Chapter 4: **Genetic Assessment**

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RECOMMENDATIONS

4.1 | Introduction

Recommendation 4.1.1

For people with hemophilia, the WFH recommends that genetic testing be offered to identify the specific underlying genetic variant associated with their disorder. **CB**

Recommendation 4.1.2

For obligate carriers of hemophilia and "at-risk" female relatives of people with hemophilia or potential carriers of hemophilia, the WFH recommends that genetic testing be offered for the previously identified genetic variant in the *F8* or *F9* gene. **CB**

Recommendation 4.1.3

For females with low phenotypic coagulation FVIII or FIX levels, the WFH recommends that investigation of the genetic/epigenetic basis of the phenotype be offered. **CB**

Recommendation 4.1.4

For obligate carriers of hemophilia and "at-risk" female relatives of people with hemophilia or potential carriers of hemophilia, the WFH recommends the inclusion of a detailed family pedigree to support the genetic testing referral. **CB**

Recommendation 4.1.5

For individuals with suspected hemophilia and potential carriers of hemophilia, the WFH strongly recommends that phenotypic screening for FVIII or FIX levels, von Willebrand factor (VWF) antigen, and VWF activity testing be performed prior to referral for genetic testing. **CB**

Recommendation 4.1.6

For people with hemophilia, obligate carriers of hemophilia, "at-risk" female relatives, or individuals with low coagulation factor levels, the WFH strongly recommends detailed genetic counselling prior to offering genetic testing.

- REMARK: Genetic counselling should include a discussion of the experimental limits of the molecular results according to the availability of practical approaches.
- REMARK: Genetic counselling should include a discussion of the possibility of incidental findings in genes other than *F8* or *F9*, if the methodology used by the investigating laboratory (e.g., next generation sequencing [NGS]) may detect such genetic variations.
- REMARK: Genetic counselling should be performed by a genetic counsellor when available. If no genetic counsellor is available, a medical professional with knowledge of genetics in hemophilia can provide genetic counselling. **CB**

Recommendation 4.1.7

For all patients referred for genetic testing, the WFH strongly recommends that informed consent be obtained from the patient, parent, or legal guardian. This requires both permission to carry out testing and education to ensure that they fully understand the testing procedure, the benefits and limitations of the test, and possible consequences of the test results.

- REMARK: Written informed consent may need to be obtained and documented by the clinician or genetic counsellor in compliance with local policies and practices. **CB**

4.2 | Indications for genetic assessment

Recommendation 4.2.1

For people with suspected or established hemophilia undergoing genetic testing, the WFH recommends that the index case (proband) be genotyped to identify the underlying genetic variant. **CB**

Recommendation 4.2.2

For obligate carriers of hemophilia and "at-risk" female relatives of the affected proband or potential carrier of hemophilia, the WFH recommends genetic counselling about their risk of being a carrier. **CB**

Recommendation 4.2.3

For all obligate carriers of hemophilia and "at-risk" female relatives of people with hemophilia or potential carriers of hemophilia, the WFH recommends that phenotypic coagulation factor levels be measured. **CB**

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Recommendation 4.2.4

For all obligate carriers of hemophilia and “at-risk” female relatives of people with hemophilia, the WFH recommends that genetic testing be offered for the previously identified genetic variant in the *F8* or *F9* gene. **CB**

Recommendation 4.2.5

For females with low phenotypic coagulation FVIII or FIX levels, the WFH recommends that investigation of the genetic/epigenetic basis of the phenotype be offered. **CB**

Recommendation 4.2.6

For pregnant females who are carriers of an *F8* or *F9* variant and are carrying a male fetus, the WFH recommends that prenatal diagnosis (PND) be offered to determine the hemophilia status of the fetus.

• REMARK: Genetic counselling should include a discussion of the risk of the PND procedure to the pregnancy.

• REMARK: It is important to be aware of and follow the relevant laws governing such procedures in the country where the service is being provided. **CB**

Recommendation 4.2.7

For families who wish to be prepared for a child with hemophilia before birth or who wish to terminate an affected fetus, the WFH recommends that prenatal diagnosis (PND) by chorionic villus sampling or amniocentesis be offered.

• REMARK: It is important to be aware of and follow the relevant laws governing such procedures in the country where the service is being provided.

• REMARK: PND may be offered in early pregnancy or in late pregnancy by late-gestation amniocentesis in order to guide the management of the delivery of an affected child. **CB**

Recommendation 4.2.8

For people with suspected or established hemophilia, the WFH recommends that genetic testing be performed; knowledge of the genetic variant may help predict the risk of inhibitor development, response to immune tolerance induction (ITI), and depth of phenotype severity, as well as determine the availability of gene manipulation techniques. **CB**

4.3 | Strategy for genetic testing of probands

Recommendation 4.3.1

For male probands, the WFH recommends that genetic testing be directed by the proband ' s baseline phenotypic coagulation factor level, which indicates the severity of the disorder.

◦ In patients with severe hemophilia A (FVIII:C <1 IU/dL) or moderate hemophilia A with lower-borderline factor activity levels (FVIII:C 1-3 IU/dL), analysis of the *F8* intron 22 inversion and the *F8* intron 1 inversion should be performed first.

◦ Patients with severe hemophilia A in whom recurrent inversions (i.e., *F8* intron 22 and intron 1 inversions) cannot be detected should undergo screening and characterization of small variants, including single nucleotide variants (SNV) and small insertion, duplication, or deletion variants covering the essential regions of *F8* including the 26 exons, exon/intron boundaries, and 5' and 3' untranslated regions. If these tests are still uninformative, patients should be screened for copy number variants (CNV) including large *F8* deletions, duplications, or complex rearrangements.

◦ In patients with moderate (FVIII:C 1-5 IU/dL) or mild (FVIII:C 5-40 IU/dL) hemophilia A, screening and characterization of small variants (i.e., SNV and small insertions, duplications, or deletions) covering the essential regions of *F8* including the 26 exons, exon/intron boundaries, and 5' and 3' untranslated regions should be performed first. If these tests are still uninformative, patients should be screened for *F8* CNV.

◦ In all patients with hemophilia B (i.e., patients with severe [FIX:C <1 IU/dL], moderate [FIX:C 1-5 IU/dL], and mild [FIX:C 5-40 IU/dL] hemophilia B), screening and characterization of small variants (i.e., SNV and small insertions, duplications, or deletions) covering the essential regions of *F9* including the 8 exons, exon/intron boundaries, and 5' and 3' untranslated regions should be performed first. If these tests are still uninformative, patients should be screened for *F9* CNV. **CB**

4.4 | Techniques for genetic assessment

Recommendation 4.4.1

For people with severe hemophilia A, or moderate hemophilia A with lower-borderline factor activity levels (FVIII:C 1-3 IU/dL), the WFH recommends testing for the *F8* intron 22 inversion and *F8* intron 1 inversion in the first line of genetic testing.

• REMARK: Different techniques can be used for detection of the *F8* intron 22 inversion and intron 1 inversion depending on the available technical expertise and resources.

• REMARK: All results should be confirmed by independent analytical testing of the DNA sample. **CB**

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Recommendation 4.4.2

For people with severe hemophilia A who are negative for the common *F8* intron 22 inversion and *F8* intron 1 inversion variants, the WFH recommends full gene screening of the essential regions of *F8* , including the 26 exons, splice boundaries, promoter, and 5' and 3' untranslated regions.

• REMARK: For example, depending on the availability of resources, full *F8* gene screening may take the form of polymerase chain reaction (PCR) and Sanger sequencing or next generation sequencing (NGS). Where resources are limited, laboratories may choose a cost-effective screening approach prior to Sanger sequencing.

• REMARK: When choosing an analytical technique, laboratories must be aware of the sensitivity and specificity of the approach used and the turn-around time for producing an interpretive report.

• REMARK: The presence of a variant should be confirmed in both 5' (forward) and 3' (reverse) directions, specifically in heterozygous carriers, when analyzing variants detected using Sanger sequencing.

• Remark : All results should be confirmed by independent analytical testing of the DNA sample. **CB**

Recommendation 4.4.3

For people with hemophilia B, the WFH recommends full gene screening of the essential regions of *F9* , including the 8 exons, splice boundaries, promoter, and 5' and 3' untranslated regions.

• REMARK: For example, depending on the availability of resources, full *F9* gene screening may take the form of polymerase chain reaction (PCR) and Sanger sequencing or next generation sequencing (NGS). Where resources are limited, laboratories may choose a cost-effective screening approach prior to Sanger sequencing.

• REMARK: When choosing an analytical technique, laboratories must be aware of the sensitivity and specificity of the approach used and the turn-around time for producing an interpretive report.

• REMARK: The presence of a variant should be confirmed in both 5' (forward) and 3' (reverse) directions, specifically in heterozygous carriers, when analyzing variants detected using Sanger sequencing.

• REMARK: All results should be confirmed by independent analytical testing of the DNA sample. **CB**

Recommendation 4.4.4

For people with hemophilia A or B in whom no variant is detectable on inversion analysis or full gene sequencing, the WFH recommends that a large deletion or duplication event be investigated.

• REMARK: Copy number variation (CNV) analysis may be performed using various validated techniques dependent on the resources available to the laboratory. According to the practical limitations of the technique, results should be provided with an estimation of error, if applicable.

• REMARK: All results should be confirmed by independent analytical testing of the DNA sample. **CB**

Recommendation 4.4.5

For prenatal testing, the WFH recommends maternal cell contamination testing of the fetal sample.

• REMARK: Different techniques can be used for maternal cell contamination testing depending on the available technical expertise and resources. For example, multiple autosomal short tandem repeat (STR) markers may be used.

• REMARK: When choosing an analytical technique, laboratories must be aware of the sensitivity and specificity of the approach used and the turn-around time for producing an interpretive report. **CB**

4.5 | Classification and description of variants

Recommendation 4.5.1

The WFH recommends that variants be classified per the American College of Medical Genetics and Genomics (ACMG) guidelines.

• REMARK: ClinGen, a U.S. National Institutes of Health-funded resource dedicated to building a central resource that defines the clinical relevance of genes and variants, has assembled an international expert committee to apply ACMG recommendations to *F8* and *F9* variants, which should produce more hemophilia-specific recommendations. **CB**

Recommendation 4.5.2

The WFH recommends that variants be described using the Human Genome Variation Society (HGVS) nomenclature. **CB**

4.6 | Interpretative report

Recommendation 4.6.1

The WFH recommends that interpretive reports contain:

◦ patient information including patient name, date of birth, ordering clinician, date of specimen collection, diagnosis, baseline factor level, and family pedigree;

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◦ description of the assay(s), references to the literature (if applicable), limitations of the test, and the genome reference sequence used for analysis;
◦ results including DNA variant(s) in Human Genome Variation Society (HGVS) nomenclature and American College of Medical Genetic and Genomics (ACMG) variant classification; and
◦ interpretation of test results in a format useful to the ordering clinician, including recommendations for follow-up testing if indicated, implications of test results for patients and family members, and the role of genetic counselling. **CB**

Recommendation 4.6.2

For all interpretive reports for all individuals undergoing genetic testing for hemophilia, the WFH recommends that the ordering clinician and reporting scientist be available to discuss the potential phenotypic consequences of the reported genotype, as required. **CB**

4.7 | Strategies if causative variant is not detected

Recommendation 4.7.1

For people in whom a strong diagnosis of hemophilia is certain but no *F8* or *F9* variant is detected using current diagnostic genetic testing, the WFH recommends that other genetic causes be considered (e.g., deep intronic variants).
• REMARK: Current testing techniques are expected to evolve in the near future to include next generation sequencing (NGS) and whole genome sequencing (WGS).
• REMARK: NGS and WGS techniques should only be used after it is established that structural variants can be detected by the technique. **CB**

Recommendation 4.7.2

For "at-risk" female relatives of people with hemophilia in whom the familial variant is not detected using standard diagnostic genetic testing, particularly in females with one affected child, the WFH recommends that the possibility of mosaicism be considered and discussed during genetic counselling. **CB**

Recommendation 4.7.3

For people with hemophilia A in whom the mode of inheritance is not conclusive, and in whom no inversion or variant is detected by current diagnostic testing, the WFH recommends that other potential diagnoses be investigated, including type 2N von Willebrand disease (VWD), combined FV and FVIII deficiency, or other types of VWD. **CB**

Recommendation 4.7.4

For symptomatic females with low phenotypic coagulation
FVIII or FIX levels in whom just one pathogenic variant is found, the WFH recommends performing investigative tests for an X-chromosome inactivation pattern, if locally available. **CB**

4.8 | Quality assurance

Recommendation 4.8.1

The WFH recommends that genetic diagnostic laboratories should undergo periodic accreditation, if available, by an approved body. **CB**

Recommendation 4.8.2

The WFH recommends that internal quality control (IQC) of genetic tests be performed and recorded routinely within the laboratory. **CB**

Recommendation 4.8.3

The WFH recommends that laboratories participate in external quality assessment schemes (EQAS) for the genetic tests they provide.
• REMARK: Participation in an EQAS ensures the provision of a test that is robust and reliable. This may be through participation in a formal EQAS or an informal sample exchange between laboratories. **CB**

CB, consensus based; VWF, von Willebrand factor; NGS, next generation sequencing; PND, prenatal diagnosis; ITI, immune tolerance induction; CNV, copy number variants; SNV, single nucleotide variants; DNA, deoxyribonucleic acid; PCR, polymerase chain reaction; STR, short tandem repeat; ACMG, American College of Medical Genetics and Genomics; HGVS, Human Genome Variation Society; WGS, whole genome sequencing; IQC, internal quality control; EQAS, external quality assessment schemes.

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For more information on the WFH Guidelines for the Management of Hemophilia, visit www.WFH.org/TGResourceHub