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Decoding hereditary spherocytosis: Unveiling the genes as a potential causative agent

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Abstract:

Hereditary spherocytosis (HS) is the most common inherited hemolytic anemia in populations of northern European descent, arising from mutations in genes encoding key erythrocyte membrane proteins, including *ANK1*, *SPTB*, *SLC4A1*, *SPTA1*, and *EPB42*. These genetic defects disrupt the vertical linkages between the red blood cell (RBC) plasma membrane and its underlying cytoskeleton, reducing RBC deformability and leading to characteristic spherocytosis, premature hemolysis, and accelerated splenic clearance. Inheritance is predominantly autosomal dominant, although autosomal recessive forms occur, particularly involving SPTA1 and EPB42. Clinically, HS manifests with anemia, jaundice, splenomegaly, and complications such as cholelithiasis. This study aimed to identify and characterize pathogenic variants in primary HS genes, establish genotype–phenotype correlations, and enhance diagnostic accuracy to guide personalized management within a defined cohort. Diagnostic advancements, notably the tests of next-generation sequencing and the eosin-5-maleimide binding test, have improved the detection of genetic heterogeneity, yet regional underdiagnosis persists. A comprehensive understanding of the HS genetic spectrum is therefore critical not only for precise diagnosis and optimized patient care but also for informing the development of future gene-targeted therapies, including CRISPR-based approaches. Regional epidemiological investigations, including limited data from Iraq, suggest that HS remains underdiagnosed despite its significant clinical impact. A comprehensive understanding of the HS genetic spectrum is imperative for accurate diagnosis, optimized personalized management, and the eventual development of gene-based therapeutic interventions.

Keywords:

ANK1, EPB42, hereditary spherocytosis, *SLC4A1, SPTA1, SPTB*

Introduction

Ordinary congenital hemolytic anemia, including hereditary spherocytosis (HS), is most frequently transmitted as an autosomal dominant disorder (approximately 75% of cases) with the remainder inherited in an autosomal recessive manner.[1] In autosomal dominant inheritance, a single copy of the pathogenic variant on one of the autosomes is sufficient to cause the disease, and each affected individual has a 50% likelihood of transmitting the mutation to their offspring.[2] HS represents a prevalence of approximately 1 in 2000 individuals in northern European populations,[3] while the reported incidence in China is 1.39 per 100,000 individuals.[4]

Clinically, HS is characterized by anemia, jaundice, and splenomegaly.[5] Mild-to-severe illness of HS depends on the bone marrow compensation degree; in mild HS, increased erythropoiesis counterbalances hemolysis, whereas severe HS can result in hemolytic crises and, in extreme cases, mortality.[6] Diagnosis

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is typically based on heredity, clinical examination, and the spherocyte investigation on peripheral blood smears. Supportive diagnostic methods include the tests of flow cytometry, eosin-5-maleimide (EMA) binding, osmotic fragility, protein electrophoresis, or mass spectrometry and autohemolysis assays.[7,8] Current diagnostic guidelines emphasize the incorporation of next-generation sequencing (NGS), which enables comprehensive detection of disease-causing mutations in red blood cell (RBC) membrane protein genes, which proves particularly valuable in complex or diagnostically challenging cases.[9] The implementation of targeted NGS panels has significantly advanced the understanding of the molecular basis of HS, providing deeper insights into genotype–phenotype correlations.[10] Concurrently, developments in precision medicine underscore gene therapy as a promising approach for the definitive correction of inherited hematological disorders. Consequently, the discovery of novel HS-associated mutations remains crucial for clarifying genotype–phenotype relationships and for informing the design of future targeted therapeutic strategies.[11] To date, five major genes encoding RBC cytoskeletal proteins have been implicated in HS pathogenesis: *ANK1* (ankyrin), *SLC4A1* (band 3), *SPTA1* (α-spectrin), *SPTB* (β-spectrin), and *EPB42* (band 4.2).[12] Mutational heterogeneity is well documented, with variations in both frequency and mutation type observed across populations. Globally, mutations in both *SPTB* and *ANK1* genes are considered the most prevalent HS-inducing factors, followed by *SLC4A1*, whereas mutations in SPTA1 and EPB42 are rare and reported primarily in East Asian cohorts, such as Japanese and Korean populations.[1] Thus, HS demonstrates striking clinical and genetic heterogeneity.[13]

Despite its relative frequency among hereditary hemolytic anemias, HS remains challenging to diagnose accurately. Many patients are misdiagnosed or underdiagnosed, leading to an underestimation of their true prevalence. Consequently, establishing a definitive diagnosis continues to represent a significant clinical challenge.[14]

Global and Regional Epidemiology of Hereditary Spherocytosis

The HS is characterized by spherical erythrocytes that are prone to splenic destruction. Globally, its prevalence varies, with almost 1 in 5000 people affected in the United States, where 75% of cases follow an autosomal dominant inheritance pattern and the remainder are autosomal recessive.[15] In the United Kingdom, HS prevalence ranges from 1 in 2000 to 1 in 5000, predominantly autosomal dominant. In East Asia, HS is relatively rarer, with China reporting 1.27 cases per 100,000 males and 1.49 per 100,000 females, commonly caused by *ANK1* gene mutations, while Japan and South Korea show prevalence estimates similar to Europe, approximately 1 in 2000–1 in 5000 individuals, with autosomal dominant inheritance predominating.[16] In Australia, prevalence is comparable to that in Europe and North America, ranging from 1 in 2,000 to 1 in 5,000.[17] HS is a worldwide disorder, but its prevalence is notably higher in northern Europe, reaching approximately 1 in 2,000 individuals. In East Asia, studies from Korea and Japan have demonstrated that spherocytosis represents the most frequent form of inherited blood disorder.[18] In China, there are about 1.27 in 100,000 males and 1.49 in 100,000 females who were diagnosed in adolescence.[19]

In Iraq, official nationwide statistics are lacking; however, based on a descriptive, file-based survey of 589 patients with inherited hemoglobin disorders attending the Hematological Disease Center in Misan Governorate in 2024, among 589 patients diagnosed with inherited hemoglobin disorders in 2024, only 15 patients (2%) were diagnosed with HS, including 10 males and 5 females, reflecting the relative rarity of this condition in southern Iraq. These findings underscore the need for increased awareness, early diagnostic strategies, and genetic counseling to better characterize the epidemiology and clinical impact of HS in Iraqi populations.[20] The age distribution and clinical severity among the HS cases were not fully detailed in this dataset; however, the identification of cases through the centralized hematological disease center highlights the utility of hospital-based surveillance in detecting rare hemolytic disorders in Iraq. These findings align with regional reports suggesting that HS, although underdiagnosed, contributes a minor yet clinically significant burden to the spectrum of inherited red cell disorders in the Iraqi population. Given the low prevalence and potential for complications such as anemia, jaundice, and splenomegaly, these results emphasize the need for increased awareness, early diagnostic screening, and genetic counseling in both urban and rural populations of Misan. No national registry or population-based incidence study for HS in Iraq was identified. Local and regional reports indicate that HS represents a small fraction of inherited hemolytic disorders in the country: a descriptive study from Erbil reported spherocytosis among ≈2% of inherited hemoglobin/hemolytic disorders diagnosed at a regional center. Individual hospital reports and surgical series document only sporadic HS cases, suggesting that the true incidence is uncertain and HS may be under-recognized in some settings. These observations indicate a need for systematic surveillance or a national registry to obtain accurate incidence and prevalence estimates.[21]

Future studies with larger, multicenter cohorts are warranted to delineate better the epidemiology, clinical spectrum, and genetic variations of HS in Iraq.

Pathogenesis: Membrane Instability and Microparticle Formation

Molecular abnormalities in one or more genes encoding RBC membrane proteins disrupt the vertical interactions between the cytoskeleton and the phospholipid bilayer, compromising membrane stability and ultimately altering RBC morphology. In addition, horizontal interactions within the cytoskeletal network, particularly between spectrin and actin, may also be affected, further promoting the gradual loss of membrane fragments during circulation.[22] This progressive membrane loss reduces the surface area-to-volume ratio, resulting in the formation of rigid, less deformable spherocytes.

Due to their decreased deformability, spherocytes struggle to pass through the narrow interendothelial slits of the splenic cords. The spleen functions as a mechanical filter, retaining these abnormal erythrocytes, where they are subsequently phagocytosed by splenic macrophages, leading to premature destruction and chronic hemolysis.[22,23] Furthermore, the instability of the membrane, combined with oxidative stress, contributes to the release of microparticles (MPs) from erythrocytes.[17] MPs are small extracellular vesicles shed from blood cells in response to oxidative stress, cellular activation, or apoptosis and are considered indicators of ongoing membrane damage.[24] Table 1 demonstrated the risks and complications associated with HS.

Genetic Causes

Key genes involved in hereditary spherocytosis

Spherocytosis is a genetically heterogeneous erythrocyte membrane disorder predominantly caused by mutations in genes encoding structural proteins responsible for maintaining RBC membrane stability. Classic genetic studies indicate that mutations in *ANK1* (ankyrin-1) are the most frequently identified and account for approximately 50% of all cases, followed by mutations in SPTB (β-spectrin), EPB42 (protein 4.2), and SPTA1 (α-spectrin).[28] These genes collectively contribute to the integrity of vertical and horizontal membrane–cytoskeleton linkages. Table 2 summarizes the main genes associated with HS.

Population-specific mutation patterns

More recent investigations have shown that the mutation spectrum varies among different populations, highlighting the genetic diversity of the disease. In a Chinese cohort, mutations were most commonly detected in *ANK1* (46%), followed by *SPTB* (42%), *SLC4A1* (11%), and *SPTA1* (1%), with no mutations detected in *EPB42*.[29] Conversely, a European cohort demonstrated a different distribution – *SPTA1* was the most frequent mutation (36.5%), followed by *ANK1* (27.1%), *SPTB* (20.0%), *SLC4A1* (15.2%), and *EPB42* (1.2%).[30] The frequency of the mutated gene is displayed in Figure 1. These findings underscore the clinical importance of considering genetic background and ethnicity when evaluating patients or interpreting mutational spectra. Understanding these variations assists in optimizing diagnostic strategies and may guide future genotype–phenotype correlation studies.

The listed genes encode proteins crucial for both the structure and function of the erythrocyte membrane

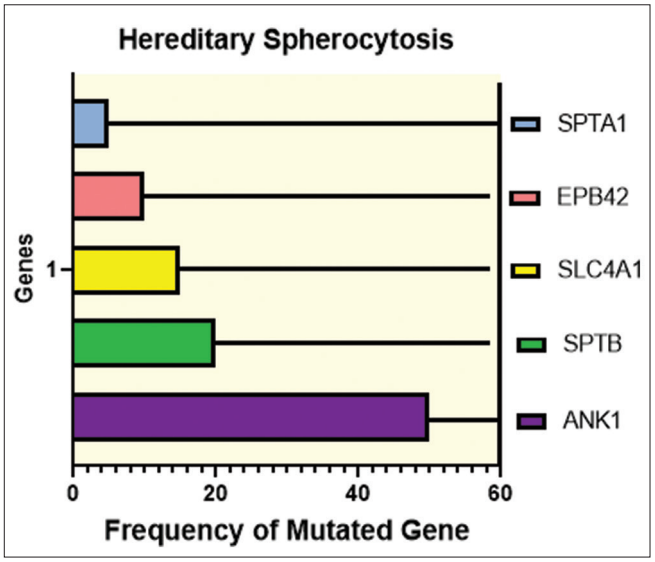

Figure 1: Frequency of mutated gene in hereditary spherocytosis, designed according to the current investigation using GraphPad Prism software (Dotmatics Company, San Diego, California, United States), according to previous statistics

Table 1: Risks and complications of hereditary spherocytosis

Risk factors	Description	References
Hemolytic anemia	Premature destruction of spherocytosis causes anemia and fatigue	[23]
Splenomegaly	Enlargement of the spleen due to increased removal of abnormal RBCs	[25]
Jaundice	Increased bilirubin levels from hemolysis cause yellowing of the skin and eyes	[26]
Gallstones	Formation of pigment gallstones due to chronic hemolysis and increased bilirubin secretion	[27]
Iron deficiency	May result from chronic hemolysis or blood loss related to disease or treatment	[26]
Fatigue and weakness	General symptoms related to anemia and decreased oxygen delivery to tissues	[26]

RBCs=Red blood cells

Table 2: Gene-related risk of hereditary spherocytosis

Gene	Variant type	Inheritance pattern	Clinical significance
ANK1	Missense (p.Asn32Ser)	AD	Associated with HS with variable severity (most common cause of HS)
SPTB	Missense (p.Arg4Gln)	AD	Associated with HS
SLC4A1	Frameshift (c. 238_242del)	AD Variable	Typically associated with SAO, which is often mild/asymptomatic (though SLC4A1 mutations in general cause HS in the AD pattern)
SPTA1	Splicing variant (Alpha-Lepra)	AR Complex	Causes severe HS primarily when inherited in compound heterozygosity, leading to severe α-spectrin deficiency
EPB42	Missense (p.Asp80Tyr)	AR	Associated with HS Type 2 (Protein 4.2 deficiency), typically following an AR pattern

AD=Autosomal dominant, AR=Autosomal recessive, HS=Hereditary Spherocytosis, SAO=Southeast Asian Ovalocytosis

Table 3: Genetic variants associated with erythrocyte membrane skeleton defects

Gene	Protein product	Role in the RBC membrane	Pathogenic mechanism
ANK1	Ankyrin-1	Major vertical anchor, linking the integral membrane protein Band 3 (encoded by SLC4A1) to the underlying spectrin skeleton	Loss of vertical support causes membrane loss and microspherocyte formation
SPTB	β-Spectrin	A component of the spectrin heterodimer (αβ spectrin) that forms the structural lattice (skeleton) lining the inner side of the membrane	Defects destabilize the entire membrane scaffold
SPTA1	α-Spectrin	The other component of the heterodimer of spectrin	Defects destabilize the entire membrane scaffold
SLC4A1	Band -3	An integral membrane protein and AE1 that is the primary binding site for ANK1	Disrupts the vertical linkage to the skeleton. The c. 238_242del variant listed is often associated with a form of dRTA in addition to HS
EPB42	Protein 4.2	Binds ANK1 to Band 3 and is crucial for stabilizing the ternary complex	Loss of stabilization leads to membrane loss and HS

dRTA=Distal renal tubular acidosis, ANK1=Ankyrin-1, AE1=Anion exchanger, HS=Hereditary spherocytosis, RBC=Red blood cell

skeleton, as explained in Table 3. Mutations in these genes disrupt the stability, shape, or elasticity of the RBCs, leading to their premature destruction (hemolysis), primarily in the spleen.

ANK1 gene

The *ANK1* gene, located on chromosome 8p11.2, encodes the protein ankyrin-1, which is expressed in erythrocytes, muscles, and brain cells. In erythrocytes, ankyrin-1 protein localizes to the plasma membrane, where it anchors the cytoskeleton to integral membrane proteins, particularly the spectrin, thereby maintaining the stability, structure, and flexibility of erythrocytes as they traverse narrow capillaries.[31] Structurally, ankyrin-1 consists of three major domains: an N-terminal membrane-binding domain containing the "band 3" connecting site, a C-terminal regulatory domain, and a central spectrin-binding domain harboring a domain of death.[19] By mediating high-affinity vertical linkages between the plasma membrane and the primary cytoskeleton of spectrin with band 3, ankyrin-1 represents one of the principal proteins essential for erythrocyte membrane stabilization.[32]

To date, at least 55 mutations in the *ANK1* gene have been reported in association with HS. These mutations include deletions, frameshifts, nonsense, and splice-site variants, with an autosomal dominant manner mostly inherited, though it has also been described as autosomal recessive inheritance.[33] Notably, nonsense mutations account for the majority of reported *ANK1* variants.[29] Functional studies have demonstrated that *ANK1* mutations increase erythrocyte osmotic fragility, destabilize ankyrin-1 protein structure, impair localization to the plasma membrane, and interfere with its interactions with β-spectrin (SPTB) and band 3 (SLC4A1) proteins.[34] In certain cases, *ANK1* mutations result in the production of a nonfunctional protein incapable of binding cytoskeletal partners such as spectrin, leading to cytoskeletal instability and abnormal erythrocyte morphology.[35] The resulting spherocytes are fragile, spherical red cells that are prematurely secluded and damaged in the spleen, causing the characteristic spherocytosis.[25]

The ankyrin-1 protein complex of erythrocytes also incorporates several additional membrane-associated components, including the band 3 chloride/bicarbonate exchanger, protein 4.2, glycophorins A and B (GPA and GPB), and Rhesus proteins – RhAG and RhCE, further highlighting its central role in RBC membrane architecture and function.[2]

ANK1 gene genomic location

This gene is mapped to the short arm of chromosome 8 at cytogenetic band 8p11.21, as consistently reported by the HUGO Gene Nomenclature Committee, NCBI Gene, and the Ensembl Genome Browser. This precise chromosomal localization underscores the gene's conserved genomic position across multiple authoritative databases, reflecting its fundamental biological role. *ANK1* encodes the ankyrin-1 protein, a key structural molecule of erythrocyte membrane

Figure 2: Genomic localization of *ANK1* on chromosome 8p11.21

cytoskeleton, which is also expressed in muscle and neural tissues [Figure 2].

SPTB gene

The β-spectrin protein, a product of the SPTB gene, constitutes a critical structural element of the cytoskeletal network situated beneath the erythrocyte plasma membrane. Spectrin and actin, as a structured net, mechanically give stability and shape to fragile cellular membranes. The periodic spectrin–actin cytoskeleton was first identified in erythrocytes. Mutations within the *SPTB* gene are linked to HS type 2, as they compromise the vital linkage of the lipid bilayer and the cytoskeletal proteins, a primary factor governing erythrocyte integrity, consequently inducing blood disorder in affected individuals. Such genetic alterations may end with the synthesis of a malfunctioning or a deficiency of the spectrin protein.[36]

The β-spectrin protein participates in forming the erythrocyte plasma membrane's cytoskeletal superstructure through its interactions with band 4.1 and actin. The chromosomal location of the *SPTB* gene is on 14q23.3. *SPTB* variants have been identified in various ethnic populations, including Korean,[1] northern European,[37] Chinese,[12] and Brazilian cohorts,[38] and are estimated to account for approximately 15%–30% of HS cases. Moreover, quantitative real-time PCR (qRT-PCR) assays have revealed attenuated messenger RNA expression levels of both *ANK1*and *SPTB* genes in mutant erythroblasts relative to normal control cells, underscoring the molecular impact of these mutations on gene expression and protein stability.

EPB42 gene

EPB42-associated HS (EPB42-HS) is a chronic nonimmune hemolytic anemia typically presenting with mild-to-moderate severity. Neonates at risk should be closely monitored during the 1st week of life, with regular assessment of serum bilirubin, hemoglobin (HGB), and ferritin concentrations, particularly in those requiring frequent transfusions or iron chelation therapy.[39] The prevalence of EPB42-HS varies considerably across populations; in Japan, it accounts for approximately 40%–50% of HS cases,[40] whereas in other populations, it represents 5% or fewer of cases.[25] Laboratory findings characteristically include low hemoglobin levels, reticulocytosis, and the appearance of spherocytes in blood smears, markedly reduced or nil haptoglobin, and mildly elevated osmotic fragility, leading to hemolysis in nearly 50% of erythrocytes.[39]

EPB42-associated HS exhibits an autosomal recessive inheritance pattern. When both parents are heterozygous carriers of an *EPB42* pathogenic variant, the progeny faces a 25% risk of being ill with, a 50% probability of being a clinically silent carrier, and a 25% likelihood of inheriting neither variant. Following the identification of a familial genetic mutation, carrier detection among at-risk relatives can be achieved through the confirmation of biallelic *EPB42* mutations.[39] Genetic testing strategies encompass sequence analysis of *EPB42* to identify small intragenic deletions, insertions, along with nonsense, missense, and splice-site variants. Furthermore, gene-targeted deletion and duplication analysis utilizing techniques such as long-range PCR, quantitative PCR, multiplex ligation-dependent probe amplification, or gene-targeted microarray is used to investigate single-exon deletions or duplications;[39] however, to date, the sole gross deletion reported is a (32-base-pair) deletion.[41]

Although numerous studies have characterized mutations in erythrocyte membrane genes such as *EPB42*, *ANK1*, and *SPTB*, their clinical significance often varies between populations.[29,42] Certain variants that are rare globally may be enriched in specific ethnic groups, influencing disease severity and diagnostic yield. Despite advances in molecular diagnostics, gaps remain in understanding genotype-phenotype correlations, particularly for rare or novel mutations, which can lead to underdiagnosis or misclassification.[43] Current laboratory and genetic tools, while valuable, do not capture all mutation types, highlighting the need for more comprehensive screening strategies. Furthermore, therapeutic research remains largely exploratory; emerging approaches such as gene-targeted therapies and personalized treatment strategies hold promise but have yet to be widely implemented.[44] Addressing these gaps requires population-specific studies, longitudinal follow-up, and integration of genomic data with clinical outcomes to optimize diagnosis, management, and future therapeutic development.

Conclusion

HS is a genetically heterogeneous, lifelong blood disorder primarily caused by mutations in erythrocyte

cytoskeletal genes, most commonly *ANK1* and *SPTB*. These mutations disrupt RBC membrane stability, resulting in hemolysis and a variable clinical spectrum ranging from asymptomatic carriers to severe anemia. Current diagnostic protocols, including NGS, are essential not only for precise diagnosis but also for understanding population-specific mutational patterns. Despite these advances, challenges remain in detecting rare or novel variants, underscoring the need for more comprehensive and accessible diagnostic strategies. Looking forward, ongoing progress in molecular characterization of HS may facilitate the development of gene-targeted therapies and personalized management approaches, including the potential use of CRISPR-based gene editing to correct pathogenic variants. Integration of genomic data with longitudinal clinical studies will be critical for optimizing patient outcomes, improving prognostic accuracy, and ultimately guiding the translation of innovative therapeutic strategies from research to clinical practice.

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Conflicts of interest
There are no conflicts of interest.

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