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ANKYRIN DEFICIENCY IS THE MOST COMMON DEFECT IN DOMINANT AND NON DOMINANT HEREDITARY SPHEROCYTOSIS

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ABSTRACT

Background and Methods. Hereditary spherocytosis (HS) is one of the most common hereditary hemolytic anemias in Caucasians. The primary defect resides in red blood cell (RBC) membrane skeleton proteins. Using polyacrylamide gel electrophoresis and densitometric quantitation of solubilized skeleton membrane proteins we investigated biochemical abnormalities in 20 Italian kindreds.

Results and conclusions. We detected an ankyrin deficiency combined with decreases in both spectrin and protein 4.2 as the most common defects in our HS patients. Reticulocytosis may strongly influence ankyrin evaluation. An isolated spectrin deficiency was present in 15% of patients and combined band 3 and protein 4.2 deficiencies were also detected. These results underline the importance of ankyrin in the pathogenesis of HS and point out the extreme heterogeneity of molecular defects in the disease.
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Keyword: hereditary spherocytosis, spectrin, ankyrin, band 3, protein 4.2

Hereditary spherocytosis (HS) is the most commonly inherited hemolytic anemia in Caucasians. In 75% of cases HS is inherited as a dominant trait, but a subset of patients shows autosomal recessive inheritance or may carry a *de novo* mutation. Various red blood cell (RBC) membrane skeletal protein abnormalities are responsible for HS. The clinical features are heterogeneous and the severity of hemolysis and anemia differs markedly among the mild, typical or severe forms of the disease.[1]

Ankyrin mutations are a major cause of dominant and recessive hereditary spherocytosis;[2] however, molecular abnormalities in the genes for α and β spectrin, band 3 and protein 4.2 have also been observed.[1] In this report we present the results of densitometric quantitation of RBC membrane skeleton proteins in 20 kindreds performed in order to identify the underlying defects and estimate their frequency.

Materials and Methods

We investigated RBC protein abnormalities in 14 HS families with clear autosomal dominant inheritance patterns (32 patients) and 6 additional patients belonging to families in which the propositus was the only one affected. We could not establish whether recessive inheritance or a new dominant mutation was present, so we referred to them as *isolated patients*. The age of the propositi ranged from a few days to 14 years. All the other affected relatives were adults. We report only data concerning the affected parents (Table 1). Healthy adult volunteer donors were used as controls.

Venous blood was collected in ACD solution and processed within a few hours.

Erythrocyte ghosts were prepared by hypotonic lysis with the addition of PMSF as proteolytic inhibitor.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli (linear gradient 4-12%)[3] and Fairbanks (linear gradient 3.5-17%)[4] was carried out in all subjects. Gels were stained with Coomassie brilliant blue and scanned using a laser densitometer (Ultroscan XL, LKB). The amount of the major RBC membrane skeleton proteins was calculated as the ratio between the integrated area of the peak protein and that of band 3, and expressed as a percentage of the same protein ratio obtained in normal controls run on the same gel. At least six determinations in different gels were made for each patient and control (variation coefficient ± 5%).

Results and Discussion

Clinical data and results are shown in Table 1. An ankyrin deficiency was detected in 8 kindreds (#1, 2, 4, 7, 8, 9, 10, 13) and 2 isolated patients. In 8 of them it was combined with a similar decrease of both spectrin and protein 4.2. Ankyrin and spectrin deficiencies were found in family #13; family #10 was characterized by a remarkable decrease in protein 4.2 along with a reduction in ankyrin.

In 3 families (#2, 7, 9) the propositi showed an apparently normal or higher ankyrin/band 3 ratio compared to the lower ratio found in their affected parents. Presumably, this was due to the presence of a high level of immature erythrocytes, which strongly influences ankyrin evaluation.[5,6]

Unfortunately we could not repeat the analysis when the reticulocytes reached normal values

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Table 1. Hematological data and RBC membrane skeleton protein studies of HS patients.

KINDRED	HB G/DL	RET %	GLT	AGLT	SP %	ANK %	4.1 %	4.2 %	Detected protein deficiencies
Family 1									
Propositus	9.7	12	19″	37″	84	84	98	85	Sp-Ank-p 4.2
Father*	12.3	1.2	13″	29″	84	84	98	79	Sp-Ank-p 4.2
Grandfather*	13.4	1	11″	22″	79	69	102	85	Sp-Ank-p 4.2
Family 2									
Propositus	9.9	28	8″	17″	82	102	116	81	Sp-p 4.2
Mother*	12.4	1.3	8″	15″	89	85	100	77	Sp-Ank-p 4.2
Family 3									
Propositus	9.3	15	11″	17″	119	138	132	103	B3-p 4.2
Mother	9.6	16	21″	38″	121	136	132	104	B3-p 4.2
Family 4									
Propositus	11.8	13	9″	30″	85	94	101	92	Sp-Ank-p 4.2
Father*	15.9	1	11″	29″	85	88	98	90	Sp-Ank-p 4.2
Family 5									
Propositus	13.1	10	18″	49″	124	134	117	96	B3-p 4.2
Sister	13	5.6	19″	53″	124	138	113	101	B3-p 4.2
Mother	12	4	20″	74″	132	141	118	103	B3-p 4.2
Family 6									
Propositus	12.1	4.2	17″	27″	110	113	110	81	B3-p 4.2
Brother	11.9	4.7	13″	26″	115	109	113	78	B3-p 4.2
Father	17.3	2.0	7″	19″	114	106	117	73	B3-p 4.2
Family 7									
Propositus	8.8	16	18″	47″	83	110	107	75	Sp-p 4.2
Father	14.6	2.8	14″	27″	80	80	96	77	Sp-Ank-p 4.2
Family 8									
Propositus	7.7	5	18″	36″	85	78	102	82	Sp-Ank-p 4.2
Father	15.4	0.8	20″	39″	88	73	100	81	Sp-Ank-p 4.2
Family 9									
Propositus	8.6	23	17″	39″	86	99	127	84	Sp-p 4.2
Brother	9.7	12	15″	41″	84	98	119	84	Sp-p 4.2
Mother*	11.2	1.6	16″	45″	81	80	99	69	Sp-Ank-p 4.2
Family 10									
Propositus*	13.8	1	14″	30″	100	82	100	70	Ank-p 4.2
Father*	15.6	1.4	17″	35″	100	69	100	63	Ank-p 4.2
Family 11									
Propositus	9.5	15	19″	53″	83	108	108	100	Sp
Mother*	13.1	1.1	20″	55″	83	103	98	101	Sp
Family 12									
Propositus	10.4	4.6	16″	46″	85	104	102	101	Sp
Father	16.7	2.3	13″	30″	85	98	100	99	Sp
Family 13									
Propositus	10.8	3.8	15″	64″	81	81	100	101	Sp-Ank
Mother	11.7	2	16″	65″	83	87	103	97	Sp-Ank
Family 14									
Propositus	12.9	7	18″	45″	98	101	103	99	Normal
Mother*	13.4	1.1	18″	36″	99	103	101	100	Normal
Isolated patients									
CO	10	18	9″	32″	82	86	110	85	Sp-Ank-p 4.2
PL*	15.8	1.3	8″	23″	88	77	101	70	Sp-Ank-p 4.2
RF	9.3	12	22″	46″	88	108	100	97	Sp
CR	10.9	7	11″	22″	101	97	98	97	Normal
FV	11.4	12	16″	40″	100	108	102	99	Normal
SC	8.8	12	16″	42″	98	107	104	98	Normal

**Splenectomized. Sp: spectrin; Ank: ankirin; B3: band 3; 4.1: protein 4.1; 4.2: protein 4.2; GLT: normal value ≥ 23″; AGLT: normal value ≥ 180″; ↑ increased.*

because the patients were no longer available.

An isolated spectrin deficiency was present in 2 kindreds (#11, 12) and in 1 isolated patient. In 3 families (#3,5,6) dominantly inherited HS was associated with band 3 reduction, as deduced from the increase of spectrin, ankyrin and protein 4.1 to band 3 ratios. In two of these families the protein 4.2/band 3 ratio was in the normal range, which means that protein 4.2 was decreased to the same extent as band 3. Since protein 4.2 binds to the cytoplasmic domain of band 3, the protein 4.2 deficiency was probably secondary to the band 3 decrease.

In family #6 the ratio of protein 4.2/band 3 was lower by between 19% and 27% with respect to the normal control, which can be explained by a large decrease in protein 4.2. In this case the primary defect may reside in protein 4.2 or the disease could be due to a combination of molecular defects of both protein 4.2 and band 3.

We did not detect any protein abnormalities in family #14 or in 3 isolated patients. This may be due to very mild protein deficiencies not revealed by SDS-PAGE.

In our experience, defective erythrocyte ankyrin combined with spectrin and protein 4.2 deficiency is the primary and most common defect in dominant and non-dominant HS patients. This finding is consistent with previous data asserting that ankyrin deficiency affects 35%-65% of HS patients.[2,7]

We underline the importance of proper ankyrin evaluation in laboratory investigation of HS abnormalities, particularly in the presence of reticulocytosis. The central role of ankyrin in the RBC membrane skeleton structure may explain the high frequency of ankyrin defects. In fact, decreased ankyrin synthesis leads to decreased assembly, of a similar magnitude, of spectrin on the RBC membrane despite normal spectrin synthesis.[8,9] Since ankyrin stabilizes the assembly of protein 4.2 in the RBC cytoskeleton,[10] a similar mechanism may also be responsible for protein 4.2 deficiency as a consequence of ankyrin defects. By combining the data regarding ankyrin with those involving band 3 deficiencies we identified most of the protein deficiencies in our patients.

The present data corroborate the prevailing theory[1] that HS is caused by lesions involving vertical interactions between the bilayer and skeletal complex that are crucial for maintaining the mechanical integrity of the erythrocyte membrane.

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