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HEMOPHILIA: GENETICS, DIAGNOSIS AND TREATMENT

Genetics

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ABSTRACT

Hemophilia is an X linked disorder which is caused by a deficiency of Factor VIII and Factor IX. The worldwide prevalence of this disorder is approximately half a million. Hemophilia families are always under emotional and financial stress, as the treatment is very expensive. Genetic studies of Hemophilia are an essential for the management of this disorder. Carrier detection and genetic counselling in affected families can significantly help in reducing the incidence. This review article summarizes genetic aspects of Hemophilia with components of genetic evaluation and management of Hemophilia.

KEYWORDS

Factor VIII, Factor IX, Genetic diagnosis, Hemophilia,

Introduction:

Hemophilia is an X-linked bleeding disorder which is caused by a deficiency of blood coagulation factor VIII (F8) and factor IX (F9). F8 protein is essential for coagulation pathway. There are two types of Hemophilia. A (deficiency of F8) which is more common and occurs in about 1 in 5000 births. Hemophilia B (deficiency of F9) is less common and occurs in approximately 1 in 20,000 births.[1,2] India is the second highest number of patients with hemophilia A. According to an Annual global survey conducted by the World Federation of hemophilia, approximately 50 per cent of the world's hemophilia population lives in India. There are an estimated 10,000 hemophilia in India and currently only 15 per cent of the total hemophilia population has been identified in India and the rest lay undiagnosed. The worldwide prevalence of this syndrome is approximately half a million.[3-5] Internal bleeding is serious concern in hemophilic patients and this severity is correlated with the level of the coagulation protein in their blood. Joints bleeding is more common in such patients caused by injury. This review article summarizes genetic aspects of hemophilia with components of genetic evaluation and management of hemophilia.

Molecular basis of Hemophilia:

F8 and F9 are the only known gene which is associated with hemophilia. Both F8 and F9 genes map to the distal end of the long arm of the X chromosome at Xq28 and Xq27 respectively.[6] F8 gene has 26 exons spanning 186 KB. It encodes a 2351 amino acids precursor polypeptide. The F9 gene is comparatively smaller (34 KB) and has 8 exons. Exon and intron sizes of F8 and F9 are given in table 2. The mature F8 protein consists of different domains which are arranged in the order A1-A2-B-A3-C1-C2 (Fig. 1). These domains are very important for proper function of F8 protein and genetic defects can cause hemophilia.[7-9]

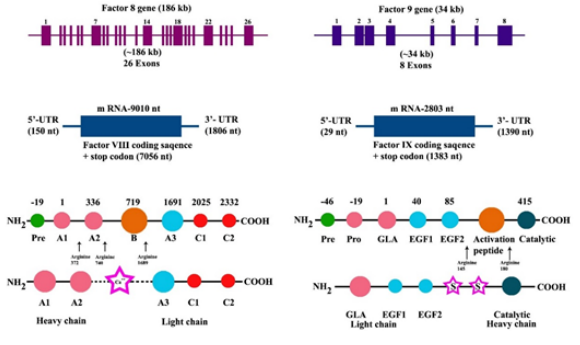

Fig. 1: Genomic organization of F8, and F9 genes.

A large number of mutations have been identified in F8 gene. The intron 22 inversion and intron 1 inversion is most common mutations of the F8 gene. Point mutation or small rearrangement is also associated with severe hemophilia. Presently, more than 1209 mutations have been identified within the F8 coding and untranslated region in the F8 gene.[10-12]

Severity of hemophilia A and B are based on in vitro clotting activity as shown in Table 1. The approximate percentage of severe hemophilia is 70%.

Table 1: Severity of Hemophilia A and B with related symptoms.[13]

Type	Severity	Clotting factor activity (%) (IU/ml)	Symptoms	Age of Diagnosis	Investigations
Hemophilia A	Severe	<1%	Spontaneous bleeding, predominantly in joints and muscles	≤1 year	APTT (increased), Factor VIII activity decreased, vWF levels (normal)
	Moderate	1-5%	Occasional spontaneous bleeding. Severe bleeding with trauma, surgery	Before age 5–6 years	
	Mild	>5-40%	Severe bleeding with major trauma or surgery	Late onset of life	
Hemophilia B	Severe	<1%	Frequent spontaneous bleeding; excessive and/or prolonged bleeding after minor injuries, surgery, or tooth extractions	≤2 years	APTT (increased), Factor IX activity decreased, vWF levels (normal)
	Moderate	1%-5%	Spontaneous bleeding rare; excessive and/or prolonged bleeding after minor injuries, surgery, or tooth extractions	<5-6 years	
	Mild	>5%-40%	No spontaneous bleeding; excessive and/or prolonged bleeding after major injuries, surgery, or tooth extractions	Often later in life	

Table 2: Exons and Introns in the gene for Human Factor IX [14-15]

Exon	Nucleotide Length (bp)	Nucleotide position	Intron	Nucleotide Length (bp)	Nucleotide position
Exon 1	117	1-117	Intron 1	6,206	118-6,325
Exon 2	164	6,326-6,489	Intron 2	188	6,490-6,677

Exon 3	25	6,678-6,702	Intron 3	3,689	6,703-10,391
Exon 4	114	10,392-10,505	Intron 4	7,163	10,506-17668
Exon 5	129	17,669-17,797	Intron 5	2,565	17,798-20,362
Exon 6	203	20,363-20,565	Intron 6	9,473	20,566-30,038
Exon 7	115	30,039-30,153	Intron 7	668	30,154-30,821
Exon 8	1935	30,822-32,757			

Inheritance of Hemophilia:

Hemophilia is the most common inherited bleeding disorder in India. It is an X linked single gene disorder which is caused by heterogeneous mutation in the blood coagulation factor 8 and factor 9. Inherited bleeding disorder is a condition that may be passed from parents to their offspring through their genes. Gene contains coded information that determines the traits an individual will express. Genetic disorders differ from other medical problems in that they tend to recur within families. These single gene defects are the easiest type of genetic abnormality to be identified by pedigree analysis. They are subdivided into autosomal dominant, autosomal recessive and X linked (sex linked) conditions. Each person has 2 genes for any trait and randomly passes on one of them to each child. In the autosomal dominant type, a person who has inherited one abnormal gene will express symptoms of the disorder. In the autosomal recessive type, a person who has inherited 2 abnormal genes will express the symptoms where as a person with one abnormal and one normal gene will be a carrier.[16] Hemophilia A and hemophilia B are single gene disorder which is an X linked. The chance of transmitting the defective X linked gene from a carrier mother to child will be 50%. Molecular genetic diagnosis is an important and integral part for carrier detection and evaluation of hemophilic population.[17,18]

Table 3: Genetics of rare clotting factors deficiencies.[19,20]

Factor	Incidence	Gene and chromosome location	Inheritance patterns	Severity of bleeding	Treatment
Factor I (Fibrinogen)	1 in 100000	FGA, FGB, FGG (4q28)	Autosomal recessive, Autosomal	Usually mild/ Severe	Fibrinogen concentrate Cryoprecipitate Fresh frozen plasma
Factor II (Prothrombin)	1 in 200000	F2 (11p11-q12)	Autosomal recessive	Usually mild	Prothrombin complex concentrate Fresh frozen plasma
Factor V (Proaccelerin)	1 in 100000	F5 (1q24.2)	Autosomal recessive	Usually mild	Fresh frozen plasma
Combined factor V and Factor VIII	1 in 100000	LMAN 1 (18q21.3-q22) MCFD2 (2p21-p16.3)	Autosomal recessive	Usually mild	Fresh frozen plasma Factor VIII concentrate Desmopressin
Factor VII	1 in 500000	F7 (13q34)	Autosomal recessive	Severe (when factor levels are low)	Recombinant factor VIIa concentrate Factor VII concentrate Prothrombin complex concentrate Fresh frozen plasma
Factor X	1 in 100000	F10 (13q34)	Autosomal recessive	Moderate to severe	Prothrombin complex concentrate Fresh frozen plasma
Factor XI	1 in 100000	F11 (4q35.2)	Autosomal recessive, Autosomal dominant	Mild to moderate	Factor XI concentrate Antifibrinolytic drugs Fibrin glue Fresh frozen plasma
Factor XIII	1 in 300000	F13A1 (6p24-p25) F13B (1q31-q32.1)	Autosomal recessive	Severe	Factor XIII concentrate Cryoprecipitate Fresh frozen plasma

Genetic diagnosis of Hemophilia

There are two different approaches to the genetic evaluation of an individual with a suspected hemophilia disorder. Analysis of single nucleotide polymorphism (SNPs) or microsatellite variable number tandem repeats (VNTR) markers in the F8 or F9 gene to track the abnormal X chromosome in the family. SNPs and VNTRs are commonly detected by PCR amplification and conventional polyacrylamide gel electrophoresis. Intron 1 or intron 22 inversions are the most common mutations in the F8 gene.[21, 22] Direct mutation detection has a near 100% accuracy and is informative in over 95% of familial hemophilia. Various mutation detection techniques also be used for hemophilia such as long distance polymerase chain reaction, multiplex ligation dependent pure amplification, capillary electrophoresis and direct sequencing. Multiplexing of amplification and conformation sensitive gel electrophoresis (CSGE) has significantly reduced the cost and time for direct mutation screening in hemophilia and other hemostasis.[23-25]

Prenatal diagnosis for Hemophilia

Prenatal diagnosis and genetic counselling are very useful method for prevention and control of the disease. Carrier detection for mutant gene is another method of prevention. Prenatal diagnosis is generally indicated in severe or moderate forms of hemophilic families.[26] It can be done for carrier women by chorionic villus sampling (10-12 weeks' gestation) or amniocentesis (approximately 15-18 weeks' gestation). If the karyotype is normal (46,XY/46XX), DNA extracted from fetus cells and analysed for the known disease causing mutation.[27-29]

Gene Therapy

Gene therapy is promising curative option for hemophilia because its can prevent above 1% spontaneous bleeding and substantially enhance the quality of life in patients with hemophilia.[30]

In vivo and ex vivo appearances have been proposed for gene therapy for hemophilia. For in vivo delivery studies, viruses have been more efficient as compared to other methods. [31] Chuah et al. 2002 reported that gene therapy has been successful in hemophilic dogs and in mice with knockout mutations for the clotting factor genes.[32] There are physical and chemical methods have been developed for gene integration into cells. In this purpose viruses have generally much more efficient. Presently the adeno-associated virus bases gene therapy is potentially safer as compared to retroviral and adenoviral vectors.[33-35]

Adeno associated virus serotype 2 has become a more preferred prototype vector for in vivo viral gene transfer due to its wide tissue tropism. It has been tested in many monogenic disorders with therapeutic success.[36-38]

Conclusion:

Hemophiliac families are always under emotional and financial stress, as the treatment is very expensive. Therefore, it is important that potential carriers should have access to precise information on the consequences of having children with an X-linked disorder. Assessment of the carrier status is an important step as the carrier females are responsible for the transmission of trait to the next generation.

Genetic study of hemophilia is an essential for the management of this disorder. Further research of hemophilia needs to be addressed on a priority basis. In India, the burden of hemophilia is heavy because of due to lack of social support and expensive treatments. In these circumstances, carrier detection and genetic counselling in affected families can significantly help in reducing the incidence. Due to the high population density, large number of hemophilia cases are reported in the country. Genetic studies of the mutation in intron 22 of factor VIII gene in severely affected hemophilic patients are an essential part for management of this condition. Causative mutation is still not identified in the F8 gene in several cases with severe hemophilia A. Newer strategies are needed for such patients for proper management of this disorder. Further advancement in gene therapy vectors is also needed for patients with hemophilia.

References:

1. O'Mahoney B. Global hemophilia care challenge and opportunities: World Federation of Hemophilia. 2002.
2. Bhattacharya DK 1987. A study of bleeding episodes of hemophilia A. Cal Med J, 84: 1-8.
3. Jones P 1991. Guidelines for the development of a national programme for hemophilia

world federation of hemophilia. Thromb Haemost, I:2051-2054.
4. Dharmarajan S, Phadnis S, Gund P, Kar A. Treatment decisions and usage of clotting factor concentrate by a cohort of Indian hemophilia patients. Hemophilia 2012; 18 : e27-9.
5. Hemophilia Federation (India). Available from: http://www.hemophilia.in/, accessed on October 3, 2017.
6. Bowen DJ. Hemophilia A and hemophilia B: molecular 22. insights. Mol Pathol 2002; 55 : 127-44.
7. White GC II, Rosendaal F, Aledort LM, Lusher JM, Rothschild C, Ingerslev J (2001) Definitions in hemophilia: recommendation of the scientific subcommittee on factor VIII and factor IX of the scientific and standardization committee of the International Society on Thrombosis and Haemostasis. Thromb Haemost 85: 560.
8. Genetics of Bleeding Disorders. Available from: https://www.researchgate.net/publication/ 228364614_Genetics_of_Bleeding_ Disorders [accessed Jan 20 2018].
9. Jayandharan G78. R, Srivastava A, Srivastava A. Role of molecular genetics in hemophilia: from diagnosis to therapy. Semin Thromb Hemost 2012; 38 : 64-78.
10. Kemball-Cook G, Gomez K. Molecular basis of hemophilia A. In: Lee C, Berntorp E, Hoots K, editors. Textbook of hemophilia, 2nd ed. West Sussex: Wiley-Blackwell; 2010. p. 24-32.
11. Ghosh K, Quadros L, Shetty S. Spectrum of factor IX gene mutations causing hemophilia B from India. Blood Coagul Fibrinolysis 2009; 20 : 333-6.
12. Ahmed R, Kannan M, Choudhry VP, Saxena R. Mutation reports: intron 1 and 22 inversions in Indian haemophilics. Ann Hematol 2003; 82 : 546-7.
13. Santagostino E, Mancuso ME, Tripodi A, et al. Severe hemophilia with mild bleeding phenotype: molecular characterization and global coagulation profile. J Thromb Haemost. 2010;8:737–743.
14. Shetty S, Pathare A, Ghosh K, Jijina F, Mohanty D. Intron 22 inversions in factor VIII gene in Indian hemophiliacs. Thromb Haemost 1998; 79 : 881-2.
15. Gitschier J, Wood WI, Goralka TM, et al. Characterization of the human factor VIII gene. Nature 1984;312:326–30.
16. McKusick VA (ed): Mendelian Inheritance in Man, 6th ed. Baltimore, The Johns Hopkin University Press, 1983, p 1044
17. Yoshitake S, Schach BG, Foster DC, et al. Nucleotide sequence of the gene for human factor IX (antihemophilic factor B). Biochemistry 1985;24:3736–50.
18. Peake IR, Lillicrap DP, Boulyjenkov V, Briet E, Chan V, et al. (1993) Hemophilia: strategies for carrier detection and prenatal diagnosis. Bull World Health Organ 71: 429-458.
19. Pavlova A, Brondke H, Musebeck J, Pollmann H, Srivastava A, et al. (2009) Molecular mechanisms underlying hemophilia A phenotype in seven females. J Thromb Haemost 7: 976-982.
20. Bolton-Maggs PH. The rare coagulation disorders. World 34. Federation of Hemophilia 2006. Available from: http://www1.wfh.org/docs/en/About_Bleeding_ Disorders/RBD_Characteristics_Table.pdf, accessed on October 10, 2017.
21. Gomez. ez K. Hemophilia B-molecular basis. In: Lee C, Berntorp E, Hoots K, editors. Textbook of hemophilia, 2nd ed. West Sussex: Wiley-Blackwell; 2010. p. 88-93.
22. Peyvandi F (2005) Carrier detection and prenatal diagnosis of hemophilia in developing countries. Semin Thromb Hemost 31: 544-554.
23. Lalloz MR, Schwaab R, McVey JH, Michaelides K, Tuddenham EG (1994) Hemophilia A diagnosis by simultaneous analysis of two variable dinucleotide tandem repeats within the factor VIII gene. Br J Haematol 86: 804-809.
24. Jayandharan G, Shaji RV, George B, Chandy M, Srivastava A (2004) Informativeness of linkage analysis for genetic diagnosis of hemophilia A in India. Hemophilia 10: 553-559.
25. Williams IJ, Abuzenadah A, Winship PR, Preston FE, Dolan G, et al. (1998) Precise carrier diagnosis in families with hemophilia A: use of conformation sensitive gel electrophoresis for mutation screening and polymorphism analysis. Thromb Haemost 79: 723-726.
26. Mannucci PM (2003) Hemophilia: treatment options in the twenty-first century. J Thromb Haemost 1: 1349-1355.
27. Peyvandi F (2005) Carrier detection and prenatal diagnosis of hemophilia in developing countries. Semin Thromb Hemost 31: 544-554.
28. Peake IR, Lillicrap DP, Boulyjenkov V, et al. Hemophilia: strategies for carrier detection and prenatal diagnosis. Bull World Health Organ 1993;71(3-4):429–458.
29. Kogan SC, Doherty M and Gitschier J: An improved method of prenatal diagnosis of genetic disease by analysis of amplified DNA sequences-application to hemophilia A. N Engl J Med 317: 985-990, 1987.
30. High KA. The Jeremiah Metzger Lecture: gene therapy for inherited disorders: from Christmas disease to Leber's amaurosis. Trans Am Clin Climatol Assoc 2009; 120;331–359.
31. Batorova A, High KA, Gringeri A. Special lectures in hemophilia management. Hemophilia 2010;16(Suppl. 5):22–8.
32. Chuah MK, Collen D, VandenDriessche T. Clinical gene transfer studies for hemophilia A. Seminars in Thrombosis and Hemostasis. 2004;30:249–256.
33. Donahue RE, Kessler SW, Bodine D, et al. Helper virus induced T cell lymphoma in nonhuman primates after retroviral mediated gene transfer. J Exp Med 1992; 176(4):1125–1135.
34. Kohn DB, Sadelain M, Dunbar C, et al; American Society of Gene Therapy (ASGT). American Society of Gene Therapy (ASGT) ad hoc subcommittee on retroviral-mediated gene transfer to hematopoietic stem cells. Mol Ther 2003;8(2):180–187.
35. Kaplitt MG, Feigin A, Tang C, et al. Safety and tolerability of gene therapy with an adeno-associated virus (AAV) borne GAD gene for Parkinson's disease: an open label, phase I trial. Lancet 2007;369(9579):2097–2105
36. Bainbridge JW, Smith AJ, Barker SS, et al. Effect of gene therapy on visual function in Leber's congenital amaurosis. N Engl J Med 2008;358(21):2231–2239.
37. Flotte TR. Recent developments in recombinant AAV-mediated gene therapy for lung diseases. Curr Gene Ther 2005;5(3):361–366.
38. Liras A, Olmedillas S. Gene therapy for hemophilia...yes, but...with non-viral vectors? Hemophilia 2009;15(3):811-6.