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Recent scenario on the genetic abnormalities in patients suffering from Haemophilia A: A review

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ABSTRACT

This review aims at compiling the different mutational changes undergone by the factor VIII gene leading to hemophilia A. It has been difficult to characterize completely a genetic disorder like hemophilia A where the gene of concerns is large, its tissue-specific expression, and all affected individuals have different mutational patterns. In this review, point mutational, insertional and deletional patterns are sited. The data are collected from all round the world, although "Western" sources are predominate.

Keywords: Hemophilia A, Mutation, X-linked inheritance, Coagulating factor VIII

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INTRODUCTION

Haemophilia is a genetic disorder in which blood loss occurs as a complication of to impaired clotting mechanism due to deficiency or lower levels of clotting factors. It is generally classified into two types-

(i) **Heamophilia -A**: It is caused by low level or no clotting factor VIII (affects about1 in 4000-10,000 of the population) [1]

(ii) **Heamophilia -B:** It is caused by low level or no clotting factor IX (affects about 1 in 20,000 of the population) [1]

Heamophilia is an X-linked recessive inheritance and affected person are predominantly males.When the mother is a carrier of a faulty X-linked clotting factor gene and the father has a working copy of the gene, in every pregnancy, the risks of having an affected child is different for their sons and daughters. Their son has 1 chance in two or a 50% chance of inheriting a faulty gene for haemophilia and having the condition. Their daughters have 1 chance in 2 or a 50% chance of inheriting the clotting factor gene and being a genetic carrier for haemophilia. Carrier would usually be unaffected [2,3]. The severity of heamophilia A (HA) which is predominant takes place when activity of factor VIII is less than 1% and this prevails with 40% of the patients with HA[4].

In most of cases of severe HA, the disease may originate from large DNA inversions which results into truncated FVIII. HA causative mutation also includes a spectrum FVIII defects that are compiled in International databases (HAMSTeRS, http://europium.csc.mrc.ac.uk) [5]. The spectrum of clinical severity approximately correlates with the assayed level of coagulation factor VIII in the plasma i.e. severely affected individuals have <0.01 IU/dL (<1% of normal) while moderate have 0.01-0.05 IU/dL (1%-5% of normal), and mild have 0.05-0.04 IU/dL (>5 %-< 40% of normal) of coagulation factor in blood [6,7, 8, 9].

Molecular structure of factor VIII gene:

The factor viii gene is located on the long arm of the X chromosome (Xq29), approximately 500 Mb telomeric to the glucose-6-phosphate dehydrogenase gene (figure: 1) the factor VIII gene's structure is typical. It is 186 kilo base (kb) with 24 relatively short exons, ranging from 69 to 262 base pairs (bp), and 2 long exons, exon 14 (3106 bp) and exon 26 (958bp). The resulting messenger RNA (mRNA) is approximately 9 kb, the coding sequence of which is 7053 bp. The intron-exon boundaries correlate roughly with the factor VIII domains. The introns are large (14-32 kb). Intron 22 is the largest (32 kb) and is of interest given that the most common mutation in hemophilia A occurs as a result of an inversion involving intron 22. The molecule circulates in plasma as a heterodimer with structural domains A1-A2-B and A3-C1-C2. The heavy chain consists of the A1-A2-B domains with a molecular mass ranging from 90 to 200 kDa due to variability of the B domain. The 80 kDa light chain consists of the A3-C1-C2 domains. The two chains are held together by a divalent metal ion bridge. Factor VIII has

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several sites important for its function. Activation of factor VIII starts by thrombin cleavage at Arg 372 and Arg 740 in the heavy chain and at Arg 1689 in the light chain. Factor VIII circulates in human plasma non-covalently linked to Von Will brand factor (vWf).

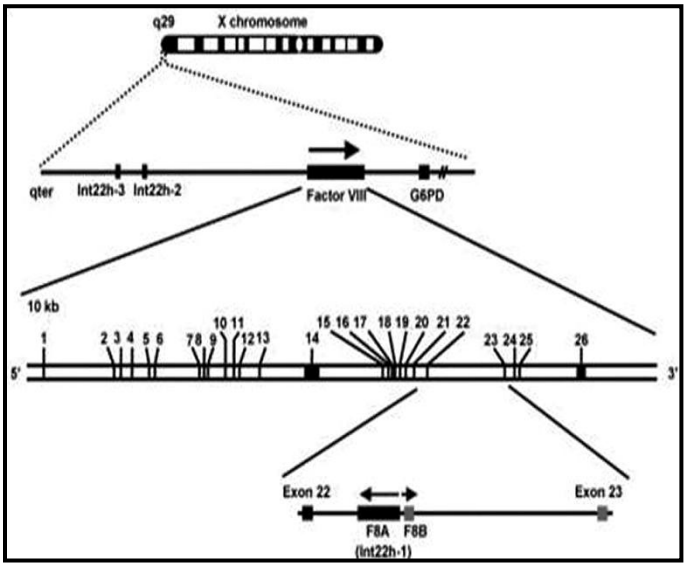

Figure 1: Factor VIII gene locus in X-chromosome.

The human in vivo half-life of factor VIII is 10-15 h and it has been suggested that the half-life is highly dependent on the factor VIII-vWf Complex formation. Factor VIII functions in the blood coagulation system as an essential cofactor for activation of factor X to factor Xa on a suitable phospholipid surface, thus amplifying the clotting stimulus many-fold. The molecule circulates in plasma as a heterodimer with structural domains A1-A2-B and A3-C1-C2. The heavy chain consists of the A1-A2-B domains with a molecular mass ranging from 90 to 200 kDa due to variability of the B domain. The 80 kDa light chain consists of the A3-C1-C2 domains. The two chains are held together by a divalent metal ion bridge. Analysis of the factor VIII gene reveals 26 exons, 24 of which vary in length from 69 to 262 base pairs (bp): the remaining much larger exons, 14 and 26, contain 3106 and 1958 bp respectively (the large majority of exon 26 is 3' untranslated sequence). The spliced FVIII mRNA is approximately 9kb in length and predicts a precursor protein of 2351 amino acids. FVIII gene defects may be broadly split into several categories: (i) gross gene rearrangements of DNA sequence involving the FVIII gene (ii) single DNA base substitutions leading to either amino-acid replacement ('missense'), premature peptide chain termination ('nonsense' or stop mutations) or mRNA splicing defects (iii) deletions of genetic sequence of a size varying from one base-pair up to the entire gene (iv) insertions of DNA of varying size. Deficiency of or defects in this protein results in the bleeding disorder Hemophilia A. At this moment we are depicting data of point mutation (missense and non-sense) [6, 9,10,11,12, and 13]

Biosynthesis and secretion of factor VIII

Factor VIII is synthesized by various tissues, including liver, kidney, and spleen, as an inactive single-chain protein. After extensive posttranslational processing, factor VIII is released

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into the circulation as a set of heterodimeric proteins. This heterogeneous population of factor VIII molecules readily interacts with vWF, which is produced and secreted by vascular endothelial cells. Upon triggering of the coagulation cascade and subsequent generation of serine proteases, factor VIII is subject to multiple proteolytic cleavages. These cleavages are associated with dramatic changes of the molecular properties of factor VIII, including dissociation of vWF and development of biological activity. After conversion into its active conformation, and participation in the factor X activating complex, activated factor VIII rapidly loses its activity. This process is governed by both enzymatic degradation and subunit dissociation [14, 15].

Possible kinds of mutation causing hemophilia:

Table 1: Point mutation leading to Hemophilia

Exon or Intron	Exon Number	Aminoacid [1]	NT Change: From, To	Aminoacid Change: From, To	Missense or Stop	Severity [5]
Promoter	-	-	-42 C>T	-	-	Mild
Exon	1	-19	ATG ATA	Met Ile	missense	Severe
Exon	1	-19	ATG AGG	Met Arg	missense	Severe
Exon	1	-5	CGA TGA	Arg Stop	stop	Severe/Moderate
Exon	1	-1	AGT AGG	SerArg	missense	Severe
Exon	1	3	AGA ACA	ArgThr	missense	Severe
Exon	1	3	AGA ATA	Arg Ile	missense	Mild
Exon	1	4	AGA GGA	ArgGly	missense	Mild
Exon	1	5	TAC TGC	Tyr Cys	missense	Mild/Moderate
Exon	1	6	TAC TGC	Tyr Cys	missense	Mild
Exon	1	7	CTG CGG	LeuArg	missense	Severe
Exon	1	7	CTG CCG	Leu Pro	missense	Severe
Exon	1	10	GTG GGG	Val Gly	missense	Severe
Exon	1	11	GAA GTA	Glu Val	missense	Mild
Exon	1	14	TGG GGG	TrpGly	missense	Moderate
Exon	1	14	TGG TAG	Trp Stop	stop	Severe
Exon	1	16	TAT CAT	Tyr His	missense	Severe

Table 2: Insertion in factor viii leading to Hemophilia

Exon	Nature of Insertion	Comments
1	1bp (T at codon 24)	frameshift
2	10bp (TTCCATTCAA at codon 38)	frameshift
2	1bp (A in 5As at codons 47-48)	frameshift
2	2bp (GA at codon 48-9) nt 203-4	frameshift
2	del G ins TT (GT>TTT at codon 103) nt 358-9	indelframeshift
4	9bp(GTCTTCCCT at codon 130)	in frame deletion
4	8bp (TCAAGATA at nt 514)	frameshift
5	1bp (T at codon 195)	frameshift
6	2bp (GTA>GTCCA at codon 238)	frameshift
6	23bp (GGTTATGTAAACAGGTCTCTGCC at codon 243)	frameshift
7	1bp (A at codon 267) nt 858	frameshift

8	1bp (T at codon 339)	frameshift
9	1bp (C at codons 426-427)	frameshift
9	1bp (A at codon 429)	frameshift
9	1bp (C at codon 453)	frameshift
9	1bp (A at nt 1441, codon 462)	frameshift
9	2bp (TG at nt 1443, codon 462)	frameshift

Table 3: Large deletion in factor viii leading to hemophilia

Exon(s) deleted	Size of Deletion (kb)
promoter - exon 1	?
promoter - exon 5	?
promoter - exon 6	?
1	>1
1	>2
1	?
1	?
1	?
1-5	>35
1-6	>55
1-7	>55
1-8	>55
1-14	?
1-22	?
1-26	>210
1-26	>210
Exon 1/Intron 1	13

Table 4: Small deletion in factor viii leading to hemophilia

Exon/Intron	Size in bp (nucleotides deleted)	Comments
Exon 1	**1 (T)**	**Frameshift**
Exon 1	**1 (C)**	**Frameshift**
Exon 2	**1**	**Frameshift**
Exon 2	**2 (AA)**	**Frameshift**
Exon 2	**6 (ACTCTG)**	**in frame del Thr49 & Leu50**
Exon 2	**c.205_206delCT p.L50fsX13**	**Frameshift**
Exon 2	**c.205_206delCT p.L50fsX13**	**Frameshift**
Exon 2	**4 in nt 206-212 (TGTTTGT)**	**Frameshift**
Exon 2	**4 in nt 206-212 (TGTTTGT)**	**Frameshift**
Exon 2	**4 in nt 206-212 (TGTTTGT)**	**Frameshift**
Exon 2	**4 in nt 206-212 (TGTTTGT)**	**Frameshift**
Exon 2	**4 in nt 206-212 (TGTTTGT)**	**Frameshift**
Exon 2	**4 in nt 206-212 (TGTTTGT)**	**Frameshift**
Exon 2	**4 in nt 206-212 (TGTTTGT)**	**Frameshift**
Exon 2	**4 in nt 206-212 (TGTTTGT)**	**Frameshift**
Exon 2	**c.224delA p.D56fsX17**	**Frameshift**
Exon 2	**6 (AGGCCA)**	**In frame del of Arg65-Pro66**

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Discussion

Mutation is a heritable change in the DNA sequence which brings about genetic variations in human being. From the above mentioned data base (Table: 1), the predominance of missense mutation over non-sense mutation is observed. Missense mutation is a kind of point mutation where a single nucleotide base is being substituted by another one, coding an entirely different amino acid [16].

Transitional mutation

The data of Table 1 suggests the prevalence of transitional point mutation. It is also observed that arginine is the amino acid which undergone mutation for the highest number of times. glycine is next in frequency. The incidence of mutation of cysteine is lowest among the twenty amino acids [17].

Insertion and deletion

Insertions can be hazardous if they occur in an exon, the amino acid coding region of a gene. A frameshift mutation is an alteration in the normal reading frame of a gene. Nineteen different insertions or deletions of which fourteen are novel are identified among HA patients. In the insertion mutation Table 2 , it is observed that insertion frameshift mutation had occurred which involved inclusion of one base to a maximum of 23 base pairs leading to severe hemophilia A, and exon 9 experienced the maximum mutation[17].

Large and small deletion mutation

Tables 3 & 4 contain large and small deletion data. Large is seen in a range of >1 to > 210 bases in one or multiple exons simultaneously. While in case of small deletions it is seen that mutation generally occurs only in one exon and does not affect more than one exon simultaneously. The 14^{th} exon is the most affected one [17].

Missence -Nonsence mutation

Forty-four different point (missense and nonsense) mutations were found of which eighteen of these are novel mutations. It is also reported that replacement triggers the disruption of a conserved disulphide bridge of (C153-C179) in the A1 domain due to C153F substitution.The D163A substitution identified in a patient with moderate hemophilia A, lies at the A1/A3 interface [18].

Splice site mutation

Four splice site mutations have also been identified at IVS 6 and 11 donor splice sites (both novel) and IVS 4 and 24 acceptor splice sites. Reports are available which states the splicing of exon 19 in ectopic F VIII mRNA in normal control samples as well as in patients with undetected E VIII mutations [18].

Inversion mutation

40-50% of severe HA cases arises from intron 22 inversion which is the mutation hot spot of factor VIII gene. A 9.5 kb region is present outside the factor VIII gene near the telomere of the X chromosome in two additional copies [19].

17 data are being stated for each pattern of mutation in tables 1-4 and for more mutational patterns refer the following internet site http://hadb.org.uk/web.

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