Review Article

Advancements and Challenges in Hemophilia B: Understanding Genetic, Clinical, and Therapeutic Dimensions

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ABSTRACT

Hemophilia B, a recessive X-linked bleeding disorder, arises from vitamin K-dependent coagulation factor IX (FIX) deficiency. Despite using FIX activity levels to gauge disease severity, they often do not align with the bleeding phenotype due to complex regulatory processes in coagulation. With rapid advancements in gene therapy and the availability of long-acting medications, the treatment landscape for hemophilia B is evolving rapidly. Understanding the factors influencing hemostatic and clinical outcomes and therapeutic responses is crucial in this era of progress. Unlike factor VIII (FVIII), FIX pathways' synthesis, cellular interactions, and clearance mechanisms remain less studied. Significant aspects include the roles of vascular collagen in platelet adhesion at hemostatic sites and magnesium's dual impact on FIX activation and platelet function. Enhanced comprehension of these mechanisms through biochemical and translational research promises to develop relevant diagnostic tests that assess treatment efficacy and hemostasis. In contemporary hemophilia care, personalized treatment strategies tailored to individual patient needs are increasingly vital. This review underscores the importance of advancing our understanding of hemophilia B's biological intricacies to facilitate the development of more effective and economical therapies.

Keywords: Hemophilia B, F9, Mutation Analysis, Carrier Testing.

INTRODUCTION

Hemophilia is a rare bleeding disorder linked to the X chromosome, characterized by deficient clotting factor VIII in hemophilia A or factor IX in hemophilia B. Inhibitors against these clotting factors are prevalent in 20%–33% of severe hemophilia A cases and 1%–6% of hemophilia B cases, posing significant challenges in treatment options, particularly in resource-limited settings where access to factor-replacement therapies is limited or absent, contributing to higher mortality and morbidity rates. Early initiation of factor replacement therapy, either as primary prevention or soon after the first bleeding episode, has been shown to reduce joint bleeding and musculoskeletal complications in inhibitor-free patients. Hematuria is the most frequent side effect and indicator of a severe hemophilia condition.

BRIEF OVERVIEW OF HEMOPHILIA B AS A SUBTYPE OF HEMOPHILIA

Hemophilia B, a recessive X-linked bleeding disorder caused by a deficiency in vitamin K-dependent coagulation factor IX (FIX), is less common than hemophilia A, affecting approximately one in thirty thousand male births globally. The severity of hemophilia is classified based on clotting activity levels: normal levels are defined as 100 IU/dL, with mild (>5% to <40% of normal), moderate (1%–5% of normal), and severe (<1% of normal) phenotypes recognized. However, these laboratory classifications do not always correlate with clinical severity due to regulatory mechanisms and additional hemostatic variables. The global prevalence of hemophilia A and B combined has been reported at 62% among those with bleeding disorders, totaling more than 304,000 individuals as per the latest World Federation of Hemophilia survey. Advances in gene therapy and extended half-life medications aim to align therapeutic targets with factor activity levels, particularly benefiting those with moderate-to-mild phenotypes and unknown family histories.

IMPORTANCE OF MUTATIONAL ANALYSIS IN UNDERSTANDING THE GENETIC BASIS OF HEMOPHILIA B

Hemophilia B manifests with spontaneous or post-traumatic bleeding episodes, ranging from joint bleeds in severe cases to prolonged oozing after surgeries or tooth extractions. The frequency and severity of bleeding episodes often correlate with the level of factor IX clotting activity and the age of diagnosis. Mutational analysis plays a crucial role in deciphering the genetic underpinnings of hemophilia B, aiding in precise diagnosis and management strategies.

Severe hemophilia B is often diagnosed within the first year of life or following procedures associated with childbirth or neonatal care (Kulkarni et al., 2009). Untreated toddlers with severe hemophilia B commonly present with

noticeable symptoms such as large "goose eggs" from minor head injuries and bleeding from minor oral wounds. Head traumas can also lead to intracranial hemorrhage. Subcutaneous hematomas are nearly always observed in untreated children, sometimes prompting investigations for potential non-accidental harm. As they grow and become more active, these children are prone to spontaneous joint bleeds or deep muscle hemorrhages, causing pain or limping due to swelling. Without prophylactic treatment, individuals with severe hemophilia B often experience two to five uncontrollable bleeding episodes per month, most commonly affecting joints, though bleeding can also occur in the gastrointestinal tract, muscles, kidneys, nose, and brain. Following minor injuries, surgeries, or tooth extractions, these patients may suffer prolonged bleeding, intense pain, and swelling if preventive measures are not administered.

Table 1: Shows the Types and Symptoms Based on severity.

Types based on severity	Factor IX clotting Factor	Symptoms
Severe	<1%	Rare-organizations of spontaneous bleeding Excessive and/or protracted bleeding following dental extractions, minor surgeries, or injuries
Moderate	1%-5%	Unexpected bleeding is rare. Excessive and/or protracted bleeding following dental extractions, minor surgeries, or injuries
Mild	6%-40%	Absence of unprovoked bleeding Excessive and/or protracted bleeding following dental extractions, serious injuries, or surgeries

In contrast, individuals with moderate hemophilia B typically experience less frequent spontaneous bleeding, although they may exhibit delayed or prolonged bleeding following minor injuries. Diagnosis typically occurs before the age of five or six years, often without prior preparation for elective invasive procedures. Treatment with factor IX concentrates is essential for managing bleeding episodes, which may occur from once a year to once a month. Otherwise, their bleeding symptoms resemble those of severe hemophilia B patients[1,2].

DESCRIPTION OF HEMOPHILIA B AND ITS CLINICAL MANIFESTATIONS

Hemophilia B results from a deficiency in factor IX clotting activity, leading to prolonged bleeding after surgeries, dental extractions, or injuries until complete healing occurs. The frequency of bleeding episodes and the severity of symptoms correlate with the age at diagnosis and the level of factor IX clotting activity. Children and adolescents typically experience more frequent bleeding episodes than adults. Severe hemophilia B is usually diagnosed within the first two years of life. Without preventive treatment, these patients may experience up to five spontaneous bleeding episodes per month, often involving joints, muscles, or prolonged bleeding following minor injuries or procedures. Moderate hemophilia B patients are typically diagnosed before the age of five or six. They rarely experience spontaneous bleeding but may have delayed or prolonged bleeding after minor injuries, occurring monthly or annually, requiring treatment with factor IX concentrates. Mild hemophilia B patients may not have spontaneous bleeding episodes but can experience excessive bleeding during surgeries or procedures without adequate pre- and postoperative treatment. Diagnosis often occurs later in life, even if there is a family history of the condition. Female carriers of hemophilia B, with factor IX clotting activity below forty percent, have a risk of femicide greater than thirty percent, but symptoms are usually mild. They may experience prolonged or profuse bleeding following major injuries or invasive procedures, irrespective of the severity of hemophilia B[3].

EXPLANATION OF THE UNDERLYING PATHOPHYSIOLOGY OF HEMOPHILIA B

Hemophilia B shares a clinical resemblance with hemophilia A, but differentiation between the two disorders is straightforward through routine laboratory tests and measurements of factor VIII (FVIII) and factor IX (FIX) activity. The FIX gene, located on the long arm of the X chromosome, is situated approximately 10 centimorgans away from the FVIII gene. Hemophilia B accounts for about 20–25% of all hemophilia cases. FIX, a serine protease, is encoded by eight exons within a 34 kb gene, synthesizing a precursor protein of 461 amino acids. Together with nonenzymatic cofactor VIII, FIX forms the "Xase" complex, crucial for activating factor X (FX) in the coagulation cascade. Post-translational carboxylation by γ-carboxylase converts specific glutamic acid residues in the pro-peptide domain to γ-carboxyglutamic acid (GLA), essential for FIX's full enzymatic function. The liver is the primary site of FIX synthesis, with robust γ-carboxylase activity ensuring efficient production of functional FIX, circulating at approximately 10 µg/ml—nearly 100 times more abundant than FVIII in plasma. The FIX Leyden variant, characterized by severe hemophilia B in childhood with FIX levels improving markedly during puberty, has shed light on the gene's transcriptional regulation. Mutations in the promoter region of the FIX gene are implicated in this

phenomenon, elucidating mechanisms governing FIX expression[4,5].

Research into FIX gene mutations has revealed that approximately one-third of hemophilia B cases result from point mutations at CpG dinucleotides[6]. These mutations arise spontaneously at a rate estimated at 1.3 mutations per 1.14 x 10^8 base pairs per generation, reflecting a global human mutation rate. Understanding these mutation patterns enhances our comprehension of hemophilia B's genetic basis and informs diagnostic and therapeutic strategies[7].

ROLE OF FACTOR IX DEFICIENCY IN THE DEVELOPMENT OF HEMOPHILIA B

Hemophilia has a rich historical background, evident even in fifteenth-century Rabbinic texts[8]. John C. Otto authored the first medical report on hemophilia in 1803[9]. In 1952, it was discerned that hemophilia A (classic hemophilia) and hemophilia B (Christmas disease) are distinct disorders sharing a common inheritance pattern[10]. Christmas disease owes its name to the first patient described with hemophilia B. Hemophilia A is characterized by a deficiency in factor VIII (FVIII), also known historically as the Christmas factor. In contrast, hemophilia B results from factor IX (FIX)11 deficiency. Recent genetic investigations in the Russian royal family, descendants of Queen Victoria, identified a specific mutation in the F9 gene associated with hemophilia B. Overall, these historical and genetic insights underscore the distinct nature of hemophilia A and B, despite their shared inheritance patterns and historical recognition.

CLINICAL PRESENTATION:

Apart from milder forms, hemophilia B (HB) prolongs activated partial thromboplastin time (APTT). Diagnosis involves measuring FIX activity in plasma using clot-based or chromogenic substrate assays, reported as a percentage of normal or units per deciliter (U/dL). HB severity is categorized as mild (<1 U/dL), moderate (1% to 5% or 1-5 U/dL), or severe (>5% to <40% or >5 to <40 U/dL). Some publications use <50% for severe classification. Factors like vitamin K deficiency, neonatal age, genetic disorders, and certain medications also affect FIX and APTT.

DIAGNOSIS CHALLENGES:

Diagnosing HB in neonates is challenging due to naturally low FIX levels, which reach adult levels by six months. Interpretation of FIX levels in infants requires expertise to prevent misdiagnosis. Genetic testing for F9 gene variants confirms the diagnosis.

CLINICAL MANAGEMENT:

Untreated severe HB leads to spontaneous joint and muscle hemorrhages, causing pain, immobility, and joint damage. Prophylactic treatment with recombinant FIX products is standard for severe cases, reducing long-term complications. Treatment protocols aim for consistent FIX levels; severe patients require more infusions annually compared to moderate and mild cases.

TREATMENT MODALITIES:

Recombinant FIX products, including extended half-life versions, are used to maintain FIX levels. Treatment frequency varies based on severity, with severe patients receiving the most infusions. Cost considerations influence healthcare decisions, particularly in the US[12,13].

EPIDEMIOLOGY:

In epidemiological terms, HB is less prevalent compared to HA. According to a global survey, the prevalence of HA stands at 17.1 per 100,000 males, whereas HB accounts for 3.8 cases per 100,000 individuals, indicating that HB constitutes approximately 18% of all hemophilia cases. The incidence rates further underscore this disparity, with HB occurring at 4.7 per 100,000 male births, while HA has a higher incidence at 23.2 per 100,000 males, suggesting HB is roughly one-fifth as common as HA. Similar data from US hemophilia patients show HB representing 24% of cases, with specific states reporting higher prevalence possibly due to a founder effect.

Regarding inheritance, HB follows X-linked patterns as the F9 gene resides on the X chromosome. Females, with two X chromosomes, can be carriers. In contrast, males, with one X and one Y chromosome, are hemizygous and manifest the full hemophilic phenotype due to the lack of a compensatory Y allele. Female carriers exhibit varied expressivity due to X chromosome inactivation. In sporadic cases of hemophilia, where there is no family history, new mutations in the F9 gene can occur in either sperm or egg cells before conception. Such cases account for about one-third of male births with HB. Subsequent testing may reveal carrier status in mothers initially considered non-carriers due to incomplete family history or mosaicism. In summary, HB shows lower prevalence and incidence than HA, primarily due to its X-linked inheritance and sporadic mutations contributing to its occurrence[14,15].

GENETIC BASIS OF HEMOPHILIA B

overview of the f9 gene and its role in hemophilia b

Hemophilia B (HB) is an X-linked recessive genetic disorder predominantly affecting males, with an incidence of approximately 1 in 30,000 live male births. This condition leads to prolonged clotting times, resulting in recurrent bleeding episodes starting in childhood, which can sometimes lead to disabilities or life-threatening situations. Currently, effective treatments for HB are limited, contributing to significant mortality and morbidity rates. Consequently, carriers of the condition are recommended to undergo prenatal diagnosis and receive genetic counseling[16].

The F9 gene, located on the long arm of the X chromosome at Xq27.1, is responsible for hemophilia B. Mutations in this gene disrupt the production of factor IX, crucial for blood clotting. Due to its relatively short length and simple structure, Sanger sequencing is commonly employed for diagnosing mutations in the F9 gene. This method is known for its accuracy, cost-effectiveness, and reliability in clinical

settings, providing essential data for genetic counseling and medical management[17].

TYPES OF MUTATIONS IN THE F9 GENE ASSOCIATED WITH HEMOPHILIA B

Hemophilia B (HB) is an X-linked recessive disorder primarily affecting males, with an incidence of approximately 1 in 30,000 live male births. It results in prolonged clotting times and recurrent bleeding episodes, posing significant health risks. Treatment options are limited, underscoring the importance of prenatal diagnosis and genetic counseling for carriers. The F9 gene, located at Xq27.1 on the X chromosome, is responsible for HB. Various mutations in this gene have been cataloged in public databases such as the Coagulation Serine Protease Mutation Database, the Human Gene Mutation Database, and the Hemophilia B Mutation Database, amounting to about 900 different variants. These mutations can be categorized into six types: nonsense/missense (64%), minor splicing (9%), significant insertions or deletions (6%), regulatory (2%), and complex rearrangements (1%). Point mutations are predominant, constituting 64% of all mutations, with certain nucleotides showing higher mutation frequencies, particularly in CpG islands, due to enhanced mutation rates during male gamete formation[18].

Missense mutations, which alter specific amino acids, can lead to two main types of defects:

- Type I mutations (60% of cases) reduce the amount of normal protein produced due to issues like impaired secretion or transcription, resulting in severe HB.
- Type II mutations (about 60% of cases among coagulation factor deficiencies) affect the protein's structure, often occurring at the active site or pro-peptide region, leading to functional deficiencies and potentially severe forms of HB. Nonsense mutations introduce premature stop codons, resulting in truncated or non-functional proteins. Splicing errors, accounting for 9% of mutations, can cause varying disease severities by disrupting mRNA processing. The founder effect has been observed in specific populations, where certain mutations become more prevalent due to historical isolation. For instance, mutations associated with HB have been documented in regions like Ireland, the UK, and North America. In summary, mutations in the F9 gene responsible for HB vary widely in type and location, influencing disease severity and clinical manifestations, thereby necessitating tailored diagnostic approaches and therapeutic strategies[18].

FREQUENCY AND DISTRIBUTION OF MUTATIONS IN DIFFERENT POPULATIONS

Mutations in the Factor 9 gene (F9) result in defective Factor IX protein (FIX), causing hemophilia B (HB), characterized by recurring bleeding. Severity is categorized based on FIX activity: <1% is severe, 1-5% is moderate, and 5-40% is mild. F9, located on Xq27, spans eight exons totaling 33.5 kb, encoding a 2.8 kb mRNA translated into a 461 amino acid liver-expressed protein, essential in coagulation. Current HB treatments include recombinant FIX or plasma-derived products. However, 1-3% develop inhibitory antibodies, rising to 20% in complete FIX gene deletions. Missense mutations pose a lower risk. HB genotyping aids inhibitor prediction, guiding personalized therapies. Over 1,000 HB-causing mutations are documented, mostly point mutations (70%) and deletions (16%). Large deletions, nonsense mutations, frameshifts, and missense mutations correlate with disease severity. Certain populations exhibit higher incidences due to founder effects, notably mutation c.1025C>T. The F8 and F9 genes on X chromosome (Xq27.6 and Xq28.5 respectively) vary in size and complexity, impacting genetic etiology and therapeutic strategies for hemophilia A and B[19,20,21,22,23,24,25,26,27].

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