Hereditary Elliptocytosis: Spectrin and Protein 4.1R

Patrick G. Gallagher

Hereditary elliptocytosis (HE) is a common disorder of erythrocyte shape, occurring especially in individuals of African and Mediterranean ancestry, presumably because elliptocytes confer some resistance to malaria. The principle lesion in HE is mechanical weakness or fragility of the erythrocyte membrane skeleton due to defects in α-spectrin, β-spectrin, or protein 4.1. Numerous mutations have been described in the genes encoding these proteins, including point mutations, gene deletions and insertions, and mRNA processing defects. Several mutations have been identified in a number of individuals on the same genetic background, suggesting a "founder effect." The majority of HE patients are asymptomatic, but some may experience hemolytic anemia, splenomegaly, and intermittent jaundice.

HEREDITARY elliptocytosis (HE) is a group of disorders characterized by the presence of elliptical-shaped erythrocytes on peripheral blood smear. HE and its related disorders are characterized by clinical, biochemical, and genetic heterogeneity. Manifestations range from the asymptomatic carrier state to severe, transfusion-dependent hemolytic anemia. Abnormalities of various membrane protein defects contribute to mechanical defects of the erythrocyte membrane skeleton.

Prevalence

HE has a worldwide distribution but it is more common in areas of endemic malaria, particularly in people of African and Mediterranean ancestry. In parts of Africa, the prevalence has been estimated between 0.6 and 3%.[1-3] In the United States, HE occurs in one in 2,000 to 4,000 individuals.[4,5] The true incidence of HE is unknown as its clinical severity is variable and many patients are asymptomatic.

Inheritance and Genetics

HE is inherited in an autosomal dominant fashion, with only rare instances of de novo mutation.[6-11] Typically, individuals heterozygous for an elliptocytocytic variant have asymptomatic elliptocytosis. Individuals homozygous or compound heterozygous for HE variants experience mild to severe hemolysis with moderate to marked anemia. Variation in severity among families and in individuals of the same family has been attributed to different molecular lesions and/or modifier alleles or to other defects that alter clinical expression.[12-15] In one kindred with Alport syndrome, mental retardation, midface hypoplasia, and elliptocytosis, inheritance was X-linked and associated with a contiguous gene syndrome due to a submicroscopic chromosome X deletion.[16]

Genetically, HE is heterogeneous with multiple genetic loci.[17,18] A wide variety of mutations have been described in the α-spectrin, β-spectrin, protein 4.1, and glycophorin C genes; these include point mutations, gene deletions and insertions, and mRNA processing defects. Several mutations have been identified in many individuals on the same genetic background.[3,19,20] Genetic haplotyping studies suggest that these common mutations may have a "founder effect" with origins in central Africa, similar to that attributed to hemoglobin S, Benin-type. These observations support the hypothesis that there has been genetic selection for elliptocytosis, as these red cells confer some resistance to malaria.

Pathophysiology

The principle defect in HE is mechanical weakness or fragility of the erythrocyte membrane skeleton, unsurprising due to qualitative and quantitative defects in several membrane skeleton proteins, α-spectrin, β-spectrin, protein 4.1, and glycophorin C, identified in HE patients.[17]

The majority of HE-associated defects occur in spectrin, the primary structural protein of the erythrocyte membrane skeleton. Spectrin is composed of two homologous nonidentical proteins, α- and β-spectrin, encoded by separate genes.[21-23] Both α- and β-spectrin are composed primarily of triple helical repeats connected by nonhelical segments. The proteins assemble side to side in an antiparallel position, forming a flexible, rod-like $\alpha\beta$ heterodimer in which the NH_2-terminus of α-spectrin and the

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Supported in part by grants from the National Institutes of Health and the March of Dimes Birth Defects Foundation.

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0037-1963/04/4102-0003$30.00/0
doi:10.1053/j.seminhematol.2004.01.003

COOH-terminus of β-spectrin form the head region of the heterodimer.[24-32] The αβ spectrin heterodimers self-associate head-to-head to make spectrin tetramers,[33-44] which are interconnected into a highly ordered two-dimensional lattice through binding, at their tail ends, to actin oligomers at the junctional complex, facilitated by protein 4.1.[45-53] Spectrin tetramers and higher order oligomers are critical for erythrocyte membrane stability, as well as for erythrocyte shape and function.[39,54,55] Local dissociation and reassociation of tetramers and dimers, respectively, may provide the membrane the ability to accommodate the distortions required to negotiate passage through the microvasculature.[33]

Defects that weaken or disrupt the interactions involved in the self-association of spectrin dimers into tetramers and oligomers, or structural and functional defects of protein 4.1 that interrupt spectrin-actin interactions in the spectrin/protein 4.1/protein 4.2/p55 junctional complex, disturb the integrity of the membrane skeleton.[56,57] Ultrastructural examination of the elliptocyte membrane skeleton reveals alteration of the normally uniform hexagonal lattice. Consequently, membrane skeletons, cell membranes, and erythrocytes are mechanically unstable, leading to red cell fragmentation and hemolysis.

An emerging concept is the influence of spectrin mutations outside the αβ-spectrin self-association contact site on membrane structure and function; recent evidence suggests that spectrin self-association, spectrin-ankyrin binding, and ankyrin-band 3 binding are coupled in a positively cooperative manner.[58] Three types of spectrin mutations that perturb this cooperative coupling between self-association and other membrane protein interactions have been proposed: (1) mutations of linker sequences joining helices C and A that block repeat-to-repeat transfer of conformational information between repeats; (2) mutations in α-spectrin repeats 4 to 6 that diminish the ability of this region to *trans*-regulate ankyrin binding by the adjacent β-spectrin repeats 14 and 15; and (3) truncating mutations that cause repeats 4 to 6 to fall out of register with the ankyrin binding region of β-spectrin. A significant number of HE/hereditary pyropoikilocytosis (HPP) mutations associated with impaired spectrin self-association are located outside the self-association contact site in repeats 2, 3, 4, and 5, and a few are in repeats 8 and 9. The majority of these mutations are located in linker sequences joining helices C and A. Mutations in repeats 4 to 6 are less common; they not only exhibit decreased spectrin self-association but also defective ankyrin binding. Spectrin$^{St.\ Claude}$, an in-frame deletion in repeat 9 that exhibits abnormal spectrin self-association and altered spectrin-ankyrin binding, is an example of a truncating mutation predicted to shift the register of α-spectrin repeats 4 to 6 and to disturb the ankyrin binding region of β-spectrin.[59,60] The pathologic consequences of truncating mutations may be generalizable to other large, α-helical repeat proteins. For example, truncating mutations of type I collagen shift the register of collagen α-chains across the entire molecule, impairing incorporation of mutant helices into fibrils and extracellular matrix, leading to severe or lethal osteogenesis imperfecta.[61]

HE membranes, which are less tolerant to shear stress, are likely to undergo permanent deformation.[62,63] Abnormal membrane skeletal interactions facilitate a shear stress-induced rearrangement of skeletal proteins after prolonged or repetitive cellular deformation that precludes recovery of the normal biconcave shape. This pathophysiology is consistent with the observation that HE red blood cell precursors are round, gradually becoming more elliptical with aging after release into the circulation.[5,64,65]

Molecular Defects of Spectrin and Protein 4.1R

Studies of erythrocytes and erythrocyte membranes from patients with elliptocytosis syndromes before cloning and determination of the primary structure of the principle erythrocyte membrane proteins focused on biochemical and mechanical properties of the membrane and its skeleton.

Electrophoretic Separation of Solubilized Membrane Proteins

One-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) may reveal qualitative or quantitative defects in erythrocyte membrane proteins, observations which may be confirmed by Western blotting. In cases of HE and HPP, SDS-PAGE has identified shortened α- and β-spectrin chains, shortened and elongated protein 4.1, and both spectrin and protein 4.1 deficiency.

Ektacytometry

The ektacytometer is an instrument that examines erythrocyte membrane deformability and stability.[66-68] Isolated red blood cell ghosts are subjected to a high shear stress in a laser diffraction viscometer, and the "deformability index" (DI; a measure of the average elongation of the sheared ghosts) is recorded as a function of time. Fragile ghosts fragment more quickly than normal, causing their DI to fall. The ektacytometer can also be used to measure deformability at different osmolalities.[69,70] The resulting curves rely on cell volume and membrane surface area and are a sensitive measure of the membrane surface loss accompanying many membrane skeletal defects.

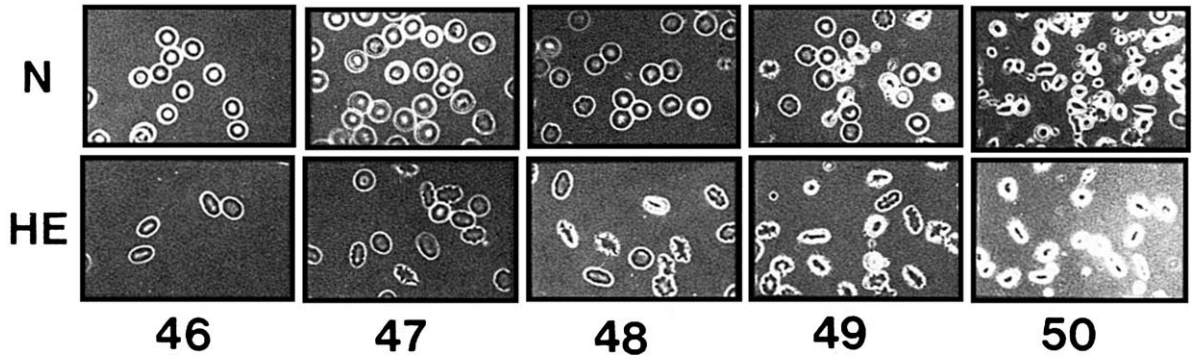

Figure 1. Effects of heat on the morphology of normal (N) and HE red blood cells. Crenation and membrane budding are first evident in HE red cells at 47° to 48°C but do not appear in normal cells until they are heated to 49°C. (Reprinted with permission.[186])

Thermal Sensitivity of Red Blood Cells and Spectrin

Normal spectrin denatures at 49°C and normal red blood cells fragment spontaneously at the same temperature, almost certainly due to spectrin denaturation.[71-73] Some HE and almost all HPP patients have thermally sensitive red blood cells, fragmenting between 44 and 48°C (Fig 1). Spectrin isolated from these red blood cells is also heat-sensitive. The molecular basis of this thermal sensitivity is unknown.

In Vitro Studies of Spectrin Self-Association

Self-association of spectrin dimers into tetramers is an interaction critical for normal membrane structure and function. Due to mutations affecting spectrin self-association, many HE and most HPP patients are unable to convert spectrin dimers to tetramers and higher oligomers in vitro or on the membrane.[74-76] Tetramer formation is readily assessed in vitro (Fig 2). Spectrin dimer-tetramer interconversion has a high activation energy and it is kinetically immobilized at approximately 0°C. As a result, the percentage of spectrin dimers and tetramers in 0°C crude spectrin extract reflects their relative distribution in the red cell membrane in vivo. Mutations in or near the $\alpha\beta$-spectrin heterodimer self-association site lead to an increase in the fraction of dimeric spectrin in the crude 0°C spectrin extract. A significant amount of unassembled dimeric spectrin correlates well with clinical severity and predicts unusually severe mutations.[13]

Tryptic Peptide Mapping of Spectrin

Limited tryptic digestion of spectrin extracted from erythrocytes followed by SDS-PAGE with or without isoelectric focusing produces a characteristic, reproducible map with five major proteolytically resistant domains of α-spectrin and four proteolytically resistant domains of β-spectrin.[41,77-79] An 80-kd αI-domain peptide encodes the NH_2-terminus of α-spectrin, the region that interacts with the COOH-terminus of β-spectrin to form the binding site for spectrin self-association. Most spectrin mutations are located in the 80-kd αI-domain peptide and yield maps containing one or more abnormal tryptic peptides that are fragments of this domain (Fig 3). The cleavage sites of the most common abnormal tryptic peptides reside in the third helix, helix C, of a given triple helical repetitive segment.[57,80] The corresponding mutations are at or near the cleavage sites either in the same helix or, less commonly, in helix A or B of a given repetitive segment. HE mutations near the COOH-terminus of β-spectrin may also alter tryptic cleavage of the interacting 80-kd αI-domain peptide.

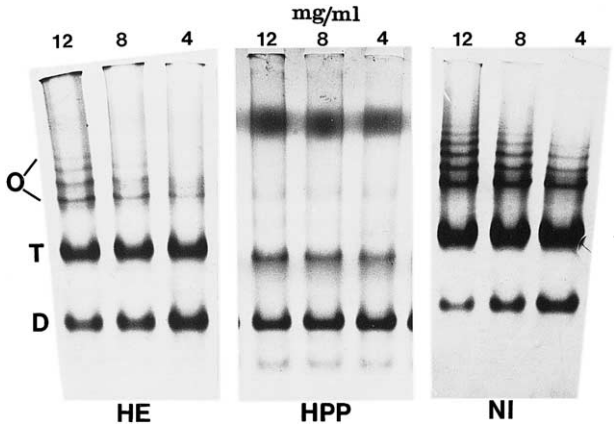

Figure 2. Association of spectrin dimers into tetramers and higher order oligomers. Low ionic strength spectrin extracts from erythrocytes of HE and HPP patients and normal controls (NI) were concentrated to 12.5, 8, and 4 mg/mL, equilibrated at 30°C for 3 hours, and subjected to nondenaturing gel electrophoresis. Reduced formation of tetramers (T) and oligomers (O) at each concentration of spectrin is seen in the HPP patient. Spectrin from the HE patient shows intermediate effects between HPP and normal control. The diffuse band at the top of the HPP patient lanes is hemoglobin. (Reprinted with permission.[74])

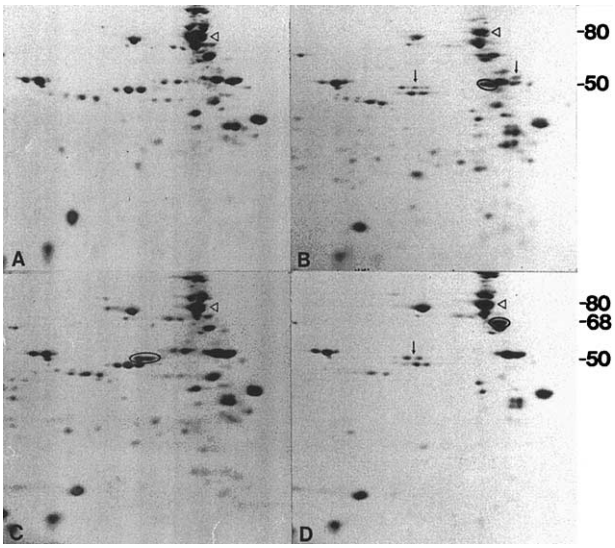

Figure 3. Two-dimensional peptide maps after partial trypsin digestion of normal and HE spectrin. The numbers on the right indicate apparent molecular weight in kilodaltons. The 80-kd αI-domain peptide is denoted by the open triangle. (A) Spectrin from a normal control. (B to D) Spectrin from 3 unrelated HE individuals with abnormal αI peptides denoted by molecular weight and migration: 50a (B), 50b (C), and 68 (D). The small arrows in (B) and (D) point to polymorphic αII- and αIII-domain peptides unrelated to the disorder. (From Marchesi SL, et al. Abnormal spectrin in hereditary elliptocytosis. *Blood* 1986;67:141-151. © American Society of Hematology, used with permission.)

Genetic Defects

Cloning and sequencing of the spectrin and protein 4.1 genes and knowledge of their primary structure allowed for determination of the precise genetic defect in individuals with elliptocytosis syndromes. In most cases, nucleotide sequence analysis of amplified genomic DNA isolated from peripheral blood leukocytes or reverse-transcribed reticulocyte or bone marrow mRNA identified the causative mutation. In some geographic regions where common elliptocytogenic mutations have been identified, multiplex polymerase chain reaction (PCR) techniques have been developed to rapidly screen for HE mutations.

Spectrin Self-Association Contact Site Mutations

The αβ-spectrin heterodimer self-association contact site between the opposed α- and β-spectrin chains is a combined "atypical" triple helical repeat in which two helices (helices A and B in the crystallographic structure of the repeat) are contributed by the COOH-terminus of β-spectrin, while the third helix is a portion of the NH_2-terminus of α-spectrin (helix C).[28,78,81] Confirmation of the existence and functional importance of this "atypical repeat," initially proposed by Tse and colleagues, has been provided by study of human mutants, biochemical studies of wild-type and mutant recombinant peptides, and molecular modeling.[36,37,40,41,82-85] Mutations of the αβ contact site, which produce the αI74 phenotype on spectrin tryptic mapping, markedly disrupt self-association and are typically the most severe mutations that cause HE/HPP.

α-Spectrin self-association contact site mutations. Mutations in the NH_2-terminal region of α-spectrin are among the most common defects in HE and HPP (Fig 4 and Table 1).[57] They are all missense mutations. One common in African-Americans, Arg28His, occurs at codon 28, a CpG dinucleotide, that is a "hot spot" for mutation.[14] Mutations in this region are commonly associated with spectrin deficiency and the presence of elliptocytes, poikilocytes, and microspherocytes on peripheral blood smear.[86] Patients homozygous for mutations in this region have severe hemolytic anemia.[12,13]

β-Spectrin self-association contact site mutations. Mutations in the COOH-terminal region of β-spectrin associated with HE and HPP are truncations or point mutations that disrupt the formation of the combined αβ atypical triple helical repeat and impair spectrin self-association (Fig 4 and Table 2). Truncation mutations, which delete one or more of

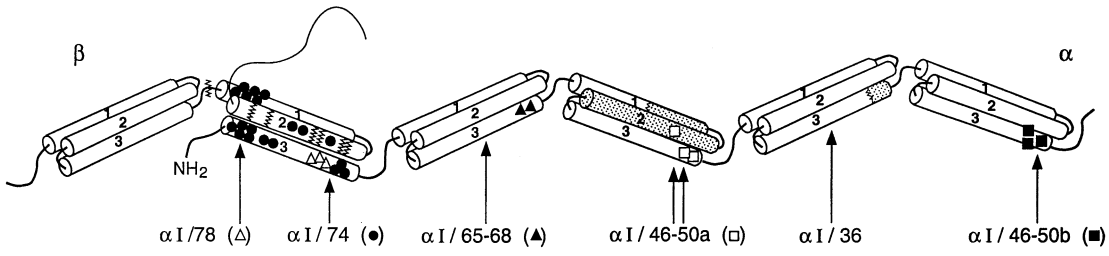

Figure 4. Triple helical model of the αβ-spectrin self-association contact site and four adjacent α-spectrin repeats. Symbols denote genetic defects identified in patients with HE or HPP. Limited tryptic digestion of spectrin followed by two-dimensional gel electrophoresis identifies abnormal cleavage sites (arrows) in spectrin associated with different mutations. (Reprinted with permission from Gallagher PC, Ferriera JD: Molecular basis of erythrocyte membrane disorders. Curr Opin Hematol 4:128-135, 1997.)

Table 1. α-Spectrin Mutations Associated With Hereditary Elliptocytosis and Hereditary Pyropoikilocytosis

Variant	Tryptic Phenotype	Systematic Name	Protein	Repeat-Helix	References
Lograno	αI/74	c71G	Ile24Ser	α1-C	290
Los Angeles	αI/74	c71C	Ile24Thr	α1-C	291
Corbeil	αI/74	c83T	Arg28His	α1-C	14,180
Unnamed	αI/74	c83T	Arg28Leu	α1-C	14,200
Unnamed	αI/74	c82A	Arg28Ser	α1-C	14,200
Unnamed	αI/74	c82T	Arg28Cys	α1-C	14
Marseille	αI/74	c92C	Val31Ala	α1-C	8
Genova	αI/74	c100T	Arg34Trp	α1-C	292
Tunis	αI/78	c121T	Arg41Trp	α1-C	169,170
Clichy	αI/78	c134T	Arg45Ser	α1-C	209
Anastasia	αI/78	c133C	Arg45Thr	α1-C	293
Culoz	αI/74	c137T	Gly46Va1	α1-C	294
Unnamed	αI/74	c142G	Lys48Arg	α1-C	200
Lyon	αI/74	c145T	Leu49Phe	α1-C	294
Ponte de Sôr	αI/65	c452A	Gly151Asp	α2-C	295
Unnamed	αI/65	c464TTG465ins	154Ins Leu	α2-C	98
Dayton	αI/50-46a	Insertion in intron 4*	178–226Del	α3-C	296
St. Louis	αI/50-46a	c620C	Leu207Pro	α3-B	20
Nigerian	αI/50-46a	c779C	Leu260Pro	α3-C	297
Unnamed	αI/50-46a	c781C	Ser261Pro	α3-C	297
Sfax	αI/36	c1086G†	362–371Del	α4-C	183
Alexandria	αI/50-46b	c1405-1407del	His469Del	α5-C	298
Barcelona	αI/50-46b	c1406C	His469Pro	α5-C	185
Unnamed	αI/50-46b	c1412C	Gln471Pro	α5-C	297
Jendouba	αII/31	c2473A	Asp791Glu	α7-C	101
Oran	αII/21	ivs 17-1A†	822–863Del	α8	99,100
St. Claude	αII/46	ivs 19-13 AT → TAG§	935–965Del	α9-B	59,60

*Skipping of exon 5.
†Creation of cryptic splice site, partial in-frame skipping of exon 8.
‡Skipping of exon 18.
§See text for details.

the β-spectrin phosphorylation sites, have included insertions, deletions, nonsense mutations, and exon skipping. Studies of missense mutations with recombinant mutant β-spectrin peptides correlate clinical severity with the degree of disruption of self-association in vitro.[37,83,87] Mutations in this region have been associated with variable clinical severity. In the homozygous state, they have been fatal or near fatal.[88,89] In some cases, spherocytic elliptocytosis has been prominent.[90] One interesting patient was a compound heterozygote for α- and β-spectrin self-association contact site mutations.[91]

Pathobiology of αβ-spectrin self-association contact site mutations. Determination of the structure of a spectrin repeat by biochemical, crystallographic, and spectroscopic techniques has provided insight into spectrin structure and led to the development of several models of spectrin flexibility.[25-27,37,83-85,92,93] Development followed of dynamic molecular modeling, which predicts the conformational rearrangements in the calculated repeat structure induced by HE/HPP-associated point mutations. This technique revealed that every HE/HPP-associated missense mutation of the αβ spectrin self-association contact site studied led to some degree of conformational change and disrupted interactions (such as salt bridges, hydrophobic interactions, and H-bonds) that stabilize the self-association unit (Fig 5).[85] This generalization held true for even conservative substitutions such as glycine for alanine, alanine for valine, and lysine for arginine. Reaffirmed by nuclear magnetic resonance studies, the various interactions between specific residues in the atypical repeat helices are critical to the self-association process. The predicted degree of structural deviation observed in modeling studies has correlated well with clinical severity.

α-Spectrin Defects in Repeats 2 to 5

Mutations in repeats 2 to 5 are heterogeneous and variable in severity. Typically, they are less severe than mutations of the self-association contact site (Fig 5 and Table 1). The 154InsLeu mutation in α-spectrin repeat 2 is widely distributed in blacks in

Table 2. β-Spectrin Mutations Associated With Hereditary Elliptocytosis and Hereditary Pyropoikilocytosis

Variant Name	Tryptic Phenotye	Systematic Name	Protein	Repeat-Helix	References
Cagllari	αI/74	c6052C	Ala2018Gly	β17-A	228
Kuwaitino	αI/74	c6052A	Ala2018Asp	β17-A	91
Providence	αI/74	c6055C	Ser2019Pro	β17-A	89
Paris	αI/74	c6068T	Ala2023Val	β17-A	290
Linguere	αI/74	c6070A	Trp2024Arg	β17-A	290
Buffalo	αI/74	c6074G	Leu2025Arg	β17-A	88
Tandil*	αI/74	c6124-6130del	PCT†	β17-B	299
Nice*	αI/74	c6136GA6137ins	PCT	β17-B	300,301
Kayes	αI/74	c6157C	Ala2053Pro	β17-B	81
Napoli*	αI/74	c6160-6167del	PCT	β17-B	302
Tokyo*	αI/74	c6177del	PCT	β17-B	303
Cotonou	αI/74	c6181A	Trp2061Arg	β17-B	3
Cosenza	αI/74	c6191C	Arg2064Pro	β17-B	304
Nagoya*	αI/74	c6205T	Glu2069X	β17-B	305
Prague*	αI/74	ivs 29 −1C†	PCT	β17-B	90
Göttinge*	αI/74	ivs 31 +2A†	PCT	β17-B	306,307
Le Puy*	αI/74	ivs 31 +4G†	PCT	β17-B	308,309
Rouen*	αI/74	ivs 32 +3T§	PCT	β17-B	310,311
Campinas*	αI/74	ivs 30 +1A†	PCT	β17-B	312

*Truncated β-chain detectable on gel.
†PCT, premature chain termination due to a frameshift.
‡Skipping of exon 30.
§Skipping of exon 31.

West, Central, and North Africa and in their descendants in the West Indies and North America,[19,94-98] and it has also been found in Arab populations. This mutation is associated with a mild clinical phenotype,[3] either typical elliptocytosis or the silent carrier state (see below). Homozygotes have only mild to moderate hemolysis. The frequency of this allele supports the hypothesis that there has been genetic selection for elliptocytosis, perhaps by providing some protection against malaria infection. In vitro, the growth of *Plasmodium falciparum* is inhibited in 154InsLeu erythrocytes. Spectrin$^{St.Louis}$, aLeu207Pro mutation in repeat 3, is particularly common in black populations. It is milder than the α-spectrin self-association contact site mutations but more severe than 154InsLeu.20 Unlike most α-spectrin HE/HPP mutations, spectrin$^{St.Louis}$ is located in helix B of the spectrin triple helical repeat.

Spectrin Defects Remote From the αβ-Spectrin Self-Association Site

Cases of HE and HPP have been associated with spectrin mutations remote from the αβ heterodimer self-association site in repeats 7, 8, and 9 of the αII domain. These mutations, spectrin^{Jendouba}, spectrin^{Oran}, and spectrin$^{St.\ Claude}$, are asymptomatic in the simple heterozygous state but cause HE or HPP in homozygous patients.[59,60,99-101] In vitro, they are associated abnormal tryptic maps of spectrin and defective spectrin self-association.

Spectrin^{Jendouba} ($\alpha^{II/31}$) is associated with asymptomatic HE, a mild defect in spectrin self-association, and abnormal tryptic cleavage after Lys^{788} due to an Asp-Glu mutation at codon 791.[101] Spectrin^{Oran} is due to a mutation of the acceptor splice site upstream of exon 18 (−1, a to g) that causes skipping of exon 18.[100] The in-frame deletion of codons 822 to 863 from the mature α-spectrin peptide probably changes the conformation of the αII domain, resulting in the abnormal pattern of tryptic digestion.

Spectrin$^{St.\ Claude}$ is due to a splice junction mutation that leads to two variant mRNA species. One species encodes a truncated α-spectrin chain that is not assembled on the membrane.[60] The other species encodes a protein that lacks exon 20 but is attached to the membrane and exhibits reduced spectrin-ankyrin binding.[59,60] Because α-spectrin is produced in excess, the heterozygous parents are clinically and biochemically asymptomatic. This variant was identified in 3% of asymptomatic individuals from Benin, Africa and in a Caucasian family of Afrikaans origin in South Africa, suggesting that it could be a production-defective allele in HPP patients. Spectrin$^{St.\ Claude}$ allele was not present in 18 African-American HPP patients heterozygous for structural mutations of α-spectrin.[102]

A large β-spectrin chain variant, spectrin^{Detroit}, with an estimated molecular weight of 330 kd, was isolated from a child with HE.[103] Further study of this patient and his family members demonstrated that

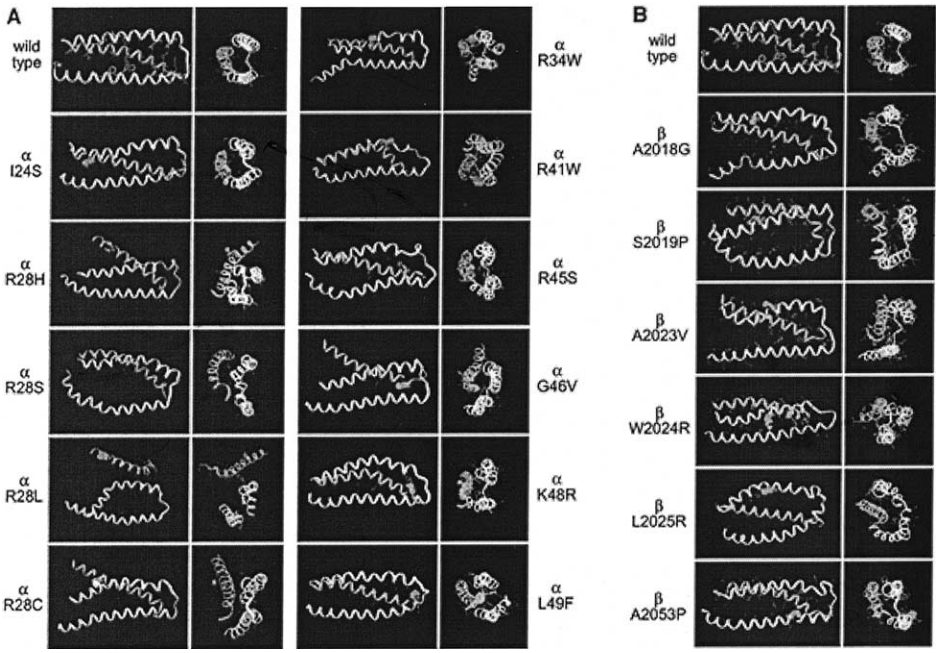

Figure 5. Molecular modeling of pathogenic mutations of the spectrin self-association contact site. Mutations involving (A) α-spectrin and (B) β-spectrin. Note the predicted disruption, sometimes severe, that accompanies the substitution of even a single native residue. Longitudinal and end-on views are shown for each mutation. The mutated residue is depicted in each case using a solid-filled representation. (From Zhang Z, et al. Dynamic molecular modeling of pathogenic mutations in the spectrin self-association domain. *Blood* 2001;98:1645-1653. © American Society of Hematology, used with permission.)

HE was caused by coinheritance of an α-spectrin mutation, 154InsLeu, and not by the β-spectrin abnormality. Family members heterozygous for the elongated β-spectrin without the α-spectrin mutation have normal erythrocytes and no clinical abnormalities, but their erythrocyte membranes are more rigid and fragile than normal. The fragility is probably a consequence of both weaker spectrin dimer association and spectrin deficiency. A similar kindred has been reported from Brazil.[104] The underlying molecular defect is unknown.

Low-Expression α-Spectrin Alleles

In many cases, HE/HPP patients heterozygous for a mutation of α-spectrin that perturbs normal spectrin self-association have an unexpectedly severe phenotype. These patients also have spectrin deficiency and have hemolytic HE, poikilocytic HE, or HPP. They may carry a second defect of β-spectrin that affects its production or accumulation. Parents who transmit the defect are clinically and biochemically normal. Studies to identify the second defect have focused on associated α-spectrin polymorphisms and of α-spectrin chain synthesis in vitro.

Alpha^{LELY}. The best-characterized low-expression allele of α-spectrin is α^{LELY} (low-expression Lyon). Initially discovered as an abnormality in tryptic digests of spectrin,[105] the α^{LELY} allele is defined by the two abnormalities linked in *cis*: (1) a C to T mutation at −12 of intron 45, which leads to partial (~50%) in-frame skipping of the 18-bp encoding exon 46, and (2) a Leu1857Val substitution in exon 40.[106,107] The α^{LELY} allele is common, found in approximately 20% to 30% of all individuals, with a worldwide distribution.[108] Deletion of the six amino acids in exon 46 disrupts the folding of α-spectrin repeat 21, which participates in αβ-spectrin nucleation,[29,109] thus inhibiting assembly of the shortened peptide into stable spectrin dimers and proteolysis. As a result, the α^{LELY} allele is associated with a 50% decrease in spectrin available for membrane assembly.

The α^{LELY} allele is clinically silent in normal individuals, even in the homozygous state, presumably because α-spectrin is synthesized in three- to fourfold excess.[110,111] HE patients heterozygous for mutations of α-spectrin on one allele and α^{LELY} *in trans* are typically more severely affected than anticipated.[57] The decreased amount of α^{LELY}-spectrin incorporated into the membrane increases the relative incorporation of spectrin containing the mutation *in trans*. Conversely, when the α^{LELY} allele is *in cis* to an α-spectrin mutation, it may ameliorate the elliptocytic phenotype. Despite these general observations,

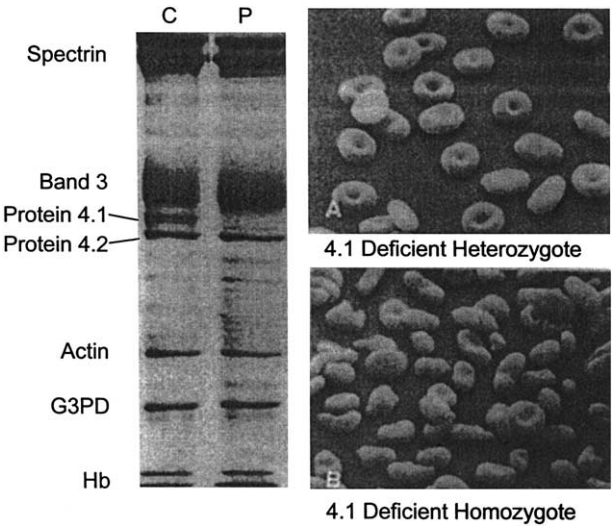

Figure 6. Deficiency of protein 4.1 in HE. Left: SDS-PAGE of red cell membrane proteins from a normal control (C) and a patient with homozygous HE who lacks protein 4.1 (P). Right: Scanning electron micrographs of red blood cells in 4.1-deficient patients. (A) Elliptocytes in a patient with heterozygous HE and 50% protein 4.1. (B) Elliptocytes, poikilocytes, and fragmented red cells in a patient with homozygous HE and no 4.1. (Reprinted with permission.[151])

in some cases, α^{LELY} *in trans* does not worsen the clinical severity or is only associated with an increase in the number of elliptocytes on peripheral blood smear.[112] In other severely affected patients, α^{LELY} is *in cis* to an α-spectrin mutation, suggesting the coinheritance of a non-α^{LELY} production-defective α-spectrin allele *in trans*.[113]

Clearly there are non-α^{LELY} thalassemia-like defects of α-spectrin synthesis that, when coinherited with α-spectrin mutations, produce a phenotype of hemolytic HE or HPP. These defects are characterized by reduced α-spectrin mRNA levels and diminished α-spectrin synthesis.[114,115] The molecular basis of this production-defective α-spectrin allele is unknown.

Protein 4.1R Mutations

Protein 4.1R

Protein 4.1R is a multifunctional protein that participates in the linkage of the spectrin-actin–based membrane skeleton to the lipid bilayer.[116-119] Protein 4.1R interacts with the membrane skeleton by linking a region of β-spectrin in the distal end of the spectrin $\alpha\beta$ heterodimer to actin, markedly increasing the binding of spectrin to oligomeric actin.[15,120-130] Protein 4.1R also interacts with the plasma membrane via interactions with glycophorin C, band 3, p55, phosphatidylinositol, and phosphatidylserine.

Protein 4.1R has many different cell type–, tissue-, and developmental stage-specific isoforms, including multiple erythroid cell isoforms, created primarily by alternate splicing and possibly by the use of tissue-specific promoters.[131-137] Alternate splicing at the 5′ end of the protein 4.1R mRNA generates isoforms with distinct NH_2-termini that utilize different translation initiation sites.[131,138] In early erythroid cells and in most nonerythroid tissues, the downstream initiator methionine is spliced out and the upstream methionine is utilized, creating a 135-kd protein 4.1R isoform. During erythropoiesis, developmentally regulated splicing switches to remove the upstream initiator methionine and to utilize a downstream initiator methionine,[132,134,135,139,140] leading to production of the 80-kd mature erythroid protein 4.1R isoform. Tissue-specific and erythroid differentiation-dependent alternate splicing in the region encoding the spectrin/actin binding domain also creates functionally important protein 4.1R isoforms.[140-145]

Protein 4.1R and Hereditary Elliptocytosis

Abnormalities of protein 4.1R are much less common than spectrin mutations in the etiology of HE. Partial deficiency of protein 4.1R is associated with mild, dominant HE, while complete deficiency leads to severe hemolytic disease. Quantitative and qualitative defects of protein 4.1R have been associated with HE.

Quantitative defects of protein 4.1R. HE and partial protein 4.1R deficiency, known as protein

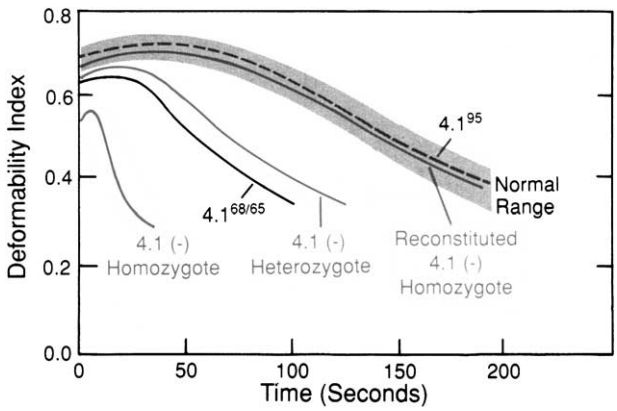

Figure 7. Erythrocyte membrane stability in defects of protein 4.1. Red cell membranes were subjected to shear stress in an ektacytometer and deformability was measured as a function of time. A fall in deformability occurred as the membranes fragmented. Cells completely lacking protein 4.1, (−/−), have a very fragile membranes, and normal fragility can be restored by reconstitution with normal protein 4.1. Heterozygous mutant cells (+/− and 68/65) have intermediate stability. (From Mohandas N, et al. A technique to detect reduced mechanical stability of red cell membranes: Relevance to elliptocytic disorders. *Blood* 1982;59: 768-774. © American Society of Hematology, used with permission.)

4.1R(−) trait, is a common cause of HE in some Arab and European populations.[10,146-149] Partial protein 4.1R deficiency occurs in heterozygotes that have mild HE with little or no hemolysis, prominent elliptocytosis, and minimal red blood cell fragmentation.

Complete protein 4.1R deficiency is associated with significant hemolytic anemia, which may require transfusions or even splenectomy.[20,150,151] Elliptocytes and fragmented poikilocytes are seen on peripheral smear (Fig 6). Erythrocytes completely lacking protein 4.1R are osmotically fragile with normal thermal stability. Their membranes fragment much more rapidly than normal at moderate sheer stresses,[66] indicative of their intrinsic instability (Fig 7). Membrane mechanical stability can be completely restored by reconstituting the deficient red blood cells with normal protein 4.1 or the protein 4.1/spectrin/actin binding site.[152] Protein 4.1–deficient membranes also lack protein p55 and are deficient in glycophorin C and D.[150,153-156]

The markedly altered skeletal network with disruption of the intramembrane particles on electron micrographs of erythrocyte membranes completely lacking protein 4.1R[157] indicates that protein 4.1R participates in the maintenance of the membrane skeletal network and the structure of the integral proteins.

Etiology of protein 4.1R deficiency. In the original protein 4.1R-deficient HE Algerian kindred, a 318-bp deletion removes the downstream translation initiation site.[158] In other families, point mutations of the downstream initiator methionine have been found.[150,159] Defects of the downstream translation initiation site utilized in reticulocytes result in erythrocyte protein 4.1R deficiency because the corresponding protein 4.1R mRNA is not translated. Expression of protein 4.1R is unimpaired in nonerythroid tissues and early erythroblasts because most of the protein 4.1R isoforms in these tissues initiate translation at the alternatively spliced, upstream methionine.[56,131,132,137,138]

Qualitative defects of protein 4.1R. Protein 4.1R variants with abnormal molecular weights have also been described in association with HE, primarily deletions or duplications of the exons encoding the spectrin-binding domain.[10,160-162] A truncated protein 4.1R was discovered in erythrocytes from a kindred with dominant, typical HE and mechanically unstable red blood cells (Fig 7).[10,160,161] The mutant protein 4.1R, which migrates as a doublet of 65 and 68 kd on SDS gels, is presumed to be nonfunctional. The genetic defect is the deletion of two exons encoding the spectrin/actin binding domain.

An elongated protein 4.1R, protein $4.1^{\text{Hurdle-Mills}}$, was found in the erythrocytes of a Scottish-Irish kindred with dominant, typical HE. The variant protein 4.1R migrates at approximately 95 kd due to duplication of three exons that include the spectrin/actin binding domain.[10,160,161] As erythrocyte membranes from affected patients have normal mechanical stability, it is uncertain how this mutant causes HE.

Mutations in the COOH-terminus of protein 4.1R associated with HE have been identified. These mutants are characterized by heterogeneity in clinical phenotype and degree of protein 4.1 deficiency.[162,163] In several of the variants, a truncated protein 4.1R assembled on the membrane, indicating that this region was not necessary for protein 4.1R membrane assembly in erythroid cells.[162] In other cases, the mutant protein 4.1R mRNA was unstable.

A large genomic deletion in the protein 4.1R gene was identified in a kindred with protein 4.1-deficient HE. A stable, truncated protein 4.1R mRNA was produced and unaltered tissue-specific alternative splicing was observed.[163]

Glycophorin C Defects

HE occurs in patients with glycophorin C deficiency. This subset of disorders is discussed in the review by Reid et al in this issue of *Seminars*.

Clinical Syndromes

The clinical presentation of HE is heterogeneous, ranging from asymptomatic carrier to severe, life-threatening anemia. The overwhelming majority of HE is asymptomatic, but approximately 12% of patients will become symptomatic from their anemia at sometime during their life. Typically, patients are only diagnosed incidentally. Asymptomatic carriers, with the same molecular defect as an affected HE relative, have normal peripheral blood smears. The erythrocyte lifespan is normal in most patients, and decreased in only about 10%. It is this subset of HE patients with decreased red cell lifespan who experience hemolysis, anemia, splenomegaly, and intermittent jaundice. Many of these patients have parents with typical HE and thus are homozygotes or compound heterozygotes for defects inherited from each of the parents. Symptomatology may vary among members of the same family, and indeed, in the same individual over time.

Classification

Most cases of HE can be classified into one of three categories: common HE, spherocytic HE, and Southeast Asian ovalocytosis (SAO). Common HE is further categorized based on clinical features. With the exception of SAO, which is homogeneous in molecular genetic terms (see below), these classifications denote clinical phenotypes and not specific molecular etiologies, although in some cases correlations do exist.

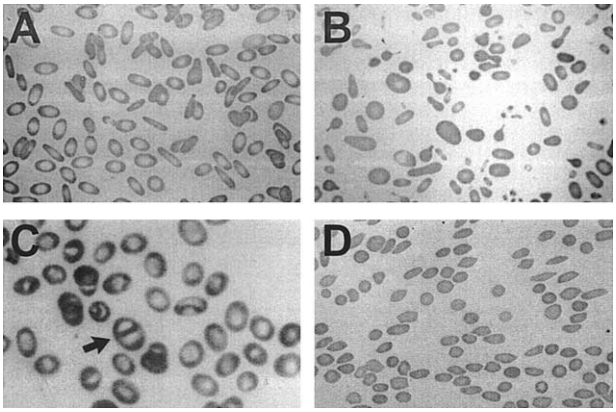

Figure 8. Peripheral blood smears. (A) Typical HE. Smooth, cigar-shaped elliptocytes are seen. (B) HPP. Pronounced microcytosis, poikilocytosis, fragmentation of erythrocytes, and elliptocytes are seen. (C) SAO. The majority of cells are oval, some of them containing either a longitudinal slit or a transverse ridge (arrow). (D) Pseudoelliptocytosis. An artifact of peripheral blood smear preparation, the long axes of pseudoelliptocytes are parallel, whereas the axes of true elliptocytes (A) are distributed randomly.

Common HE. Common HE is by far the most prevalent form of HE, particularly in African populations. The clinical characteristics vary enormously, defining several clinical subtypes detailed below. Of note, different members of the same family may exhibit different clinical patterns, and even single individuals may exhibit temporal variability in their signs and symptoms. Thus the clinical subtypes defined below are probably more useful for illustrating the spectrum of common HE than for classifying the disease.

Typical HE (also known as mild HE or heterozygous common HE). This is the most common clinical form of HE.[10,43,65,80,95,147,149,151,164-171] Patients are asymptomatic and are frequently not diagnosed until undergoing screening for an unrelated condition. Peripheal blood smears show prominent elliptocytosis, up to 100% of erythrocytes (Fig 8). There is no anemia and red blood cell survival may be normal.[43] A subset of typical HE patients exhibits very mild, compensated hemolysis with a slight reticulocytosis and a decreased haptoglobin level.[12,91,147,166]

Silent carrier state. The silent carrier state in HE was identified by study of asymptomatic members of HE kindreds. Erythrocyte morphology is normal and there is no hemolysis. In-depth studies identified thermal instability and decreased mechanical properties of isolated membranes, abnormal spectrin self-association, and abnormal tryptic peptide maps of spectrin in these asymptomatic individuals.[81,149,172-174] Some silent carriers have the same genetic defect as family members with typical HE, highlighting the clinical variability of the elliptocytosis syndromes.[174,175]

HE with sporadic hemolysis. Patients with common HE may develop uncompensated hemolysis in response to stimuli that cause hyperplasia of the reticuloendothelial system, particularly if the spleen is involved: examples include viral hepatitis, cirrhosis, infectious mononucleosis, bacterial infections, hypersplenism, and malaria.[167,176-178] Hemolysis has also been observed with disseminated intravascular coagulation and thrombotic thrombocytopenic purpura, in which presumably worsening hemolysis is due to microcirculatory damage superimposed on the underlying mechanical instability of the red blood cell membrane.[179] Elliptocytes may be particularly susceptible to microangiopathic damage. Development or worsening of hemolysis has also been described in pregnancy and vitamin B_{12} deficiency.

HE with chronic hemolysis. Some HE patients experience moderate to severe compensated hemolysis.[14,180-182] Elliptocytes, poikilocytes, and red blood cell fragments are present on blood smear. In some families, hemolytic HE has been transmitted through several generations. In others, some HE members experience chronic hemolysis and others do not, presumably due to modifier alleles. Splenectomy has been curative. The genetics of hemolytic HE are variable. Some patients are heterozygous for a spectrin mutation associated with severe disease.[14] Others have inherited the low expression allele, α^{LELY}, or an as yet undiscovered production-defective α-spectrin allele, *in trans*, worsening the clinical phenotype.[91,94,183-186] Other patients are homozygous or compound heterozygous for α-spectrin mutations, typically not associated with severe disease in the heterozygous state.[12,105]

Homozygous and compound heterozygous HE. Homozygotes or compound heterozygotes for HE-related spectrin mutations have been described.[60,81,88,89,99,100,187-197] Some patients experience moderate hemolysis (hemoglobin, 7 to 11 g/dL), and others suffer from severe, transfusion-dependent hemolytic anemia (hemoglobin, 2 to 6 g/dL) with marked fragmentation, poikilocytosis, spherocytosis, and elliptocytosis. The variable clinical severity is primarily due to the nature of the causative mutation but is also influenced by the degree of spectrin deficiency and the coinheritance of modifier alleles.[12,13,146] For example, patients homozygous for the mild α-spectrin Ins154Leu mutation experience mild to moderate hemolysis and rarely require therapy, whereas patients homozygous for the severe Arg28His or Leu206Pro mutations have moderate to severe hemolysis and frequently require splenectomy. In one interesting case, a patient with moderate HE inherited an α-spectrin self-association site mutation from his mother and a β-spectrin self-association site mutation from his father.[91] Peripheral blood smears of some homozygous HE patients contain

elliptocytes, poikilocytes, and microspherocytes. The laboratory findings and clinical course are indistinguishable from HPP (below), emphasizing the considerable overlap between these disorders.

Hereditary pyropoikilocytosis. HPP presents in infancy with neonatal jaundice or severe hemolytic anemia (hemoglobin, 4 to 8 g/dL) characterized by striking red cell findings.[12,198-204] Similar to the morphology of the blood smear in severe thermal burns, poikilocytes, red blood cell fragments, spherocytes, triangulocytes, and other bizarre-shaped cells are seen in HPP (Fig 8).[201,203,204] Elliptocytes are frequently present. Microspherocytosis is prominent and contributes to the characteristic microcytosis (mean corpuscular volume, 25 to 75 fL). Erythrocyte osmotic fragility is increased, particularly after incubation.[189,203] Thermal instability of red blood cells and spectrin, initially described as diagnostic of HPP203 as noted above is not unique to HPP but is also found in some patients with common HE. Spectrin deficiency is typically found in HPP erythrocytes but is uncommon in HE erythrocytes.[12,86,115] Most HPP cases have been of African origin, but HPP has also been found in Arabs and whites. There may be complications of severe anemia, including growth retardation, frontal bossing, marked splenomegaly, and early gallbladder disease.[202,203] Splenectomy is palliative, significantly decreasing but not eliminating hemolysis[12,202,203]; postsplenectomy, the hemoglobin typically ranges from 10 to 14 g/dL with 3% to 10% reticulocytes.[12]

Although HPP was initially described as a recessive disorder, its genetics suggest that patients typically fall into one of three categories: (1) homozygotes for a structural variant of spectrin, typically located in the region of spectrin $\alpha\beta$ heterodimer self-association, (2) compound heterozygotes for structural variants of the self-association site, and (3) heterozygotes for a single structural variant of spectrin self-association who possess a second, unknown defect that contributes to spectrin deficiency. This third group has a production-defective or "thalassemia-like" α-spectrin allele *in trans* to the structural variant. Because α-spectrin chains are normally produced in excess, the parent transmitting the production-defective allele is clinically and biochemically normal. The molecular basis of this production-defective allele is unknown.

Subtypes of HE and HPP show considerable overlap in clinical, biochemical, and genetic abnormalities.[13,115] HPP is clinically and morphologically similar to the homozygous and compound heterozygous subtypes of HE. In some HPP patients, a parent or sibling has typical HE, and in some kindreds, the identical spectrin mutation, Arg28His, has been detected in siblings with phenotypically different disorders, typical HE and HPP. In other HPP kindreds, all first-degree relatives are phenotypically normal.

HE with infantile poikilocytosis. Infants born to a parent with typical HE may experience neonatal hemolytic anemia and jaundice.[12,80,173,201,204-209] The peripheral blood smear reveals poikilocytosis, red blood cell fragmentation, pyknocytosis, and in many cases, elliptocytosis. Phototherapy, packed red blood cell transfusion, or even exchange transfusion may be required. Neonatal HE can easily be distinguished by family history or detection of elliptocytosis on one of the parents' peripheral blood smears. However, it is frequently impossible to distinguish HE with neonatal poikilocytosis from HPP in the neonatal period. Resolution of hemolysis and poikilocytosis and evolution into typical HE between 6 and 12 months of age clarify the diagnosis.[201] Such patients have usually inherited a single mutant α-spectrin allele. Worsening of hemolysis in the neonatal period has been attributed to high levels of 2,3-diphosphoglycerate (due to elevated hemoglobin F), which destabilize spectrin–protein 4.1–actin interactions in the membrane skeleton.[210,211]

HE with dyserythropoiesis. In a few Italian families with typical HE, sporadic hemolysis has been attributed to dysplastic and ineffective erythropoiesis.[212,213] Anemia and erythroid dysplasia typically appear in adolescence and gradually worsen with age. Splenectomy is not curative for the dyserythropoiesis. Apparently HE and dyserythropoiesis are coinherited, as all dyserythropoietic patients have HE.

Spherocytic Elliptocytosis

Spherocytic elliptocytosis, also known as spherocytic HE, HE with spherocytosis, or hereditary hemolytic ovalocytosis, shares features of HE and hereditary spherocytosis (HS).[167,214-217] An uncommon, dominantly inherited condition, it has been found primarily in Caucasian families of European descent, particularly Italians. The diagnosis is made when elliptocytes, sometimes called "fat" elliptocytes, *and* spherocytes or round sphero-ovalocytes are found on blood smear. Cells of other shapes, such as rod-shaped cells, poikilocytes, and fragments, are absent. Numbers of elliptocytes and spherocytes vary, even in affected members of the same family.[214,218] Erythrocyte osmotic fragility is increased, especially after incubation.[216,217,219]

Most patients suffer from mild to moderate hemolysis that is partially compensated, a clinical picture that resembles mild to moderate HS. Splenomegaly and gallstones are common.[220,221] Aplastic crisis may occur. Splenectomy ameliorates or cures the hemolysis.[219]

The molecular bases of this group of disorders are heterogeneous. Patients with mutations in the COOH-terminus of β-spectrin, particularly trunca-

tions of the self-association site (see above), have features of spherocytic HE. Patients who lack glycophorin C have rounded, smooth elliptocytes and features of spherocytic HE including abnormal erythrocyte osmotic fragility.[222,223] However, this condition is recessively inherited (patients with recessively inherited defects of protein 4.2 also may exhibit features of spherocytic HE[224]; because erythrocyte morphology and clinical features associated more closely resemble HS, it is typically classified as type of recessive HS).

HE in pregnancy. Pregnancy may precipitate hemolysis in HE, even in patients who are completely asymptomatic before and after pregnancy.[217,225-227] Transfusion support may be required. Megaloblastic changes due to folate deficiency can also develop during pregnancy.

HE in fetus and newborn. In newborns, anemia and jaundice may be seen but clinical symptoms of elliptocytosis are uncommon. Typically, mild anemia is noted at 4 to 6 months of age with the appearance of elliptocytes on peripheral blood smear. Uncommon subtypes of HE, including HE with infantile poikilocytosis and HPP, present with severe hemolytic anemia and jaundice in the neonatal period. Red blood cell transfusion, exchange transfusion, and splenectomy early in life have been needed. Even in patients with severe hemolysis, red blood cell production decreases during the first year of life, leading to evolution of typical HE with mild anemia.

Several cases of hydrops fetalis accompanied by in utero or early neonatal death due to unusually severe forms of HE have been described.[88,89,228] One severely affected hydropic infant salvaged by intrauterine transfusions and early exchange transfusion has remained transfusion-dependent.

Models of hereditary elliptocytosis. *sph*^{Dem} is a murine model of severe hemolytic HE caused by the in-frame deletion of exon 11 of the α-spectrin gene.[229] *sph*^{Dem} erythrocytes are spectrin-deficient, exhibit abnormal spectrin self-association, and they have increased adhesion to thrombospondin and laminin in vitro.[230] Mice with targeted disruption of protein 4.1R suffer from a moderate hemolytic anemia and subtle neurologic defects.[231,232] Their erythrocytes exhibit increased osmotic fragility and spherocytic morphology attributed to concomitant spectrin deficiency, not seen in humans with protein 4.1 deficiency.

The zebrafish *riesling* variant, due to mutation of erythroid β-spectrin, exhibits a severe hereditary spherocytosis phenotype, similar to human β-spectrin gene mutations outside the self-association site.[233] The zebrafish *merlot* and *chablis* mutants, due to mutation of protein 4.1, exhibit severe hemolytic anemia.[234] Erythrocytes exhibit abnormal morphology with disruption of the cortical membrane and increased osmotic fragility.

Laboratory Characteristics

Peripheral blood smear. Cigar-shaped elliptocytes on peripheral blood smear are the diagnostic characteristic of HE (Fig 8). Normochromic, normocytic elliptocytes vary in number from few to 100%; the degree of hemolysis does not correlate with the number of elliptocytes. Ovalocytes, spherocytes, stomatocytes, and fragmented cells may also be found. Elliptocytes do not typically appear in the peripheral blood of most infants with HE until 4 to 6 months of age. Blood smear evaluation is essential both for the diagnosis of HE and for the classification of the disorder into the three major subtypes outlined above. In patients in whom elliptocytosis is the only morphologic abnormality, hemolysis is characteristically minimal or absent, with the exception of spherocytic elliptocytosis, in which the presence of round "fat" ovalocytes is associated with accelerated red blood cell destruction. In patients with hemolytic forms of common HE, poikilocytosis is characteristically found on the blood film. In severe forms of HE, particularly in homozygous HE, many red cells circulate as cell fragments, producing a marked decrease in mean corpuscular volume. The finding of red blood cell fragments together with a striking microspherocytosis and often only occasional elliptocytes is characteristic of HPP.

Pseudoelliptocytosis is an artifact of blood smear preparation (Fig 8). Pseudoelliptocytes are found only in certain areas of the slide, usually near the tail of the smear, and the long axes of pseudoelliptocytes are parallel, whereas the axes of true elliptocytes are distributed randomly.

Osmotic fragility testing. In cases of typical HE, the incubated osmotic fragility is normal. In severe HE, spherocytic HE, and HPP, osmotic fragility is increased.

Other laboratory findings. Other laboratory findings in HE are similar to those found in the hemolytic anemias. The reticulocyte count generally is less than 5% but may be higher when hemolysis is severe. Increased serum bilirubin, increased urinary urobilinogen, and decreased serum haptoglobin are markers of accelerated erythrocyte destruction. Haptoglobin may be low or absent in neonates and thus is an unreliable marker of hemolysis in the newborn.

Specialized laboratory procedures are available to study the erythrocyte membranes of HE and HPP patients, including ektacytometry, analysis of membrane proteins by one-dimensional gel electrophoresis, tryptic mapping of spectrin, spectrin self-association studies, and cDNA/genomic DNA analyses. These studies are not routinely required for diagnosis

Table 3. Disorders Where Elliptocytosis May be Prominent

Hereditary elliptocytosis
Iron deficiency
Leukemia
Megaloblastic anemia
Myelofibrosis
Myelophthisic anemias
Myelodysplastic syndromes
Polycythemia
Pyruvate kinase deficiency
Sickle cell disease
Thalassemia

of HE or HPP but may be helpful in problematic cases and in the determination of precise genetic defects. Prenatal diagnoses of HE and HPP have been made using ektacytomtery, tryptic mapping of spectrin, and spectrin self-association studies.[235,236] As these disorders are rarely life-threatening, routine prenatal diagnosis is not required.

Differential Diagnosis

Several acquired and inherited disorders are associated with elliptocytosis and poikilocytosis (Table 3). While the percentage of elliptocytes in these conditions rarely exceeds 50%, some HE subjects also may have a relatively low percentage of elliptocytes. In normal individuals, the percentage of elliptocytes is less than 5%. Older diagnostic criteria of HE, based on the percentage of elliptocytes and their axial ratio, are not useful. The most reliable differentiation of HE is based on a positive family history.

Elliptocytosis has been found in previously unaffected individuals who develop myelodysplasia; schistocytosis may also be present.[237-240] Abnormalities of 20q have been detected in some patients but this is not a constant finding.[241,242] In one case, protein 4.1 deficiency was observed.[241]

Treatment

Therapy is rarely needed in HE. In severe cases, red blood cell transfusions may be required, particularly during the neonatal period and with intercurrent illness. Patients with significant hemolysis should receive daily folic acid supplementation. They need close observation for signs of decompensation during acute illnesses and should receive serial interval ultrasound examinations to detect gallstones.

Splenectomy to decrease hemolysis, improve anemia, and avoid the formation of bilirubin gallstones has been the cornerstone of therapy for cases of severe hemolytic HE and HPP, as the spleen is the site of erythrocyte sequestration and destruction.[215] The same indications for splenectomy in HS should be applied to hemolytic HE and HPP. Postsplenectomy, most patients with HE or HPP experience resolution of anemia and reticulocytosis with improvement in clinical symptoms. If hemolysis is still active after splenectomy, folate should be administered daily. Recommendations for antibiotic prophylaxis, immunization, and monitoring during intercurrent illnesses are similar to those for HS patients (see Eber and Lux in this issue).

Neonates should be managed as any patient with hemolytic anemia. Phototherapy and exchange transfusions are warranted in cases of severe anemia and pathologic hyperbilirubinemia. Splenectomy is rarely necessary in the neonatal period.

Hemolytic Elliptocytosis and Malaria

Epidemiologic and genetic studies in African populations have revealed that several mutations, including 154InsLeu, have a prevalence much higher than would be expected at random.[1-3] These mutations always associate with the same α-spectrin haplotype.[243] Thus a selective advantage for elliptocytes, conferring some resistance to malaria, has been hypothesized. In vitro studies have demonstrated decreased malarial parasite entry or growth in HE erythrocytes with α-spectrin mutations, homozygous protein 4.1 deficiency, and glycophorin C deficiency (Leach phenotype).[244-246]

Molecular Determinants of Clinical Severity

The molecular determinants of clinical severity are the degree of decreased cellular deformability, erythrocyte spectrin content, and the percentage of dimeric spectrin in crude spectrin extracts.[11-13,247] These determinants are interrelated.

Spectrin deficiency occurs by several mechanisms. Several of the elliptocytogenic α-spectrin mutants are unstable, reducing the amount of spectrin available for membrane assembly. Patients who are homozygous or compound heterozygous for these spectrin mutations will be more severely affected. In other patients, coinheritance of a low-production α-spectrin allele *in trans* contributes to spectrin deficiency, increases the relative expression of the mutant allele, and worsens the clinical phenotype.[105,107]

The fraction of dimeric spectrin depends on the dysfunction induced by the spectrin mutation and on the amount of mutant spectrin in the erythrocyte. Mutations of the spectrin self-association contact site lead to a greater degree of spectrin dysfunction and a more severe clinical phenotype than do mutations in more distant spectrin repeats.[57] The amount of the mutant spectrin in the cells is determined by the gene dose (heterozygote *v* homozygote or compound heterozygote) or the presence of genetic defects leading to decreased α-spectrin production *in trans*. Undis-

covered alleles of spectrin or protein 4.1R or as yet unknown modifier genes that influence membrane protein structure and/or function may also influence clinical severity. Acquired factors may influence clinical severity in HE and HPP. In fetal and neonatal erythrocytes, increased free 2,3-DPG destabilizes spectrin-actin-protein 4.1 interactions. Acquired conditions that alter the microcirculatory stress to the cells, such as thrombotic thrombocytoenic purpura, can precipitate or worsen hemolysis in HE/HPP patients.[248]

Southeast Asian Ovalocytosis

SAO (also known as Melanesian elliptocytosis or stomatocytic elliptocytosis) is found in Melanesia, Malaysia, the Philippines, and Indonesia.[249-255] SAO is very common in lowland tribes of Melanesia where malaria is endemic, affecting 5% to 25% of individuals.[250,251,256] Rarely, SAO has been detected in Caucasian and African-Americans.[128,257,258] SAO is classified with the elliptocytosis disorders due to the presence of oval-shaped erythrocytes, many with one or two transverse ridges or a longitudinal slit, on blood smear (Fig 8). Inherited in an autosomal dominant manner, only heterozygotes have been identified,[259,260] and homozygosity may lead to embryonic or fetal lethality.

SAO apparently provides some protection against all forms of malaria, particularly against heavy infections and cerebral infection. In these malaria-endemic regions, there is a decrease in the prevalence and severity of malaria as well as reduced numbers of intracellular parasites in vivo in patients with SAO compared to controls.[251,252,261-265] The prevalence of SAO increases with age in these populations, suggesting that SAO individuals have a selective survival advantage.[263]

Membrane pathobiology. SAO red cells are unique among elliptocytes in that they are rigid and hyperstable rather than unstable.[75,266-268] Thus the SAO mutation in band 3 (see below) is a defect of an integral membrane protein that leads to membrane rigidity, previously attributed only to membrane skeletal proteins. Band 3 SAO is made and assembled into the membrane but is nonfunctional: it does not transport anions.[269-271] There is increased tyrosine phosphorylation of band 3, and tighter binding of band 3 to ankyrin.[272-274] The formation of linear aggregates in the membrane is thought to significantly restrict lateral and rotational mobility of band 3.[274,275]

There are many hypotheses for the etiology of the membrane rigidity in SAO erythrocytes, but the precise basis is unknown. Perhaps conformational changes of the cytoplasmic domain of band 3 SAO preclude lateral movement of the membrane skeleton during deformation.[75,267,276] The tendency of band 3 SAO to form linear arrays also would decrease its lateral mobility. Solution nuclear magnetic resonance of the first transmembrane domain of band 3 revealed that a bend or hinge-like region between the cytoplasmic domain and the first transmembrane domain present in wild-type cell was absent in SAO cells, leading to formation of a stable helix (Fig 9).[277] Increased numbers of band 3 oligomers formed by band 3 SAO could lead to tighter band 3–ankyrin interactions, secondarily influencing skeleton-membrane interactions.[274,278] Finally, band 3 SAO may adhere to the membrane skeleton nonspecifically, possibly due to denaturation of the membrane-spanning domain.[279]

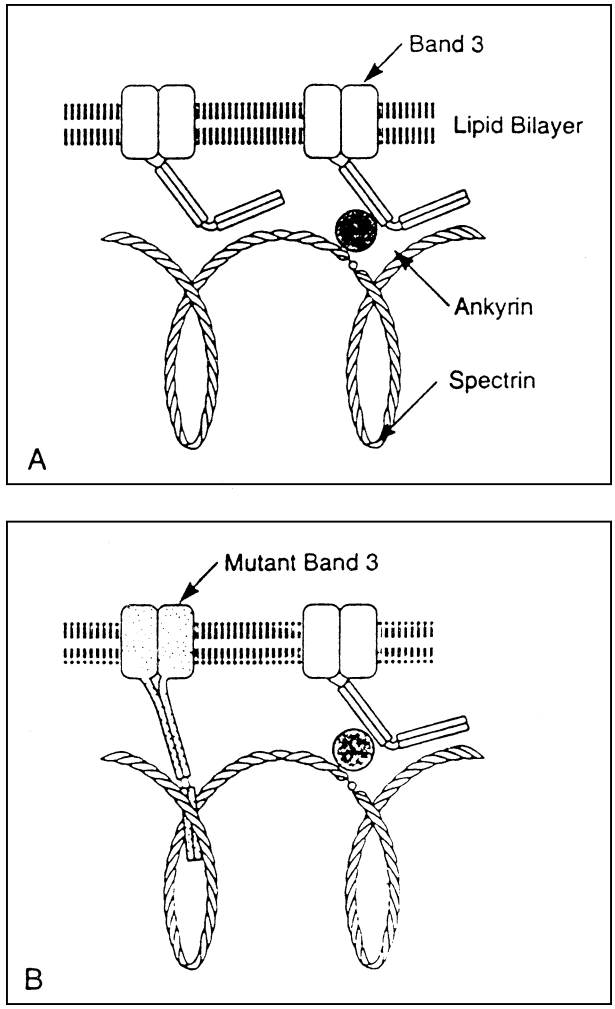

Figure 9. Model of the band 3 configuration in association with the membrane and cytoskeletal lattice. (A) The "interhinge" domain permits it to act flexibly and nonobstructively with ankyrin and spectrin. The SAO defect abolishes the hinge enhancing the interactions between the cytoplasmic domain, ankyrin, and the spectrin lattice. (Reprinted with permission.[275])

Genetic defects. Identification of linkage of abnormal proteolysis of erythrocyte band 3 to SAO led to the detection of the underlying molecular defect.[274] In all SAO cases, patients were found to be heterozygous for two band 3 mutations *in cis*: genomic deletion of 27 bp encoding amino acids 400 to 408 located at the boundary of the cytoplasmic and membrane domains of band 3, and a missense mutation, Lys56Glu.[267,275,280,281] The missense mutation is an asymptomatic polymorphism known as band $3^{Memphis}$.[272,273,282,283] Deletion of 27 bp from the band 3 gene allows rapid PCR-based diagnosis in genomic DNA, producing a single band in normals and a doublet with the second band shorter by 27 bp in SAO individuals.

Clinical characteristics. Most SAO patients experience no or minimal hemolysis[249,252] although neonatal hyperbilirubinemia has been described.[284] One patient had compensated hemolysis without anemia, mild splenomegaly, mild hyperbilirubinemia, and gallstones. SAO erythrocytes are oval, many with one or two transverse ridges or a longitudinal slit; occasional cells contain one or two transverse bars that divide the central clear space (Fig 8C).[285] These stomatocytic elliptocytes are unique to SAO. Other characteristics of SAO erythrocytes are increased rigidity, decreased osmotic fragility, increased thermal stability, and reduced expression of many red cell antigens.[75,250,255,266,268]

Identification of large numbers of characteristic oval-shaped elliptocytes with transverse bars on peripheral blood smear in the absence of clinical and laboratory hemolysis in a subject from Southeast Asia is essentially diagnostic of SAO. Although the resistance of SAO erythrocytes or their ghosts to changes in shape induced by metabolic depletion, exposure of ghosts to salt solutions, or other treatments that produce spiculation in normal cells has been suggested to differentiate SAO cells from normal, these are not well characterized or standard assays. Since the underlying cause of SAO is the deletion of 27 bp from the band 3 gene the most specific diagnostic test is isolation of genomic or reticulocyte cDNA with subsequent amplification of the deletion-containing region, producing a single band in control cells and a doublet in SAO, with the second band shorter by 27 bp. When genetic assays are impractical, the unique erythrocyte morphology combined with the strong resistance of SAO cells to crenation, even after simple storage for several days,[276] provides strong support for a diagnosis of SAO.

Renal tubular acidosis has been discovered in some SAO patients, in whom the SAO deletion is accompanied by additional band 3 mutations.[276,286,287]

Molecular basis of malaria resistance in SAO. The resistance of SAO erythrocytes to malaria is thought to be due to alterations in SAO band 3 (see Cooke et al in this issue of *Seminars*). Band 3 serves as one of the malaria receptors. Band 3–containing liposomes inhibit malaria invasion in vitro.[288] In normal erythrocytes, malaria invasion is associated with marked membrane remodeling and redistribution of band 3–containing intramembrane particles.[289] At the site of malaria invasion, intramembrane particles cluster and form a ring around the orifice through which the parasite enters the cell. The invaginated membrane surrounding the invading parasite lacks intramembrane particles. The reduced lateral mobility of band 3 SAO may preclude band 3 receptor clustering and thus prevent attachment or entry of the malarial parasite.[274,275] Resistance of SAO erythrocytes to malaria has also been attributed to diminished anion exchange and to adenosine triphosphate (ATP) depletion due to increased utilization.[268,270] However, these appear to be in vitro phenomena. Band 3–deficient hereditary spherocytes are considerably less resistant to malaria invasion than are SAO erythrocytes, even though both have similarly decreased anion transport and SAO erythrocytes maintain their resistance to malaria invasion at high ATP levels.

Conclusion

Advances in molecular biology have provided the tools to carry out detailed study of the proteins of the erythrocyte membrane and their corresponding genes. These studies have provided significant insight into the pathogenesis of the hereditary elliptocytosis syndromes. They have also provided insight into the structure and function of the membrane skeleton in erythroid and nonerythroid cells. Future advances will hopefully expand this knowledge of membrane pathobiology in inherited disorders of erythrocyte shape and provide additional insight into phenotype and genotype correlation.

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