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
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
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].

| 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.
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.Ile1213Asmsfs*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.438C>T    | p.Arg15*           | Nonsense       | Substitution| 1    | Signal | X                | X                    |                 |
| c.4796G>A   | p.Trp1599*         | Nonsense       | Substitution| 14   | B      | X                | X                    |                 |
| c.4825dupA  | p.Thr1609Asns*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.5371dupA  | p.Asn1824Lysfs*6   | Frameshift     | Duplication | 16   | A3     | X                | X                    |                 |
| c.5356A>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_9976del | 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                   | X                |
| c.602-?_787+?del |              | Large structural change (>50 bp) | Deletion      | 5–6  |        | X               | X                   |                |
| c.6046C>T       | p.Arg2161Trp | Missense Substitution           |               | 19   | A3     | X               | X                   | X                |
| c.6133G>A       | p.Gly2045Arg | Missense Substitution           |               | 20   | C1     | X               | X                   | X                |
| c.6172G>C       | p.Ala2058Pro | Missense Substitution           |               | 20   | C1     | X               | X                   | X                |
| c.6274-?_6429+?del |              | Large structural change (>50 bp) | Deletion      | 22   | C1     | X               | X                   | X                |
| c.6403C>T       | p.Arg2135*   | Nonsense Substitution           |               | 22   | C1     | X               | X                   | X                |
| c.6429+?_6430-?inv |              | Large structural change (>50 bp) | Inversion     | 23   | C1     | X               | X                   | X                |
| c.6481C>T       | p.Pro2161Ser | Missense Substitution           |               | 23   | C1     | X               | X                   | X                |
| c.6485C>T       | p.Pro2162Leu | Missense Substitution           |               | 23   | C1     | X               | X                   | X                |
| c.6496C>T       | p.Arg2166*   | Nonsense Substitution           |               | 23   | C1     | X               | X                   | X                |
| c.6544C>T       | p.Arg2182Cys | Missense Substitution           |               | 23   | C1     | X               | X                   | X                |
| c.6593G>T       | p.Gly2198Val | Missense Substitution           |               | 24   | C2     | X               | X                   | X                |
| c.6682C>G       | p.Arg2228Gly | Missense Substitution           |               | 24   | C2     | X               | X                   | X                |
| c.6682C>T       | p.Arg2228*   | Nonsense Substitution           |               | 24   | C2     | X               | X                   | X                |
| c.670+5G>A      |              | Splice site change              | Substitution   | 25   | C2     | X               | X                   | X                |
| c.6742T>A       | p.Trp2248Arg | Missense Substitution           |               | 25   | C2     | X               | 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                   | X                |
| c.6977G>T       | p.Arg2326Leu | Missense Substitution           |               | 26   | C2     | X               | X                   | X                |
| c.6994T>C       | p.Trp2322Arg | Missense Substitution           |               | 26   | C2     | X               | X                   | X                |
| c.764G>C        | p.Gly255Ala  | Missense Substitution           |               | 6    | A1     | X               | 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               |                   |                 |
| c.787+3A>G |              | Splice site change | Substitution | Intron 6 |       | X               | X                 | X               |
| c.822G>C  | p.Trp274Cys  | Missense      | Substitution | 7    | A1     | X               |                   |                 |
| c.901C>T  | p.Arg301Cys  | Missense      | Substitution | 7    | A1     | X               |                   |                 |
| c.954_955del | p.Leu319Aspfs*18 | Frameshift | Deletion | 7    | A1     | X               |                   |                 |
| c.991_992del | p.Ile331Leufs*6 | Frameshift | Deletion | 7    | A1     | X               |                   |                 |
| c.1043G>A  | p.Cys348Tyr  | Missense      | Substitution | 8    | A1     | X               |                   |                 |
| c.121G>T   | p.Gly41Cys   | Missense      | Substitution | 1    | A1     | X               |                   |                 |
| c.1409C>T  | p.Pro470Leu  | Missense      | Substitution | 9    | A2     | X               |                   |                 |
| c.1751A>G  | p.Gln584Arg  | Missense      | Substitution | 11   | A2     | X               |                   |                 |
| c.1910A>G  | p.Asn637Ser  | Missense      | Substitution | 13   | A2     | X               |                   |                 |
| c.3870dupA | p.Gly1291Argfs*29 | Frameshift | Duplication | 14   | B      | X               |                   |                 |
| c.437A>C   | p.Lys146Thr  | Missense      | Substitution | 4    | A1     | X               |                   |                 |
| c.5150A>G  | p.Tyr1717Cys | Missense      | Substitution | 14   | A3     | X               |                   |                 |
| c.5183A>G  | p.Tyr1728Cys | Missense      | Substitution | 14   | A3     | X               |                   |                 |
| c.6273+1G>T |              | Splice site change | Substitution | Intron 21 |       | X               |                   |                 |
| c.677G>T   | p.Ser226Leu  | Missense      | Substitution | 6    | A1     | X               |                   |                 |
| c.696C>G   | p.Arg232Gly   | Missense      | Substitution | 26   | C2     | X               |                   |                 |
| c.902G>T   | p.Arg301Leu  | Missense      | Substitution | 7    | A1     | X               |                   |                 |
| c.923C>T   | p.Ser308Leu  | Missense      | Substitution | 7    | A1     | X               |                   |                 |
| c.1293G>T  | p.Leu431Phe  | Missense      | Substitution | 9    | A2     | X               | X                 |                 |
| c.1348T>A  | p.Tyr450Asn  | Missense      | Substitution | 9    | A2     | X               | X                 | X               |
| c.1408C>A  | p.Pro470Thr  | Missense      | Substitution | 9    | A2     | X               | 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.Thr137Leu  | 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.Met1842Leu | 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                   | X                |
| c.127C>T       | p.Arg43Trp        | Missense Substitution | 2         | PRO  | X      | X                | X                   | X                |
| c.128G>A       | p.Arg43Gln        | Missense Substitution | 2         | GLA  | X      | X                | X                   | X                |
| c.172G>A       | p.Gly58Arg        | Missense Substitution | 2         | GLA  | X      | X                | X                   | X                |
| c.173G>A       | p.Gly58Glu        | Missense Substitution | 2         | GLA  | X      | X                | X                   | X                |
| c.191G>A       | p.Cys64Tyr        | Missense Substitution | 2         | GLA  | X      | X                | X                   | X                |
| c.259T>G       | p.Phe87Val        | Missense Substitution | 3         | GLA  | X      | X                | X                   | X                |
| c.301C>G       | p.Pro101Ala       | Missense Substitution | 4         | EGF1 | X      | X                | X                   | X                |
| c.316G>A       | p.Gly106Ser       | Missense Substitution | 4         | EGF1 | X      | X                | X                   | X                |
| c.412A>C       | p.Asn138His       | Missense Substitution | 5         | EGF2 | X      | X                | X                   | X                |
| c.415G>A       | p.Gly139Ser       | Missense Substitution | 5         | EGF2 | X      | X                | X                   | X                |
| c.571C>T       | p.Arg191Cys       | Missense Substitution | 6         | Linker | X    | X                | X                   | X                |
| c.572G>A       | p.Arg191His       | Missense Substitution | 6         | Linker | X    | X                | X                   | X                |
| c.720G>T       | p.Trp240Cys       | Missense Substitution | 6         | Protease | X  | X                | X                   | X                |
| c.755G>A       | p.Cys252Tyr       | Missense Substitution | 7         | Protease | X  | X                | X                   | X                |
| c.797C>T       | p.Ala266Val       | Missense Substitution | 7         | Protease | X  | X                | X                   | X                |
| c.833G>A       | p.Ala279Thr       | Missense Substitution | 7         | Protease | X  | X                | X                   | X                |
| c.838G>C       | p.Gly280Arg       | Missense Substitution | 7         | Protease | X  | X                | X                   | X                |
| c.881G>A       | p.Arg294Gln       | Missense Substitution | 8         | Protease | X  | X                | X                   | X                |
| c.914A>G       | p.Tyr305Cys       | Missense Substitution | 8         | Protease | X  | X                | X                   | X                |
| c.987C>G       | p.Ser329Arg       | Missense Substitution | 8         | Protease | X  | X                | X                   | X                |
| c.1009G>A      | p.Ala337Thr       | Missense Substitution | 8         | Protease | X  | 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.1135G>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                   | X                   | X                 |
| c.-35G>A       | Promoter Substitution | 5’UTR       |         | X      | X                 | X                   | X                 |
| c.-35G>C       | Promoter Substitution | 5’UTR       |         | X      | 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.Arg73Stop       | 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                | X                   |                   |
| c.260T>G       | p.Phe87Cys        | Missense Substitution  | 3             | GLA  | X      | X                | X                   |                   |
| c.263G>A       | p.Trp88*          | Nonsense Substitution  | 3             | GLA  | X      | X                | X                   |                   |
| c.291T>G       | p.Cys97Trp        | Missense Substitution  | 4             | EGF1 | X      | X                | X                   |                   |
| c.304T>C       | p.Cys102Arg       | Missense Substitution  | 4             | EGF1 | X      | X                | X                   |                   |
| c.305G>A       | p.Cys102Tyr       | Missense Substitution  | 4             | EGF1 | X      | X                | X                   |                   |
| c.350G>A       | p.Cys117Tyr       | Missense Substitution  | 4             | EGF1 | X      | X                | X                   |                   |
| c.383G>A       | p.Cys128Tyr       | Missense Substitution  | 4             | EGF1 | X      | X                | X                   |                   |
| c.392delA      | p.Asp131fs        | Frameshift Deletion    | 5             | EGF2 | X      | X                | X                   |                   |
| c.414T>A       | p.Asn138Lys       | Missense Substitution  | 5             | EGF2 | X      | X                | X                   |                   |
| c.422G>A       | p.Cys141Tyr       | Missense Substitution  | 5             | EGF2 | X      | X                | X                   |                   |
| c.423C>A       | p.Cys141*         | Nonsense Substitution  | 5             | EGF2 | X      | X                | X                   |                   |
| c.423G>A       | p.Cys141Tyr       | Missense Substitution  | 5             | EGF2 | X      | X                | X                   |                   |
| c.427C>G       | p.Gln143Glu       | Missense Substitution  | 5             | EGF2 | X      | X                | X                   |                   |
| c.434G>A       | p.Cys145Tyr       | Missense Substitution  | 5             | EGF2 | X      | X                | X                   |                   |
| c.446C>T       | p.Cys155Phe       | Missense Substitution  | 5             | EGF2 | X      | X                | X                   |                   |
| c.470G>A       | p.Cys157Tyr       | Missense Substitution  | 5             | EGF2 | X      | X                | X                   |                   |
| c.470G>C       | p.Cys157Ser       | Missense Substitution  | 5             | EGF2 | X      | X                | X                   |                   |
| c.479G>T       | p.Gly160Val       | Missense Substitution  | 5             | EGF2 | X      | X                | X                   |                   |
| c.482A>G       | p.Tyr161Cys       | Missense Substitution  | 5             | EGF2 | X      | X                | X                   |                   |
| c.484C>T       | p.Arg162*         | Nonsense Substitution  | 5             | EGF2 | X      | X                | X                   |                   |
| c.509G>A       | p.Ser170Tyr       | Missense Substitution  | 5             | EGF2 | 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.520G>A       | p.Val174Met      | Missense Substitution | 5             | EGF2 | X      | X                 | X                   |               |
| c.532T>C       | p.Cys178Arg      | Missense Substitution | 6             | Linker | X      | X                 | X                   |               |
| c.535G>A       | p.Gly179Arg      | Missense Substitution | 6             | Linker | X      | X                 | X                   |               |
| c.545_546del   | p.Ser182Cysfs*6  | Frameshift Deletion | 6             | Linker | X      | X                 | X                   |               |
| c.547delG      | p.Val183fs       | Frameshift Deletion | 6             | Linker | X      | X                 | X                   |               |
| c.676C>T       | p.Arg226Trp      | Missense Substitution | 6             | Activation | X      | X                 | X                   |               |
| c.677G>A       | p.Arg226Gln      | Missense Substitution | 6             | Activation | X      | X                 | X                   |               |
| c.677G>T       | p.Arg226Leu      | Missense Substitution | 6             | Activation | X      | X                 | X                   |               |
| c.688_690del   | p.Gly230del      | Small structural change (in-frame, < 50 bp) Deletion | 6             | Protease | X      | X                 | X                   |               |
| c.706G>T       | p.Gly236Cys      | Missense Substitution | 6             | Protease | X      | X                 | X                   |               |
| c.707G>A       | p.Gly236Asp      | Missense Substitution | 6             | Protease | X      | X                 | X                   |               |
| c.711A>G       | p.Gln237Gln      | Synonymous Substitution | 6             | Protease | X      | X                 | X                   |               |
| c.719G>A       | p.Trp240*        | Nonsense Substitution | 6             | Protease | X      | X                 | X                   |               |
| c.719G>T       | p.Trp240Leu      | Missense Substitution | 6             | Protease | X      | X                 | X                   |               |
| c.721C>T       | p.Gln241*        | Nonsense Substitution | 6             | Protease | X      | X                 | X                   |               |
| c.723G>A       | p.Gln241Gln      | Synonymous Substitution | 6             | Protease | X      | X                 | X                   |               |
| c.727_728delinsA | p.Val243fs        | Frameshift Insertion/deletion | 7             | Protease | X      | X                 | X                   |               |
| c.757G>A       | p.Gly253Arg      | Missense Substitution | 7             | Protease | X      | X                 | X                   |               |
| c.789_790delnsT | p.Thr264fs       | Frameshift Insertion | 7             | Protease | X      | X                 | X                   |               |
| c.799C>T       | p.His267Tyr      | Missense Substitution | 7             | Protease | X      | X                 | X                   |               |
| c.839G>T       | p.Gly280Val      | 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.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.Arg379Glu      | 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                   | X                |
| c.1204G>A      | p.Gly402Arg       | Missense        | Substitution | 8    | Protease| X                | X                   | X                |
| c.1217C>G      | p.Ser406*         | Nonsense        | Substitution | 8    | Protease| X                | X                   | X                |
| c.1217C>T      | p.Ser406Leu       | Missense        | Substitution | 8    | Protease| X                | X                   | X                |
| c.1219T>C      | p.Cys407Arg       | Missense        | Substitution | 8    | Protease| X                | X                   | X                |
| c.1226G>A      | p.Gly409Glu       | Missense        | Substitution | 8    | Protease| X                | X                   | X                |
| c.1228G>A      | p.Asp410Asn       | Missense        | Substitution | 8    | Protease| X                | X                   | X                |
| c.1228G>C      | p.Asp410His       | Missense        | Substitution | 8    | Protease| X                | X                   | X                |
| c.1232G>A      | p.Ser411Asn       | Missense        | Substitution | 8    | Protease| X                | X                   | X                |
| c.1237G>A      | p.Gly413Arg       | Missense        | Substitution | 8    | Protease| X                | X                   | X                |
| c.1241C>T      | p.Pro414Leu       | Missense        | Substitution | 8    | Protease| X                | X                   | X                |
| c.1245T>A      | p.His415Gln       | Missense        | Substitution | 8    | Protease| X                | X                   | X                |
| c.1256T>A      | p.Val419Glu       | Missense        | Substitution | 8    | Protease| X                | X                   | X                |
| c.1258G>T      | p.Glu420*         | Nonsense        | Substitution | 8    | Protease| X                | X                   | X                |
| c.1291T>C      | p.Trp431Arg       | Missense        | Substitution | 8    | Protease| X                | X                   | X                |
| c.1293G>T      | p.Trp431Cys       | Missense        | Substitution | 8    | Protease| X                | X                   | X                |
| c.1294G>A      | p.Gly432Ser       | Missense        | Substitution | 8    | Protease| X                | X                   | X                |
| c.1295G>A      | p.Gly432Asp       | Missense        | Substitution | 8    | Protease| X                | X                   | X                |
| c.1295G>C      | p.Gly432Ala       | Missense        | Substitution | 8    | Protease| X                | X                   | X                |
| c.1295G>T      | p.Gly432Val       | Missense        | Substitution | 8    | Protease| X                | X                   | X                |
| c.1297G>A      | p.Glu433Lys       | Missense        | Substitution | 8    | Protease| X                | X                   | X                |
| c.1298A>C      | p.Glu433Ala       | 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.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       | X                 | X                   |                 |
| c.252+3_252+6del|                  | Splice site change| Deletion    | Intron 2 | X       | X                 | X                   |                 |
| c.252+6T>C     |                  | Splice site change| Substitution| Intron 2 | X       | X                 | X                   |                 |
| c.253-25A>G    |                  | Splice site change| Substitution| Intron 2 | X       | X                 | X                   |                 |
| c.277+2T>C     |                  | Splice site change| Substitution| Intron 3 | X       | X                 | X                   |                 |
| c.277+5G>A     |                  | Splice site change| Substitution| Intron 3 | X       | X                 | X                   |                 |
| c.392-1G>C     |                  | Splice site change| Substitution| Intron 4 | X       | X                 | X                   |                 |
| c.392-2A>G     |                  | Splice site change| Substitution| Intron 4 | X       | X                 | X                   |                 |
| c.521-3T>G     |                  | Splice site change| Substitution| Intron 5 | X       | X                 | X                   |                 |
| c.-55G>A       |                  | Promoter        | Substitution| 5' UTR| X       | X                 | X                   |                 |
| c.723+1G>A     |                  | Splice site change| Substitution| Intron 6 | X       | X                 | X                   |                 |
| c.839-4A>G     |                  | Splice site change| Substitution| Intron 7 | X       | X                 | X                   |                 |
| c.88+1_88+4del |                  | Splice site change| Deletion    | Intron 1 | X       | X                 | X                   |                 |
| c.88+1G>T      |                  | Splice site change| Substitution| Intron 1 | X       | X                 | X                   |                 |
| c.88+5G>C      |                  | Splice site change| Substitution| Intron 1 | X       | X                 | X                   |                 |
| c.88+5G>T      |                  | Splice site change| Substitution| Intron 1 | X       | 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.Asn13Lys        | Missense      | Substitution | 4    | EGF1   | X                 |                     | X               |
| c.466T>C       | p.Ser156Phe       | Missense      | Substitution | 5    | EGF2   | X                 |                     | X               |
| c.675C>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                   | X               |
| c.572G>C      | p.Arg191Pro      | Missense          | Substitution| 6    | Linker | X                | X                   | X               |
| c.785T>C      | p.Ile262Thr      | Missense          | Substitution| 7    | Protease| X                | X                   | X               |
| c.786T>G      | p.Ile262Met      | Missense          | Substitution| 7    | Protease| X                | X                   | X               |
| c.839G>C      | p.Gly280Ala      | Missense          | Substitution| 8    | Protease| X                | X                   | X               |
| c.872A>G      | p.Glu291Gly      | Missense          | Substitution| 8    | Protease| X                | X                   | X               |
| c.950C>T      | p.Ala317Val      | Missense          | Substitution| 8    | Protease| X                | X                   | X               |
| c.997C>A      | p.Pro333Thr      | Missense          | Substitution| 8    | Protease| X                | X                   | X               |
| c.1067G>T     | p.Trp356Leu      | Missense          | Substitution| 8    | Protease| X                | X                   | X               |
| c.1097C>T     | p.Ala366Val      | Missense          | Substitution| 8    | Protease| X                | X                   | X               |
| c.1127T>C     | p.Leu376Pro      | Missense          | Substitution| 8    | Protease| X                | X                   | X               |
| c.1180A>G     | p.Met394Val      | Missense          | Substitution| 8    | Protease| X                | X                   | X               |
| c.1187G>T     | p.Cys396Phe      | Missense          | Substitution| 8    | Protease| X                | X                   | X               |
| c.1193G>C     | p.Gly398Ala      | Missense          | Substitution| 8    | Protease| X                | X                   | X               |
| c.1348T>C     | p.Tyr450His      | Missense          | Substitution| 8    | Protease| X                | X                   | X               |
| c.-48G>C      |                  | Promoter          | Substitution| 5'UTR|        | X                | X                   | X               |
| c.-49T>A      |                  | Promoter          | Substitution| 5'UTR|        | X                | X                   | X               |
| c.520+13A>G   |                  | Splice site change| Substitution| Intron 5|       | X                | X                   | X               |
| c.88+5G>A     |                  | Splice site change| Substitution| Intron 1|       | X                | 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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