Genetics 101: understanding transmission and genetic testing of inherited bleeding disorders

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Haemophilia is an X-linked inherited disorder that affects males and females, though the bleeding risk in girls and women has traditionally been underrecognised. About one third of haemophilia cases occur in individuals where there is no known family history. The gene mutations for rare bleeding disorders are not carried on the X chromosome and are therefore not sex-linked; however, the risk of passing on the condition is greatly increased for consanguineous parents where both parents may carry a copy of the fault in the genetic code which causes the condition. Genetic testing should be offered to every prospective mother, ideally before conception. This should be supported by counselling as the implications for family planning are profound.

Von Willebrand factor (VWF) has an important role in primary and secondary haemostasis. Loss of function or low levels of VWF are associated with spontaneous bleeding causing nosebleeds, heavy periods and bruising as well as post-surgical bleeding. Joint bleeding and intracranial haemorrhage can also occur in those with a severe type of VWF. Diagnosis depends on bleeding assessment, family history and measurement of VWF. There are three types of VWD: Types 1 and 3 are caused by low or absent levels of VWD; Type 2 is caused by loss of function. Of these, Type 3 VWD is associated with the most severe bleeding risk but there is wide variation in bleeding phenotype among the other sub-types. The correlation between genetic mutation and bleeding phenotype is weak in VWD; therefore genetic testing is mainly useful for interpreting the risk when planning a family and to allow prenatal diagnosis in severe bleeding disorders.

Genetic testing is essential for prospective parents to make fully informed decisions about having a family and how or whether to proceed with a pregnancy. The rationale for prenatal testing is to determine the bleeding status of the foetus and to inform decisions about managing delivery. Women may choose to terminate a pregnancy to avoid having a child with severe haemophilia. For some couples the option of adoption or not having children may be explored. Options for prenatal diagnostic testing

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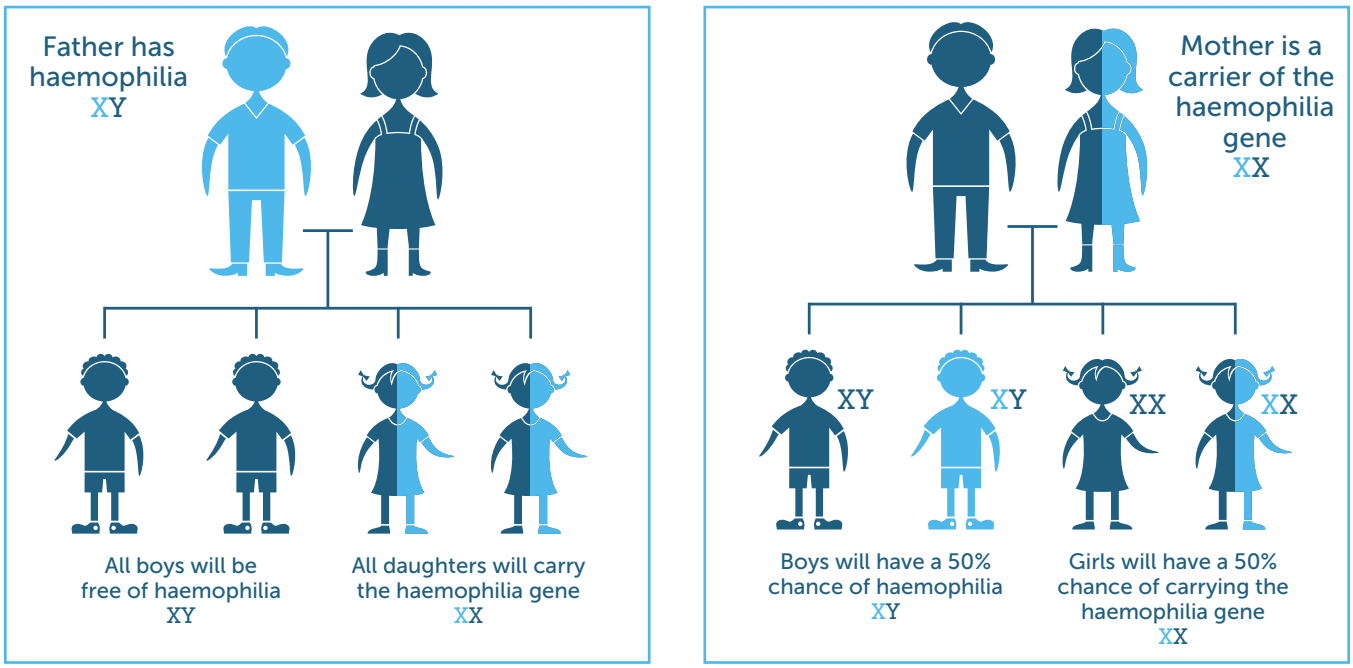
Figure 1. Pattern of inheritance of haemophilia (X-linked recessive inheritance)



include non-invasive methods, e.g. assessment of free foetal DNA in maternal plasma to determine the sex of a baby from 10 weeks in pregnancy, and invasive methods, e.g. chorionic villus sampling or amniocentesis, to determine the inheritance of the genetic mutation. Invasive methods are associated with a very small increased risk of pregnancy loss or early labour, which many couples feel is an unacceptable risk. Advanced techniques such as preimplantation screening also available, but require a huge commitment as this involves an IVF technique.

Keywords: *Women with bleeding disorders, haemophilia, von Willebrand disease, transmission, inheritance, diagnosis, prenatal testing*

HAEMOPHILIA AND RARE BLEEDING DISORDERS: TRANSMISSION, INHERITANCE AND CARRIER DETECTION

Haemophilia

Haemophilia is traditionally described as a disorder associated with excessive bleeding or with being a carrier. Those affected by haemophilia are known as haemophiliacs and are usually assumed to be male; carriers are assumed to be female and not affected by excessive bleeding, but have the haemophilia gene. The distinction of bleeding and non-bleeding status is becoming increasingly blurred as it is now recognised that girls and women who have the haemophilia gene can have a bleeding disorder. Although it is very rare for them to be severely affected, many experience heavy menstrual bleeding and other bleeds.

The gene for haemophilia is carried on the X chromosome and may therefore be present in males (who have an X and a Y chromosome) and females (who have two X chromosomes). A man who has the haemophila gene mutation cannot pass it to a son (who receives his Y chromosome), but always transmits it to a daughter (who receives his X chromosome). A woman who has the gene has a 50% chance of transmitting it to a daughter (who receives one X chromosome from each parent) and a 50% chance of transmitting to a son (who receives an X chromosome from his mother) (Figure 1).

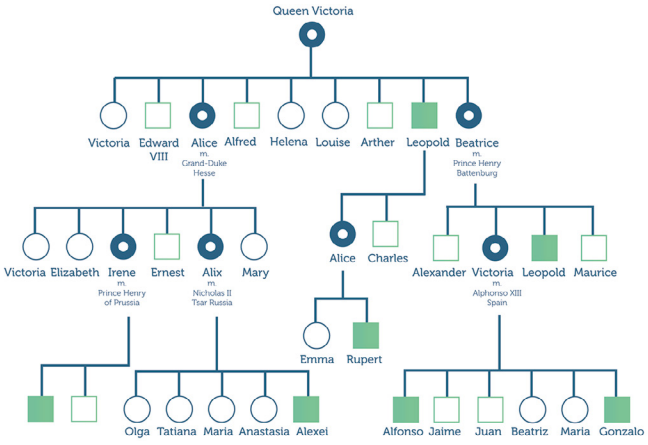


Figure 2. Transmission of haemophilia from Queen Victoria. Victoria had four sons (squares) and five daughters (circles). One son had haemophilia (solid square) and two daughters were known to carry the gene (dotted circle), passing it on to boys in the Spanish and Russian royal families.

Although haemophilia is a rare disorder, many people have heard of it (though they may know little about it). Haemophilia affected several members of. the European royal families in the 19th and early 20th centuries through the descendants of Queen Victoria of Great Britain and, perhaps most famously, Alexei, son of Tsar Nicholas II of Russia. The royal family tree of Queen Victoria shows how this pattern of genetic inheritance can affect successive generations (Figure 2).

Not all cases of haemophilia have a known family history. Spontaneous gene mutations of any type are very common (it is estimated that every adult has approximately 10^{13}–10^{14} mutations) and can occur in several ways (Table 1). Both haemophilia A and B occur spontaneously in families with no history of the disorder, affecting only one (isolated case) or two or more (sporadic cases) individuals in a single generation. These isolated and sporadic cases each account for 20–25% of haemophilia. A higher proportion of familial haemophilia is of mild to moderate severity compared with isolated and sporadic cases [1].

A mutation that causes what appears to be an isolated or sporadic case does not necessarily originate in the individual who has come to clinical attention. Studies of family trees in people with haemophilia A or B have shown the mutation to be present in apparently unaffected mothers in about 60% of cases, who in turn had acquired the mutation from their father or mother [2,3]. It is therefore important to carry out genetic testing in the family of a person newly diagnosed with haemophilia in order to identify other family members affected. The genes responsible for genetic transmission of haemophilia are mutated forms of the F8 (for haemophilia A) and F9 (for haemophilia B) genes. Both molecules present many different mutations that can cause haemophilia. In haemophilia A, the most common are the Intron 22 inversion, found in about 40% of people, and the Intron 1 inversion, which occurs in 3–6%. This information is useful when carrying out genetic testing. Knowing the genetic mutation in a family may also help to assess the risk of inhibitor (antibody) formation.

Other rare bleeding disorders

Familial rare bleeding disorders (RBDs), excluding platelet function disorders, may be mild or severe, depending on the deficiency of a particular clotting factor; severe RBDs are very rare. The genes responsible for these disorders are not carried on the X chromosome, and transmission is therefore not sex-linked. For example, factor VII deficiency is inherited in an autosomal recessive pattern: acquiring the mutated gene from one parent means the child is a carrier and may have a slightly reduced level, but a child with abnormal genes from both parents will have a severe bleeding disorder. The sons and daughters of a father with factor VII deficiency and an unaffected mother therefore each have a 50% chance of carrying the gene but are unlikely to have symptoms. The offspring of a father and mother who were both carriers would have a 25% chance of being unaffected, a 50% chance of being a carrier and a 25% chance of a bleeding disorder. Consequently, consanguineous marriage increases the risk that children will inherit a severe RBD, and as marriage between near-relatives is common in some communities, the occurrence of RBDs is often culturally determined.

Table 1. Types of DNA mutation

The changes in the letters/words in the left hand column demonstrate the type, mechanism and results of DNA mutation. Every letter of the words that make up the sentence represents a DNA base. Three consecutive letters codify an amino acid of the protein.

THE MAN SAW THE DOG HIT THE CAN	Normal DNA	
THE MAN SAW THE DOT HIT THE CAN	Missense	A change in one DNA base pair resulting in substitution of one amino acid for another in the protein made by the gene
THE MAN SAW THE DOG *	Nonsense	A change in one DNA base pair that prematurely signals the cell to stop building a protein, resulting in a shortened protein
THE MAN SAW THE DOG * THE CAN	Deletion	Removes a piece of DNA
THE MAN SAW THE FAT DOG HIT THE CAN	Insertion	Changes the number of DNA bases in a gene by adding a piece of DNA
THE MAN SAW THE *OGH ITT HEC AN	Frameshift	Occurs when the addition or loss of DNA bases changes a gene's reading frame. The groups of three bases that code a single amino acid are shifted, thereby changing the code

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Where an individual is found to have an RBD, permission should be sought to inform all relatives and invite them to have an assessment. Having a normal level of the coagulation factor does not exclude the possibility of being a carrier. Genetic testing in the person first identified as having the RBD can require time and it is important not to delay this analysis.

VON WILLEBRAND DISEASE: TRANSMISSION, INHERITANCE AND DETECTION

Von Willebrand factor (VWF) has actions in both primary and secondary haemostasis. In primary haemostasis (the constriction of blood vessels and the formation of a platelet plug), it is released by damaged endothelial cells and promotes platelet activation and aggregation. In secondary haemostasis (activation of the clotting cascade and deposition of fibrin), it acts as a chaperone to factor VIII, inhibiting its breakdown in blood but releasing factor VIII in response to thrombin. A deficiency in VWF activity or loss of function therefore has profound effects on bleeding risk. Although there is wide overlap, defects in primary haemostasis are associated with easy or spontaneous bruising, mucosal bleeding, nose bleeds, heavy menstrual bleeding, bleeding in surgery and postpartum haemorrhage; whereas defects in secondary haemostasis cause deep tissue bleeding, joint and muscle bleeding, prolonged bleeding after trauma and intracranial bleeding.

The diagnosis of VWD is made from a personal and family history supported by a formal evaluation of bleeding risk using a structured instrument such as the ISTH Bleeding Assessment Tool (ISTH-BAT). This is confirmed by screening assays (aPTT, PFA, platelet count), measurement of VWF activity (as inferred by an antigen-based assay) and levels, and factor VIII activity [4]. There are three types of VWD [5]:

- Type 1 is the most common type of VWD. It is usually associated with mild bleeding and is caused by low activity of VWF (<30%); its prevalence is 1/100 – 1/10,000. Individuals with a VWF activity of 30% – 50% are normally described as having low VWF but are not considered to have VWD.
- Type 2 is caused by loss of function of VWF and accounts for about 20% of cases of VWD. There are four subtypes (2A, 2B, 2M, 2N), differentiated by levels of VWF multimers, altered VWF function and level in relation to factor VIII.
- Type 3 VWD is characterised by the absence of VWF activity and a low level of factor VIII; it accounts for <5% of VWD.

The pattern of inheritance of VWD differs according to type. Types 1 and 2 follow an autosomal dominant pattern, whereas in Type 3 it is autosomal recessive; none is sex-linked (Figure 3). For Types 1 and 2, the children of a parent with VWD and an unaffected parent have a 50% chance of having VWD and 50% chance of being unaffected. For Type 3, the children of parents who are both carriers have a 25% chance of having VWD, a 25% chance of being unaffected, and a 50% chance of being a carrier. Some cases of VWD are not inherited but acquired – for example, due to myeloproliferative disease.

The nature of the gene mutation strongly determines the type of VWD [6], but genetic testing is not always necessary. Testing determines the subtype of Type 2 VWD and differentiates between congenital and acquired VWD; it also facilitates diagnosis in family members and is valuable for prenatal diagnosis. However, the presence of a mutation correlates poorly with symptoms and is not invariably associated with VWD [7]. This is especially true for Type 1 VWD: one third of people carrying the mutation do not have symptoms.

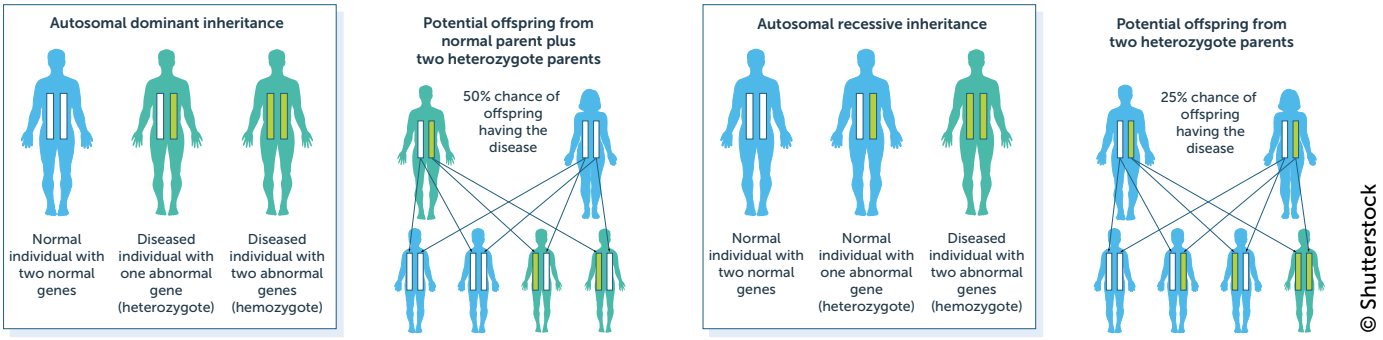
The diagnosis of VWD should be made with several caveats in mind:

- There are clinically significant differences between assays for VWF activity, leading to misclassification in up to 20% of patients [8].
- People with blood group O have low levels of VWF and there is a large natural variation in VWF level even within an individual a population.
- VWF level is increased by inflammation, stress, pregnancy, oestrogen therapy and comorbidity [9]. VWF level rises with age and comorbidities [9].

The distinction between a diagnosis of Type 1 VWD and low VWF is not absolute [10,11]. In people with low VWF, genetic variation of the VWF gene is about 40% and VWF activity is 30–50%. They may have a high bleeding score, but the family history may be positive or negative (and their bleeding risk is not explained by another bleeding disorder or platelet defect). About 90% of this population have blood group O. Among people diagnosed with Type 1 VWD, VWF activity is <30% and genetic variation is 50–80%. The family history is positive but the bleeding score is variable. About 70% of this population is blood group O. There is considerable overlap between these groups due normal biological variation and the effects on VWF of ageing and comorbidity, such as diabetes [9].

In summary, VWD mainly causes a defect in primary haemostasis due to its strong interaction with platelets, but it also affects secondary haemostasis due to its

Figure 3. Von Willebrand disease Types 1 and 2 follow an autosomnal dominant inheritance pattern; Type 3 follows an autosomnal recessive pattern



association with factor VIII. It is detected by measurement of low VWF activity in blood and further subtyped by more specific assays and factor VIII level. It is most frequently transmitted in an autosomal dominant inheritance pattern, but genetic testing but genetic testing is indicated only when the outcome may influence treatment choices, such as in prenatal diagnosis.

PRENATAL TESTING AND DIAGNOSIS

Couples affected by haemophilia who are considering whether to have a child are faced with a difficult decision. The choices they make are influenced by cultural and religious factors, their experience of haemophilia and that of their family, the risk of complications during and after delivery, access to care, and how much they trust the services available. Even those who are well informed about the odds of having a child with haemophilia will be uncertain about what will transpire for them. The time at which they seek counselling – before or after conception – is also important.

Receiving a diagnosis can be highly emotive, and it may be that the individual is unable to fully understand or remember what they are being told in the first instance. For this reason, a separate appointment should be arranged at which counselling can be provided, preferably by a team comprising an obstetrician/gynaecologist, haematologist and a clinical genetics specialist. The aim of counselling is to explain:

- The genetic risk of having an affected child
- The care and treatment available if the child is affected
- Reproductive options
- Methods for prenatal testing
- The choices available to the parents if the foetus is affected.

Being aware of carrier status can have profound implications. One survey of women who were known or potential carriers of haemophilia A or B received responses from 197 of 545 individuals approached (36%) [12]. Of those who responded, 106 (54%) had decided not to have a child, or not to have any more, 60 of whom (57%) stated that this was due to factors related to haemophilia. Of these, 47 women (44%) cited the risk of passing on haemophilia, six (6%) said they could not cope or did not want to repeat previous experiences, and seven (7%) said they want to avoid the stress of prenatal testing. Of the 41 women who had terminated a pregnancy, 11 (27%) said haemophilia was the main reason and 25 (61%) had done so for social reasons.

Other research shows that women who have experienced the complications of haemophilia or its treatment are more likely to favour prenatal diagnosis and termination of pregnancy than others [13]. However, it should not be assumed, that everyone accepts the potential benefits of genetic testing. A study of women in the Xhosa-speaking community of South Africa who were mothers or caregivers of boys with haemophilia found little understanding of the genetics and causes of haemophilia, and some women preferred explanations founded in traditional medicine [14]. Other factors contributing to not receiving genetic counselling include being unaware of the service, a focus on people with haemophilia but not potential carriers, not understanding the risks associated with carrier status, fear of knowing, and non-disclosure in families [15].

Techniques for prenatal diagnosis

Prenatal diagnostic testing may be performed in early pregnancy to diagnose an affected male foetus in time to consider termination, or at a later stage to support safe obstetric management. Current options include chorionic villus sampling (CVS), amniocentesis

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(sampling amniotic fluid), and cordocentesis (sampling cord blood). CVS can be carried out at 11–14 weeks' gestation, whereas amniocentesis is performed at 15–20 weeks; both are associated with a one percent risk of miscarriage. Cordocentesis is performed after 20 weeks' gestation to assess foetal factor level when the causative mutation is unknown or genetic analysis is not available. It is associated with a higher risk of miscarriage compared to CVS and amniocentesis, and is little used for prenatal diagnosis in Western Europe.

Most women with haemophilia consider non-invasive prenatal diagnosis to determine the foetal sex [16]. Studies have shown that women with haemophilia are less likely to choose prenatal diagnosis than women whose foetus is at risk of Down's syndrome (23% vs 71%) [12]. About one in three women decline an invasive procedure due to the risk of pregnancy loss [16].

Foetal sex determination allows female foetuses to be excluded from subsequent invasive testing. Options for non-invasive testing to assess foetal sex include ultrasound examination of foetal genitalia, with 98–100% accuracy in the second trimester. In the first trimester, analysis of free foetal DNA in maternal plasma for the detection of Y chromosome sequence offers 99–100% accuracy for foetal sex determination from 8–10 weeks' gestation [17,18]. Experience at the Royal Free Hospital in London shows that uptake of prenatal diagnosis in 2005–2018 was twice that in 1985–1995. This was associated with a corresponding increase in knowledge of foetal sex to the attending obstetrician; uptake of invasive testing declined over the same period.

Late prenatal diagnosis

Decisions about the management of labour and the mode and place of delivery can be supported by amniocentesis during the third trimester; confirmation that the pregnancy is unaffected means delivery can take place in the local maternity unit. This timing avoids the risk of miscarriage but there is a one percent chance of early labour, raising the possibility that delivery could occur before the results of the test are known. In one London centre, late amniocentesis in nine pregnancies identified three males with haemophilia, of whom two were delivered vaginally and one by emergency Caesarean section (for foetal bradycardia). Five foetuses were confirmed normal, of whom four were delivered vaginally and one assisted by vacuum extraction. One amniocentesis failed and an unaffected boy was delivered vaginally [19].

New developments in prenatal diagnosis

Analysis of free foetal DNA in maternal plasma has been shown to determine non-invasively whether a male foetus has inherited a haemophilia gene from his mother by identifying an imbalance between the mutant and wild-type gene in maternal plasma using quantitative PCR technology. In one study, haemophilia mutations were correctly identified in all of 12 at-risk pregnancies as early as 11 weeks' gestation [20]. In another study, foetal DNA analysis in maternal plasma was also used successfully for prenatal diagnosis of haemophilia caused by gene mutations most frequently associated with severe haemophilia [21]. It is hoped that these non-invasive approaches are soon validated for use in clinical practice in the near future and can be used to identify affected male pregnancies after early determination of sex, offering a potentially safer alternative to CVS and amniocentesis, and avoiding the risk of miscarriage associated with these procedures.

Preimplantation genetic diagnosis

Preimplantation genetic diagnosis is a very early form of prenatal diagnosis, in which embryos created in vitro are analysed for the causative genetic mutation and only unaffected embryos are transferred to the uterus.

Preimplantation genetic diagnosis is a challenging process for potential parents, both emotionally and physically. It involves hormone therapy to induce ovulation followed by a procedure to obtain eggs, which are then fertilised. The embryo is biopsied to obtain genetic material; embryos found to be female or unaffected by a gene mutation are then returned to the uterus. This procedure has largely been used to select female embryos for embryo transfer, but has recently been used to identify male embryos without haemophilia. In the UK, the application of preimplantation diagnosis for women with X-linked disorders is associated with a pregnancy rate of 29% and a live birth rate of 27%; 9% of pregnancies have been twins. There are concerns about safety because the removal of genetic material for analysis at such an early stage may affect foetal viability and development, and there is a risk of misdiagnosis.

Comprehensive chromosome screening can be used to identify embryos with chromosomal abnormalities; this technique, along with selective single embryo transfer, increases the rates of implantation and live birth [22], and reduces multiple pregnancies.

Other reproductive options

All options should be considered when counselling potential parents affected by haemophilia about their reproductive choices. These include assisted fertility techniques, such as egg or embryo donation, offering more approaches for parents affected by haemophilia. There is also the possibility of adoption and fostering, or of having no children. All the procedures for prenatal diagnosis and assisted pregnancy are challenging and may affect mental and physical health. Counselling and support should be available so that women in particular have opportunities to talk about their feelings.

DISCUSSION

Members of the audience and the panel discussed how the available evidence is being implemented in practice. One common theme was how best to manage differences between people, whether those with a bleeding disorder or the health professionals providing care. For example, one member of the audience pointed out that it can take 16 years to receive a diagnosis of VWD in the United States, only for that step to be reversed by a second physician who defines the condition by different threshold levels of VWF. There appears to be a lack of international consensus on VWD diagnosis, and the introduction of the term "low VWF" has caused great confusion. Diagnosis is not solely about VWF levels and function, the panel responded, and there is a need for haematologists to be better educated on the impact of bleeding disorders. Some countries have agreed national diagnostic criteria – although this, of course, not solve the problems faced by people with unclassified bleeding disorders.

Individual choice is of paramount importance when considering reproductive issues. The option of not having children should be raised so that women can make a fully informed decision, and information should be given as early as possible as they may want to reconsider at a later stage. Genetic counselling is not an isolated intervention but part of a process that should be offered in the context of ethics and religious belief, together with social and family support. The nature of counselling should reflect the treatment options available in a particular country. Diagnostic testing is not available in some countries; cross-border cooperation should address this.

Knowing the mutation that affects a foetus predicts the likely bleeding severity in haemophilia, and this is useful information to provide when counselling. However, the correlation between the mutation, VWF levels and bleeding severity in VWD is less consistent. Timely prenatal diagnosis depends on knowing which mutation affects the family so that attention can be focused on the relevant part of the gene.

Most women choose not to have invasive testing. In many countries, haemophilia management is now good – hence, the risk is no longer justified. When invasive testing is used, timing is critical. For example, there is a window of opportunity for chorionic villus sampling between 11 and 14 weeks' gestation. Earlier than 10 weeks, there is an increased risk of foetal limb malformations; amniocentesis before 15 weeks doubles the risk of miscarriage. It is important that clinicians providing care for these women are up-to-date and knowledgeable on the available reproductive

KEY MESSAGES

- Haemophilia is an X-linked inherited disorder, but about half of cases occur in a family with no apparent history of a bleeding disorder
- Bleeding risk in women with haemophilia is under-recognised
- Unlike haemophilia, von Willebrand disease and other inherited bleeding disorders are not X-linked
- There is marked variation in bleeding risk among people with von Willebrand disease or low von Willebrand factor
- Genetic testing should be offered to potential parents, ideally before conception
- It is important to know which mutation affects a family so that testing can be targeted on the relevant part of the gene
- Prenatal diagnosis informs decisions about safe obstetric management and whether to continue pregnancy
- A non-invasive technique for genetic testing does not increase the risk of pregnancy loss and is preferred by most women when it is available
- Genetic testing and the prospect of assisted pregnancy can raise challenging issues for potential parents

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options, and their risks and benefits, to provide timely and accurate information, assisting women to make an informed choice.

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