Chapter

Genotype-Phenotype Heterogeneity in Haemophilia

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Abstract

Haemophilia was previously regarded as a classical example of Mendelian inheritance, with mutation in only a single gene (F8 or F9) causing the disease phenotype. The disease manifests complete penetrance. Studies, however, revealed the striking genetic and phenotypic heterogeneities of the disease. With further sophistication of clinical and molecular techniques, the disease was also found to have allele heterogeneity, phenotypic plasticity and variation in expressivity. The variations are more pronounced in F9 variants with five distinct phenotypes. All these phenomena advocate a rather complex genotype-phenotype relationship for the disease. A keen insight into the matter may unveil new avenues of therapeutics.

Keywords: genotype-phenotype correlation, genotype-phenotype heterogeneity, haemophilia

1. Phenotypic variation

1.1 Background

A phenotype is defined as an observable characteristic which is expressed by an underlying genotype interacting with the environment [1]. Phenotype, in clinical scenario, hence represents the observable interface of the disease in terms of clinical features (laboratory findings, signs and symptoms) [2]. In contrast to genotype which is a stable entity, phenotype is dynamic and influenced by both the genotype and environment [3, 4]. Hence, in strict terms, the exact disease phenotype may be difficult to ascertain in many cases. This uncertainty usually underlies contemporary processes, directly or indirectly affecting the disease, with their own genetic and/or environmental influences [2]. Precise definition for a specific phenotype, therefore, needs development of a standardised comprehensive checklist of signs, symptoms and laboratory findings [3]. This is considerably convenient in case of monogenic disorders. Phenotypes for multigenic disorders or genetic diseases significantly influenced by environmental interactions are difficult to delineate [5].

1.2 Phenotypic variation in haemophilia

Haemophilia is known to mankind since ancient times with references from Babylonian history [6]. The first vague description of cases appeared in the tenth century [7]. The first modern description of the disease was made in the eighteenth

century, and the term haemophilia was first used in 1828 by Johann Lukas Schönlein and his student Friedrich Hopff [6].

The two diseases, haemophilia A (HA) and haemophilia B (HB), were initially regarded as the same and attributed to fragility of vessels [8]. The idea later shifted to abnormalities in platelets in the 1930s. It was in 1937, when Patek and Taylor found the 'anti-haemophilic globulin', extracted from plasma, to be the factor responsible. The two diseases were, however, first discriminated in 1944 by Pavlosky of Buenos Aires [8].

In haemophilia, the phenotype is expressed at three distinct levels: the coagulation activity, the factor antigen level and the clinical outcome in terms of bleeding and its complications. Plasma procoagulant level, determined by coagulation activity, is the most important clinical entity determining severity of the disease. Employing this parameter, the Scientific and Standardisation Committee classified haemophilia A and haemophilia B into three major classes, that is, mild, moderate and severe [9]. Each phenotype has a distinct clinical impact (**Table 1**). Patients with severe phenotype (plasma factor level < 0.01 IU/ml; <1% of normal) commonly present with frequent (two to five bleeding episodes per month) spontaneous bleeding into the joints or deep muscles. Patients with moderate severity of the disease (plasma factor level 0.01–0.05 IU/ml; 1–5% of normal) would bleed following mild trauma; spontaneous bleeding is seen uncommonly. Diagnosis is usually established in the first 5–6 years of life. Bleeding frequency ranges from once a month to once a year. In mild severity of the disease (plasma factor level >0.05 to <0.40 IU/ml; >5 to <40% of normal), bleeding occurs as a result of major trauma, e.g., surgery or accident. Bleeding is infrequent in these patients [10, 11].

This is, however, noteworthy that patients with a specific severity of the disease do not always behave as anticipated. Studies have reported a significant number of severe haemophilia cases with a milder phenotype [1, 12, 13]. In such cases, bleeding phenotype resembles that of moderate severity. These cases are hence treated like moderate haemophilia; prophylactic treatment is often not needed.

Severity	FVIII:C/FIX:C level (%)	Age at diagnosis	Bleeding and haemarthroses
Severe	≤1	≤2 years	Spontaneous haemorrhages and haemarthroses since early childhood
Moderate	2–5	<6 years	Haemorrhage are usually secondary to minor trauma or surgery; spontaneous haemarthrosis is unusual
Mild	6–40	Subject to haemostatic challenge	Haemorrhage secondary to surgery or major trauma; spontaneous bleedings are rare

FVIII:C, factor VIII coagulation activity; FIX:C, factor IX coagulation activity.

Table 1.
Haemophilia severity classification on the basis of FVIII:C/FIX:C levels.

2. Genetic heterogeneity in haemophilia

Haemophilia was previously regarded as a classical example of Mendelian inheritance, with mutation in only one gene (F8 or F9) causing the disease phenotype. The concept, however, has significantly evolved in the last couple of decades, and the two diseases are now recognised to have a heterogeneous spectrum of mutations. More than 2800 mutations are reported in F8, whereas more than 1200

Mutation type	F8	F9
Missense/nonsense	1674	748
Splicing	193	101
Regulatory	10	28
Small deletions	489	161
Small insertions	160	52
Small indels	38	17
Gross deletions	260	75
Gross insertions/duplications	40	7
Complex rearrangements	20	13
Repeat variations	0	0
Total	**2884**	**1202**

F8, factor VIII gene; F9, factor IX gene.

Table 2.
Frequency of different types of mutations reported in F8 and F9.

mutations are reported in F9 [14]. These mutations, summarised in **Table 2**, include all the major types of mutations. Point mutations are the most frequent, followed by small indel mutations. Repeat variants are not yet reported to associate with the disease. In majority of the cases, specific mutations result in the same disease severity, a phenomenon referred to as genotype-phenotype correlation [13, 15].

2.1 Disease penetrance and expressivity

Penetrance refers to the appearance of disease in affected individuals, whereas expressivity is the degree of severity of disease in patients [16]. Haemophilia is an X-linked recessive genetic disorder with complete penetrance in most of the cases, that is, male individuals with pathogenic variants in F8 or F9 are mostly fated to have haemophilia. This stands true particularly in case of F8. Patients from the same family have approximately the same severity status. However, the severity, as described earlier, is not the same in all patients. Cases with the same mutations exhibiting different levels of coagulation factor activity advocate variable expressivity for the specific genotype. This variation is believed to be the outcome factors including genetic alterations or polymorphisms in other genes (especially those related to haemostasis, inflammation and immune response) and environmental factors [17]. It has been established that the same genotype subjected to different environments expresses diverse phenotypes [18]. This interaction between genotype and environment is called gene–environment interaction [19, 20].

Large structural changes in the protein, by default, tend to generate a severe phenotype. Nonsense mutations, particularly those occurring in the early gene segments, have a similar tendency. Almost all the nonsense mutations reported within the initial part of the gene are associated with severe disease phenotype. Frameshift mutations in F8/F9 gene are again usually associated with an adverse phenotype [21].

Approximately 30% of the female individuals with heterozygous mutation have a coagulation factor activity less than 40% [22]. Increased bleeding tendency among the carriers, in comparison to normal females, is well documented [23, 24].

In case of F9 sequence variants, besides classical HB, four other phenotypes are reported. These are described in the following sections.

2.1.1 Haemophilia B Leyden

Haemophilia B Leyden is a specific type of HB in which the patient presents with decreased FIX:C levels in the early childhood, but the levels progressively increase after puberty. The disease is postulated to occur as a result of mutation in the 50 bp region that spans the transcriptional start site [25]. A total of 23 promoter region mutations have been identified until now (**Table 3**).

The mutation at c.-55G>C (or c. -26G>C in legacy nucleotide numbering) found in the promoter region of F9 gene is also called the haemophilia B Brandenburg mutation [38]. Unlike HB Leyden this variant does not exhibit improvement in FIX: C levels with age. The promoter region sequence located at c.-34 to -10 of the F9 gene serves as a binding site for the hepatocyte nuclear factor 4 (HNF4). The liver-enriched HNF4 is a member of the steroid hormone receptor superfamily of transcription factors (also called the nuclear receptor superfamily). Mutation at HNF4

HGVS cDNA name	Legacy nucleotide no.	Nature of mutation	Disease severity	Reference
c.-55G>A	-26	Substitution	Moderate	[26]
c.-55G>C	-26	Substitution	Severe	[27]
c.-55G>T	-26	Substitution	Severe	[28]
c.-53A>G	-24	Substitution	Not reported	[21]
c.-52C>G	-23	Substitution	Not reported	[21]
c.-52C>T	-23	Substitution	Not reported	[29]
c.-50T>G	-21	Substitution	Not reported	[30]
c.-49T>A	-20	Substitution	Moderate/mild	[31]
c.-49T>C	-20	Substitution	Mild	[32]
c.-48G>C	-19	Substitution	Moderate/mild	[29]
c.-35G>A	-6	Substitution	Mild	[33]
c.-35G>C	-6	Substitution	Mild	[34]
c.-34A>G	-5	Substitution	Mild	[26]
c.-34A>T	-5	Substitution	Moderate	[35]
c.-24T>A	6	Substitution	Mild	[34]
c.-23T>C	7	Substitution	Not reported	[21]
c.-22T>C/c	8	Substitution	Mild	[36]
c.-22delT	8	Deletion	Moderate	[21]
c.-21C>G	9	Substitution	Not reported	[21]
c.-18A>G	12	Substitution	Moderate	[21]
c.-17A>C	13	Substitution	Severe	[26]
c.-17A>G	13	Substitution	Mild	[37]
c.-17delA	13	Deletion	Mild	[37]

HGVS, Human Genome Variation Society; no., number.

Table 3.
F9 promoter site mutations associated with HB Leyden (mutation c.-55G>C is an exception).

disrupts the binding site to variable extents of severity. The mutation c.-55G>C, however, occurs at a site which is overlapped by the HNF4 binding site and another regulatory region, the androgen-responsive element (ARE) [39].

2.1.2 Thrombophilia

The F9 mutation c.1151G>T is associated with several fold increase in FIX:C activity [40]. The mutant FIX has leucine substituted for arginine at p.Arg384Leu. This alteration increases the affinity for FX to bind at this site. Patients might present with thromboembolic complications. This variant was named 'factor IX Padua'. Studies have also demonstrated that Arg-338 is part of an exosite (a secondary binding site) that binds factor X and heparin at the same time [41].

People with FIX:C levels more than 129 U/dL are 2–3 times more at risk of developing DVT in comparison to those with lower FIX:C levels. The risk is higher in females [42]. Variations in F9-associated single-nucleotide polymorphisms (SNPs) do not explain this raise in FIX antigen levels [43].

2.1.3 Protection against DVT

The Malmo polymorphism, c.580G>A (p. Ala194Thr), has an allele frequency of 0.32 in the Western population. It has been found that people with the G allele (F9 Malmo) have a 15–43% decreased risk of developing DVT in comparison to those with A allele [44]. This protective role of F9 Malmo has been extensively studied and confirmed [45]. The biochemical mechanisms behind this phenomenon are still obscure.

2.1.4 Warfarin sensitivity

All vitamin K-dependent clotting factors [including FII, FVII, FIX, FX, protein C (PC), protein S (PS) and protein Z (PZ)] possess an 18 amino acid propeptide sequence which serves as a binding site for the γ-glutamyl carboxylase enzyme. This enzyme catalyses modification of certain glutamate residues in the amino terminus of the mentioned clotting factors [46]. It has been determined that mutations at this site reduce the affinity vitamin K-dependent γ-carboxylase for the proteins.

3. Phenotypic plasticity

Phenotypic plasticity is defined as 'the ability of individual genotypes to produce different phenotypes when exposed to different environmental conditions' [47]. In the current scenario, this refers to presentation of the same mutation with different severities of the disease.

3.1 Genetic basis of phenotypic plasticity

It has been found that the mutations with varying phenotypes (MVPs) mostly occur at the less conserved sites with Arg being the usual mutated residue. It is also noted that these mutations commonly occur at the CpG dinucleotides. In comparison, mutations with uniform phenotypes (MUPs) occur in more conserved sites, with cysteine as the most frequently mutated amino acid residue. Intrinsic protein structural changes have been reported with reduced severity in cases of MVPs. No significant structural variations are identified between the two groups. The phenomenon is hypothesised to be a function of multiple factors including modifier

HGVS cDNA	HGVS protein	Mutation type	Mechanism	Exon	Domain	Severe (<1 U/dL)	Moderate (1–5 U/dL)	Mild (>5 U/dL)
c.1171C>T	p.Arg391Cys	Missense	Substitution	8	a1	X	X	X
c.1172G>A	p.Arg391His	Missense	Substitution	8	a1	X	X	X
c.1492G>A	p.Gly498Arg	Missense	Substitution	10	A2	X	X	X
c.396A>C	p.Glu132Asp	Missense	Substitution	4	A1	X	X	X
c.4380delT	p.Asn1460Lysfs*5	Frameshift	Deletion	14	B	X	X	X
c.5122C>T	p.Arg1708Cys	Missense	Substitution	14	a3	X	X	X
c.5219+3A>G		Splice site change	Substitution	Intron 14		X	X	X
c.5399G>A	p.Arg1800His	Missense	Substitution	16	A3	X	X	X
c.5663G>T	p.Arg1888Ile	Missense	Substitution	17	A3	X	X	X
c.590T>G	p.Val197Gly	Missense	Substitution	4	A1	X	X	X
c.6356A>G	p.Gln2119Arg	Missense	Substitution	22	C1	X	X	X
c.6371A>G	p.Tyr2124Cys	Missense	Substitution	22	C1	X	X	X
c.6506G>A	p.Arg2169His	Missense	Substitution	23	C1	X	X	X
c.6545G>A	p.Arg2182His	Missense	Substitution	23	C1	X	X	X
c.6683G>A	p.Arg2228Gln	Missense	Substitution	24	C2	X	X	X
c.6977G>A	p.Arg2326Gln	Missense	Substitution	26	C2	X	X	X
c.902G>A	p.Arg301His	Missense	Substitution	7	A1	X	X	X
c.1063C>T	p.Arg355*	Nonsense	Substitution	8	A1	X	X	
c.1226A>G	p.Glu409Gly	Missense	Substitution	8	A2	X	X	
c.1316G>T	p.Gly439Val	Missense	Substitution	9	A2	X	X	
c.143+1567A>G		Splice site change	Substitution	Intron 1		X	X	
c.1475A>G	p.Tyr492Cys	Missense	Substitution	10	A2	X	X	

HGVS cDNA	HGVS protein	Mutation type	Mechanism	Exon	Domain	Severe (<1 U/dL)	Moderate (1–5 U/dL)	Mild (>5 U/dL)
c.1639T>C	p.Cys547Arg	Missense	Substitution	11	A2	X	X	
c.1702G>A	p.Gly568Ser	Missense	Substitution	11	A2	X	X	
c.1754T>C	p.Ile585Thr	Missense	Substitution	12	A2	X	X	
c.1804C>T	p.Arg602*	Nonsense	Substitution	12	A2	X	X	
c.1809C>G	p.Ser603Arg	Missense	Substitution	12	A2	X	X	
c.2015_2017del	p.Phe672del	Small structural change (in-frame, <50 bp)	Deletion	13	A2	X	X	
c.2048A>G	p.Tyr683Cys	Missense	Substitution	13	A2	X	X	
c.206_212del	p.Leu69Glnfs*21	Frameshift	Deletion	2	A1	X	X	
c.2090T>A	p.Val697Asp	Missense	Substitution	13	A2	X	X	
c.2114-?_5219+?del		Large structural change (>50 bp)	Deletion	14	A3	X	X	
c.2159G>A	p.Gly720Asp	Missense	Substitution	14	A2	X	X	
c.2182delT	p.Ser728Leufs*23	Frameshift	Deletion	14	A2	X	X	
c.2373G>A	p.Trp791*	Nonsense	Substitution	14	B	X	X	
c.2440C>T	p.Arg814*	Nonsense	Substitution	14	B	X	X	
c.266G>A	p.Gly89Asp	Missense	Substitution	3	A1	X	X	
c.2945dupA	p.Asn982Lysfs*9	Frameshift	Duplication	14	B	X	X	
c.296T>A	p.Val99Asp	Missense	Substitution	3	A1	X	X	
c.3143G>A	p.Trp1048*	Nonsense	Substitution	14	B	X	X	
c.3300dupA	p.Glu1101Argfs*17	Frameshift	Duplication	14	B	X	X	
c.353A>G	p.His118Arg	Missense	Substitution	3	A1	X	X	
c.3637delA	p.Ile1213Phefs*5	Frameshift	Deletion	14	B	X	X	
c.3637dupA	p.Ile1213Asnfs*28	Frameshift	Duplication	14	B	X	X	

HGVS cDNA	HGVS protein	Mutation type	Mechanism	Exon	Domain	Severe (<1 U/dL)	Moderate (1–5 U/dL)	Mild (>5 U/dL)
c.3702_3705del	p.His1234Glnfs*2	Frameshift	Deletion	14	B	X	X	
c.388G>C	p.Gly130Arg	Missense	Substitution	3	A1	X	X	
c.421G>A	p.Glu141Lys	Missense	Substitution	4	A1	X	X	
c.4296_4300del	p.His1434Serfs*6	Frameshift	Deletion	14	B	X	X	
c.4379dupA	p.Asn1460Lysfs*2	Frameshift	Duplication	14	B	X	X	
c.43C>T	p.Arg15*	Nonsense	Substitution	1	Signal	X	X	
c.4796G>A	p.Trp1599*	Nonsense	Substitution	14	B	X	X	
c.4825dupA	p.Thr1609Asnfs*4	Frameshift	Duplication	14	B	X	X	
c.491G>A	p.Gly164Asp	Missense	Substitution	4	A1	X	X	
c.5113C>T	p.Gln1705*	Nonsense	Substitution	14	a3	X	X	
c.515G>T	p.Cys172Phe	Missense	Substitution	4	A1	X	X	
c.5219G>T	p.Arg1740Met	Missense	Substitution	14	A3	X	X	
c.5471dupA	p.Asn1824Lysfs*6	Frameshift	Duplication	16	A3	X	X	
c.5536A>T	p.Lys1846*	Nonsense	Substitution	16	A3	X	X	
c.556G>T	p.Asp186Tyr	Missense	Substitution	4	A1	X	X	
c.5606G>T	p.Gly1869Val	Missense	Substitution	17	A3	X	X	
c.5685delT	p.Phe1895Leufs*50	Frameshift	Deletion	17	A3	X	X	
c.5719A>T	p.Ser1907Cys	Missense	Substitution	17	A3	X	X	
c.5878C>T	p.Arg1960*	Nonsense	Substitution	18	A3	X	X	
c.5953C>T	p.Arg1985*	Nonsense	Substitution	18	A3	X	X	
c.5973_5976del	p.Met1992Hisfs*37	Frameshift	Deletion	18	A3	X	X	
c.5998+1G>A		Splice site change	Substitution	Intron 18		X	X	

HGVS cDNA	HGVS protein	Mutation type	Mechanism	Exon	Domain	Severe (<1 U/dL)	Moderate (1–5 U/dL)	Mild (>5 U/dL)
c.5999-?_6429+?dup		Large structural change (>50 bp)	Duplication	19–22		X	X	
c.602-?_787+?del		Large structural change (>50 bp)	Deletion	5–6		X	X	
c.6046C>T	p.Arg2016Trp	Missense	Substitution	19	A3	X	X	
c.6133G>A	p.Gly2045Arg	Missense	Substitution	20	C1	X	X	
c.6172G>C	p.Ala2058Pro	Missense	Substitution	20	C1	X	X	
c.6274-?_6429+?del		Large structural change (>50 bp)	Deletion	22	C1	X	X	
c.6403C>T	p.Arg2135*	Nonsense	Substitution	22	C1	X	X	
c.6429+?_6430-?inv		Large structural change (>50 bp)	Inversion	Intron 22		X	X	
c.6481C>T	p.Pro2161Ser	Missense	Substitution	23	C1	X	X	
c.6485C>T	p.Pro2162Leu	Missense	Substitution	23	C1	X	X	
c.6496C>T	p.Arg2166*	Nonsense	Substitution	23	C1	X	X	
c.6544C>T	p.Arg2182Cys	Missense	Substitution	23	C1	X	X	
c.6593G>T	p.Gly2198Val	Missense	Substitution	24	C2	X	X	
c.6682C>G	p.Arg2228Gly	Missense	Substitution	24	C2	X	X	
c.6682C>T	p.Arg2228*	Nonsense	Substitution	24	C2	X	X	
c.670+5G>A		Splice site change	Substitution	Intron 5		X	X	
c.6742T>A	p.Trp2248Arg	Missense	Substitution	25	C2	X	X	
c.6875_6876del	p.Phe2294Serfs*90	Frameshift	Deletion	25	C2	X	X	
c.6967C>T	p.Arg2323Cys	Missense	Substitution	26	C2	X	X	
c.6977G>T	p.Arg2326Leu	Missense	Substitution	26	C2	X	X	
c.6994T>C	p.Trp2332Arg	Missense	Substitution	26	C2	X	X	
c.764G>C	p.Gly255Ala	Missense	Substitution	6	A1	X	X	

HGVS cDNA	HGVS protein	Mutation type	Mechanism	Exon	Domain	Severe (<1 U/dL)	Moderate (1–5 U/dL)	Mild (>5 U/dL)
c.785C>T	p.Pro262Leu	Missense	Substitution	6	A1	X	X	
c.787+3A>G		Splice site change	Substitution	Intron 6		X	X	
c.822G>C	p.Trp274Cys	Missense	Substitution	7	A1	X	X	
c.901C>T	p.Arg301Cys	Missense	Substitution	7	A1	X	X	
c.954_955del	p.Leu319Aspfs*18	Frameshift	Deletion	7	A1	X	X	
c.991_992del	p.Ile331Leufs*6	Frameshift	Deletion	7	A1	X	X	
c.1043G>A	p.Cys348Tyr	Missense	Substitution	8	A1	X		X
c.121G>T	p.Gly41Cys	Missense	Substitution	1	A1	X		X
c.1409C>T	p.Pro470Leu	Missense	Substitution	9	A2	X		X
c.1751A>G	p.Gln584Arg	Missense	Substitution	11	A2	X		X
c.1910A>G	p.Asn637Ser	Missense	Substitution	13	A2	X		X
c.3870dupA	p.Gly1291Argfs*29	Frameshift	Duplication	14	B	X		X
c.437A>C	p.Lys146Thr	Missense	Substitution	4	A1	X		X
c.5150A>G	p.Tyr1717Cys	Missense	Substitution	14	A3	X		X
c.5183A>G	p.Tyr1728Cys	Missense	Substitution	14	A3	X		X
c.6273+1G>T		Splice site change	Substitution	Intron 21		X		X
c.677G>T	p.Ser226Ile	Missense	Substitution	6	A1	X		X
c.6967C>G	p.Arg2323Gly	Missense	Substitution	26	C2	X		X
c.902G>T	p.Arg301Leu	Missense	Substitution	7	A1	X		X
c.923C>T	p.Ser308Leu	Missense	Substitution	7	A1	X		X
c.1293G>T	p.Leu431Phe	Missense	Substitution	9	A2		X	X
c.1348T>A	p.Tyr450Asn	Missense	Substitution	9	A2		X	X
c.1408C>A	p.Pro470Thr	Missense	Substitution	9	A2		X	X

HGVS cDNA	HGVS protein	Mutation type	Mechanism	Exon	Domain	Severe (<1 U/dL)	Moderate (1–5 U/dL)	Mild (>5 U/dL)
c.1569G>T	p.=	Synonymous	Substitution	11	A2		X	X
c.1636C>T	p.Arg546Trp	Missense	Substitution	11	A2		X	X
c.1648C>T	p.Arg550Cys	Missense	Substitution	11	A2		X	X
c.1660A>G	p.Ser554Gly	Missense	Substitution	11	A2		X	X
c.1834C>T	p.Arg612Cys	Missense	Substitution	12	A2		X	X
c.2044G>T	p.Val682Phe	Missense	Substitution	13	A2		X	X
c.2149C>T	p.Arg717Trp	Missense	Substitution	14	A2		X	X
c.2167G>A	p.Ala723Thr	Missense	Substitution	14	A2		X	X
c.274G>A	p.Gly92Ser	Missense	Substitution	3	A1		X	X
c.311T>A	p.Val104Asp	Missense	Substitution	3	A1		X	X
c.410C>T	p.Thr137Ile	Missense	Substitution	4	A1		X	X
c.5096A>T	p.Tyr1699Phe	Missense	Substitution	14	a3		X	X
c.5143C>G	p.Arg1715Gly	Missense	Substitution	14	A3		X	X
c.5339C>A	p.Pro1780Gln	Missense	Substitution	15	A3		X	X
c.5393C>T	p.Ala1798Val	Missense	Substitution	16	A3		X	X
c.5398C>G	p.Arg1800Gly	Missense	Substitution	16	A3		X	X
c.541G>A	p.Val181Met	Missense	Substitution	4	A1		X	X
c.5428T>C	p.Ser1810Pro	Missense	Substitution	16	A3		X	X
c.5526G>A	p.Met1842Ile	Missense	Substitution	16	A3		X	X
c.5557G>A	p.Ala1853Thr	Missense	Substitution	16	A3		X	X
c.5618C>T	p.Pro1873Leu	Missense	Substitution	17	A3		X	X
c.5825G>C	p.Gly1942Ala	Missense	Substitution	18	A3		X	X
c.5879G>A	p.Arg1960Gln	Missense	Substitution	18	A3		X	X

HGVS cDNA	HGVS protein	Mutation type	Mechanism	Exon	Domain	Severe (<1 U/dL)	Moderate (1–5 U/dL)	Mild (>5 U/dL)
c.5921C>T	p.Ser1974Phe	Missense	Substitution	18	A3		X	X
c.5954G>A	p.Arg1985Gln	Missense	Substitution	18	A3		X	X
c.601+1632G>A		Splice site change	Substitution	Intron 4			X	X
c.6113A>G	p.Asn2038Ser	Missense	Substitution	19	A3		X	X
c.6119G>A	p.Cys2040Tyr	Missense	Substitution	20	C1		X	X
c.6212G>C	p.Arg2071Thr	Missense	Substitution	21	C1		X	X
c.6278A>G	p.Asp2093Gly	Missense	Substitution	22	C1		X	X
c.6350T>G	p.Ile2117Ser	Missense	Substitution	22	C1		X	X
c.6413C>A	p.Ser2138Tyr	Missense	Substitution	22	C1		X	X
c.6443A>G	p.Asn2148Ser	Missense	Substitution	23	C1		X	X
c.6520C>G	p.His2174Asp	Missense	Substitution	23	C1		X	X
c.6532C>T	p.Arg2178Cys	Missense	Substitution	23	C1		X	X
c.668A>C	p.Glu223Ala	Missense	Substitution	5	A1		X	X
c.670+6T>C		Splice site change	Substitution	Intron 5			X	X
c.6744G>T	p.Trp2248Cys	Missense	Substitution	25	C2		X	X
c.67A>G	p.Arg23Gly	Missense	Substitution	1	A1		X	X
c.6915T>G	p.Asn2305Lys	Missense	Substitution	26	C2		X	X
c.6920A>C	p.Asp2307Ala	Missense	Substitution	26	C2		X	X
c.6956C>T	p.Pro2319Leu	Missense	Substitution	26	C2		X	X
c.755C>T	p.Thr252Ile	Missense	Substitution	6	A1		X	X
c.871G>A	p.Glu291Lys	Missense	Substitution	7	A1		X	X

Table 4.
List of F8 mutations reported with phenotypic plasticity.

HGVS cDNA name	HGVS protein name	Mutation type	Mechanism	Exon	Domain	Severe (< 1 U/dL)	Moderate (1–5 U/dL)	Mild (>5 U/dL)
c.87A>G	p.Thr29Thr	Synonymous	Substitution	1	PRO	X	X	X
c.127C>T	p.Arg43Trp	Missense	Substitution	2	PRO	X	X	X
c.128G>A	p.Arg43Gln	Missense	Substitution	2	PRO	X	X	X
c.172G>A	p.Gly58Arg	Missense	Substitution	2	GLA	X	X	X
c.173G>A	p.Gly58Glu	Missense	Substitution	2	GLA	X	X	X
c.191G>A	p.Cys64Tyr	Missense	Substitution	2	GLA	X	X	X
c.259T>G	p.Phe87Val	Missense	Substitution	3	GLA	X	X	X
c.301C>G	p.Pro101Ala	Missense	Substitution	4	EGF1	X	X	X
c.316G>A	p.Gly106Ser	Missense	Substitution	4	EGF1	X	X	X
c.412A>C	p.Asn138His	Missense	Substitution	5	EGF2	X	X	X
c.415G>A	p.Gly139Ser	Missense	Substitution	5	EGF2	X	X	X
c.571C>T	p.Arg191Cys	Missense	Substitution	6	Linker	X	X	X
c.572G>A	p.Arg191His	Missense	Substitution	6	Linker	X	X	X
c.720G>T	p.Trp240Cys	Missense	Substitution	6	Protease	X	X	X
c.755G>A	p.Cys252Tyr	Missense	Substitution	7	Protease	X	X	X
c.797C>T	p.Ala266Val	Missense	Substitution	7	Protease	X	X	X
c.835G>A	p.Ala279Thr	Missense	Substitution	7	Protease	X	X	X
c.838G>C	p.Gly280Arg	Missense	Substitution	7	Protease	X	X	X
c.881G>A	p.Arg294Gln	Missense	Substitution	8	Protease	X	X	X
c.914A>G	p.Tyr305Cys	Missense	Substitution	8	Protease	X	X	X
c.987C>G	p.Ser329Arg	Missense	Substitution	8	Protease	X	X	X
c.1009G>A	p.Ala337Thr	Missense	Substitution	8	Protease	X	X	X

HGVS cDNA name	HGVS protein name	Mutation type	Mechanism	Exon	Domain	Severe (< 1 U/dL)	Moderate (1–5 U/dL)	Mild (>5 U/dL)
c.1025C>T	p.Thr342Met	Missense	Substitution	8	Protease	X	X	X
c.1135C>T	p.Arg379*	Nonsense	Substitution	8	Protease	X	X	X
c.1136G>A	p.Arg379Gln	Missense	Substitution	8	Protease	X	X	X
c.1187G>C	p.Cys396Ser	Missense	Substitution	8	Protease	X	X	X
c.1235G>A	p.Gly412Glu	Missense	Substitution	8	Protease	X	X	X
c.1240C>A	p.Pro414Thr	Missense	Substitution	8	Protease	X	X	X
c.1275A>C	p.Leu425Phe	Missense	Substitution	8	Protease	X	X	X
c.1304G>A	p.Cys435Tyr	Missense	Substitution	8	Protease	X	X	X
c.1306G>A	p.Ala436Thr	Missense	Substitution	8	Protease	X	X	X
c.1328T>C	p.Ile443Thr	Missense	Substitution	8	Protease	X	X	X
c.*2545A>G		3′UTR	Substitution	3′UTR		X	X	X
c.-17A>G		Promoter	Substitution	1		X	X	X
c.-35G>A		Promoter	Substitution	5′UTR		X	X	X
c.-35G>C		Promoter	Substitution	5′UTR		X	X	X
c.50T>A	p.Ile17Asn	Missense	Substitution	1	Signal peptide	X	X	
c.83G>A	p.Cys28Tyr	Missense	Substitution	1	Signal peptide	X	X	
c.128G>T	p.Arg43Leu	Missense	Substitution	2	PRO	X	X	
c.138G>T	p.Arg47Ser	Missense	Substitution	2	PRO	X	X	
c.190T>C	p.Cys64Arg	Missense	Substitution	2	GLA	X	X	
c.199G>A	p.Glu67Lys	Missense	Substitution	2	GLA	X	X	
c.219A>C	p.Glu73Asp	Missense	Substitution	2	GLA	X	X	
c.223C>T	p.Arg75Stop	Nonsense	Substitution	2	GLA	X	X	

HGVS cDNA name	HGVS protein name	Mutation type	Mechanism	Exon	Domain	Severe (< 1 U/dL)	Moderate (1–5 U/dL)	Mild (>5 U/dL)
c.226G>A	p.Glu76Lys	Missense	Substitution	2	GLA	X	X	
c.260T>G	p.Phe87Cys	Missense	Substitution	3	GLA	X	X	
c.263G>A	p.Trp88*	Nonsense	Substitution	3	GLA	X	X	
c.291T>G	p.Cys97Trp	Missense	Substitution	4	EGF1	X	X	
c.304T>C	p.Cys102Arg	Missense	Substitution	4	EGF1	X	X	
c.305G>A	p.Cys102Tyr	Missense	Substitution	4	EGF1	X	X	
c.350G>A	p.Cys117Tyr	Missense	Substitution	4	EGF1	X	X	
c.383G>A	p.Cys128Tyr	Missense	Substitution	4	EGF1	X	X	
c.392delA	p.Asp131fs	Frameshift	Deletion	5	EGF2	X	X	
c.414T>A	p.Asn138Lys	Missense	Substitution	5	EGF2	X	X	
c.422G>A	p.Cys141Tyr	Missense	Substitution	5	EGF2	X	X	
c.423C>A	p.Cys141*	Nonsense	Substitution	5	EGF2	X	X	
c.423C>G	p.Cys141Trp	Missense	Substitution	5	EGF2	X	X	
c.427C>G	p.Gln143Glu	Missense	Substitution	5	EGF2	X	X	
c.434G>A	p.Cys145Tyr	Missense	Substitution	5	EGF2	X	X	
c.464G>T	p.Cys155Phe	Missense	Substitution	5	EGF2	X	X	
c.470G>A	p.Cys157Tyr	Missense	Substitution	5	EGF2	X	X	
c.470G>C	p.Cys157Ser	Missense	Substitution	5	EGF2	X	X	
c.479G>T	p.Gly160Val	Missense	Substitution	5	EGF2	X	X	
c.482A>G	p.Tyr161Cys	Missense	Substitution	5	EGF2	X	X	
c.484C>T	p.Arg162*	Nonsense	Substitution	5	EGF2	X	X	
c.509G>A	p.Ser170Tyr	Missense	Substitution	5	EGF2	X	X	

HGVS cDNA name	HGVS protein name	Mutation type	Mechanism	Exon	Domain	Severe (< 1 U/dL)	Moderate (1–5 U/dL)	Mild (>5 U/dL)
c.520G>A	p.Val174Met	Missense	Substitution	5	EGF2	X	X	
c.532T>C	p.Cys178Arg	Missense	Substitution	6	Linker	X	X	
c.535G>A	p.Gly179Arg	Missense	Substitution	6	Linker	X	X	
c.545_546del	p.Ser182Cysfs*6	Frameshift	Deletion	6	Linker	X	X	
c.547delG	p.Val183fs	Frameshift	Deletion	6	Linker	X	X	
c.676C>T	p.Arg226Trp	Missense	Substitution	6	Activation	X	X	
c.677G>A	p.Arg226Gln	Missense	Substitution	6	Activation	X	X	
c.677G>T	p.Arg226Leu	Missense	Substitution	6	Activation	X	X	
c.688_690del	p.Gly230del	Small structural change (in-frame, <50 bp)	Deletion	6	Protease	X	X	
c.706G>T	p.Gly236Cys	Missense	Substitution	6	Protease	X	X	
c.707G>A	p.Gly236Asp	Missense	Substitution	6	Protease	X	X	
c.711A>G	p.Gln237Gln	Synonymous	Substitution	6	Protease	X	X	
c.719G>A	p.Trp240*	Nonsense	Substitution	6	Protease	X	X	
c.719G>T	p.Trp240Leu	Missense	Substitution	6	Protease	X	X	
c.721C>T	p.Gln241*	Nonsense	Substitution	6	Protease	X	X	
c.723G>A	p.Gln241Gln	Synonymous	Substitution	6	Protease	X	X	
c.727_728delinsA	p.Val243fs	Frameshift	Insertion/ deletion	7	Protease	X	X	
c.757G>A	p.Gly253Arg	Missense	Substitution	7	Protease	X	X	
c.789_790InsT	p.Thr264fs	Frameshift	Insertion	7	Protease	X	X	
c.799C>T	p.His267Tyr	Missense	Substitution	7	Protease	X	X	
c.839G>T	p.Gly280Val	Missense	Substitution	8	Protease	X	X	

HGVS cDNA name	HGVS protein name	Mutation type	Mechanism	Exon	Domain	Severe (< 1 U/dL)	Moderate (1–5 U/dL)	Mild (>5 U/dL)
c.871G>A	p.Glu291Lys	Missense	Substitution	8	Protease	X	X	
c.880C>T	p.Arg294*	Nonsense	Substitution	8	Protease	X	X	
c.881G>T	p.Arg294Leu	Missense	Substitution	8	Protease	X	X	
c.892C>T	p.Arg298*	Nonsense	Substitution	8	Protease	X	X	
c.946A>T	p.Ile316Phe	Missense	Substitution	8	Protease	X	X	
c.990C>A	p.Tyr330*	Nonsense	Substitution	8	Protease	X	X	
c.1004G>T	p.Cys335Tyr	Missense	Substitution	8	Protease	X	X	
c.1009G>C	p.Ala337Pro	Missense	Substitution	8	Protease	X	X	
c.1068G>C	p.Trp356Cys	Missense	Substitution	8	Protease	X	X	
c.1069G>A	p.Gly357Arg	Missense	Substitution	8	Protease	X	X	
c.1070G>A	p.Gly357Glu	Missense	Substitution	8	Protease	X	X	
c.1076T>G	p.Val359Gly	Missense	Substitution	8	Protease	X	X	
c.1097C>A	p.Ala366Asp	Missense	Substitution	8	Protease	X	X	
c.1108C>T	p.Gln370*	Nonsense	Substitution	8	Protease	X	X	
c.1113C>A	p.Tyr371*	Nonsense	Substitution	8	Protease	X	X	
c.1120G>T	p.Val374Glu	Missense	Substitution	8	Protease	X	X	
c.1135C>G	p.Arg379Gly	Missense	Substitution	8	Protease	X	X	
c.1144T>C	p.Cys382Arg	Missense	Substitution	8	Protease	X	X	
c.1147C>T	p.Leu383Phe	Missense	Substitution	8	Protease	X	X	
c.1150C>T	p.Arg384*	Nonsense	Substitution	8	Protease	X	X	
c.1168A>T	p.Ile390Phe	Missense	Substitution	8	Protease	X	X	
c.1169T>G	p.Ile390Ser	Missense	Substitution	8	Protease	X	X	

HGVS cDNA name	HGVS protein name	Mutation type	Mechanism	Exon	Domain	Severe (< 1 U/dL)	Moderate (1–5 U/dL)	Mild (>5 U/dL)
c.1181T>A	p.Met394Lys	Missense	Substitution	8	Protease	X	X	
c.1204G>A	p.Gly402Arg	Missense	Substitution	8	Protease	X	X	
c.1217C>G	p.Ser406*	Nonsense	Substitution	8	Protease	X	X	
c.1217C>T	p.Ser406Leu	Missense	Substitution	8	Protease	X	X	
c.1219T>C	p.Cys407Arg	Missense	Substitution	8	Protease	X	X	
c.1226G>A	p.Gly409Glu	Missense	Substitution	8	Protease	X	X	
c.1228G>A	p.Asp410Asn	Missense	Substitution	8	Protease	X	X	
c.1228G>C	p.Asp410His	Missense	Substitution	8	Protease	X	X	
c.1232G>A	p.Ser411Asn	Missense	Substitution	8	Protease	X	X	
c.1237G>A	p.Gly413Arg	Missense	Substitution	8	Protease	X	X	
c.1241C>T	p.Pro414Leu	Missense	Substitution	8	Protease	X	X	
c.1245T>A	p.His415Gln	Missense	Substitution	8	Protease	X	X	
c.1256T>A	p.Val419Glu	Missense	Substitution	8	Protease	X	X	
c.1258G>T	p.Glu420*	Nonsense	Substitution	8	Protease	X	X	
c.1291T>C	p.Trp431Arg	Missense	Substitution	8	Protease	X	X	
c.1293G>T	p.Trp431Cys	Missense	Substitution	8	Protease	X	X	
c.1294G>A	p.Gly432Ser	Missense	Substitution	8	Protease	X	X	
c.1295G>A	p.Gly432Asp	Missense	Substitution	8	Protease	X	X	
c.1295G>C	p.Gly432Ala	Missense	Substitution	8	Protease	X	X	
c.1295G>T	p.Gly432Val	Missense	Substitution	8	Protease	X	X	
c.1297G>A	p.Glu433Lys	Missense	Substitution	8	Protease	X	X	
c.1298A>C	p.Glu433Ala	Missense	Substitution	8	Protease	X	X	

HGVS cDNA name	HGVS protein name	Mutation type	Mechanism	Exon	Domain	Severe (< 1 U/dL)	Moderate (1–5 U/dL)	Mild (>5 U/dL)
c.1307C>T	p.Ala436Val	Missense	Substitution	8	Protease	X	X	
c.1318A>G	p.Lys440Glu	Missense	Substitution	8	Protease	X	X	
c.1324G>A	p.Gly442Arg	Missense	Substitution	8	Protease	X	X	
c.1357T>C	p.Trp453Arg	Missense	Substitution	8	Protease	X	X	
c.1361T>C	p.Ile454Thr	Missense	Substitution	8	Protease	X	X	
c.*1157A>G		3′UTR	Substitution	3′UTR		X	X	
c.252+3_252+6del		Splice site change	Deletion	Intron 2		X	X	
c.252+6T>C		Splice site change	Substitution	Intron 2		X	X	
c.253-25A>G		Splice site change	Substitution	Intron 2		X	X	
c.277+2T>C		Splice site change	Substitution	Intron 3		X	X	
c.277+5G>A		Splice site change	Substitution	Intron 3		X	X	
c.392-1G>C		Splice site change	Substitution	Intron 4		X	X	
c.392-2A>G		Splice site change	Substitution	Intron 4		X	X	
c.521-3T>G		Splice site change	Substitution	Intron 5		X	X	
c.-55G>A		Promoter	Substitution	5′UTR		X	X	
c.723+1G>A		Splice site change	Substitution	Intron 6		X	X	
c.839-4A>G		Splice site change	Substitution	Intron 7		X	X	
c.88+1_88+4del		Splice site change	Deletion	Intron 1		X	X	
c.88+1G>T		Splice site change	Substitution	Intron 1		X	X	
c.88+5G>C		Splice site change	Substitution	Intron 1		X	X	
c.88+5G>T		Splice site change	Substitution	Intron 1		X	X	
c.19A>T	p.Ile7Phe	Missense	Substitution	1	Signal peptide	X		X

HGVS cDNA name	HGVS protein name	Mutation type	Mechanism	Exon	Domain	Severe (< 1 U/dL)	Moderate (1–5 U/dL)	Mild (>5 U/dL)
c.164T>G	p.Phe55Cys	Missense	Substitution	2	GLA	X		X
c.339T>A	p.Asn113Lys	Missense	Substitution	4	EGF1	X		X
c.466T>C	p.Ser156Phe	Missense	Substitution	5	EGF2	X		X
c.676C>G	p.Arg226Gly	Missense	Substitution	6	Activation	X		X
c.685G>A	p.Gly229Ser	Missense	Substitution	6	Protease	X		X
c.907C>T	p.His303Tyr	Missense	Substitution	8	Protease	X		X
c.942T>G	p.His314Gln	Missense	Substitution	8	Protease	X		X
c.1045G>T	p.Gly349*	Nonsense	Substitution	8	Protease	X		X
c.1072A>G	p.Arg358Gly	Missense	Substitution	8	Protease	X		X
c.1079T>C	p.Phe360Ser	Missense	Substitution	8	Protease	X		X
c.1109A>C	p.Gln370Pro	Missense	Substitution	8	Protease	X		X
c.1174A>G	p.Asn392Asp	Missense	Substitution	8	Protease	X		X
c.1238G>A	p.Gly413Glu	Missense	Substitution	8	Protease	X		X
c.252+5G>A		Splice site change	Substitution	Intron 2		X		X
c.839-1G>A		Splice site change	Substitution	Intron 7		X		X
c.82T>C	p.Cys28Arg	Missense	Substitution	1	Signal peptide		X	X
c.151A>G	p.Lys51Glu	Missense	Substitution	2	GLA		X	X
c.163T>A	p.Phe55Ile	Missense	Substitution	2	GLA		X	X
c.279T>A	p.Asp93Glu	Missense	Substitution	4	EGF1		X	X
c.335T>C	p.Ile112Thr	Missense	Substitution	4	EGF1		X	X
c.479G>A	p.Gly160Glu	Missense	Substitution	5	EGF2		X	X
c.479G>C	p.Gly160Ala	Missense	Substitution	5	EGF2		X	X

HGVS cDNA name	HGVS protein name	Mutation type	Mechanism	Exon	Domain	Severe (< 1 U/dL)	Moderate (1–5 U/dL)	Mild (>5 U/dL)
c.484C>A	p.Arg162Arg	Synonymous	Substitution	5	EGF2		X	X
c.572G>C	p.Arg191Pro	Missense	Substitution	6	Linker		X	X
c.785T>C	p.Ile262Thr	Missense	Substitution	7	Protease		X	X
c.786T>G	p.Ile262Met	Missense	Substitution	7	Protease		X	X
c.839G>C	p.Gly280Ala	Missense	Substitution	8	Protease		X	X
c.872A>G	p.Glu291Gly	Missense	Substitution	8	Protease		X	X
c.950C>T	p.Ala317Val	Missense	Substitution	8	Protease		X	X
c.997C>A	p.Pro333Thr	Missense	Substitution	8	Protease		X	X
c.1067G>T	p.Trp356Leu	Missense	Substitution	8	Protease		X	X
c.1097C>T	p.Ala366Val	Missense	Substitution	8	Protease		X	X
c.1127T>C	p.Leu376Pro	Missense	Substitution	8	Protease		X	X
c.1180A>G	p.Met394Val	Missense	Substitution	8	Protease		X	X
c.1187G>T	p.Cys396Phe	Missense	Substitution	8	Protease		X	X
c.1193G>C	p.Gly398Ala	Missense	Substitution	8	Protease		X	X
c.1348T>C	p.Tyr450His	Missense	Substitution	8	Protease		X	X
c.-48G>C		Promoter	Substitution	5′UTR			X	X
c.-49T>A		Promoter	Substitution	5′UTR			X	X
c.520+13A>G		Splice site change	Substitution	Intron 5			X	X
c.88+5G>A		Splice site change	Substitution	Intron 1			X	X

Table 5.
List of F9 mutations reported with phenotypic plasticity.

genes, epigenetic influences and environmental effects. These factors may act individually or in combination [48].

Tables 4 and **5** depict F8 and F9 mutations, respectively, reported with phenotypic plasticity [49, 50]. A total of 351 mutations are presented here with cases reported from at least two severity classes. The most significant are the 85 cases (32 from F8 and 53 from F9) wherein patients from both severe and mild categories are reported.

Taking into account the significant amount of phenotypic plasticity in haemophilia, researchers have proposed to recognise the disease phenotype, in terms of coagulation activity, a continuous variable and abandoning of the classical categorical classification [51]. With the evolving concepts of personalised medicine, this may prove realistic... and the future.

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