detected with primer 2 in many patients, including the missense mutation at position 142472 that altered Arginine to Glutamic acid (Arg to Gln), the second missense mutation found at position 142302 that originated from the change of Glutamic acid to Histidine (Gln to His), the third missense mutation at position 142533, which changed Glutamic acid to Histidine (Gln to His), the last missense mutation at position 142015, which change leucine to Arginine (Leu to Arg) and all of these mutations resulted from substitution mutation(Figure 6).

The current study revealed two novel mutations with primer 3, one of which was a silent mutation at position 143222 (Thr to Thr), while the second mutation belongs to the missense type

at position 142717 (Gln to Leu) (Figure 7). The primer 4 result was one novel mutation, including nonsense (Substitution (Trp to *)) mutation at position 143276 (Figure 8).

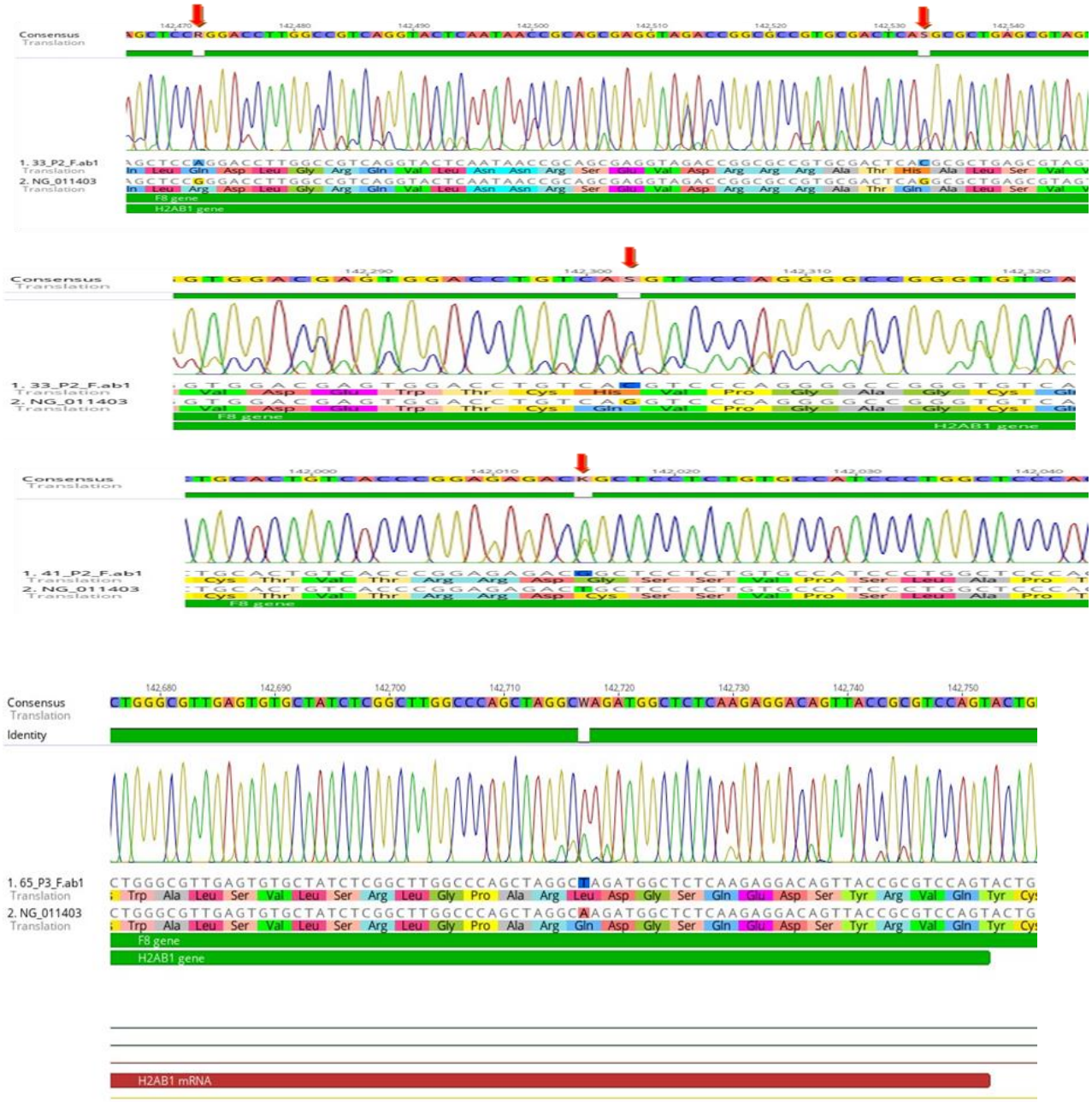


Figure (6): Alignment of haemophilia A intron 22 of primer 2 with reference genes.

Figure (7): Alignment of haemophilia A intron 22 of primer 3 with reference genes.

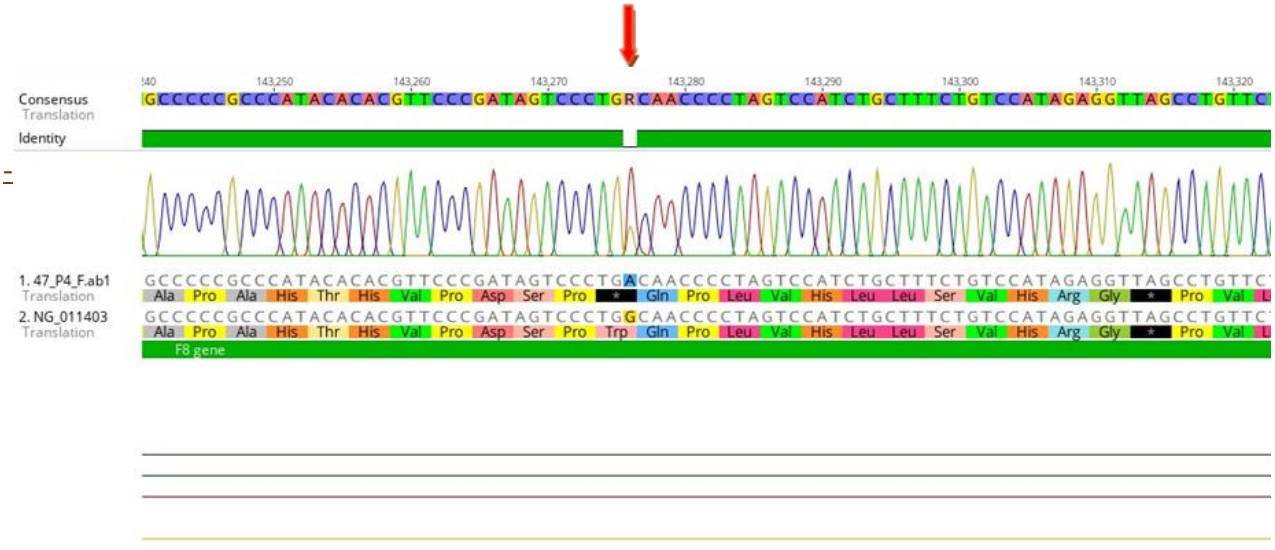
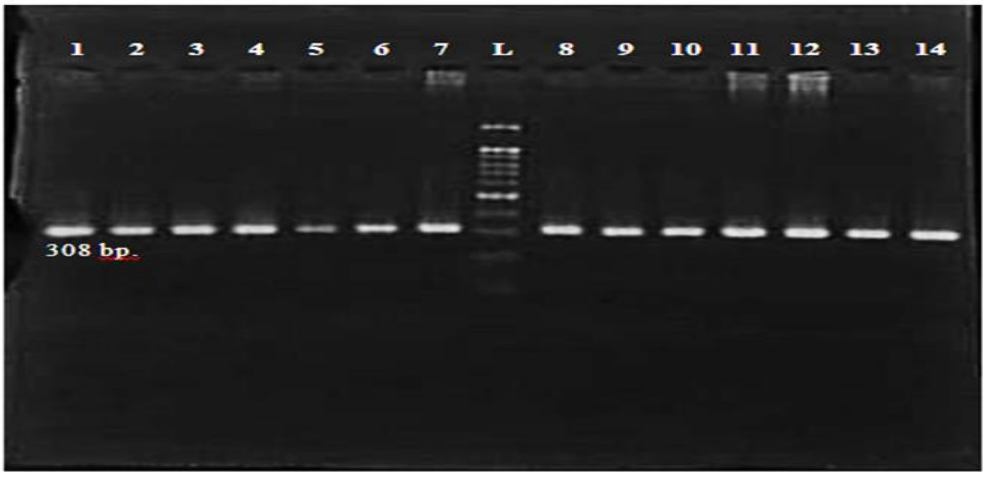


Figure (8): Alignment of haemophilia A intron 22 of primer 4 with reference genes.

Detection of Hemophilia B

Three primers sets were synthesized to amplify 4,5, and 7exons of *F9* gene. Screening for mutation within*F9* gene in samples of hemophilia B; 13 patients and 9 health samples was adopted. PCR and sequencing of the entire coding sequence of *F9* gene resulted in the identification of all pathologic mutations. A successful amplification was obtained for patients and control samples, the amplified fragment size was 308 bp in the Exon 4 region, 229 bp in the Exon 5 region and



218bp in the exon 7 region of *F9* (FIX) gene, as shown in figure 9, 10, 11, respectively.

Figure (9): Amplification product of exon 4 of F9(FIX) gene Lanes -1_14 with 308 bp. L is a DNA ladder(100bp). PCR products run on 2 % agarose.

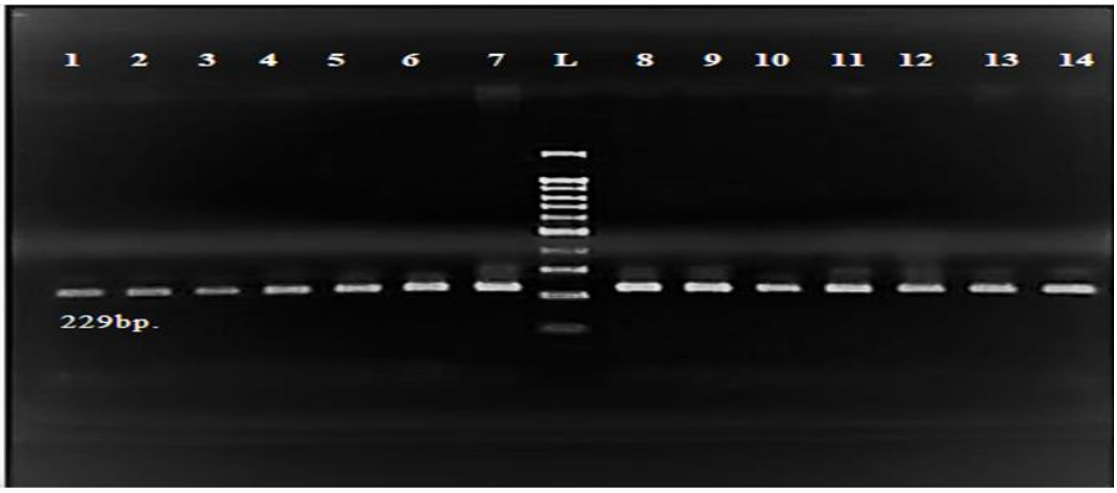
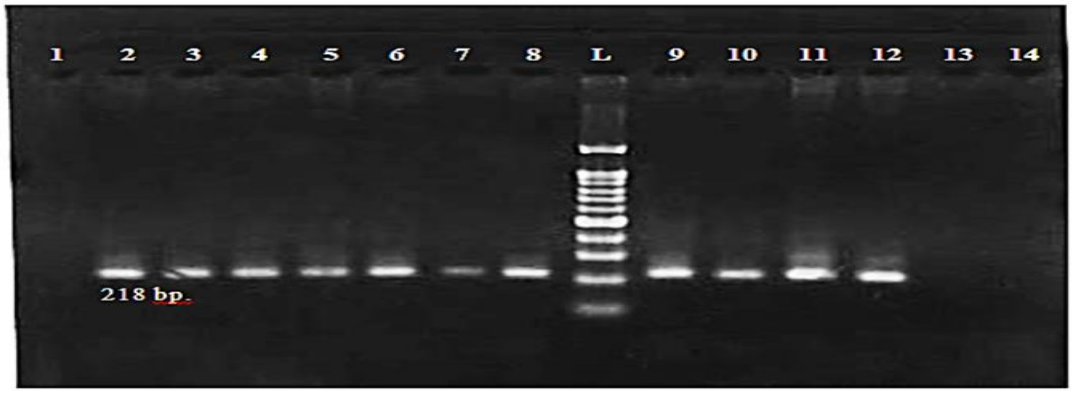


Figure (10): Amplification product of exon 5 of F9 (FIX)gene Lanes 1-14 with 229 bp.L is a DNA ladder(100bp).PCR products run on 2 % agarose.



Figure(11): Amplification product of exon 7 of F9 (FIX)gene Lanes 2_12 with 218bp. L is a DNA ladder(100bp). PCR products run on 2 % agarose.

Sequencing analysis of exons of FIX

A total of 13 patients with hemophilia B and 9 healthy samples were analyzed for exons 4,5, and 7 of the *F9* gene using PCR amplification and direct sequencing. The resulting mutation was single-substitution mutations with exon 4 in patients with hemophilia B, while those with exon 5 and 7 did not detect mutations. Single substitution mutation was detected in the sequence review at position 15445 T > C with exon four, which switches the amino acid leucine to proline (Figure 12).

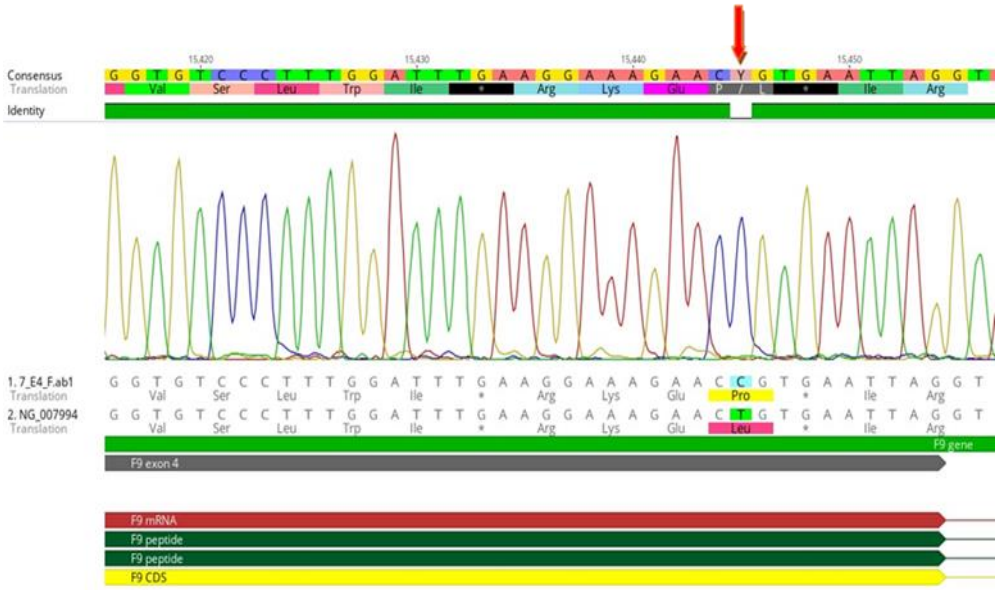


Figure (12): Alignment of heamophilia B patients' sequence with reference genes.

Discusion

Detection of intron 1 of hemophilia A

The discovery aligns with several studies, such as Mantilla-Capacho et al[8]., which established a null intron ratio of 1 (0%). Allaf et al[9]. identified 1.8% of inv-1 positive cases among Saudi Arabian hemophilia patients. Similarly, other research has noted approximately 2-3% of hemophilia instances associated with the intron-1 inversion. For example, in the UK, Cumming[10] found intron 1-linked hemophilia A at a rate of 1.8%, while Salviato et al.[11] reported a 1.7% inv1 occurrence in the Italian population. Additionally, a separate study by Sattar et al.[6] documented a lower rate of 0.77% for Inv 1 compared to the average.

The intron-1 inversion mutation in the factor VIII gene stands as the second most prevalent mutation in hemophilia A. Pinpointing the exact distance between int1h-1 and int1h-2 remains challenging, yet available data suggest a minimum of 136 kb. Initially, the presence of the Intron-1 inversion mutation was estimated at around 1-5% of cases.[6]

Most instances of the Inv1 mutation originate in the germline, primarily traced back to male germ cell meiosis. Since the X chromosome in male cells lacks a paired homologous chromosome, it tends to undergo turnover, leading to a crossover between homologous copies of FVIII.[12]

Detection of intron 22 of Hemophilia A

Until 1998, successful polymerase chain reaction (PCR) spanning the 9.5 kb region of int22h had not been reported due to the complexities within this area. This specific region comprises a 3.5 kb island with a GC content of 65% and a 1 kb region within the GC island with a GC content of 79%, rendering it challenging to investigate using traditional PCR techniques. The primary difficulty stemmed from the lack of adequate knowledge about flanking sequences necessary for designing PCR primers[13].

In this study, the incidence of intron-22 inversion among Dohuk hemophiliacs aligns with rates reported in other countries such as India[14], Austria[15], and Jordan [16]. They found that all patients demonstrated mutations linked to hemophilia A. Specifically, intron-22 and -1 inversions were observed in 52% and 2% of families, respectively. The higher percentage of intron 22 observed in our population could potentially be attributed to differences among various ethnic groups or, perhaps, due to the relatively smaller cohort investigated in this study.

The most prevalent mutation identified in hemophilia A was the intron 22 mutation within the factor VIII gene. This mutation, causing the severe form of the disease, disrupts the gene's continuity through intrachromosomal recombination between the region at intron 22 and two copies positioned 400 kb towards the telomeric region. This rearrangement accounts for 40-50% of hemophilia A cases[17].

Sequencing analysis of intron 22 FVIII gene

Abu-Amero et al. [18] conducted research on 20 unrelated hemophilia A patients from Syria, Jordan, Yemen, and Saudi Arabia, revealing no FVIII gene deletion within this Arab patient

cohort. This absence of deletion mirrors previous findings in other populations where such deletions are infrequent, aligning with the outcomes of this study. Djambas Khayat et al. [19] discovered both substitution and deletion mutations related to intron 22, noting missense and nonsense mutations alongside deletion mutations predominantly observed in smaller patients.

However, the current study's results were inconsistent with Akkarapatumwong et al.'s [20] findings, which highlighted frame shift mutations primarily observed in patients displaying moderate clinical phenotypes and other severe cases.

The frequencies of pathogenic variants observed in our study resemble those reported in other populations. For instance, in Lebanon, Djambas Khayat et al.[21,19] noted intron 22 frequencies at 23/79, while in Costa Rica, Salazar-Sánchez et al. [21] found Inv22 in 21/34 severe patients.

Intron 22 mutations potentially alter the structure of the F8 heterodimer or the stability of the overall F8 complex by disrupting or modifying hydrogen bonds and hydrophobic interactions. Three-dimensional modeling highlighted a distinct conformational change in the F8 protein surface compared to the wild-type, as indicated by Xia et al.[22]

Nair et al. [23] emphasized the significance of not only specific alterations but also the positioning of these changes within the protein's 3D structure in determining a patient's phenotype. Thus, the same mutation type can yield different phenotypes, underscoring the importance of considering the mutation type as a crucial predictor for clinical phenotypes. Genotyping hemophilia patients holds significance for physicians, enabling improved clinical management of severe bleeding disorders.

.

Detection of Hemophilia B

Haemophilia B, a extensively researched genetic disorder, presents identifiable mutations in over 97% of cases, yet a minority remain elusive. Investigations into pathogenicity modes through in vitro expression have shed light on some mutations. Identifying these mutations aids in predicting inhibitor risks and identifying carriers within affected families.[24]

The mutations underlying Hemophilia B result in either a lack of functional protein or the production of a dysfunctional protein product. These mutations encompass large gene deletions, nonsense mutations, missense, and most frame shift mutations. The severity of Hemophilia B correlates with the types of mutations observed.[25]

Our findings align with Li et al.[26], highlighting missense mutations as the most common in F9, accounting for 58.4% in cases of moderate Hemophilia B. A smaller percentage (15.4%) was attributed to frame shift mutations caused by deletion in moderate Hemophilia B. However, Balraj et al.[27]argued that frame shift mutations were associated with severe, moderate, and mild cases of Hemophilia B.

Hemophilia B shows a higher incidence of missense mutations, suggesting lower severity compared to Hemophilia A, though clinical evidence supporting this claim is limited. [24]Mutations within the F9 gene may or may not impact the structure and function of the FIX protein, with certain mutations adversely affecting protein structure stability and FIX protein function.[28]

While our study supports the link between phenotype and mutation type, a definitive conclusion remains challenging due to multiple influencing variables. Larger-scale studies are necessary for validation.

The rate of F9 mutations appears unaffected by modern chemicals and remains comparable across different population groups, indicating that germline F9 mutations primarily arise from endogenous processes rather than external mutagens. Knowledge of these mutations aids in predicting inhibitor risks, carrier identification, and prenatal analysis within affected families.

Molecular genotyping for Hemophilia B contributes to effective genetic counseling and insight into the disease's causative mechanism, yet the precise impact of mutations on disease severity remains to be fully understood.[26] Molecular analysis confirms diagnoses, predicts certain treatment complications, and reliably identifies carriers.[29]

Both F8 and F9 gene sequences exhibit minimal polymorphism, and the few single nucleotide variants in the F8 gene vary in frequency among ethnic groups. This variability may partially explain differences in FVIII inhibitor production between white and nonwhite populations, pending further evidence [25]. In conclusion, this initial data will contribute to constructing an Iraqi mutation database, aiding in genetic counseling endeavors.

Reference

1. P. H. Bolton-Maggs and K. J. Pasi, "Haemophilias A and B," Lancet, vol. 361, pp. 1801-1809, 2003.
2. R. Kruse-Jarres et al., "Acquired hemophilia A: Updated review of evidence and treatment guidance," Am J Hematol, vol. 92, pp. 695-705, 2017.
3. H. Kizilocak and G. Young, "Diagnosis and treatment of hemophilia," Clin Adv Hematol Oncol, vol. 17, pp. 344-351, 2019.
4. J. Sambrook and D. W. Russell, "Molecular Cloning alabrotory manual," Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2001.
5. I. H. Aziz and A. H. Al-Musawi, "Investigation of the inherited mutations related to inversion process of intron 22 in a sample of Iraqi patients with Hemophilia A," Pakistan Journal of Biotechnology, vol. 14, pp. 817-821, 2017.
6. A. Sattar et al., "Screening of Intron 1 Inversion of the Factor VIII Gene in 130 Patients with Severe Hemophilia A from a Pakistani Cohort," Turk J Haematol, vol. 34, pp. 278-279, 2017.
7. R. D. Bagnall et al., "Recurrent inversion breaking intron 1 of the factor VIII gene is a frequent cause of severe hemophilia A," Blood, vol. 99, pp. 168-174, 2002.
8. J. M. Mantilla-Capacho et al., "Frequency of intron 1 and 22 inversions of Factor VIII gene in Mexican patients with severe hemophilia A," Am J Hematol, vol. 82, pp. 283-287, 2007.
9. F. Allaf et al., "Mutation Screening of the Factor VIII Gene in Hemophilia A in Saudi Arabia: Two Novel Mutations and Genotype-Phenotype Correlation," Journal of Molecular and Genetic Medicine, vol. 10, 2016.
10. A. M. Cumming, "The factor VIII gene intron 1 inversion mutation: prevalence in severe hemophilia A patients in the UK," J Thromb Haemost, vol. 2, pp. 205-206, 2004.
11. R. Salviato et al., "Factor VIII gene intron 1 inversion: lower than expected prevalence in Italian haemophiliac severe patients," Haemophilia, vol. 10, pp. 194-196, 2004.
12. N. J. Faridi, P. Kumar, and N. Husain, "Prevalence of intron 1 inversion of cases with hemophilia A in North Indian population," Clin Appl Thromb Hemost, vol. 18, pp. 599-603, 2012.
13. W. Iqbal, M. Raza, and M. S. Khan, "Intron 22 Inversions in Severe Hemophiliacs," American Journal of Medicine and Medical Sciences, vol. 3, pp. 190-196, 2013.

14. R. Ahmed et al., "Mutation reports: intron 1 and 22 inversions in Indian haemophilics," Ann Hematol, vol. 82, pp. 546-547, 2003.
15. S. Reitter et al., "Spectrum of causative mutations in patients with haemophilia A in Austria," Thromb Haemost, vol. 104, pp. 78-85, 2010.
16. A. Awidi et al., "Study of mutations in Jordanian patients with haemophilia A: identification of five novel mutations," Haemophilia, vol. 16, pp. 136-142, 2010.
17. J. Naylor et al., "Characteristic mRNA abnormality found in half the patients with severe haemophilia A is due to large DNA inversions," Hum Mol Genet, vol. 2, pp. 1773-1778, 1993.
18. K. K. Abu-Amero et al., "Spectrum of factor VIII mutations in Arab patients with severe haemophilia A," Haemophilia, vol. 14, pp. 484-488, 2008.
19. C. Djambas Khayat et al., "Molecular analysis of F8 in Lebanese haemophilia A patients: novel mutations and phenotype-genotype correlation," Haemophilia, vol. 14, pp. 709-716, 2008.
20. V. Akkarapatumwong et al., "Frameshift mutations with severe and moderate clinical phenotypes in Thai hemophilia A patients," Hum Mutat, vol. 16, pp. 530-531, 2000.
21. L. Salazar-Sánchez et al., "Molecular analysis of FVIII gene in severe HA patients of Costa Rica," Hamostaseologie, vol. 30, pp. 150-152, 2010.
22. Z. Xia et al., "An intronic mutation c.6430-3C>G in the F8 gene causes splicing efficiency and premature termination in hemophilia A," Blood Coagul Fibrinolysis, vol. 29, pp. 381-386, 2018.
23. S. C. Nair et al., "Tests of global haemostasis and their applications in bleeding disorders," Haemophilia, vol. 16, pp. 85-92, 2010.
24. A. C. Goodeve, "Hemophilia B: molecular pathogenesis and mutation analysis," J Thromb Haemost, vol. 13, pp. 1184-1195, 2015.
25. K. R. Viel et al., "Inhibitors of factor VIII in black patients with hemophilia," N Engl J Med, vol. 360, pp. 1618-1627, 2009.
26. T. Li et al., "The CDC Hemophilia B mutation project mutation list: a new online resource," Mol Genet Genomic Med, vol. 1, pp. 238-245, 2013.
27. P. Balraj et al., "Factor IX mutations in haemophilia B patients in Malaysia: a preliminary study," Malays J Pathol, vol. 34, pp. 67-69, 2012.

28. K. Y. Lam et al., "A Novel Missense Mutation of F9 Gene in Hemophilia B Patients," J Blood Disord Transfus, vol. 8, p. 383, 2017.
29. Z. Abla et al., "Mutations Causing Haemophilia B: Identification of a Rare Mutation in the EGF1 Domain of Coagulation Factor IX in Patients with