Chapter 4
GENETIC ASSESSMENT

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GENOTYPE ANALYSIS SHOULD BE OFFERED TO ALL PEOPLE WITH HEMOPHILIA AND THEIR “AT-RISK” FEMALE FAMILY MEMBERS.

When is genetic testing indicated?

- To define specific genetic mutation
- To establish diagnosis in difficult cases
- To predict risk of inhibitor development
- To identify female carriers
- To provide prenatal diagnosis

Referring for genetic testing

4.1.1 Suspected or established hemophilia
Test to identify the specific genetic variant

4.1.2 Obligate carriers and “at-risk” females
Test for previously identified familial genetic variant in the F8 or F9 gene

Prior to REFERRAL for genetic testing:

4.1.5 Phenotypic screening for:
FVIII or FIX levels, VWF antigen, and VWF activity testing

4.1.6 Genetic Counselling (pre and post testing, by genetic counsellor if available) for:
Limits of molecular results; possibility of incidental findings; consent; education
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Approach for genetic testing for hemophilia

- **Suspected or established hemophilia in index case**
  - Identify clinical phenotype

- **Baseline phenotypic coagulation factor**

- **Genetic counselling** (pre-test)
  - Familial genetic variant known (obligate carriers and “at-risk” females)
  - Familial genetic variant unknown
  - Test genetic variant in the F8 or F9 gene
    - Consider mosaicism

- **Suspected or established hemophilia in index case**
  - Severe or moderate hemophilia A
    - Baseline phenotypic coagulation factor
    - Genetic counselling (pre-test)
    - Screen for inversions (F8 introns 22 and 1)
    - Investigate differential diagnosis
    - Consider mosaicism
    - Repeat genetic testing to confirm result
  - Mild or moderate hemophilia A
    - Full F8 gene screening
  - Mild, moderate or severe hemophilia B
    - Full F9 gene screening

- **Screen for small variants**

- **Screen for copy number variants**

- **Screen for deep intronic variants‡**

- **Investigate differential diagnosis**

†Depending on availability, full F8 or F9 gene screening is performed by PCR and Sanger sequencing or NGS for the detection of missense, nonsense, splice-site, small and large deletions, duplications, and insertions; where resources are limited, laboratories may choose a cost-effective screening approach prior to Sanger sequencing. *Small variants includes: SNVs and small insertions, duplications, or deletions covering the essential regions of F8 for hemophilia A (including the 26 exons) or F9 for hemophilia B (including the 8 exons), exon/intron boundaries, promotor and 5’ and 3’ untranslated regions. **Copy number variants include large F8 (hemophilia A) or F9 (hemophilia B) deletions, duplications, or complex rearrangements. ‡NGS and WGS techniques may be used, but only after it is established that structural variants can be detected by the technique. Based on Recommendation 4.2.

NGS, next-generation sequencing; PCR, polymerase chain reaction; SNV, single nucleotide variants; VWD, von Willebrand disease; WGS, whole genome sequencing.

- ✔ Genotype analysis should be offered to all people with hemophilia and their “at-risk” female family members
- ✔ Genetic counselling for people with hemophilia and their families is an essential requirement prior to genetic testing
- ✔ Genetic diagnostic laboratories should adhere to strict protocols and procedures and undergo periodic accreditation
- ✔ The interpretation of the results of genetic testing should be performed by scientists who have knowledge and expertise in hemophilia genetics
- ✔ The ordering clinician and reporting scientist should be available to discuss the potential phenotypic consequences of the reported genotype