All-in-one solution for single gene disorders with diverse mutations

Hemophilia A & B

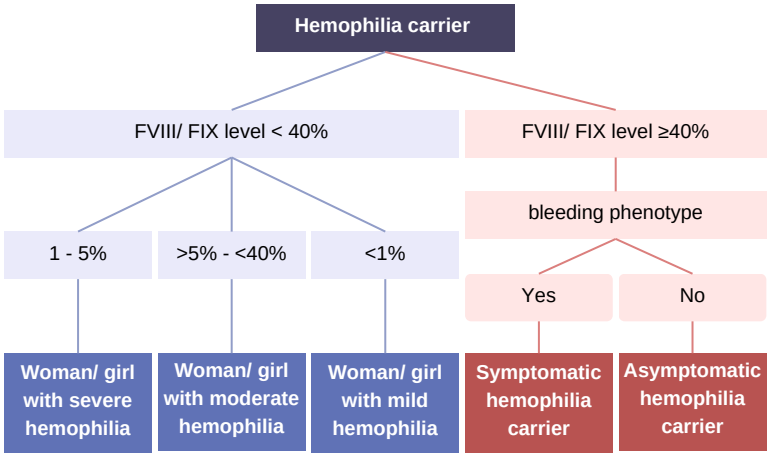
Background

Hemophilia is an inherited bleeding disorder in which blood fails to clot properly. There are two the most common types: hemophilia A (HA), caused by *F8* gene mutations resulting in reduced factor VIII (FVIII), and hemophilia B (HB), caused by *F9* gene mutations resulting in reduced factor IX (FIX). Both HA and HB are X-linked recessive disorders, primarily affecting males, with HA occurring in 1 in 5,000 male births and HB in 1 in 30,000 [1].

Refer to International Society on Thrombosis and Hemostasis (ISTH) 2001, the severity of HA and HB is determined based on the activity levels of FVIII or FIX in plasma [2].

Severity	FVIII/ FIX level of the normal
Severe hemophilia	<1%
Moderate hemophilia	1 - 5%
Mild hemophilia	>5% - <40%

Women carriers typically have a mean factor activity level of 60% [3], however, some may have levels below 40% and are classified as having mild hemophilia due to lyonization (X-inactivation). Additionally, approximately one in four women carriers with FVIII or FIX levels >50% exhibit an increased bleeding tendency [4]. In 2021, ISTH introduced the following terms for carriers with clotting factor levels ≥40%: “symptomatic hemophilia carrier” for those with bleeding symptoms and “asymptomatic hemophilia carrier” for those without [4].



Conventional blood test for hemophilia requires multiple assays. These assays are often inaccurate for carrier screening and unsuitable for prenatal testing, as they rely on analyzing the proband's fresh blood sample. For instance, about 86% of female carriers of hemophilia A (HA) have normal APTT findings [5], and only 25.7% of female carriers of HA or HB exhibit reduced factor levels below 40% [6]. Identifying carriers with normal factor levels can be particularly challenging, especially when the mutation is predominantly passed through female family members.

Conventional blood test

First screening + Mixed testing PT, APTT, and platelet count → **Factor assay** → **Rule out Von Willebrand Disease (VWD) and other conditions**

Although genetic testing for hemophilia provides a more reliable approach, it comes with significant challenges. The wide spectrum of mutation types makes the process inherently complex. While the *F8* intron 1/22 inversion test is the first-line test for severe HA patients, it is ineffective for detecting non-inversion HA variants (which account for about half of severe cases and the majority of moderate to mild cases [7]) as well as HB variants. As a result, multiple molecular assays are often required for these variants, making the process both time-consuming and expensive.

Frequencies of *F8* and *F9* variants by type and severity in male patients

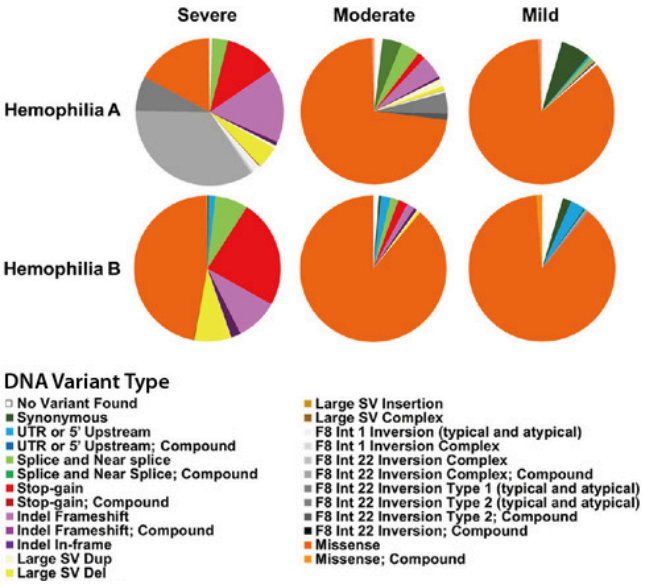


Figure adopted from reference [7] with modifications

Comprehensive Analysis of Hemophilia

Comprehensive Analysis of Hemophilia (CAHEA)

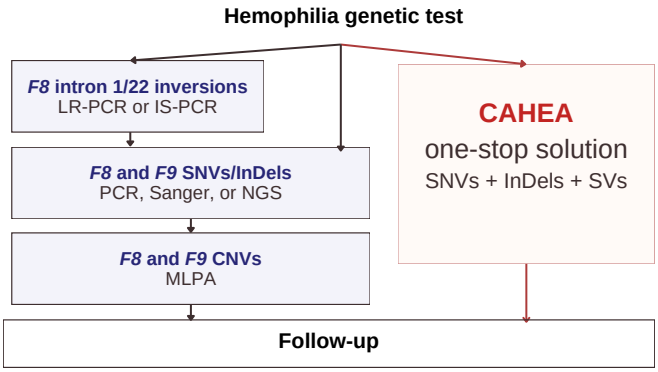
is your one-stop partner for hemophilia

CAHEA leverages long-read single molecule real-time (SMRT) sequencing to provide comprehensive hemophilia analysis, detecting diverse mutations in the *F8*, *F9*, and *VWF* genes. In addition to covering all variant types, it offers precise breakpoint identification and inversion subtyping in a single test, significantly reducing diagnostic time. In two separate studies, CAHEA demonstrated 100% accuracy in identifying various types of *F8* variants (in all 131 hemophilia A probands) [8] and *F9* variants [9]. This makes CAHEA an ideal solution for genetic testing.

Patent no. 202310224086.5

Technology: Single molecule real-time (SMRT) sequencing
Platform: PacBio Vega, Sequel II, and Sequel IIe system
Sample type: Blood, DBS, gDNA, and buccal swab
Turnaround time: 17 working days
(starting from the date of sample arrival at the testing laboratory)

One-Stop Solution for Hemophilia Genetics



Disorders (Gene)	Basic panel	Comprehensive panel	Prenatal Comprehensive Panel
Hemophilia A (*F8*)	Intron 22 inversion	Intron 22 inversion	Intron 22 inversion
	Intron 1 inversion	Intron 1 inversion	Intron 1 inversion
	Exon 22 deletions/duplications	Exon 22 deletions/duplications	Exon 22 deletions/duplications
	--	P/LP/selected VUS SNVs/InDels	P/LP SNVs/InDels
		Selected P/LP/VUS large deletions/duplications	Selected P/LP large deletions/duplications
Hemophilia B (*F9*)	--	P/LP/selected VUS SNVs/InDels	P/LP SNVs/InDels
		Selected P/LP/VUS large deletions/duplications	Selected P/LP large deletions/duplications
Von Willebrand disease (*VMF*)	--	Potentially clinically significant SNVs/InDels in exons 18-28 (the region responsible for binding factor VIII (F8))	P/LP SNVs/InDels in exons 18-28 (the region responsible for binding factor VIII (F8))

References: [1] Peyvandi F, Garagiola I, Young G. The past and future of haemophilia: diagnosis, treatments, and its complications. *Lancet*. 2016;388(10040):187-197. [2] White GC 2nd, Rosendaal F, Aledort LM, et al. Definitions in hemophilia. Recommendation of the scientific subcommittee on factor VIII and factor IX of the scientific and standardization committee of the International Society on Thrombosis and Haemostasis. *Thromb Haemost*. 2001;85(3):560. [3] Plug, Iris, et al. "Bleeding in carriers of hemophilia." *Blood*. 108.1 (2006): 52-56. [4] van Galen KPM, d'Oiron R, James P, et al. A new hemophilia carrier nomenclature to define hemophilia in women and girls: Communication from the SSC of the ISTH. *J Thromb Haemost*. 2021;19(8):1883-1887. [5] Ho SKL, Ng SYL, Yung TK, et al. Clinical and molecular characteristics of hemophilia A affected individuals and carriers: A 24 years experience from three centers. *Am J Med Genet A*. 2024;194(9):e63657. [6] Plug I, Mauser-Bunschoten EP, Bröcker-Vriends AH, et al. Bleeding in carriers of hemophilia. *Blood*. 2006;108(1):52-56. [7] Johnsen JM, Fletcher SN, Dove A, et al. Results of genetic analysis of 11 341 participants enrolled in the *My Life, Our Future* hemophilia genotyping initiative in the United States. *J Thromb Haemost*. 2022;20(9):2022-2034. [8] Liu Y, Li D, Yu D, et al. Comprehensive Analysis of Hemophilia A (CAHEA): Towards Full Characterization of the F8 Gene Variants by Long-Read Sequencing. *Thromb Haemost*. 2023;123(12):1151-1164. [9]Shi M, Ma Y, Peng X, et al. Clinical validation and application of targeted long-range polymerase chain reaction and long-read sequencing-based analysis for hemophilia: experience from a hemophilia treatment center in China. *J Thromb Haemost*. 2024;22(12):3431-3447.

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