Molecular Genetic Mechanisms of Hereditary Spherocytosis: Current Perspectives

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Keywords

Erythrocyte membrane protein · Gene mutation · Hereditary spherocytosis

Abstract

With the widespread use of genetic diagnostic technologies, many novel mutations have been identified in hereditary spherocytosis (HS)-related genes, including *SPTA1*, *SPTB*, *ANK1*, *SLC4A1*, and *EPB42*. However, mutations in HS-related genes are dispersed and nonspecific in the diagnosis of some HS patients, indicating significant heterogeneity in the molecular deficiency of HS. It is necessary to provide the molecular and genetic characteristics of these 5 genes for clinicians to examine HS. Here, we reviewed the recent proposed molecular genetic mechanisms of HS.

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Introduction

Hereditary spherocytosis (HS) is a common type of hereditary hemolytic anemia, with wide heterogeneity in the severity of its clinical symptoms, membrane protein defects, and genetic patterns. HS is characterized by anemia, jaundice, splenomegaly, and cholelithiasis. In the primary pathogenic mechanism of HS, dysregulation or

abnormal quantities of erythrocyte membrane protein can decrease the stability and deformability of erythrocytes and increase their osmotic fragility, causing them to break easily and cause hemolysis.

There are 5 genes associated with HS, including α -spectrin (*SPTA1*), β -spectrin (*SPTB*), ankyrin (*ANK1*), band 3 (*SLC4A1*), and protein 4.2 (*EPB42*), that are involved in the interaction between the erythrocyte membrane and the lipid bilayer. Mutations in ≥ 1 HS-related genes can cause membrane protein deficiency [1, 2], leading to HS. The mutations in these 5 genes differ between countries, so HS can easily be misdiagnosed and overlooked in the clinic [3–5].

We analyzed the pathogenic characteristics and genetic mechanisms of HS in the literature, focusing on its molecular genetics, in order to provide references for genetic counseling, prenatal diagnosis, and future research directions.

Molecular Genetic Mechanisms of Five HS-Related Genes

ANK1

Ankyrin-1, encoded by *ANK1*, is a major protein of erythrocytes, and it anchors transmembrane proteins to the cell membrane skeleton through band 3, spectrin, and

Fig. 1. Schematic presentation of erythrocyte membrane proteins. The erythrocyte membrane proteins regulating the elasticity and deformability in a vertical direction are band 3, ankyrin-1, protein 4.2, spectrin, and Rh complex. Those regulating the properties in a horizontal direction include spectrin, protein 4.1R, actin, adducin, tropomodulin, tropomyosin, and p55.

protein 4.2 (Fig. 1). More than 60 mutations have been identified in *ANK1* in HS patients, including 7 missense mutations [2]. However, not all polymorphisms alter the expression or levels of *ANK1*.

Park et al. [6] verified that heterozygous *ANK1* mutations are the most common mutations in these 5 HS-related genes, accounting for 52% of all Korean HS patients; the mutation types are primarily frameshift, nonsense, and splice site mutations. *ANK1* heterozygous mutations account for approximately 31% of all Japanese HS patients [7], suggesting that the proportions of heterozygous mutations in *ANK1* differ in HS patients between countries. Gallagher et al. [8] reported that the same site in exon 14 of *ANK1* was mutated in 3 HS patients from different countries and genetic backgrounds, causing a frameshift mutation and premature chain termination. The resulting mutant protein is called Ankyrin Flori-anópolis and is associated with severe HS. This mutation has been helpful in studying the molecular genetic mechanisms of HS, but no new cases at this site have been reported.

Approximately half of all HS patients are associated with mutations in *ANK1* [9]. The genetic pattern of most HS patients with *ANK1* mutations is autosomal domi-

nant, but autosomal recessive inheritance has occurred in several such patients. Mutations in *ANK1* are unique to an individual or an entire kindred [2, 10, 11], and common mutation sites in a family can be inherited. Miya et al. [12] identified a 3.7-Mb deletion from 8p11.2 in a 19-month-old HS patient with Kallmann syndrome; the deleted region included *ANK1*, but no mutation was found in any exons of *ANK1*. It was presumed that HS was induced by haploinsufficiency of *ANK1*, demonstrating that there is a complex pathogenic relationship between *ANK1* and HS.

Many studies [13–17] have demonstrated that *ANK1* mutant mice have clinical phenotypes to those of humans with HS, and have confirmed that *ANK1* mutations play an important role in the pathogenesis of HS in mice. Several Chinese case reports have identified *ANK1* mutation sites [18–20]; these are sporadic and differ among cases.

SLC4A1

SLC4A1 encodes 2 protein isomers, the Cl⁻/HCO₃⁻-anionic exchange transporter 1 in erythrocytes (band 3/eAE1) and a truncated form of anionic exchange transporter 1, which is expressed in α -intercalated kidney cells (kAE1). A variety of mutations in human *SLC4A1* can

cause 2 diseases [21], HS and distal renal tubular acidosis, due to a loss of erythrocyte membrane deformability, or a deficiency in urine acidification, respectively. Band 3 deficiency is often inherited in a dominant manner, occurring in approximately 33% of HS patients [22]. The chief types of *SLC4A1* mutations are missense and frameshift.

Studies on the association between *SLC4A1* mutations and HS have reported that these occur primarily in exons, which can lead to truncated or unstable band 3, disrupting its function [23–25]. Moreover, mutations can arise in introns, resulting in abnormal mRNA processing or a premature termination codon [23–25]. *SLC4A1* mutations also cause distal renal tubular acidosis, which has been observed along with HS in the same patient [26, 27].

Chu et al. [28] found a TGC>TGG mutation in exon 13 and a GGC>GAC mutation in exon 17 of *SLC4A1* in an HS patient with distal renal tubular acidosis. The mutation in exon 13 was detected in his father and that in exon 17 in his mother. These findings indicate that these mutations in *SLC4A1* induce the combination of HS and distal renal tubular acidosis and also that the mutations are inherited.

There are only a few reports on *SLC4A1* mutations in HS patients in China. We studied an *SLC4A1* mutation in a family [29] but did not find any reported pathogenic mutations, such as c.1582C>T, c.2624C>T, c.2623C>G, and c.2623A>G, indicating that *SLC4A1* mutation sites are diverse and appear in other exons or introns. Another of our studies confirmed that the majority of HS patients in China suffer from band 3 deficiency, and we verified the existence of sporadic mutation sites in *SLC4A1* in HS patients from various families in exons and introns [30].

The mutation sites in *SLC4A1* vary in HS patients, but most are concentrated in exons. *SLC4A1* mutations are not specific to HS, so the presence of distal renal tubular acidosis, or a combination of this with HS should be considered. Even if there is a mutation in *SLC4A1*, the clinical data and other relevant laboratory results of patients must be analyzed further.

SPTA1

α -Spectrin, encoded by *SPTA1*, plays a major role in the shape and deformability of erythrocytes. α -Spectrin deficiency might cause HS, accounting for approximately 5% of HS patients. It is transmitted primarily by autosomal recessive inheritance, and mutations in *SPTA1* are mainly splice site mutations [22, 31, 32].

Wichterle et al. [33] found compound heterozygous mutations in 2 alleles of *SPTA1* in a single patient with

severe spherocytic hemolytic anemia but no family history of HS: α LEPRA (C→T at position 99 in intron 30, leading to a frameshift mutation and a significant decrease in α -spectrin synthesis) and α PRAGUE (A→G at position 2 in intron 36, leading to a frameshift mutation and truncated α -spectrin). It is presumed that α LEPRA is a widely distributed allele that is associated with the pathogenesis of HS and a lack of spectrin.

Delaunay et al. [34] identified compound heterozygous mutations in 2 alleles in a family in which the siblings had severe HS, α LEPRA (from the mother), and α LELY-Bicêtre (from the father; resulting in G→A at position 2377 in exon 51 of the lowly expressed polymorphic allele, α LELY, essentially a null mutation of a synthetic nonfunctional protein). The α -chain yield of α LEPRA and α LELY-Bicêtre is 16 and 0% of the normal amount, respectively. Thus, total α -spectrin synthesis reaches up to 8% of normal production. Only a reduction in α -spectrin expression to <25% of the normal yield can cause spherocytosis.

Bogardus et al. [35] found a C→T homozygous mutation in exon 19 of *SPTA1* in a severe transfusion-dependent HS proband; this mutation converts arginine 891 to stop codon. His mother was heterozygous for the R891X mutation. By array-based comparative genomic hybridization, uniparental disomy was partially identified from the mother on chromosome 1 of the proband, suggesting that this deleterious mutation leads to HS and raising the possibility that uniparental disomy is a pathogenic mechanism of HS.

Christensen et al. [36] verified that *SPTA1* mutations are also present in patients with hereditary elliptocytosis, reporting 2 new mutations. There have been no reports of mutations in *SPTA1* in HS patients in China.

α LEPRA mutations are common in HS patients with *SPTA1* mutations [22, 32], but the other new mutations in *SPTA1* continue to be reported, showing its heterogeneity and also indicating that such mutations are not unique to HS; they can be found in hereditary elliptocytosis. The interaction among mutations in the same gene can lead to a variety of clinical phenotypes, eliciting inconsistent phenotypes. Therefore, in the diagnosis of HS, we should combine clinical, erythrocyte morphological, biochemical, and genomic data.

SPTB

β -Spectrin, encoded by *SPTB*, plays an important role in the stability of the erythrocyte membrane. In normal human erythrocytes, α -spectrin is synthesized at rates 3–4 times that of β -spectrin. Therefore, a mutation in an

Table 1. Molecular and genetic characteristics of 5 erythrocyte membrane protein genes

Gene	Membrane protein	Prevalent mutations	Heredity	Associated disease
<i>ANK1</i>	Ankyrin-1	frameshift, nonsense, splicing, novel mutations	AD, AR	HS
<i>SLC4A1</i>	Band 3	missense, frameshift, polymorphism	AD	HS, dRTA
<i>SPTA1</i>	α -Spectrin	Sp α LEPRA allele, splicing, frameshift	AR	HS, HE, HPP
<i>SPTB</i>	β -Spectrin	splicing, frameshift, nonsense, novel mutations	AD	HS, HE, HPP
<i>EPB42</i>	Protein 4.2	missense, nonsense	AR	HS

AD, autosomal dominant; AR, autosomal recessive; dRTA, distal renal tubular acidosis; LEPRA, low-expression allele Prague; HE, hereditary elliptocytosis; HPP, hereditary pyropoikilocytosis.

allele of β -spectrin can lead to erythrocytosis; if both alleles are mutated, the incidence of HS increases [22]. β -spectrin deficiency is usually inherited in an autosomal dominant manner, accounting for approximately 15–30% of HS patients in the northern European population [22]. Heterozygous mutations in *SPTB* represent approximately 25% of HS patients in the USA and Europe [37]. The common mutation types in *SPTB* include splice site, frameshift, and nonsense mutations, often leading to defects in mRNA processing and truncated β -spectrin [38].

Previous studies [6, 36, 38] have shown that *SPTB* mutations are associated with HS, but findings vary with regard to the site of the mutation. In families with HS, researchers have found a common mutation site in *SPTB* that is specific to each family, indicating that this site is associated with the pathogenesis of HS and is inherited. Garbarz et al. [39] detected G→C in exon 3 of *SPTB* in all members of a dominant HS family; this mutation can severely reduce the utilization of the 5' splice site, causing aberrant splicing of mRNA with intron 3 retention and ultimately decreasing β -spectrin mRNA levels. Boguslawska et al. [40] found a novel heterozygous mutation, 466insG, in exon 11 of *SPTB*, in all members of a Polish HS family, and they concluded that *SPTB* undergoes monoallelic expression, resulting in spectrin deficiency. Mutations in *SPTB* have also been identified in Chinese HS patients [19], but there are no reports on any of them, suggesting that they are novel and specific to HS patients.

EPB42

Protein 4.2, encoded by *EPB42*, is a key protein of the erythrocyte membrane and an ATP-binding protein that regulates the relationship between band 3 and ankyrin; it also has an important role in the regulation of the shape and mechanical properties of erythrocytes. Protein 4.2 deficiency is primarily recessive. Genetic variations in

EPB42 are primarily missense and nonsense mutations (Table 1). *EPB42* mutations are generally found in the Japanese population [32]. They generate variants in protein 4.2, including protein 4.2 Lisboa (265delG), protein 4.2 Komatsu (D175Y), protein 4.2 Nippon (A142T), and protein 4.2 Shiga (R317C). They cause protein 4.2 deficiency and are strongly associated with HS.

EPB42 mutation-induced HS has also been reported in other populations. Beauchamp-Nicoud et al. [41] detected 949delG in exon 7 of *EPB42* of a gypsy; it produces protein 4.2 Nancy and is associated with HS. Bruce et al. [42] reported a 41-bp frameshift loss in protein 4.2 cDNA of a Pakistani HS proband, probably leading to the truncated protein 4.2 hammersmith. By conducting genomic DNA sequencing, they found a homozygous mutation, 1747G>T, in exon 11 of *EPB42*, and the proband's mother was heterozygous for mutations. Maciag et al. [43] observed that the relative mRNA levels of *EPB42* were decreased by approximately 45% in a Polish α -thalassemia patient with a protein 4.2 mutation. They identified 2 mutations by sequencing: G1701A in exon 10 *EPB42*, leading to an A567T mutation, and IVS2nt+6T>A in *EPB42*, decreasing splice site activity and leading to mRNA instability.

EPB42 mutations in HS patients are rare in China. We found a single-nucleotide polymorphism (SNP), c.329C>T (Ser 10 Leu), in exon 1 of *EPB42* in an HS patient and in his mother, as well as other mutations in *ANK1* [18]. SNPs may affect the stability of a protein or its mRNA levels [44], suggesting that the SNP in *EPB42* is involved in the pathogenesis of HS.

Thus, mutations in *EPB42* are linked to HS. *EPB42* mutation-induced HS has been reported in several countries, but the mutation sites vary. Multiple mutation sites in the same gene or simultaneous mutations in several HS-related genes can produce complex molecular genetic mechanisms in HS.

Summary and Prospects

HS is a common type of hereditary hemolytic anemia. The prevalence of HS in North America and northern Europe is 1/2,000 [22, 32]. Wang et al. [45] measured the prevalence of HS in China from 1978 to 2013 using Dis-Mod-II software; it was 1.27/100,000 in males and 1.49/100,000 in females. Historically, experimental methods, such as peripheral blood smear, red blood cell osmotic fragility test, acidified glycerin hemolysis test, and SDS-PAGE, have had low sensitivity and specificity. HS has therefore been easily missed, and patients have been diagnosed as having G6PD deficiency or another type of autoimmune hemolytic anemia [4]. These data in China need to be confirmed. In the USA and Europe, HS patients primarily bear *ANK1* mutations, accounting for 40–65% of all HS patients; in Japan, HS patients generally harbor *EPB42* mutations, representing 40–50% of all patients with HS [22]; and in South Korea, most HS patients (52%) have *ANK1* mutations [6].

Erythrocyte morphology, a blood analyzer, or SDS-PAGE are used to diagnose sporadic HS in China. In recent years, HS-related gene mutation sites have been reported, but these sites are sporadic and diverse. We found that most Chinese HS patients are affected by band 3 deficiency [30], but because of the small number of reported cases, it is necessary to collect more data to determine the actual rates of erythrocyte membrane protein deficiency in Chinese HS patients.

The guidelines for the diagnosis and management of HS state that *ANK1*, *SLC4A1*, or *SPTB* mutations are common in autosomal dominant HS [32]. New mutations in *ANK1* and *SPTB* can be found in most recessive HS patients [32]. Therefore, research on HS continues to face new challenges. Analyzing reported HS cases and their molecular genetic mechanisms can provide the basis for genetic counseling, prenatal and postnatal care, prenatal diagnosis, and future research into HS.

With the rapid development and wide application of gene diagnostic technologies, the detection rate of HS cases is increasing. Sequencing results have shown that ≥ 1 of the genes that we have discussed, i.e., *SPTA1*, *SPTB*, *ANK1*, *SLC4A1*, and *EPB42*, are mutated. Most of these novel mutations occur in exons, but several arise in introns, suggesting that intronic mutations also play an important role in the pathogenesis of HS. However, there is no hotspot mutation in HS; most mutations are sporadic and specific to individual patients or their families.

A single heterozygous (or homozygous) or compound heterozygous mutation has occurred in the same gene in

HS patients, and mutations in ≥ 2 related genes can also develop. The mutation sites therefore present with tremendous heterogeneity. In some cases, the synergistic or inhibitory effects of multiple mutations in related genes can lead to a complex pathogenesis of HS. HS, combined with other diseases, such as β -thalassemia and Gilbert syndrome [46, 47], can lead to inconsistent HS genotypes and phenotypes, causing high rates of missed diagnoses or even misdiagnoses in clinical HS cases.

The guidelines for the diagnosis and management of HS suggest that HS can be diagnosed using molecular detection of related genes [32]; no further examination is required. This approach may be suitable for some HS patients with mild clinical symptoms and can be considered a new standard for HS diagnosis. Nevertheless, recent studies have verified that mutations in *SLC4A1* and *SPTA1* are not specific to HS patients [4, 48, 49]. To identify different types of hemolytic anemia and hepatitis, clinical histories and laboratory tests are needed [3, 4, 48–51]. Ribeiro et al. [52] reported that, in the case of some mutations that can be inherited and lead to severe HS, genetic counseling and prenatal diagnosis can play an extremely important role in prenatal and postnatal care. Thus, if ≥ 2 members of a family with HS are found to have the same mutation in the same gene, this suggests that the mutation site is hereditary. This is important when studying the molecular genetic mechanisms of HS and assessing the risk of HS for other members or descendants of the family, and can guide the future of precision medical care.

Acknowledgments

This study was funded by the National Natural Science Foundation of China, grant No. 81360263.

Disclosure Statement

We declare that we have no conflicts of interest.

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