Association of Red Cell Spherocytosis With Deletion of the Short Arm of Chromosome 8

By Robert R. Chilcote, Michelle M. Le Beau, Carlton Dampier, Eugene Pergament, Yury Verlinsky, Narla Mohandas, Henri Frischer, and Janet D. Rowley

Congenital spherocytic anemia is a common disorder, but in most cases the nature of the underlying membrane lesion is unknown and the genetic defect has not yet been unequivocally mapped to a chromosome. We studied two dysmorphic siblings with neurologic findings and hemolytic anemia. Clinical and laboratory findings in these two siblings were consistent with the diagnosis of congenital spherocytosis whereas both parents and two unaffected siblings were normal. The two affected children had an abnormal chromosomal complement as a result of a deletion of the short arm of chromosome 8 ([46,XX.del(8)(p11.1p21.1)]. These results suggest that a gene whose deletion results in a congenital spherocytic anemia phenotype resides on this region on the short arm of chromosome 8.

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MATERIALS AND METHODS

Case reports. The family was first brought to medical attention because of anemia in C.H. (Table 1). Born in 1978, this child was small (birth weight 2,500 g; length 38 cm), had hyperbilirubinemia in the second day of life and gained weight slowly. In the first months of life, intermittent splenomegaly, anemia, reticulocytosis, and microspherocytes in the peripheral smear were noticed. When the child was aged 6 months, psychomotor retardation, unusual facies, and nystagmus became evident and tonic-clonic seizures occurred. The results of osmotic fragility studies of RBCs before and after 24-hour incubation were characteristic of spherocytosis. The patient required several transfusions following respiratory infections in the first years of life. After splenectomy was performed when she was aged 4 years, her hemoglobin levels and reticulocyte counts have been normal and transfusions have not been necessary. However, microspherocytes persist on the peripheral smear, and osmotic fragility studies of both fresh and incubated erythrocytes continue to show increased fragility.

The second child, born in 1980, was also small at birth (birth weight 2,700 g; length 41 cm); she developed similar dysmorphic features with nystagmus, psychomotor retardation, and intermittent splenomegaly. Hematologic studies revealed that she also has spherocytosis. Anemia was not as severe as in the first child, and no transfusions have been required.

Both children attend schools for the educationally handicapped, have grown more slowly than their peers, and have a slight mongoloid slant, a pit located anterior to one ear, and micrognathia. The parents are unrelated and of normal appearance (mother's height 65 inches; father's height 79 inches) and are college graduates. There was no family history of congenital anomalies, anemia, jaundice, gall bladder disease, or splenectomy. On two occasions, the complete blood counts, blood films, serum bilirubins, haptoglobin levels, and osmotic fragility studies (before and after 24-hour incubation at 37 °C) on both parents were normal.

A third female child born in 1983 was normal (birth weight 3,540 g; length 45 cm) and by 3 years of age had not developed dysmorphic features, neurologic problems, or evidence of spherocytic anemia. A fourth child was born in 1986 and at 9 months of age shows normal development, is not dysmorphic, and has normal blood counts.

Laboratory studies. Using previously described techniques, RBC ektacytometric studies were performed, and carbonic anhydrase and glutathione reductase (GSSG-R) levels were measured on the two affected children and both parents. Plasma factor VII levels were measured using factor-deficient plasma (Dade Diagnostics). Peripheral blood RBCs were separated by density. Membrane proteins from RBC ghosts prepared by hypotonic lysis were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described (except 0.1% SDS gel and buffers were used to enhance resolution of band 4.1) and the two-dimensional gel technique of O'Farrell. One-dimensional gels were stained with Coomassie blue and scanned with a Hoefer GS100 scanning densitometer to estimate the area under each peak. Relevant protein bands were also excised and, after eluting with 80% methanol at 95 °C, the Coomassie blue stain in each band was quantitated at 595 nm in a spectrophotometer. RBC phospholipids...
SPHEROCYTOSIS AND CHROMOSOME 8p

Table 1. Hematologic Values for the Two Patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before Splenectomy</th>
<th>After Splenectomy</th>
<th>B.H.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>4</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>9.2</td>
<td>15.2</td>
<td>10.2</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>20</td>
<td>1.4</td>
<td>8.8</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>35.0</td>
<td>35.9</td>
<td>34.6</td>
</tr>
<tr>
<td>Microspherocytes (0-4 +)</td>
<td>2 +</td>
<td>4 +</td>
<td>2 +</td>
</tr>
</tbody>
</table>

*This patient had hyperbilirubinemia in the newborn period and, before splenectomy, four severe anemic episodes associated with splenomegaly and requiring transfusions. Hematologic values were obtained during stable periods with no recent transfusions. Two normal-appearing siblings have normal hematologic studies.

were assayed using ammonium ferrothiocyanate and silica gel (Fischer Silicon Gel G) thin-layer chromatography. Trypsin-Giemsa-banded chromosome studies were performed on phytohemagglutinin-stimulated peripheral blood lymphocytes on two occasions on each family member and on cultures established from skin biopsies of the parents. Multiple blood group and HLA markers were determined on both parents and the two older children.

RESULTS

Hematologic studies. The results of osmotic fragility studies of unincubated RBCs from one affected child (C.H.), are shown in Fig 1. Density centrifugation of fresh peripheral blood enriched the lower or denser, presumably older layers (as judged by the absence of reticulocytes), for spherocytes. Also shown in Fig 1 are the results of osmotic fragility studies obtained at the time of this child's splenectomy. RBCs taken from the splenic pulp and splenic vein had a higher proportion of osmotically fragile RBCs than did the peripheral blood, consistent with splenic "conditioning." Genetic studies. Metaphase cells from both affected children had an interstitial deletion of the short arm of chromosome 8 extending from band p11.1 to p21.1 (Fig 2): [46,XX,del(8)(p11.1p21.1)]. Peripheral blood lymphocytes from one unaffected sibling and both lymphocyte and skin fibroblast cultures from the parents had normal chromosomal complements.

RBC GSSG-R levels in the affected children were slightly lower than those of either parent, one unaffected sibling, or controls (C.H.: 8.6, 6.3; B.H.: 9.0, 11.2; unaffected sibling: 13.7; mother: 12.0, 15.2; father: 12.4; and nine controls: 13.2 ± 2.4 mmol/min/g Hb SD). Carbonic anhydrase I and II and plasma factor VII levels were not reduced. Blood group and lymphocyte HLA studies gave no evidence of nonpaternity.

Membrane studies. Ektacytometric studies of peripheral blood taken from both parents and the affected children were normal. These findings are consistent with the results noted in patients with classical hereditary spherocytosis in whom no specific skeletal protein defect can yet be identified.

SDS-polyacrylamide gels of RBC ghosts isolated from a normal control and the family members are illustrated in Fig 3. Spectrin (both α and β), actin, band 4.1, and other bands delineated by this technique were present; results were confirmed by two-dimensional gel analysis. Table 2 shows that the ratios of spectrin, band 4.1, and band 4.2 to band 3 for each of the affected children, both parents, and controls were similar. The membrane phospholipids, sphingomyelin, phosphatidylcholine, phosphatidylserine, and phosphatidylethanolamine were present in normal proportions.

Fig 1. Osmotic fragility studies of C.H.'s peripheral RBCs (separated by density) and splenic RBCs. The results of studies of peripheral blood taken during a routine visit and separated by RBC density (centrifuged at 30 °C for 60 minutes at 27,000 g (ref. 7) show that the least dense fraction with the highest proportion of reticulocytes from the top of the gradient was, as judged by osmotic fragility, the least severely damaged, whereas the denser layers (middle and bottom of the gradient, no reticulocytes) had a higher proportion of spherocytes than the spleen was damaging RBCs in transit.
resulted in the RBC membrane abnormality. The clinical strongly suggests that the loss of this chromosomal segment chromosome 8 and the absence of these findings in other family members with normal chromosomal complements two siblings with an interstitial deletion of the short arm of parents, and control. affected patients C.H. and B.H. (lanes 3 and 4). and a normal control (lane 5). There were no major differences in membrane protein composition between the affected children, parents, and control.

**DISCUSSION**

The association of congenital spherocytic anemia in these two siblings with an interstitial deletion of the short arm of chromosome 8 and the absence of these findings in other family members with normal chromosomal complements strongly suggests that the loss of this chromosomal segment resulted in the RBC membrane abnormality. The clinical course, hematologic findings, osmotic fragility studies, the role of the spleen in damaging RBCs, membrane loss with cell age, and ektacytometric measures of RBC structural stability in these two patients are compatible with previous descriptions of patients with congenital spherocytic anemias such as HS.6,12,14

Kimberling and associates described a family with spherocytosis and a balanced translocation between chromosomes 8 and 12 [t(8;12)(p11;p13)].2 whereas Bass and associates1 described another family with spherocytosis and a translocation between chromosomes 3 and 8 [t(3;8) (p21;p11)]. These data, along with our observations of an interstitial deletion involving bands p11.1 and p21.1, suggest that a functional alteration in a gene on the short arm of chromosome 8, at band p11, is associated with spherocytosis.

GSSG-R levels were slightly reduced in the two affected children relative to their parents and the unaffected sibling but did not approach half-normal values expected from GSSG-R gene dosage studies in patients trisomic for this region of chromosome 8.13 These results are ambiguous and do not confirm or deny the cytogenetic findings, which indicate that the deletion extends to 8p21, the region to which the GSSG-R gene has been assigned.16 It is unlikely that the moderate reduction in GSSG-R activity would cause hemolysis and, in a previous study of a kindred with both HS and GSSG-R deficiency, the two genes segregated independently and family members who had GSSG-R deficiency alone were asymptomatic.17 Levels of carbonic anhydrase II and factor VII, genes with structural or control loci assigned to chromosome 8,18 were not reduced, but the deletion observed in our patients probably involved a number of other genes, resulting in dysmorphology and the neurologic findings.

The pattern of inheritance in this family is unusual in that two siblings were affected whereas the parents and a third sibling were chromosomally normal. Cytoplasmic analysis on a fourth sibling has not yet been performed, but the child has a normal physical exam and hemoglobin values. Studies of blood groups and HLA antigens in this family were consistent with the presumed paternity. Although other possible mechanisms may explain our observations, the most likely explanation is that one parent is mosaic for this abnormality.

It is likely that congenital spherocytic anemias are a heterogeneous group of disorders but, other than a deficiency of α and β spectrin described in a small number of patients,19

**Table 2. Comparison of Ratios of Spectrin, Band 4.1, and Band 4.2 to Band 3**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Spectrin:Band 3</th>
<th>Band 4.1:Band 3</th>
<th>Band 4.2:Band 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Father</td>
<td>1.06 ± 0.08</td>
<td>0.24 ± 0.01</td>
<td>0.14 ± 0.02</td>
</tr>
<tr>
<td>Mother</td>
<td>1.05 ± 0.04</td>
<td>0.23 ± 0.04</td>
<td>0.14 ± 0.02</td>
</tr>
<tr>
<td>B.H.</td>
<td>1.06 ± 0.05</td>
<td>0.23 ± 0.03</td>
<td>0.14 ± 0.02</td>
</tr>
<tr>
<td>C.H.</td>
<td>1.06 ± 0.06</td>
<td>0.25 ± 0.02</td>
<td>0.14 ± 0.02</td>
</tr>
<tr>
<td>Control</td>
<td>1.08 ± 0.06</td>
<td>0.25 ± 0.02</td>
<td>0.15 ± 0.02</td>
</tr>
</tbody>
</table>

All results are means ± SD (n = 7). Results were determined by dye elution of Coomassie blue stain of 3.5% to 7.5% gradient sodium dodecyl sulfate polyacrylamide gels.
the results of SDS-PAGE of RBC ghosts derived from peripheral blood of HS patients, as in our two cases, show no qualitative abnormalities. Functional studies have detected abnormalities of spectrin-band 4.1 binding and cytoskeletal structure in small groups of patients. Hemizygosity for a gene on 8p might alter the quantity or function of a membrane protein in a manner not detectable by the semiquantitative gel systems used in this study. For example, an enzymatic deficiency resulting in a failure to modify appropriately a structural protein after translation could affect that protein's binding capacity. Additional studies will be needed to delineate the defect, but these findings may narrow the chromosomal region in which to search for a gene responsible for spherocytosis; the availability of a cell line should help in these investigations.

ACKNOWLEDGMENT

We would like to thank Drs Louis Miceli and Edward Klimek for referring the patient and Dr Ted Steck for helpful discussions. Barbara Jones provided technical assistance, and the carbonic anhydrase studies were performed by Dr Richard Tashian.

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