

# Hematologically Important Mutations: Spectrin and Ankyrin Variants in Hereditary Spherocytosis

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Hereditary spherocytosis is a group of inherited disorders characterized by the presence of spherical-shaped erythrocytes on peripheral blood smear (1-4). The principal cellular defect in HS is loss of erythrocyte membrane surface area relative to intracellular volume, accounting for the spherical shape as well as decreased deformability of the erythrocyte. Splenic destruction of these abnormal erythrocytes is the primary cause of the hemolysis experienced by HS patients. The clinical and laboratory features of HS have been the subject of recent reviews (3-6).

The primary biochemical defects in HS reside in the proteins of the erythrocyte membrane, particularly those proteins involved in the interactions between the membrane skeleton and the lipid bilayer:  $\alpha$  and  $\beta$  spectrin, ankyrin, band 3, and protein 4.2. Study of erythrocyte membranes from HS patients has revealed qualitative and/or quantitative differences in these proteins (7-13). Combined spectrin and ankyrin deficiency is the most common defect observed in these studies, followed by band 3 deficiency, isolated spectrin deficiency, and protein 4.2 deficiency. Concomitant spectrin and ankyrin deficiency is not unexpected; decreased ankyrin

synthesis, decreased ankyrin assembly on the membrane, or assembly of an abnormal ankyrin could lead to decreased assembly of spectrin on the membrane because spectrin binding sites on ankyrin may be absent or defective. This review focuses on genetic defects of spectrin and ankyrin associated with HS. A previous tabulation reviewed defects of band 3 and protein 4.2 associated with HS (14).

Erythrocyte spectrin maintains cellular shape, regulates lateral mobility of integral membrane proteins, and provides structural support for the lipid bilayer (1,5). Spectrin is composed of two subunits,  $\alpha$  and  $\beta$  spectrin, that despite some similarities are structurally distinct and are encoded by separate genes (15,16).  $\alpha$  and  $\beta$  spectrin chains intertwine in an antiparallel manner to form heterodimers, which in turn self-associate to form tetramers and oligomers. Abnormalities of the NH<sub>2</sub>-terminus of  $\alpha$  spectrin and the COOH-terminus of  $\beta$  spectrin, the regions involved in self-association, lead to the disorders hereditary elliptocytosis and hereditary pyropoikilocytosis (reviewed in 4-6,17).

Defects of spectrin outside the self-association site have been associated with hereditary spherocytosis. Erythrocytes from

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many HS patients are spectrin deficient, including both dominant and recessive forms of HS. In normal erythroid cells,  $\alpha$  spectrin is synthesized in excess, with  $\alpha$ -spectrin synthesis exceeding  $\beta$ -spectrin synthesis by a ratio of about three or four to one (18,19). Heterozygotes for  $\alpha$ -spectrin defects should still produce enough normal  $\alpha$  spectrin chains to associate with all, or nearly all, of the  $\beta$  spectrin synthesized, with assembly of normal amounts of spectrin on the membrane. Thus spectrin deficiency should be apparent only in HS patients who are homozygotes or compound heterozygotes for  $\alpha$ -spectrin gene mutations. In a similar manner, deficiency of the limiting  $\beta$ -spectrin chain due to mutations of the  $\beta$ -spectrin gene are associated with dominantly inherited HS (Table 2).

Ankyrin gene mutations are the most common cause of typical, dominant HS. Initially, cytogenetic studies of ankyrin-deficient HS patients with karyotypic abnormalities of the ankyrin gene locus on chromosome 8 implicated ankyrin in the pathogenesis of HS (reviewed in 20). Later, studies using restriction length polymorphisms linked the ankyrin gene to dominantly inherited HS (21). One third of HS patients with combined spectrin and ankyrin

deficiency were shown to express only one of their two ankyrin alleles in reticulocyte RNA, demonstrating reduced expression from a mutant ankyrin allele (22). Together, these observations suggested that a defect of ankyrin is the primary abnormality in the majority of patients with typical HS.

Genetic screening has now identified a number of mutations in spectrin and ankyrin. These are listed in Tables 1 and 2. The nucleotide numbers shown in the table are based on the cDNA sequences listed in the GenBank/EMBL databanks, the accession numbers of which are: J05244 ( $\alpha$ -spectrin cDNA) (23), J05500 ( $\beta$ -spectrin cDNA) (24), and X16609 (ankyrin 1 cDNA) (25). In accordance with recommendations for the use of systematic names in nomenclature for mutations, the nucleotide numbers of the cDNA coding sequences presented here begin with +1, which refers to A in the ATG initiation codon (26). This numbering is different from that used in the GenBank/EMBL databank files.

Keywords: hereditary spherocytosis, mutation, spectrin, ankyrin, erythrocyte

**Table 1.** Spectrin Mutations Associated with Hereditary Spherocytosis

Variant	Mutation	Amino Acid Variation	Systematic Name	Reference
<b><math>\alpha</math> Spectrin</b>				
Prague	splicing	1446-frameshift	IVS 30 -1C	(27)
LEPRA	splicing	1729-frameshift	IVS 36 -99T	(27)
<b><math>\beta</math> Spectrin</b>				
Promissão	missense	1 Met-Val	c1G	(28)
Guemene-Penfao	splicing	100-frameshift	IVS 3 -1C	(29)
Atlanta	missense	182 Trp-Gly	c544G	(30)
Unnamed	missense/splicing?	189 Gly-Ala	c567C	(31)
Ostrava	deletion	202-frameshift	c604del	(30)
Kissimmee	missense	202 Trp-Arg	c604C	(32)
Oakland	missense	220 Ile-Val	c658G	(31)
Bicetre	deletion	443 or 444-frameshift	c1328-1335del or c1330-1337del	(31)
Alger	nonsense	514 Gln-Stop	c1540T	(31)
Philadelphia	insertion	589-frameshift	c1766A1767	(30)
St. Barbara	deletion	638-frameshift	c1912del	(33)
Bergen	insertion	783-frameshift	c2351A2352ins	(30)

Variant	Mutation	Amino Acid Variation	Systematic Name	Reference
Baltimore	nonsense	845 Gln-Stop	c2533T	(30)
Winston-Salem	splicing	935-frameshift	IVS 17 +1A	(34)
Columbus	missense	1227 Pro-Ser	c3679T	(30)
Durham	deletion	1492-1614 123 AA deletion	c4473-4842del	(35)
Birmingham	missense	1684 Arg-Cys	c5050T	(30)
Sao Paulo	missense	1884 Ala-Val	c5651T	(32)
Tabor	nonsense	1946 Gln-Stop	c5836T	(30)

**Table 2. Ankyrin Mutations Associated with Hereditary Spherocytosis**

Variant	Mutation	Amino Acid Variation	Systematic Name	Reference
Unnamed	missense	5' untranslated	-204G	(36)
Unnamed	missense	5' untranslated	-153A	(37)
Unnamed	missense	5' untranslated	-108C	(36)
Unnamed	deletion	5' untranslated	-72/-73del	(36)
Bugey	deletion	146-frameshift	c556del	(38)
Osterholz	deletion	173-frameshift	c521-540del	(36)
Stuttgart	deletion	329-frameshift	c985-986del	(36)
Bari	deletion	426-frameshift	c1276del	(39)
Walsrode	missense	463 Val-Ile	c1387A	(36)
Florianopolis	insertion	506-frameshift	c1603C1604ins	(40)
Duisburg	? splicing	527-frameshift	IVS 16 -18A	(36)
Laguna	deletion	535-frameshift	c1603del	(40)
Napoli	deletion	573-frameshift	c2078del	(41)
Einbeck	insertion	573-frameshift	c2077C2078ins	(36)
Unnamed	deletion	596-frameshift	c1788del	(42)
Unnamed	nonsense	631 Glu-Stop	c1890T	(43)
Unnamed	nonsense	765 Ser-Stop	c2294A	(43)
Marburg	deletion	797-798-frameshift	c2390-2393del	(36)
Unnamed	deletion	907-frameshift	c2720del	(43)
Napoli II	deletion	933-frameshift	c2799del	(39)
Nara	missense	1046 Leu-Pro	c3137C	(44)
Unnamed	nonsense	1053 Arg-Stop	c3157T	(43)
Tubarao	missense	1075 Ile-Thr	c3223A	(40)
Porta Westfalica	deletion	1127-frameshift	c3380del	(36)
Bovendem	nonsense	1436 Arg-Stop	c4306T	(36)
Unnamed	nonsense	1488 Arg-Stop	c1502T	(43)
Prague	insertion	1512-1513 67AA insertion	c4537-201 nt insertion-4538	(45,46)
Unnamed	? splicing	1513-frameshift	IVS 38 -34T	(36)
Dusseldorf	missense	1592 Asp-Asn	c4774A	(36)
Saint-Etienne 1	nonsense	1721 Trp-Stop	c1563A	(47)
Saint-Etienne 2	nonsense	1833 Arg-Stop	c5497T	(47)
Bocholt	substitution	1879 Arg-Trp	c5635T	(36)
Rakovnik	nonsense	1669 Glu-Stop	c5005T	(48)
Anzio	deletion	983-frameshift	c2948-2949del	(39)

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