Genetic causes of haemophilia in women and girls

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Abstract

Women and girls reported as "haemophilic females" may have complex genetic causes for their haemophilia phenotype. In addition, women and girls may have excessive bleeding requiring treatment simply because they are heterozygous for haemophilia alleles. While severe and moderate haemophilia are rare in females, 16% of patients with mild haemophilia A and almost one-quarter of those with mild haemophilia B seen in U.S. haemophilia treatment centres are women and girls. A phenotypic female with a low level of factor VIII or factor IX may be classified into one of the following categories of causality: homozygosity (two identical haemophilia alleles), compound heterozygosity (two different haemophilia alleles), hemizygosity (one haemophilia allele and no normal allele), heterozygosity (one haemophilia allele and one normal allele), genetic causes other than haemophilia and non-genetic causes. Studies required for classification may include coagulation parameters, *F8* or *F9* sequencing, *F8* inversion testing, multiplex ligation-dependent probe amplification, karyotyping and X chromosome inactivation studies performed on the patient and parents. Women and girls who are homozygous, compound heterozygous or hemizygous clearly have haemophilia, as they do not have a normal allele. Heterozygous women and girls with factor levels below the haemostatic range also meet the definitions used for haemophilia treatment.

Keywords

factor IX; factor VIII; haemophilia A; haemophilia B

1 | INTRODUCTION

The dogma that haemophilia affects males and is transmitted through unaffected females has over centuries hampered the recognition that women and girls with haemophilia may bleed as significantly as affected males. The group of women who have been reported as "haemophilic females" may have complex genetic causes for their haemophilia phenotype. In addition, women and girls may have excessive bleeding requiring treatment simply because they are heterozygous for haemophilia alleles, either haemophilia A (HA), a defect

or deficiency of factor VIII (FVIII), or haemophilia B (HB), a defect or deficiency of factor IX (FIX). The gene for FVIII, *F8*, and the gene for FIX, *F9*, are both located on the tip of the long arm of the X chromosome. The role of the X chromosome in sex determination leads to the pattern of X-linked inheritance, which has been recognized for the haemophilias since ancient times.[1]

Literature in the 1800s questioned whether haemophilia could occur in women;[2] in the 1900 s, a lively discussion of the possibility ensued until the publication of cases in the 1950 s describing women with homozygous[3] and heterozygous[4] haemophilia with bleeding symptoms similar to those seen in their affected male relatives. Study of the genetic causes for haemophilia in females has kept pace with, and often informed, understanding of genetic principles, particularly those surrounding the function of the X chromosome.[5–7] Today, clarification of the molecular basis for HA and HB has provided new tools with which to address the topic. With more than 3000 unique mutations in *F8*[8] and more than 2000 in *F9*[9] reported since 1983, inheritance and new mutation can be precisely distinguished. In addition, techniques now exist for assessing the expression of X chromosome genes. The application of these tools in understanding the genetic causes of haemophilia in women and girls is the topic of this review.

2 | X CHROMOSOME GENETICS

The *F8* and *F9* genes are located on the long arm of the X chromosome at Xq28 and Xq27.1, respectively, making them subject to the unique inheritance pattern of X-linked genes. Males who have a deleterious allele on their single X chromosome exhibit its full effects and are called hemizygous. Severity of symptoms is based on the specific deleterious allele present and is classified as severe if FVIII or FIX is <1 unit/decilitre (u/dL), moderate if 1–5 u/dL and mild if >5 and less than 40 u/dL.[10] Homozygous females with two abnormal alleles will have the same phenotype as hemizygous males, while heterozygous females are usually protected by the presence of a normal allele on their second X chromosome. Phenotypic variability among heterozygotes, however, was reported as soon as factor assays became available in the 1950 s.[11,12] The distribution of factor levels in heterozygous women compared to control women is shown in Figure 1, which illustrates the wide ranges seen in both groups, with heterozygotes having a mean level near 50 u/dL and controls with a mean near 100 u/dL. The variability seen is due to the fact that X chromosome genes are subject to X chromosome inactivation (XCI).[13]

XCI is a normal process by which each cell of a female contains only a single functioning X to equalize the "dose" of X chromosome genes between males and females. XCI was first described by Mary Lyon in 1961[14] using coat colour genes in the mouse and has been called "Lyonization." In 1962, Lyon ascribed the variable expression of both HA and HB in heterozygous women to cellular mosaicism produced by this process.[6]

XCI involves three now well studied phases: initiation, spreading and maintenance.[15] *Initiation* occurs in early embryogenesis and is genetically controlled at a master regulatory locus, the X inactivation centre.[16,17] A critical element located in this region is the *XIST* gene which encodes the X inactive-specific transcript long noncoding (*XIST*) RNA which is

expressed exclusively from the silenced X chromosome.[18,19] *Spreading* occurs when the *XIST* RNA upregulated from a single X chromosome accumulates *in cis*, coating that chromosome and acting as a scaffold to help recruit factors required for stable chromosome-wide chromatin remodelling and gene silencing.[20] Once established, *maintenance* of the silenced state, including *XIST* RNA expression, DNA methylation and repressive histone modifications, is stable and is clonally inherited through somatic cell divisions. A detailed discussion of XCI is provided in a recent review.[20]

Because XCI occurs early in development and normally randomly silences either the maternally or paternally derived X chromosome at initiation, typical XX females are mosaic with two populations of cells, each expressing alleles from one chromosome or the other. The X inactivation ratios, or proportions of cells expressing alleles from one X chromosome compared to the other, can vary from an even 50:50 expression to 100:0 expression from a single chromosome. In fact, in what remains the largest study of its kind, Amos-Landgraf et al[21] used the human androgen receptor locus methylation assay to determine the X inactivation ratio in over one thousand phenotypically unaffected females and found that the ratio was normally distributed with 8% of the individuals with a greater than 80:20 ratio. Although highly skewed XCI greater than 95:5 can occur by chance, it is uncommon and may be a marker for a deleterious X-linked defect particularly when found segregating in a family. It is important to note that while XCI status is stably maintained during somatic cell division, the silenced X is reactivated in female precursor germ cells so that each haploid germ cell will contain an active X chromosome.[22,23] The process then repeats itself in each new female embryo.

3 | EPIDEMIOLOGY OF WOMEN AND GIRLS WITH HAEMOPHILIA

Approximately 250 women and girls said to have haemophilia have been reported in the literature worldwide, most with severe or moderate disease; for many of them, there is insufficient information available to fully elucidate the underlying cause for their phenotype. Early reports were limited by the inability to accurately measure factor levels, diagnose other disorders and analyse chromosome and DNA structure. Some early cases have been re-studied with newer methods. More recently, cases have been reported only if they demonstrate a new mechanism or presentation; therefore, case reports do not accurately reflect the number of affected women and girls. More useful data have come from bleeding disorder surveillance. The Community Counts programme, initiated in 2011,[24] has collected de-identified data on all patients attending 135 federally funded U.S. haemophilia treatment centres (HTCs). Table 1 shows the proportion of females among the unique HA and HB patients attending these HTCs from January 2012 through September 2020.[25] Those with known severity may be assumed to have a diagnosis of HA or HB, while those with normal levels or unknown severity may have attended the HTC for diagnosis or genetic testing. Among those with a diagnosis, 6.1% of HA patients and 8.5% of HB patients were female, for a total of 1672 females among 25,043 patients (6.7%). Severely affected and moderately affected females were rare, 51 (0.48%) and 80 (1.4%) cases in those categories, respectively; however, females made up 16.0% of mild HA patients and 23.7% of mild HB patients.

4 | CLASSIFICATION OF WOMEN AND GIRLS WITH HAEMOPHILIA

Females with a low level of FVIII or FIX may be classified into one of the following categories of causality (Table 2): homozygosity (two identical haemophilia alleles), compound heterozygosity (two different haemophilia alleles), hemizygosity (one haemophilia allele and no normal allele), heterozygosity (one haemophilia allele and one normal allele), genetic causes other than haemophilia and non-genetic causes.[13] Tables 3 and 4 list cases identified by systematic review by searching electronic databases and bibliographies of retrieved reports. Cases were included in which a specific deleterious variant has been identified in *F8* or *F9* and for which published information was sufficient for classification of the genetic explanation.

4.1 | Homozygosity

Homozygosity in a female (Table 3A, 4A) most often occurs because of a cousin marriage within a haemophilia family, with the father having haemophilia and the mother a heterozygote by inheritance. This was first reported in the large family described by Treves in 1886[2] and was confirmed with DNA analysis for *F8* in 2009.[27] Three additional cases with HA who were homozygous by inheritance have been reported.[26,28,29] In addition, one girl with severe HA was described whose mother was heterozygous for an inversion of intron 22 (inv22), the most common deleterious variant in *F8*, and whose father was unaffected with paternity confirmed; a new instance of inv22 occurred *de novo* on the paternally derived X chromosome. The paternally derived *F8* gene was not missing, since a paternally derived non-deleterious variant in *F8* was detected.[30] This is quite rare, since the inv22 variant more often originates in the gametes of the maternal grandfather.[80] A single homozygous case has been reported for FIX.[58]

4.2 | Compound heterozygosity

Compound heterozygosity (Table 3B, 4B) has been observed due to inheritance of a different haemophilia allele from each parent in the same gene.[31] More often, compound heterozygosity has resulted from the inheritance of one haemophilia allele and a *de novo* mutation on the X chromosome received from the other parent.[27,32,34,35,40] In two instances, the *de novo* variant was a deletion of all or part of the *F8* gene; this could be distinguished from homozygosity only by assessment of gene dose by techniques such as Multiplex Ligation-Dependent Probe Amplification (MLPA®).[27,31] One case was reported in which a prenatal diagnosis was performed in a woman heterozygous for a familial deletion causing severe HA, which was absent in the foetus; the newborn male, however, was found to have mild HA due to a second mutation which had occurred *de novo* in the mother, who had 7 u/dL FVIII.[39] In another case,[36] both parents were unaffected, requiring 2 new mutation events, producing either homozygosity for inv22 or inv22 in combination with a deletion. The latter seemed more likely due to lack of heterozygosity for any *F8* polymorphism, but MLPA® was not performed. Occasionally, heterozygotes are seen with an X chromosome abnormality resulting in deletion of the region containing the *F8* or *F9* gene, but these are more properly called hemizygotes.

4.3 | Hemizygosity

Hemizygosity (Table 3C, 4C) is the state of having only one allele at an X chromosome locus, the usual case in an affected male. This has occurred in phenotypic females who have Turner syndrome (45,X)[45,62] or mosaic Turner syndrome.[33] Haemophilia has also appeared in 46,XY individuals who have a female phenotype because they have complete gonadal dysgenesis[46] or androgen insensitivity syndrome.[28] Their chromosomal status has often been detected due to a work-up triggered by their haemophilia. A haemophilia allele may also be deleted as part of a larger X chromosome deletion or rearrangement, leaving a single haemophilia allele.[33,41–44,61] Clinical features other than haemophilia may be apparent, depending on the other genes involved.

4.4 | Heterozygosity with preferential X chromosome inactivation

Women and girls who are heterozygous may be functionally hemizygous because one of their two X chromosomes is preferentially inactivated in every cell (Table 3D, 4D). Total inactivation may occur if (a) one X chromosome is structurally abnormal, (b) another X chromosome gene is deleterious to cell function, or (c) an *XIST* allele is present that preferentially influences XCI. X-autosome translocations have been observed that have the normal X inactivated.[33,47,63,65] In other cases, an abnormal X chromosome with an interstitial deletion[49] and one with uniparental disomy (UPD) not including the haemophilia locus[66] were inactivated. In such cases, the presence of haemophilia is determined by whether the active or inactive X bears the haemophilia allele. In a HB female with FIX <1 u/dL and an X-autosome translocation involving preferential inactivation of the normal X, no defect in *F9* was found, and it was suggested that movement of the *F9* gene to chromosome 14 influenced its regulation.[81] In one family, a microdeletion not visible on karyotyping was proposed to be the cause of severe HA in a girl.[55] The authors suggested that such subclinical defects may be an often overlooked mechanism in affected women.

XCI can also be influenced by other genes, either those lethal at the cellular level or those controlling XCI. Women heterozygous for both HA and incontinentia pigmenti[36,82] or Coffin-Lowry syndrome[27] had X chromosomes with those disease alleles preferentially inactivated leaving the X with the HA allele active because of the deleterious effects of the other disease alleles. One family has been described with three generations of skewed XCI and a common allele at the *XIST* locus.[50]

4.5 | Heterozygosity with skewed X chromosome Inactivation

Women and girls who are heterozygous for variants that cause HA or HB have levels of FVIII or FIX determined by where they fall on the spectrum produced by XCI (Figure 1). Those with low levels are often referred to as having skewed XCI (Table 3E, 4E); however, this is not an abnormality but the result of the normal process of XCI. In some heterozygotes, just by chance, all of the normal alleles are inactivated causing the woman to have no more FVIII or FIX than her affected male relatives. In other heterozygous women, all of the haemophilia alleles may be inactivated causing totally normal factor levels. These extremes are relatively rare, and any factor level between totally normal and totally abnormal may be produced. The phenotypic range of heterozygotes is demonstrated by a family with HA (Figure 2),[83] in which three daughters of a man with 6 u/dL FVIII had 9

u/dL, 40 u/dL and 112 u/dL FVIII; the sister with the highest level had an affected son. Monozygotic twins with widely discrepant levels also demonstrate this random effect for HA[52,57] and HB.[84]

A number of women and girls with haemophilia have been reported with no explanation for their phenotype other than XCI. When no other cause can be identified, the chance occurrence of skewed XCI is presumed and can often be demonstrated, as discussed above. In cases where multiple women in a family have the same phenotype, however, the likelihood of multiple rare XCI events occurring by chance in the same family is small, and a genetic cause can be postulated, such as alleles at *XIST*[50] or a very small structural change.[55] This may explain some isolated cases as well, when complete skewing occurs, demonstrated by a female having the same level as affected males in the family.[85]

Since levels of FVIII or FIX in heterozygotes produce a normal distribution with a mean of about 50 u/dL (Figure 1),[12,86] one-half of heterozygotes would be expected to have levels below 50 u/dL. From distributions of heterozygote data, it has been estimated that 28% of heterozygous women will have levels outside the haemostatic range, that is below 40 u/dL.[87] Bleeding symptoms in heterozygotes have been recognized since the 1951 report of Merskey et al;[88] comparing 19 proven heterozygotes from haemophilia families with 100 control women, they found 47%, 16% and 11% of heterozygous women had bleeding following tooth extraction, cuts and surgery, respectively, compared to 5%, 7%, and 2% of control women. These findings were confirmed in a larger study in 1988.[89] That study and two more recent ones[90,91] provided data on bleeding symptoms of heterozygotes and unrelated or genetically proven control groups from which odds ratios could be calculated (Figure 3). Odds ratios were significantly higher for heterozygotes compared to controls for excessive bleeding from tooth extraction, surgery and delivery. Hemarthrosis occurred rarely in the groups studied and was increased, although not significantly. The findings for heavy menstrual bleeding and epistaxis were more variable, with one study[90] showing significantly increased odds ratios for both and the other two having odds ratios that were not significant.[89,91] The latter two symptoms are seen more often in disorders of primary haemostasis. Menorrhagia is not invariable even in severely or moderately affected women with haemophilia.[33] An international study[92] of the International Society on Thrombosis and Haemostasis Bleeding Assessment Tool (ISTH-BAT) in 168 women heterozygous for HA or HB found them to have a higher bleeding score than 46 age-matched control women (5.7 vs. 1.43, $p < .0001$). Heterozygotes scored higher in the categories of cutaneous, minor wound, oral cavity, menorrhagia, hemarthrosis, postdental, postsurgical and postpartum bleeding. There was a significant inverse correlation between factor level and bleeding score. Raso et al.[93] compared 44 heterozygous women with factor levels of 6–49 u/dL to 77 males with mild haemophilia with factor levels of 5–40 u/dL. The males had somewhat higher rates of bleeding than the females in most categories, the most striking difference being hemarthrosis (36% vs. 4%); however, the comparison was hampered by the significantly lower mean factor level in males (19 vs. 29 u/dL, $p < .0001$). Women and girls with <40 u/dL FVIII or FIX have been shown to have reduced joint range of motion compared to healthy female controls at all ages, suggesting that joint bleeding does occur in that group.[94]

4.6 | Other genetic disorders

Women with decreased FVIII due to HA alleles may be misdiagnosed as having von Willebrand disease (VWD), particularly type 2 variants in which von Willebrand factor (VWF) antigen is present at normal levels, although dysfunctional.[95] VWD type 2N, in particular, has been said to "masquerade" as haemophilia; it is caused by production of otherwise normal VWF with decreased ability to bind FVIII and is inherited as an autosomal recessive disease.[95] In homozygous individuals, it results in FVIII deficiency in the moderate-to-mild range with normal VWF antigen and activity. Type 2N alleles may also occur in combination with those for other types of VWD, resulting in a more variable picture, usually with reductions of VWF antigen and/or activity but much lower FVIII levels. It can be detected by measuring the binding of FVIII to VWF and identification of specific mutations.[96] VWD 2N is frequent enough to have been reported within haemophilia families.[96,97] Other VWD types resulting in intermediate FVIII levels are usually autosomal dominant disorders and can be distinguished from HA by the panel of tests commonly used to diagnose VWD. VWD type 3 is a severe disease that is usually observed in individuals who are homozygous or compound heterozygous for VWD alleles. It results in FVIII levels less than 10 u/dL but with undetectable levels of VWF antigen and activity, making it easy to distinguish from HA.[95]

Women with decreased FVIII levels also may have combined factor V (FV) and FVIII deficiency, an autosomal recessive disorder characterized by mildly decreased levels of both FVIII and FV, caused by mutations in the *LMAN1* and *MCFD2* genes.[98] This is usually detected by prolongation of both the prothrombin time and the partial thromboplastin time. No vertical transmission of this rare trait should be seen in the family. Heterozygous parents are unaffected.

FIX deficiency also occurs in combined vitamin K-dependent clotting factor deficiency, an autosomal recessive disorder with decrease in factors II, VII, IX and X due to defects in the vitamin K pathway.[98] This is easily differentiated from HB by the decrease in other clotting factors but must be distinguished from acquired deficiencies due to lack of vitamin K.

4.7 | Non-genetic causes

Acquired HA, due to an autoantibody directed against FVIII in a person without a genetic bleeding disorder, occurs most often in the elderly or postpartum.[99] It can be distinguished by the absence of a lifelong history of bleeding symptoms and with laboratory tests. Acquired HB has been reported much more rarely.[100,101]

Vitamin K deficiency resulting in decreased FIX levels, as well as the other vitamin K-dependent factors, is a source of bleeding most often seen in the neonate or later in infancy but possible at any age.[102] It will result in a prolonged prothrombin time due to decreased factor VII levels, which is not a feature of HB.

5 | DIFFERENTIAL DIAGNOSIS FOR HAEMOPHILIA IN A FEMALE

The differential diagnosis for a female with a low FVIII or FIX activity level and the tests to be considered as part of the work-up (Table 5) are guided by the presence or absence of

haemophilia in the family and the age, clinical status and history of the patient. If haemophilia is present in the family, it is likely that the patient has inherited at least one haemophilia allele, and the reason for her haemophilia phenotype can usually be elucidated by further testing. Most genetic explanations apply similarly to HA and HB. A complete genetic work-up including karyotyping is important for clinical management in severe and moderate cases. Mild cases are less likely to have a complex genetic cause. A FVIII or FIX level higher than that of affected male relatives usually means that at least one normal allele is present. Gene studies are required in all cases to determine definitively how many disease-causing alleles are present, both for clinical purposes and to predict reproductive outcomes. Tests required to establish genotype may include sequencing of the affected gene and testing for the common intron 22 inversion in *F8*, as conducted in affected males, as well as MLPA for copy number to detect gene deletions in the heterozygous state. Non-paternity is more common than new mutation, and paternal mosaicism has also been observed.[70] For women and girls with severe or moderate disease, genotyping of both parents, along with measurement of their factor levels, is warranted, even in the absence of a paternal history of bleeding, to establish paternity and mutation source, since inheritance from both parents has been reported. Parental testing may be necessary in mild cases if inheritance is unclear, to inform other family members. XCI studies are less commonly performed but can provide important genetic information in some cases. It should be noted that other disorders, such as VWD, may also occur in families transmitting haemophilia and their correct diagnosis is necessary for appropriate treatment. When there is no family history of haemophilia, both haemophilia and other disorders must be considered. Disorders also exhibiting FVIII deficiency include von Willebrand disease, combined FV and FVIII deficiency, and acquired haemophilia. FIX deficiency can occur in genetic deficiencies of multiple factors, acquired haemophilia and vitamin K deficiency. Acquired haemophilia is usually ruled out by the presence of a lifelong history of bleeding. Other disorders can be ruled out by appropriate laboratory tests, as discussed above.

6 | SYMPTOMS OF HAEMOPHILIA IN WOMEN AND GIRLS

Women with low FVIII or FIX levels may be classified using the same categories of severity as for affected males based on factor level.[10] Women with levels in the severe and moderate range have had significant joint disease.[27,33] Those in the mild range have experienced traumatic hemarthrosis.[28,74,78,103] In heterozygotes, factor levels rise with age.[104] Joint impairment observed in heterozygotes with currently normal levels[94] may have occurred when they were younger and had lower factor levels. Women with no normal allele (homozygous, compound heterozygous and hemizygous women) are equivalent to affected males and would not be expected to show this change. In addition to the symptoms seen in affected males, women with haemophilia have the additional risks of excessive bleeding with menstruation, childbirth, and spontaneous or induced abortion.[105] In HA heterozygotes, FVIII levels usually rise gradually during pregnancy and may reach normal levels at term.[106,107] HB heterozygotes usually show no change.[107] In both, prenatal diagnostic procedures may result in bleeding and potential pregnancy loss if performed without treatment.[105] Odds ratios for symptoms commonly seen in heterozygotes are shown in Figure 3 and discussed above. Women and girls with haemophilia should receive treatment based on their factor

levels and clinical history at specialized haemophilia treatment centres. In addition, each affected woman warrants genetic counselling in order to understand her disorder and her potential reproductive outcomes, which may differ from those of other family members depending on the cause of her haemophilia.

Inhibitors to FVIII have been reported in three women with haemophilia who had been previously documented to have low FVIII levels. A girl diagnosed at age 10 months with severe HA (FVIII <1 u/dL) had a *de novo* inv 22 and also a large X chromosome deletion starting at Xq22 and including *F8*, making her hemizygous. She was placed on prophylaxis with plasma-derived FVIII concentrate and after 25 exposure days developed a high-titre inhibitor.[49] The other two cases had family history of HA. A 31-year-old heterozygous for an inv22 with FVIII level of 20 u/dL developed a low-titre inhibitor after two surgeries with B-domain-deleted FVIII replacement.[108] A 42-year-old with 30 u/dL FVIII developed a high-titre inhibitor after total hip arthroplasty treated with B-domain-deleted FVIII.[109] She was heterozygous for an exon 3 missense variant, p.Pro114His, which has been reported to cause mild disease and is not known to predispose to inhibitors.[8] All three women successfully underwent immune tolerance induction therapy. Two additional women heterozygous for *F8* variants developed FVIII inhibitors later in life, but neither had a history of excessive bleeding, low FVIII or treatment prior to inhibitor development; they may represent acquired haemophilia.[40,110] Development of an inhibitor in a woman with one normal allele is unexpected and may relate to the type of product used.

7 | CONCLUSIONS

Women with FVIII or FIX levels below the haemostatic range can be expected to have the same degree of bleeding symptoms as males with haemophilia having similar factor levels, as well as gynaecologic and obstetrical bleeding. Identification of women who have levels comparable to affected males is important to assure that they receive appropriate treatment based on factor level and clinical history, particularly for surgery, dental procedures and childbirth. It has been estimated that there are 5–6 potentially heterozygous women for every affected male in the population.[111] Testing of factor levels in women and girls from haemophilia families and review of their history is important for their clinical care, even when DNA analysis is used to assess their genetic status. The more severely affected women will present with unexpected bleeding and require more extensive evaluation. Review of reported cases shows that affected women occur both in families transmitting HA or HB and through *de novo* mutation. Even if no family history of haemophilia is known, women with low FVIII or FIX levels potentially 'carry' at least one haemophilia allele[112] and should be investigated in order to provide appropriate treatment and genetic counselling.

Women heterozygous for haemophilia alleles have traditionally been called "carriers," and those with symptoms called 'symptomatic carriers'. These terms do not reflect our current knowledge of haemophilia and genetics. The rare women who are homozygous, compound heterozygous or hemizygous clearly have haemophilia; they do not have a normal allele. Women who have been shown to be heterozygous but have factor levels below the haemostatic range also meet the definitions used for haemophilia treatment. One in six patients with mild HA and almost one-quarter of those with mild HB seen in HTCs in the

United States are women and girls (Table 1). For genetic purposes, it is appropriate to assign zygosity and to use scientifically accurate terminology in addressing families. The presence of bleeding symptoms in a proportion of heterozygous women and girls, and the rare instances of more severely affected females, should be discussed in genetic counselling for haemophilia. For treatment purposes, it is appropriate to apply a classification based on factor level rather than genetic status. The term "carrier" should not be used, because it lumps affected and unaffected women together and is viewed by some as pejorative or dismissive;[113] its use does not aid women in getting appropriate medical care and reimbursement.

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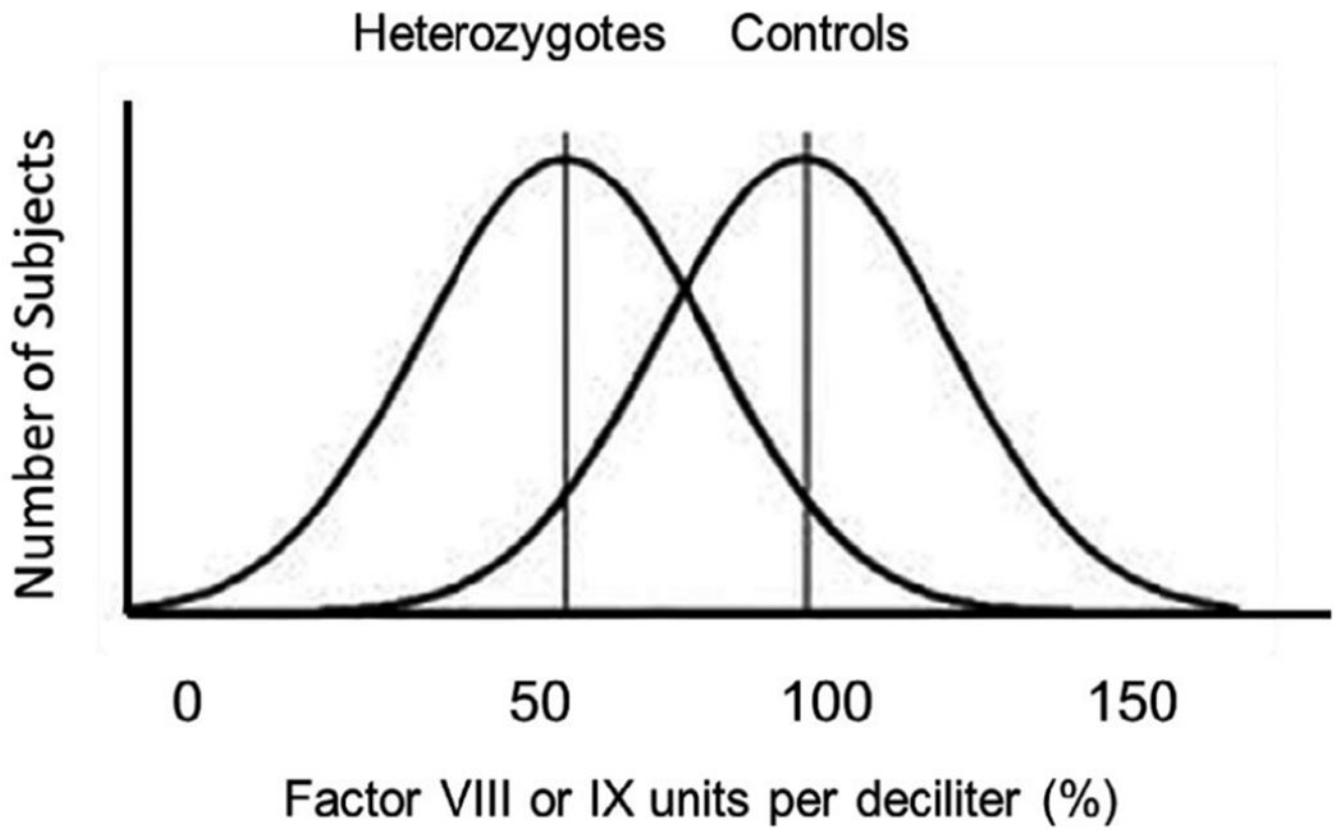
FIGURE 1.
Theoretical distribution of factor VIII or IX levels in women heterozygous for variants causing haemophilia (heterozygotes) and for women not having variants causing haemophilia (controls)
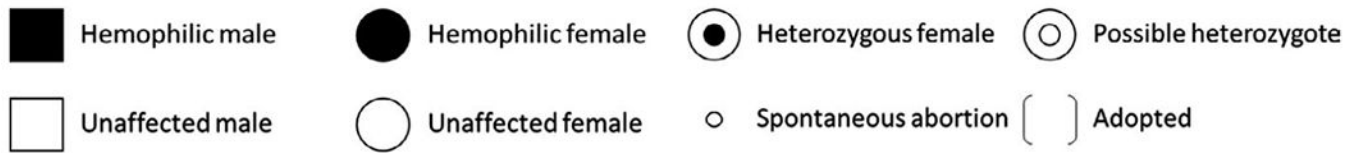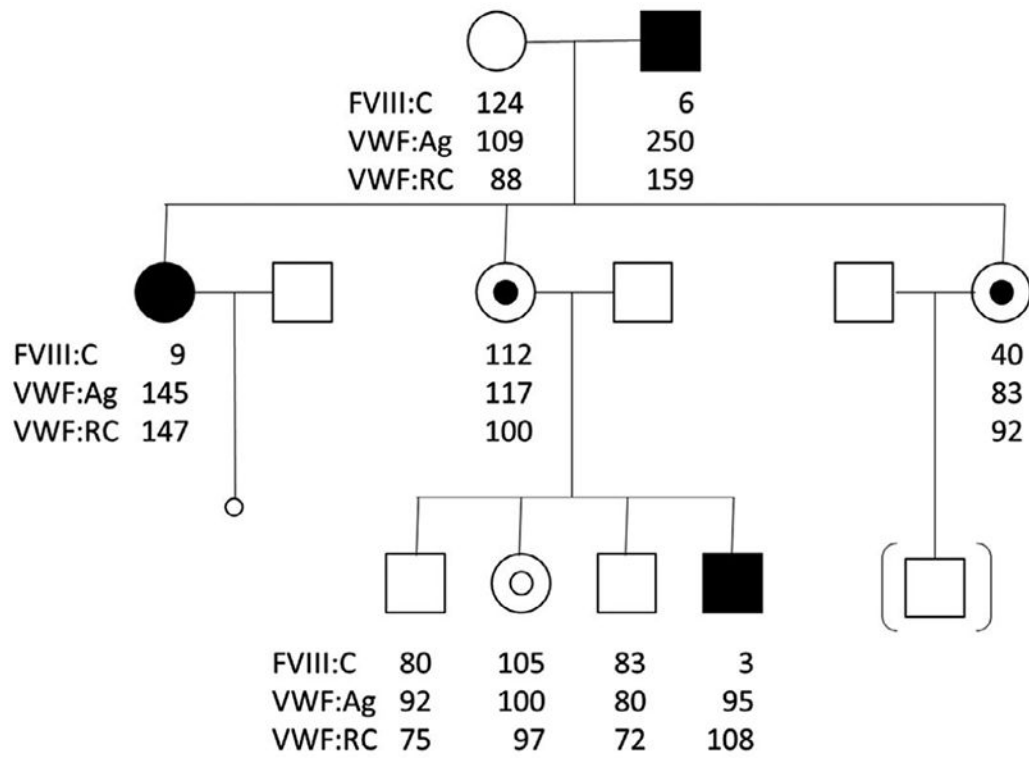
FIGURE 2.
Comparison of the phenotypes of three haemophilia heterozygotes in a family transmitting haemophilia A,[83] showing factor VIII coagulant activity (FVIII:C), von Willebrand factor antigen (VWF:Ag) and von Willebrand factor activity as ristocetin cofactor (VWF:RC) in units per decilitre
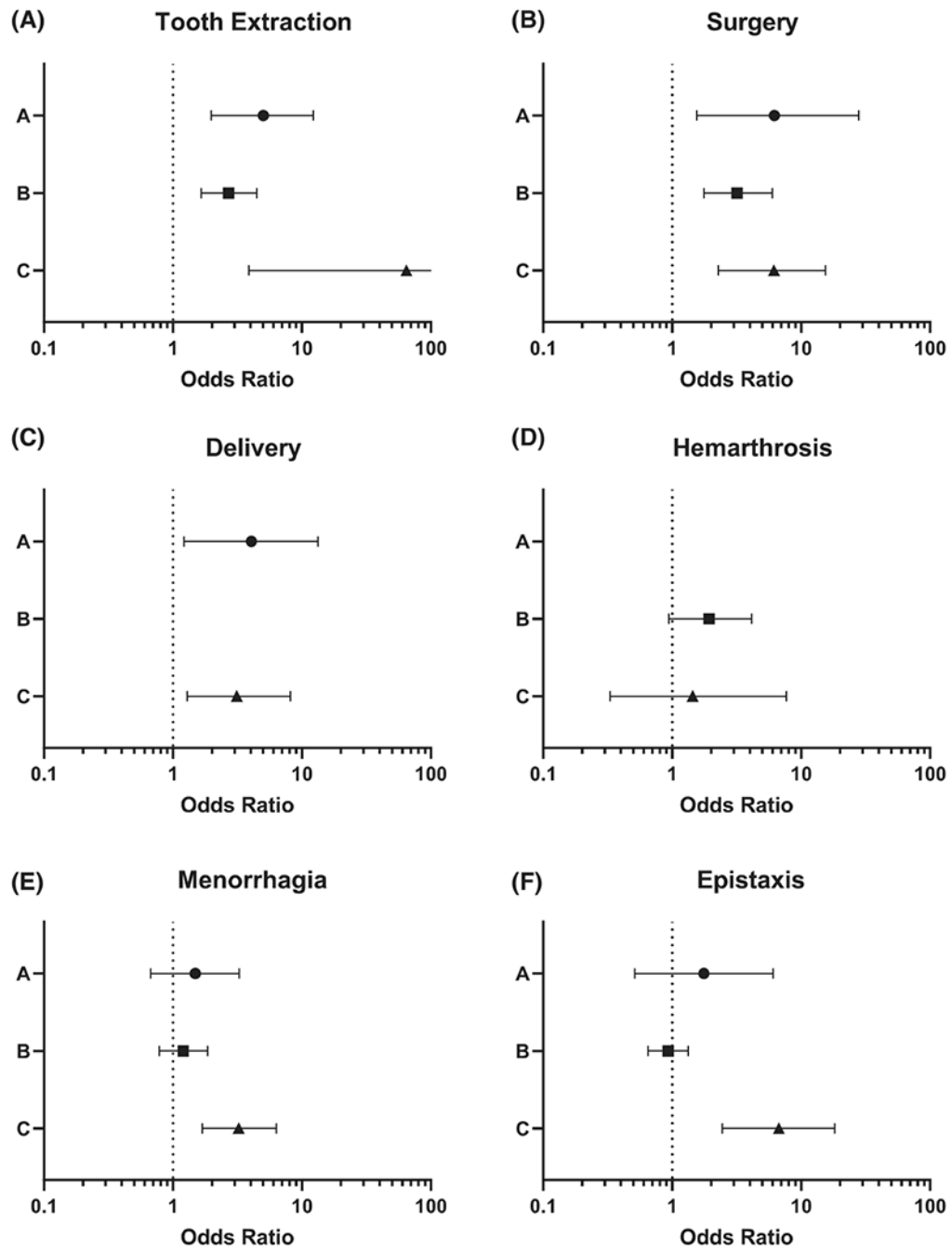
FIGURE 3.
Odds ratios and 95% confidence intervals of bleeding symptoms in women heterozygous for haemophilia A or B compared to women without haemophilia from three studies: A,[89] B[91] and C[90]

TABLE 1

Patients attending haemophilia treatment centres in the United States from January 2012 through September 2020 with factor levels known, from https://www.cdc.gov/ncbddd/hemophilia/communitycounts/data-reports/2020-9/table-2-factor.html.[25]

	Severe (<1 u/dL[a])		Moderate (1–5 u/dL)		Mild (>5 to <40 u/dL)	
	Patients n	Females n (%)	Patients n	Females n (%)	Patients n	Females n (%)
Haemophilia A	9038	44 (0.49)	3381	60 (1.8)	6616	1057 (16.0)
Haemophilia B	1651	7 (0.42)	2315	20 (0.86)	2042	484 (23.7)
Haemophilia A and B	10,689	51 (0.48)	5696	80 (1.4)	8658	1541 (17.8)

[a]Units per decilitre.

TABLE 2

Possible causes of factor VIII (FVIII) or factor IX (FIX) deficiency in women and girls.

I. Homozygosity (two identical haemophilia alleles)
Consanguinity in haemophilia family
Haemophilia in unrelated parental families
Second mutation occurring *de novo* in a woman heterozygous by inheritance
Two new mutations

II. Compound Heterozygosity (two different haemophilia alleles)
Haemophilia in unrelated parental families
Second mutation occurring *de novo* in a woman heterozygous by inheritance
Two new mutations

III. Hemizygosity (one haemophilia allele, no normal allele)
Single X chromosome: 45,X: Turner syndrome and mosaics
Male karyotype: 46,XY: Complete androgen insensitivity syndrome, complete gonadal dysgenesis
X chromosome deletion including *F8* or *F9* gene

IV. Heterozygosity (one haemophilia allele, one normal allele)
Inheritance in haemophilia family
Inheritance from a haemophilia heterozygote with no or unrecognized family history
One new mutation
Followed by:
   Skewed X inactivation
      Unknown or random
   Preferential X inactivation due to:
      X chromosome abnormality
      Cell viability disorder
      Specific allele of the X inactive-specific transcript gene *XIST*
      Other inherited skewed X inactivation

V. Other genetic causes
von Willebrand disease, particularly Type 2N (autosomal dominant or recessive)
Factor V and VIII deficiency (autosomal recessive)
Combined vitamin K-dependent clotting factor (II, VII, IX and X) deficiency (autosomal recessive)

VI. Non-genetic causes
Acquired haemophilia due to inhibitor to FVIII or FIX
Vitamin K deficiency

TABLE 3

Women and girls reported with haemophilia A with factor VIII level in units/decilitre for each case reported and for affected male relatives within the same family

Source of deleterious variant	Factor VIII level	Karyotype	Genotype	X Inactivation	Family history	Father	Mother	Affected male level	Additional diagnosis
A. Homozygosity									
Inherited[26]	<1	46,XX	c.608T>C; p.Leu203Pro[b] homozygote	ND	Parents 1st cousins	Affected	Heterozygous	<1	None
Inherited[27]	4.5	46,XX	c.5096A>T; p.Tyr1699Phe homozygote	ND	Parents 1st cousins	Affected	Heterozygous	Moderate	None
Inherited[28]	5, 7, 8[a]	46,XX	c.5428T>C; p.Ser1810Pro homozygote	ND	Parents related	Affected	Heterozygous	Mild	None
Inherited[29]	5.5	ND	c.1315G>A; p.Gly439Ser homozygote	ND	Parents related	Affected	Heterozygous	4.8–5.7	None
Inherited[27]	12	46,XX	c.1834C>T; p.Arg612Cys homozygote	ND	Parents 2nd cousins	Affected	Heterozygous	–	None
1 *de novo*[30]	<1	46,XX	inv22, distal, homozygote	Skewed, paternal X active	None	Unaffected	Heterozygous	None	None
B. Compound heterozygosity									
Inherited[31]	<1	46,XX	inv22 and whole gene deletion	Skewed, paternal X active	Paternal	Affected, inv22	Heterozygous *F8* deletion	<1	None
1 *de novo*[32,33]	<1	46,XX	inv22 Type I and inv22 Type II	Skewed, maternal X active	Maternal	Unaffected	Heterozygous inv22 Type I	<1	None
1 *de novo*[34]	3.4	46,XX	c.5981T>C; p.Leu1994Pro and c.3637dupA; p.Ile1213Asnfs*28	ND	Paternal	Affected, p.Leu1994Pro	No variant	7	None
1 *de novo*[35]	<1	ND	Exons 1–22 del and c.2014_2016delTTC; p.Phe672del	ND	None	Unaffected	Heterozygous Exons 1–22 del	<1	None
1 *de novo*[27]	5	46,XX	c, 1293G>T; p.Leu431Phe and del exons 9–22	ND	Maternal	Unaffected	Heterozygous Leu431Phe	Severe	None
2 *de novo*[36]	<1	46,XX	inv22 and *F8* deletion[c]	Skewed	None	Unaffected	No variant	–	None

Source of deleterious variant	Factor VIII level	Karyotype	Genotype	X Inactivation	Family history	Father	Mother	Affected male level	Additional diagnosis
1 or 2 de novo[37]	4.7	ND	c.1505T>A; p.Val502Asp and c.3279G>A; p.Met1093Ile[b]	ND	None	Not tested	Not tested	–	None
1 de novo[38]	9	46,XX	c.6142T>G; p.Ser2048Ala[b] and c.1281_1292del12; p.Ser428_Leu431del[b]	ND	None	Unaffected p.Ser2048Ala[b]	Heterozygous p.Ser428_Leu431del[b]	–	None
1 de novo[39]	7	ND	del exons 1–22 and c.1569G>T; p.Leu523Leu	ND	Maternal	Unaffected	Heterozygous del exons 1–22	Severe (del) and 7 u/dL (missense)	None
1 de novo[40]	8–21	ND	c.4379_4380dupA; p.Asn1460Lysfs*1 and c.6077A>G; p.His2026Arg[b]	ND	Paternal	Mild, not tested	No variant	Mild	None
C. Hemizygosity									
De novo[41]	<1	46,X,del(X)(q26)? inv(q21q25)	inv22, Type 1	ND	None	Unaffected	No variant	–	None
Inherited[42]	<1	46,X,r(X)(p22.2q13)	inv 22	ND	None	Unaffected	Heterozygous	–	None
Inherited[43]	20	46,X,del(X)(q27.3-Xq28)	c.6547A>G; p.Met2183Val and whole gene del	Maternal X active	Maternal	Unaffected	Heterozygous p.Met2183Val	18	None
Inherited[44]	22	46,X,del(X)(q28)	whole gene deletion	Skewed 79:21 paternal X active	Maternal	Unaffected	Heterozygous	Severe	Moyamoya
Inherited[45]	<1	45,X	inv22, Type 1	–	None	Unaffected	Heterozygous	–	Turner
Unknown[46]	29	46,XY	c.6932C>A; p.Pro2311His	–	None	Not tested	Not tested	–	Complete gonadal dysgenesis
Inherited[28]	45, 48[a]	46,XY	c.6437T>C; p.Phe2146Ser	–	None	Unaffected	Heterozygous	–	Androgen insensitivity syndrome
D. Heterozygosity: preferential X inactivation									
De novo[47,48]	<1	46,X,t(X;17) complex rearrangement	del exons 1–15 heterozygote	Normal X inactive	None	Unaffected	No variant	–	Developmental delay, dysmorphic features

Source of deleterious variant	Factor VIII level	Karyotype	Genotype	X Inactivation	Family history	Father	Mother	Affected male level	Additional diagnosis
Unknown[33]	<1	46,X,t(X;2)(q27;q34)	inv22 heterozygote	ND	Not reported	Not reported	Not reported	–	None
De novo[49]	<1	46,X,del(X)(q22)	inv22 heterozygote	Normal X active	None	Unaffected	No variant	–	None
Inherited[36]	6	ND	c.6371A>G; p.Tyr2124Cys heterozygote	Skewed 100:0 paternal X active	Paternal	Affected	No variant, IP heterozygote	10–12	Incontinentia pigmenti (IP)
De novo[27]	<1	46,XX	c.6872delCT; p.Thr2291fs[b] heterozygote	Skewed, paternal X active	None	Unaffected	No variant, CL heterozygote	–	Coffin-Lowry (CL)
Inherited[50]	6, 6, 6[a]	46,XX	c.1171C>G; p.Arg391Gly[b] heterozygote	Extremely skewed, same XIST allele	Maternal	Unaffected	Heterozygote	6	XIST mutation?
E. Heterozygosity: skewed inactivation									
De novo[51]	1	46,XX	c.1750delC; p.Gln584Argfs*2 heterozygote	Paternal X active, no change in XIST	None	Unaffected	No variant	–	None
Inherited[52]	<1	46,XX	c.5027C>T; p.Gln1678*[b] heterozygote	Extremely skewed, maternal X active	Maternal	Unaffected	Heterozygote	Severe	None
Unknown[27]	2	46,XX	c.1172G>A; p.Arg391His heterozygote	Skewed 83:17	None	Not tested	Not tested	–	None
Inherited[27]	<1	46,XX	c.1478delA; p.Asn493Thrfs*22 heterozygote	Skewed 96:4, paternal X active	Paternal	Affected	No variant	Severe	None
Inherited[27]	10	46,XX	c.5399G>T; p.Arg1800His heterozygote	Skewed 96:4, maternal X active	Maternal	Unaffected	Heterozygote	–	VWD 2N heterozygote
Inherited[53]	<1	46,XX	inv22 heterozygote	Extremely skewed	Maternal	Unaffected	Heterozygote	<1	None
Unknown[33]	1–5	46,XX	c.1538-2A>G; splice acceptor site heterozygote	Skewed	Not reported	Not reported	Not reported	–	None
Unknown[33]	<1	46,XX	inv22 heterozygote	Skewed	Not reported	Not reported	Not reported	–	None
Unknown[33]	<1	46,XX	inv22 heterozygote	Skewed	Not reported	Not reported	Not reported	–	None
Unknown[33]	<1	46,XX	c.5822A>G; p.Asn1941Ile heterozygote	Skewed	Not reported	Not reported	Not reported	—	None

Source of deleterious variant	Factor VIII level	Karyotype	Genotype	X Inactivation	Family history	Father	Mother	Affected male level	Additional diagnosis
Inherited[54]	<1	46,XX	c.5113C>T; p.Gln1705* heterozygote	Maternal X active	Maternal	Unaffected	Heterozygote	<1	None
Inherited[55]	<1	46,XX	c.6545G>A; p.Arg2182His heterozygote	Paternal X active	Paternal	Affected	No variant	<1	None
De novo[54,56]	<1	46,XX	inv22 heterozygote	Extremely skewed	None	Unaffected	No variant	–	None
De novo[57]	<1, 12[a]	46,XX	c.104A>G; p.Tyr35Cys[b] heterozygote	Extremely skewed, skewed	None	Unaffected	No variant	–	Monozygotic twins

ND, not done.

[a]Sisters.

[b]Variant not previously reported.

[c]MLPA not done.

TABLE 4

Women and girls reported with haemophilia B with factor IX level in units/decilitre for each case reported and for affected male relatives within the same family.

Source of deleterious variant	Factor IX Level	Karyotype	Genotype	X inactivation	Family History	Father	Mother	Affected male level	Additional diagnosis
A. Homozygosity									
Inherited[58]	<1	46,XX	c.484C>T; p.Arg162* homozygote	ND	Parents related	Affected	Heterozygous	Severe	None
B. Compound heterozygosity									
1 *de novo*[59]	1	46,XX	c.277+2T>C; splice site change and c.1169T>C; p.Ile390Thr	Not skewed	None	Unaffected	Heterozygous c.277+2T>C (mosaic)	–	None
C. Hemizygosity									
Inherited[60]	1	45,X/47,XXX mosaic	c.128G>A; p.Arg43Leu	ND	Maternal	Unaffected	Heterozygous	<1	None
Unknown[33]	<1	46,X,del(X)(q27)	c.1181T>G; p.Met394Ile	ND	Not reported	Not reported	Not reported	–	None
Unknown[61]	2	46,X,del(X)(q26.3q28)	*F9* deleted	Not skewed	None	Unaffected	Not reported	–	Intellectual disability
Unknown[62]	20	45,X	c.224G>A; p.Arg75Gln	ND	None	Not tested	Not tested	–	Turner
Unknown[33]	1–5	45,X mosaic	c.*1157A>G	ND	Not reported	Not reported	Not reported	–	Turner
D. Heterozygosity: preferential X inactivation									
Inherited[63]	<1[a]	46,X,t(X;15)(q27.1;p11.1)	del exons 5–6	Completely skewed maternal X active	None	Unaffected	Heterozygous	–	Psychomotor disability
Unknown[63,64]	2–12[a]	46,X,t(X;15)(q27.1;p11.1)	del exons 5–6	Skewed 71:29 paternal X active	None	Unaffected	Heterozygous	–	None
Unknown[65]	<1	46,X,t(X;1)(? q26.3;?q22)	Partial gene deletion	Abnormal X active	None	Unaffected	No variant	—	None
Inherited[66]	<1	46,XX UPD(X)(q27qter)mat	c.791C>A; p.Thr264Asn[b] heterozygote	Normal X active	Maternal	Unaffected	Heterozygous	<1	None
E. Heterozygosity: skewed inactivation									
Inherited[67–69]	2–4	46,XX	Complete *F9* deletion	Maternal X active	None	Unaffected	Heterozygous	–	None

Source of deleterious variant	Factor IX Level	Karyotype	Genotype	X inactivation	Family History	Father	Mother	Affected male level	Additional diagnosis
Inherited[70]	3	46,XX	c.1187G>C; p.Cys396Ser heterozygote	ND	Paternal	Affected (mosaic)	No variant	35 (mosaic)	None
Inherited[70]	3	46,XX	c.1187G>C; p.Cys396Ser heterozygote	ND	Maternal	Unaffected	Heterozygous	–	None
Inherited[71,72]	<2, <2[a]	46,XX	c.401G>A; p.Cys134Tyr heterozygote	ND	Paternal	Affected	No variant	Severe	Monozygotic twins?
Inherited[73]	15	46,XX	c.291T>G; p.Cys97Trp heterozygote	ND	None	Unaffected	Heterozygous	–	–
Inherited[74]	8	46,XX	c.1189G>C; p.Ala297Pro[b] heterozygote	Maternal X active	None	Unaffected	Heterozygous	–	None
Inherited[75]	2	46,XX	c.1217C>T; p.Ser406Leu heterozygote	Maternal X active	Maternal	Unaffected	Heterozygous	2	None
Inherited[76]	1–5	46,XX	c.251C>G; p.Thr84Arg heterozygote	Maternal X active	None	Unaffected	Heterozygous	–	None
Inherited[77]	2, 24[a]	46,XX	c.1150C>T; p.Arg384* heterozygote	Skewed 99:1 and 65:35 paternal	Paternal	Affected	No variant	<1	Dizygotic twins
Inherited[78]	16, 17[a]	46,XX	c.301C>G; p.Pro101Ala heterozygote	Extremely skewed >95:5 paternal	Paternal	Affected	No variant	14	None
Unknown[33]	1–5	45,X[7]/46,XX[109]	c.205T>C; p.Cys69Arg heterozygote	Skewed	Not reported	Not reported	Not reported	–	None
Unknown[33]	1–5	46,XX	c.796G>A; p.Ala266Thr heterozygote	Uninformative	Not reported	Not reported	Not reported	–	None
Unknown[33]	1–5	46,XX	c.1025C>T; p.Thr342Met heterozygote	Skewed	Not reported	Not reported	Not reported	–	None
Unknown[33]	1–5	46,XX	c.1327A>C; p.Ile443Leu heterozygote	Skewed	Not reported	Not reported	Not reported	–	None
Unknown[79]	6	46,XX	c.676C>T; p.Arg226Trp heterozygote	Extremely skewed	None	Unaffected	No variant	–	None

ND, not done.

[a] Sisters.

[b] Variant not previously reported.

TABLE 5

Diagnostic evaluation for a female with decreased factor VIII (FVIII) or factor IX (FIX). See text for further discussion of test choice.

	To include	Purpose
I. History and physical	Current clinical status	Characterize bleeding phenotype
	History of excessive bleeding	Determine if history is lifelong
	History of joint pain/swelling	Identify undiagnosed hemarthrosis
	History of chronic diseases	Identify non-genetic causes
	Reproductive history	Assess fertility
II. Family history and pedigree	Family history from knowledgeable relatives	Determine inheritance pattern
	Laboratory results on affected relatives	Determine severity in males
III. Laboratory studies		
Factor VIII decreased	von Willebrand disease profile	Rule out von Willebrand disease
	Factor V	Rule out factor V and VIII deficiency
	Factor VIII binding to von Willebrand factor	Rule out von Willebrand disease Type 2N
	Factor VIII inhibitor	Evaluate for possible acquired haemophilia
Factor IX decreased	Factors II, VII and X	Rule out combined vitamin K-dependent clotting factor deficiency, genetic or acquired
	Factor IX inhibitor	Evaluate for possible acquired haemophilia
IV. Genetic studies	Paternity testing	Rule out non-paternity
	Karyotype with high-resolution of X	Identify X chromosome abnormality
	Sequencing of *F8* or *F9*	Identify point mutations
	Multiplex Ligation-Dependent Probe Amplification (MLPA®)	Identify gene deletions or duplications
	X chromosome inactivation studies	Identify non-random inactivation