ORIGINAL ARTICLE

IJBC 2009; 2: 43-47

Molecular Characterization of the Factor IX Gene in 28 Iranian Hemophilia B Patients

Morteza Karimipour[1], Sirous Zeinali[1], Edward Graham Tuddenham[2], Nafiseh Nafissi[1], Manijeh Lak[3], Peter Green[4]

1. Molecular Medicine Group, Biotechnology research Centre, Pasteur Institute of Iran, Tehran, Iran, 13164.
2. Hemophilia Center and Hemostasis Unit, Royal Free Hospital, Pond Street, London, NW3 2QG, UK.
3. Hemophilia Center, Imam Khomeini Hospital, Keshavarz Blvd, Tehran, Iran.
4. GKT School of Medicine, Guy's Hospital, London SE1 9RT, UK.

Corresponding Author: Morteza Karimipour, Molecular Medicine Group, Biotechnology Research Centre, Pasteur Institute of Iran, Tehran, Iran, P.O. Box: 1316943551, Tel-Fax: +98(21)66480780, *E - mail: mortezakarimi@yahoo.com*

Abstract

Background: Heterogeneous mutations in the human coagulation factor IX gene lead to an X-linked recessive bleeding disorder known as hemophilia B. The disease is distributed worldwide with no ethnic or geographical priority.

Materials and Methods: The aim of this study was to characterize the factor IX gene mutations in 28 unrelated Iranian hemophilia B patients. Polymerase chain reaction (PCR) and direct sequencing was performed for all functionally important regions of the gene. Haplotype analysis was performed using three markers.

Results: We identified 24 point mutations and four small deletions (one novel mutation). Overall, 20 different mutations were found and patients with common mutations had identical haplotype.

Conclusion: These data confirm high molecular heterogeneity of the mutations causing hemophilia B and will enable carrier testing and prenatal diagnosis for family members.

Keywords: hemophilia B, factor IX gene, mutation, iran

Introduction

Hemophilia B, or Christmas disease, is an X-linked coagulopathy due to the deficiency or functional abnormality of clotting factor IX (FIX).[1] However, up to 30% of cases have no prior history of the disease that arises from new mutations. The incidence of hemophilia B is approximately one in 25000-30000 male live births with a nearly similar prevalence all over the world.[2] The disease is caused by different types of mutations in the factor IX gene (F9). The F9 gene maps to Xq27.1, spans about 34 kilo bases of genomic DNA and contains eight exons (a-h) encoding a 2.8 kb mRNA.[3] The gene encodes a glycoprotein of 415 amino acid residues, normally presents in plasma, which is an essential component of the clotting cascade.[4] This protein is a vitamin K-dependent serine protease and contains six major domains: signal peptide, propeptide, gla domain, two epidermal growth factor-like (EGF-like) domains, activation and catalytic domains.[5] Factor IX is synthesized in the liver as a precursor molecule and is secreted into the plasma[6] after further modification.

Different types of mutations, mainly point mutations, have been identified in all parts of the F9 gene, mainly in the exon coding for the serine protease domain.[7,8] These mutations are listed in a freely accessible database (http: // www. kcl. ac. uk/ ip/ petergreen/ haemBdatabase. html). The mutations are so heterogeneous that most unrelated patients carry different mutations.[9]

In Iran, there are 900-1000 hemophilia B patients. Many of these patients belong to large families with more than one patient and many more females are at risk of being a carrier.[10,11] A program has been started to genotype the unrelated patients and construct a national

[Downloaded from ijbc.ir on 2026-11-01]

database for providing carrier testing and prenatal diagnosis for their families. A preliminary pilot plan in the Ministry of Health is under investigation for counselling of hemophilia A and B with the help of genetic testing of potential carriers in the families with one or more hemophilia patients. Molecular diagnosis of hemophilia B (HB) in Iran has been dependent on linkage analysis rather than direct identification of mutation. In this regard, and also to develop our knowledge about molecular basis of hemophilia B in Iran, we analyzed 28 HB patients to find genotype-phenotype correlations.

Materials and Methods

Subjects

This study included 28 unrelated hemophilia B patients of Iranian origin referred from Hemophilia Centre, Imam Khomeini hospital, Tehran, Iran. All patients were male and had a prolonged activated partial thromboplastin time (aPTT), low FIX activity and normal FVIII activity. The severity of the disease was classified by residual factor IX activity. Twenty six patients were studied for the first time. Two cases, included in our previous study with a novel mutation at nt 31125 C to A, were further investigated and all of their F9 gene was sequenced to confirm previous data.[12] None of the patients had antibody against factor IX (inhibitor) during the replacement therapy. Complete pedigree data was not available for some patients and the FIX activity had been performed at the time of diagnosis. All patients included in the study or their parents in case of underage patients gave written informed consent. About 5-10 ml of peripheral blood was collected into tubes containing EDTA, and DNA was extracted by standard methods.

Mutation Analysis

The eight exons of the F9 gene were amplified in 7 amplicons as previously described.[13] and directly sequenced following treatment with ExoSapIT ™ (GE Healthcare, Amersham, UK) using BigDye v3.1 terminator chemistry (Applied Biosystems, Warrington, UK). Products were run on an ABI 3100 DNA sequencer and analyzed using Mutation Surveyor™ (SoftGenetics). Sequence variations were analyzed with reference to the wild type sequence (GenBank accession No. K02402.1). For the nomenclature of the mutations identified, we used Yoshitake et al. 1985 numbering system, that is, the first Met was numbered-46 (based on mature protein without prepropeptide)[3] . We referred to the Hemophilia B mutation database to determine whether the mutation was known or novel.

Polymorphic Markers Analysis

Haplotypes of the patients were constructed using three intragenic polymorphic markers by PCR and restriction fragment length polymorphism (RFLP): DdeI in intron one, MnlI at exon six and HhaI at the 3'-untranslated region of the F9 gene as described by Peake et al.[14]

Results

Screening for mutation within the F9 gene in 28 Iranian hemophilia B patients by PCR and sequencing of the entire coding sequence of F9 gene led to characterization of all responsible mutations. The patients seem to be unrelated by pedigree analysis and molecular analysis revealed 20 unique mutations in these cohorts. These mutations included 24 point mutations[16] missense mutations, 5 nonsense mutations, and 3 in promoters) and four small deletions (Table1). No large deletion/duplication was found. As expected, the most common mutations responsible for the disease were point mutations. This study revealed 20 different mutations and one of them (17693 – ATT) has not been reported in the hemophilia B mutation database previously. This mutation causes an in-frame deletion of residue isoleucine at position 90 and, as it is expected, causes the severe form the disease (See Table 1).

Independent recurrent mutations were observed at the following positions: A13G, C6460T, G6472A, A17690C, C31118T, and 31157 – AAC. Aside from 31118 C to T, all the patients sharing identical mutations had the same haplotype, suggesting a common origin for the mutation. The 31118 C to T (a transition in CpG position) is a frequently reported mutation in the database and is considered a hotspot for mutation.

[Downloaded from ijbc.ir on 2026-11-01]

Table 1: *Summary of clinical and mutation data in the F9 in 28 Iranian patients with hemophilia B*

Patient	FIX activity	Nucleotide change[1]	Amino acid change	Haplotype (DdeI[2], MnlI, HhaI)
1	2%	T6A	-	++-
2	7%	A13G	-	+-+
3	4%	A13G	-	+-+
4	<1%	G6365A	R-4Q	++-
5	2%	C6460T	R29X	+--
6	1%[3]	C6460T	R29X	+--
7	12%	G6461A	R29Q	+--
8	3%	G6472A	E33K	+-+
9	2%	G6472A	E33K	+-+
10	<1%	17683–AAT*	90– I	--+
11	<1%	A17690C	N92T	+-+
12	<1%	A17690C	N92T	+-+
13	<1%	C17700A	C95X	--+
14	<1%	G17755A	G114R	++-
15	3%	G20378A	G133E	--+
16	<1%	20493–C	f/s S171	++-
17	10%	T30089A	N212K	++-
18	12%	G30150A	A233T	+--
19	<1%	T30939G	L273R	-+-
20	<1%	A31092T	Q324L	+--
21	<1%	C31118T	R333X	+-+
22	<1%	C31118T	R333X	+++
23	7%	C31125A	T335K	+--
24	9%	C31125A	T335K	+--
25	1%	31157–AAC	346–N	+-+
26	3%	–AAC	–N	+-+
27	<1%	G31278A	G386D	+--
28	<1%	G31289A	A390T	--+

1. Nucleotide numbering is from Yoshitake et al. 1985.
2. For DdeI, the 369 and 319 bp alleles are shown by – and +, respectively.
* Novel change, not previously reported in the International Hemophilia B database.

Discussion

The molecular analysis of F9 gene in this cohort of hemophilia B patients with Iranian origin in all subjects with promoter, stop codons and deletions confirmed a correlation between genotype and the severity of disease. Among 16 missense mutations, seven (R-4Q, N92T, G114R, L273R, Q324L, G386D and A390T) were responsible for the severe form of the disease. R-4Q and N92T mutations in the database have caused variable severities in registered patients from moderate to severe. G386 D mutation has been reported in two patients in the database with 2% FIX activity. However, discrepancy has been reported for many missense mutations in the database.

Among the patients, three had two well-known substitutions in promoter region (T6A and A13G) which are reported in the HB mutation database as responsible for the Leiden phenotype. This phenotype is characterized by a very low level of FIX protein in plasma during childhood and a gradual rise after puberty. These two mutations disrupt the binding site for CAAT/Enhancer Binding Protein (C/EBP) transcription factor.

In our previous study on 52 Iranian hemophilia B patients (12), we found a novel missense mutation (C31125A) in four unrelated cases by

[Downloaded from ijbc.ir on 2026-11-01]

SSCP and sequencing. In this study, the whole F9 gene of two of these patients was sequenced and no other mutation was found. These patients, with a clinically mild disease and a common haplotype, probably represent a founder effect.

Carrier testing for hemophilia B is still performed in most of the developing countries by linkage analysis using Restriction Fragment Length Polymorphism. This indirect technique has well known limitations especially in sporadic cases. Hence, identification of mutations greatly improves carrier testing and genetic counselling for obligate and probable carriers, estimation of the risk for autoantibody formation in the patients, antenatal diagnosis,[15] and pre-implantation genetic selection[16] and genotype-phenotype correlation. Similar to other countries,[17,18,19] the underlying mutations causing hemophilia B in Iranian patients include base substitutions and small nucleotide deletions or insertions. The Genetics Office at Center for Disease Control in the Iranian ministry of Health plans to start a preventive nationwide program for hemophilia A and B. In this regard, a central province (Isfahan) has been chosen for a pilot study. There are about 6500 hemophilia A and B patients in Iran (Iran Hemophilia Society, personal communication) and the cost of imported coagulation factors reaches about 60 million US dollars per year, as quoted by the Deputy of the Minister of Health. For establishing genetic counseling for the females at risk of being carriers and antenatal diagnosis, finding the disease-causing mutations and establishing haplotypes would be essential. In addition, these mutations could be used to construct a national database of mutations causing the disease.

Acknowledgement

The authors wish to thank the patients for providing the samples for this study. This work was supported partly by Iranian Molecular Medicine Network and Katharine Dormandy Trust (KDT), UK.

References

1. Brownlee CG. The molecular pathology of hemophilia B. Biochem Soc Trans; 1987. 15: 1-8.

2. Biocchi MP, Pasino M, Bottini F, Lanza T, Mori PG, Acquila M. Mutation analysis is an essential strategy in the genetic counselling of sporadic hemophilia B families. Haemophilia; 2002. 8: 730-2.

3. Yoshitake S, Shach BG, Foster DC, Davie EW and Kurachi K. Nucleotide sequence of the gene for human factor IX (antihemophilic factor B). Biochemistry; 1985. 24: 3736-50.

4. Bowen DJ. Hemophilia A and hemophilia B: molecular insights. Molecular Pathology; 2002. 55: 127-440.

5. Roberts HR. Molecular biology of hemophilia B. Thromb Haemost; 1993. 70: 1-9.

6. Lillicrap D. The molecular basis of hemophilia B. Haemophilia; 1998. 4: 350-7.

7. Sommer SS, Ketterling RP. The FIX gene as a model for analysis of human germ-line mutations. An update. Hum Mol Genet; 1994. 5: 1505-14.

8. Mukherjee S, Mukhopadhyay A, Chaudrari K, Ray K. Analysis of hemophilia B database and strategies for identification of common point mutations in the factor IX gene. Haemophilia; 2003. 9: 187-920.

9. Giannelli F, Green PM, Sommer SS, Poon MC, Ludwig M, Schwaab R et al. Hemophilia B: Database of point mutations and short additions and deletions-eighth edition. Nucleic Acids Res; 1998. 26: 265-8.

10. Enayat MS, Karimi M, Chana G, Farjadian S, Theophilus BDM, Hill FGH. Mutation analysis in F9 gene of the 17 families with hemophilia B from Iran. Haemophilia; 2004. 10: 751-5.

11. Ghandil P, Ghadiri A, Farhoud D and Zeinali S. Allele frequency of two polymorphisms associated with the factor IX gene in Iranian population. Thromb Res; 2004. 113: 289-93.

12. Karimipoor M, Zeinali S, Nafissi N, Tuddenham EG, Lak M, Safaee R. Identification of factor IX mutations in Iranian hemophilia B patients by SSCP and sequencing. Thromb Res; 2007. 120: 135-9.

13. Green PM, Bentley Dr, Mibashan RS, Nilsson IM, Giannelli F. Molecular pathology of hemophilia B. The EMBO J.; 1989. 4: 1067-72.

14. Peake IR, Lillicrap DP, Boulyjenkov V, Briet E, Chan V, Ginter EK et al. Report of a WHO/WFH meeting on the control of hemophilia: carrier detection and prenatal diagnosis. Blood coagul Fibrinol; 1993; 4: 313-44.

15. Karimipoor M, Zeinali S, Lak M and Safaee R. Carrier testing and prenatal diagnosis of hemophilia B by SSCP in an Iranian family. Haemophilia; 2003; 9: 116-8.

16. Michaelids K, Tuddenham EG, Turner C, Lavender B, Lavery SA. Live birth following the first mutation

[Downloaded from ijbc.ir on 2026-11-01]

specific preimplantation genetic diagnosis of hemophilia A. Thromb Hemost; 2006. 95: 373-9.

17. Montejo JM, Magallon M, Tizzano E & Solera J. Identification of twenty-one new mutations in the factor IX gene by SSCP analysis. Human Mut; 1999. 13: P. 160.

18. Belvini D, Salviato R, Radossi P, Pierobon F, Mori P, Castaldo G, et al. Molecular genotyping of the Italian cohort of patients with hemophilia B. Haematologica; 2005. 90: 635-42.

19. Jayandharan GR, Shaji RV, Baidya S, Nair SC, Chandy M, Srivastava A. Molecular characterization of factor IX gene mutations in 53 patients with hemophilia B in India. Thromb Hemost; 2005. 94: 883-60.

[Downloaded from ijbc.ir on 2026-11-01]