Characterization of 13 Novel Band 3 Gene Defects in Hereditary Spherocytosis With Band 3 Deficiency


Hereditary spherocytosis (HS) is a common hemolytic anemia of variable clinical expression. Pathogenesis of HS has been associated with defects of several red cell membrane proteins including erythroid band 3. We have studied erythrocyte membrane proteins in 166 families with autosomal dominant HS. We have detected relative deficiency of band 3 in 38 kindred (23%). Band 3 deficiency was invariably associated with mild autosomal dominant spherocytosis and with the presence of pincerred red cells in the peripheral blood smears of unsplenectomized patients. We hypothesized that this phenotype is caused by band 3 gene defects. Therefore, we screened band 3 DNA from these 38 kindred for single strand conformational polymorphisms (SSCP). In addition to five mutations detected previously by SSCP screening of cDNA, we detected 13 new band 3 gene mutations in 14 kindred coinherited with HS. These novel mutations consisted of two distinct subsets. The first subset included seven nonsense and frameshift mutations that were all associated with the absence of the mutant mRNA allele from reticulocyte RNA, implicating decreased production and/or stability of mutant mRNA as the cause of decreased band 3 synthesis. The second group included five substitutions of highly conserved amino acids and one in-frame deletion. These six mutations were associated with the presence of comparable levels of normal and mutant band 3 mRNA. We suggest that these mutations interfere with band 3 biosynthesis leading thus to the decreased accumulation of the mutant band 3 allele in the plasma membrane.

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We and another group have identified a previously unrecognized subset of HS patients characterized by a selective deficiency of band 3, the major integral protein of the red cell membrane, involved in transport of anions across the membrane and in linkage of the red cell membrane to the underlying membrane skeleton. The existence of this subset of HS was confirmed in a number of reports. In all studied kindred, band 3 deficiency was associated with a distinct phenotype including autosomal dominant inheritance and mild hemolytic anemia. In addition, we noted that band 3 deficient HS is associated with the presence of pincerred red cells in the peripheral blood (PB) smears of unsplenectomized subjects, the finding subsequently confirmed by other groups.

In two initial studies of band 3 deficient HS, we detected a duplication of 10 nucleotides and a cluster of substitutions of highly conserved arginine residues in the membrane domain of band 3. Several additional mutations of band 3 associated with band 3 deficient HS were reported. Based on these results, we asked whether or not the band 3 mutations represent a common cause of this phenotype and whether they consistently underlie the mild hemolytic anemia.

Since we previously cloned and completely sequenced the band 3 gene, we prepared intronic primers flanking the 19 coding exons and searched for band 3 mutations by screening genomic DNA for single strand conformation polymorphisms (SSCP) in the band 3 exons. Here we report 13 new band 3 mutations coinherited with the phenotype of dominantly inherited HS and band 3 deficiency. In addition, we report that this subset of HS is associated with a distinct phenotype including dominant inheritance, mild to moderate severity of hemolytic anemia, and a specific morphological feature of the PB smears, the presence of pincerred red cells.

MATERIALS AND METHODS

Case reports. Patients for this study were selected from 166 kindred with autosomal dominant HS studied at the Department of Biomedical Research of St Elizabeth's Medical Center. The diagnosis of autosomal dominant HS was based on the presence of hemoly-
sis in the unsplenectomized subjects, including increased reticulocyte count, increased bilirubin, and decreased haptoglobin levels, on the presence of spherocytes in the PB smear, increased osmotic fragility, exclusion of other causes of spherocytic hemolytic anemia and on the family history of hemolysis.

Quantitation of erythrocyte membrane proteins. Freshly drawn blood anticoagulated in acid citrate/dextrose was shipped on ice to Boston, MA. Within 48 hours of phlebotomy, erythrocyte ghosts were prepared by the method of Dodge et al. with minor modifications described in. Erythrocyte membrane proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by densitometry as described and the relative amounts of red cell membrane proteins were expressed as ratios of integrated densitometric peaks of individuals proteins to the peak of band 3. The biochemical findings were correlated with the clinical data and the results were statistically evaluated.

Evaluation of red cell morphology. Having previously noted the presence of mushroom-shaped, pinched red cells in the PB smears of subjects with band 3 deficient HS, we asked whether this morphological feature is characteristic of this HS subset. Therefore, without prior knowledge of the biochemical defect, we compared red cell morphology among HS subsets with different protein deficiencies. Five hundred red blood cells were evaluated in each blood smear for the percentage of pinched red cells, acanthocytes, and red cells with other shape abnormalities.

SSCP analysis of band 3 genomic DNA. Genomic DNA was isolated as described. SSCP screening was performed according to with minor modifications: coding exons of band 3 were polymerase chain reaction (PCR)-amplified using pairs of intronic primers flanking the exons (Table 1), 35 cycles, 1 minute at 94°C, 1 minute at 60°C. To each 10 μL PCR reaction, 2.5 μCi 32P-dATP (3,000 Ci/mmol; ICN, Costa Mesa, CA) were added. The PCR products were diluted in formamide loading buffer (86% formamide, 300 μM EDTA, 0.25% bromophenol blue, 0.25% xylene cyanol), heat denatured by boiling for 5 minutes, and quickly cooled on ice. Samples were electrophoresed at 8 W for 16 hours at room temperature in a nondenaturing polyacrylamide MDE gel (25 mL MDE gel solution prepared according to manufacturer’s specifications (AT Biochem, Malvern, PA), 69 mL deionized water, 6 mL 10 X TBE, 0.4 mL 10% ammonium persulfate, 40 μL TEMED) in the presence or absence of 10% glycerol and the gel was exposed to Hyperfilm-MP film (Amersham, Arlington Heights, IL) overnight at 80°C with intensifying screens.

Sequence analysis of band 3 DNA. One of the intronic primers used for the initial SSCP screening was phosphorylated at the 5’-end (3 μCi, 1 mM/L ATP, 10 U T4 polynucleotide kinase in a total volume of 30 μL of 10 mM/L Tris-acetate, 10 mM/L magnesium acetate, 50 mM/L potassium acetate, pH 7.5, 30 minutes at 37°C). Single stranded DNA was prepared from the PCR product using the PCR Template kit (Pharmacia, Piscataway, NJ) and directly sequenced using the Sequenase version 2.0 DNA Sequencing Kit (USB, Cleveland, OH).

Detection of band 3 mRNA alleles in reticulocyte RNA. Total reticulocyte RNA was isolated by ammonium chloride lysis and reverse transcribed using random primers. Patient cDNA was PCR-amplified using primers flanking the site of the mutations and the PCR product was digested with the relevant restriction endonuclease.

Sequence comparisons and structural predictions. Amino acid sequences of all 12 currently known sequences of human, mouse, rat, chicken, and trout erythroid band 3 proteins (anion exchanger 1, AE1) and of the related anion exchangers AE2 from human, mouse, rat, chicken, rabbit, and AE3 from human, mouse, rat and rabbit were retrieved from Genbank and aligned using the program CLUSTAL (PCGene, Intelligenetics, Mountain View, CA). The predictions of number and position of the band 3 transmembrane helices were made using programs RAOARGOS, HELIXMEM, and SOAP (PCGene) based on the reported cDNA sequence.

RESULTS

Red cell membrane protein analysis. A total of 166 HS kindred with autosomal dominant hereditary spherocytosis were studied. Of these, 38 kindred (23%) had a relative deficiency of band 3, reflected by an increase in the spectrin to band 3, ankyrin to band 3, and protein 4.1 to band 3 ratios. The band 3 content in these 38 kindred ranged from 57% to 73% of normal, the median being 64% of normal. Band 3

<table>
<thead>
<tr>
<th>Exon</th>
<th>Sense Primer (5' to 3')</th>
<th>Antisense Primer (5' to 3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2A</td>
<td>2B</td>
</tr>
<tr>
<td>3</td>
<td>3A</td>
<td>3B</td>
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<td>4</td>
<td>4A</td>
<td>4B</td>
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<tr>
<td>5</td>
<td>5A</td>
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<tr>
<td>6</td>
<td>6A</td>
<td>6B</td>
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<tr>
<td>7</td>
<td>7A</td>
<td>7B</td>
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<tr>
<td>8</td>
<td>8A</td>
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<td>9A</td>
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<td>12</td>
<td>12A</td>
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<td>14</td>
<td>14A</td>
<td>14B</td>
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<tr>
<td>15</td>
<td>15A</td>
<td>15B</td>
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<td>19B</td>
</tr>
<tr>
<td>20</td>
<td>20A</td>
<td>20B</td>
</tr>
</tbody>
</table>
3 deficiency was in all cases invariably associated with a deficiency of protein 4.2 (63% to 82% of normal). One hundred kindred (60%) had either an isolated relative deficiency of spectrin, reflected by a concurrent decrease in the spectrin to band 3 and ankyrin to band 3 ratios. Three kindred (2%) had an isolated relative deficiency of protein 4.2 without concurrent decrease in band 3. No quantitative biochemical abnormalities were detected in the remaining 25 kindred (15%). From the kindred described above, we selected 29 families with band 3 deficiency for further study, including five with band 3 mutations detected previously in the reticulocyte cDNA, and 31 families with spectrin deficiency of which 21 cases were combined with a deficiency of ankyrin. The spectrin content in these kindred ranged from 71% to 87% without correction for band 3 loss. An additional 30 normal unrelated subjects were also included.

Correlation between the biochemical and clinical phenotypes. Evaluation of hematological parameters revealed higher average hemoglobin values and lower reticulocyte counts and bilirubin levels for unsplenectomized HS patients combined with a deficiency of ankyrin. The spectrin content and bilirubin levels for unsplenectomized HS patients with band 3 deficiency compared to those with spectrin and/or ankyrin deficiency (Table 2). The differences between the two groups of patients were statistically significant at the level.

Identification of mutations in the band 3 gene. We prepared genomic DNA from the 29 band 3 deficient and 31 spectrin/ankyrin deficient patients with HS and from 30 controls and screened all coding exons of band 3 gene for SSCP. We detected a total of 22 different SSCPs in the 90 studied individuals. The exons containing the detected SSCPs were directly sequenced. Four SSCPs corresponded to two silent polymorphisms (codon 417, CTG → TTG, Leu → Leu, and codon 441, CTG → CTA, Leu → Leu) and to two previously described band 3 mutations, band 3 Montefiore GAG → AAG (codon 40) and band 3-Memphis AAG → GAG (codon 36) that are both asymptomatic in the heterozygous state. These four substitutions were detected in the band 3 deficient, spectrin/ankyrin deficient, and control subjects. The other 18 SSCPs were detected only in the patients with band 3 deficient HS. Five of them were caused by the five previously reported mutations, whereas 13 SSCPs corresponded to 13 new mutations. Substitution E37 ACG → ATG (Thr → Met) was detected in two unrelated subjects, while every other mutation was found in a single subject. The detected mutations are summarized in Table 3. None of these mutations has been detected in an additional 181 subjects without spherocytosis whose band 3 genomic has been screened for single strand conformational polymorphisms.

Coinheritance of band 3 mutations with the HS phenotype. We obtained blood samples from the additional family members for evaluation of the coinheritance of the detected mutations with the HS phenotype. Whenever possible, we digested the PCR amplified exons with restriction endonucleases whose restriction sites were either created or abrogated by the mutations (Table 3). In the five families where no suitable restriction endonuclease could have been used, we verified the linkage by direct sequencing of genomic DNA from other family members. We confirmed the linkage of the mutations with the HS phenotype in all 14 kindred. We designated the mutations by the name of the cities of origin (Table 3). Figure 1 shows the position of the mutations in the scheme of band 3 protein based on the predictions of a number and position of the band 3 transmembrane helices obtained with programs RAO-ARGOS, HELIXMEM, and SOAP.

Nonsense and frameshift mutations are associated with decreased content of mutant mRNA. In instances when the band 3 mutation altered a restriction site, digestion of PCR-amplified reverse transcribed reticulocyte band 3 mRNA with an appropriate restriction endonuclease allowed us to compare the relative amounts of the band 3 cDNA alleles. We found two groups of patients: the first subset includes the group of patients with frameshift and nonsense mutations in whom only the normal band 3 cDNA allele is detected suggesting that these mutations most likely render the mutant mRNA unstable. Similar finding was recently reported for one kindred. The second group includes missense mutation. In this subset, reverse transcription-PCR followed by restriction digestion detected both mRNA alleles in similar quantities. A representative example is shown in Fig 2. Similar results were obtained for other rautions from the two groups.

Missense mutations affect highly conserved amino acid residues. The second subset includes patients in whom mRNAs corresponding to the normal and mutant band 3 alleles are present in similar quantities, suggesting that neither the transcription of the mutant gene nor the stability of the mutant mRNA are affected. This subset contains the missense mutations: A285D, G455E, L707P, H834P, and T837M, and the deletion of amino acids 117-121. We aligned all currently known union exchanger sequences, i.e., human, mouse, rat, chicken, chicken, and trout erythroid band 3 (union exchanger, AE1) proteins and the related union exchangers AE2 from human, mouse, rat, and rabbit, and AE3 from human. All missense mutations and the five amino acid deletion affect highly evolutionarily conserved amino acid residues (Table 3). Two of them reside in the cytoplasmic domain and four mutations are located in three different transmembrane segments (Fig 1).

Identification of pinched red cells as a specific morphological feature of band 3 deficient HS. Blood films from
Table 3. Mutations Detected in the Band 3 Deficient HS Kindred. Results of SSCP Screening and Sequencing of DNA From 29 Unrelated Patients with Hereditary Spherocytosis With Band 3 Protein Deficiency

<table>
<thead>
<tr>
<th>Family</th>
<th>Gene Mutation</th>
<th>Protein Mutation</th>
<th>Restriction Site Altered</th>
<th>Family Members*</th>
<th>Designation</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>81 TGG→TGA</td>
<td>81 Trp→Stop (premature termination of translation)</td>
<td>3/2</td>
<td>Hodonin (Prague IV)</td>
<td>Mutant mRNA not detected</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>C→A (--3) (acceptor splice site in intron 5 mutated from CAG to AAG), deletion of amino acids from the putative ankyrin binding site</td>
<td>Deletion of amino acids GTVLL (117-121) (premature termination of translation)</td>
<td>2/1</td>
<td>Nachod (Hradec Kralove II)†</td>
<td>117G and 121L conserved in all 12 known AE sequences, conservative substitutions in positions 118 through 120</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Insertion of a G into codons 170-172 (TGGGGGGT→TGGGGGGGT)</td>
<td>Frameshift leading to premature termination of translation</td>
<td>Bsl I</td>
<td>2/0</td>
<td>Worcester</td>
<td>Mutant mRNA not detected</td>
</tr>
<tr>
<td>4</td>
<td>Insertion of a C into codons 273-275 (GCCGCCAC→GCCGCCCA)</td>
<td>Frameshift leading to premature termination of translation</td>
<td>1/4</td>
<td>Princeton</td>
<td>Mutant mRNA not detected</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>285 GCT→GAT</td>
<td>285 Ala→Asp (Amino Acids 283-285 RAAA mutated to RADA)</td>
<td>Bbv I</td>
<td>7/6</td>
<td>Boston</td>
<td>Motif RAAA conserved from chicken to human AE1, RAMA in trout AE1</td>
</tr>
<tr>
<td>6</td>
<td>455 GGG→GAG</td>
<td>455 Gly→Glu</td>
<td>2/0</td>
<td>Benesov (Prague VI)†</td>
<td>10/12† (2nd transmembrane segment). Present in cis with a duplication of 37 bases of unknown significance</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>G→A (first base of intron 12, is a termination codon)</td>
<td>Abnormal 7 amino acid termination of translation</td>
<td>Taq I</td>
<td>2/0</td>
<td>Pribram (Prague VI)†</td>
<td>Small amount of mutant cDNA detected by PCR of reverse transcribed reticulocyte RNA</td>
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<tr>
<td>8</td>
<td>Deletion of a C from codon 616 (ATC→AT)</td>
<td>Frameshift leading to premature termination of translation</td>
<td>Mbo I</td>
<td>3/1</td>
<td>Smichov (Prague VII)†</td>
<td>Mutant mRNA not detected</td>
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<tr>
<td>9</td>
<td>628 TAC→TAA</td>
<td>628 Tyr→Stop (premature termination of translation)</td>
<td>Srf I</td>
<td>2/1</td>
<td>Trutnov</td>
<td>Mutant mRNA not detected</td>
</tr>
<tr>
<td>10</td>
<td>Deletion of a G from codons 646-647 (CGGGG→CGGGG)</td>
<td>Frameshift leading to premature termination of translation</td>
<td>1/4</td>
<td>Hobart</td>
<td>Mutant mRNA not detected</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>707 CTG→CCG</td>
<td>707 Leu→Pro</td>
<td>Aci I</td>
<td>2/0</td>
<td>Most (Prague VIII)†</td>
<td>12/12† (9th transmembrane segment)</td>
</tr>
<tr>
<td>12</td>
<td>834 CAC→CCC</td>
<td>834 His→Pro</td>
<td>2/2</td>
<td>Birmingham</td>
<td>10/12† (13th transmembrane segment)</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>837 ACG→ATG</td>
<td>837 Thr→Met</td>
<td>Nia III</td>
<td>3/0</td>
<td>Philadelphia</td>
<td>11/12† (13th transmembrane segment). Identical mutation detected in two apparently unrelated families</td>
</tr>
</tbody>
</table>

Mutations are shown from the 5' to the 3' end. The mutations shown in italics are those leading to a premature termination of translation, ie, frameshift and nonsense mutations, which with the exception of the band 3m'M, lead to the absence of the mutant mRNA from the reticulocytes. * Number of affected versus number of unaffected family members studied. † (n/12) . . . amino acid is conserved in n out of 12 known anion exchanger cDNA sequences. ‡ Designation of the mutation in the preliminary communication.§ Duplicated are nucleotides 2899 through 2935 according to the numbering in Lux et al.¶ Mutation detected in two apparently unrelated families.
Fig 1. Structure of band 3 and location of the detected mutations. Band 3 protein is shown here with 14 transmembrane segments (light shading) connected by endo- and ecto-plasmic loops and with a highly schematic cytoplasmic domain of 404 amino acids. The drawing is based on the reported cDNA sequence and on predictions of the secondary structure and transmembrane helices obtained using programs RAOARGOS, HELIXMEM, and SOAP (PCGene, Intelligenetics). For detailed description of the mutations see Table 3.

the 29 kindred with band 3 deficient HS and from the 31 kindred with HS with spectrin/ankyrin deficiency were screened for the presence of pincered cells. A marked morphological difference between the blood smears of unsplenectomized spectrin/ankyrin deficient and band 3 deficient individuals was the presence of the pincered cells in the peripheral blood smears of the band 3 deficient subjects (Fig 3). Between 0.2% and 2.3% of pincered red cells were seen in 28 out of 30 unsplenectomized band 3 deficient subjects whereas occasional pincered cells (<0.2%) were observed in only 2 out of 26 unsplenectomized subjects with spectrin/ankyrin deficient HS. Pincered cells in the PB smears of unsplenectomized subjects thus represent a sensitive (93%) and specific (92%) feature of HS with band 3 deficiency. However, this morphological finding is not detected after splenectomy, when the principal morphological feature of the PB smears in both band 3 deficient and spectrin/ankyrin deficient HS patients is the presence of acanthocytes.

DISCUSSION

We describe the clinical, morphological, biochemical, and molecular genetic features of a distinct subset of HS, which include: (1) a dominantly inherited mild hemolytic disease, (2) the presence of pincered red cells in the peripheral blood film, (3) deficiency of band 3 protein, and (4) diverse mutations involving one allele of the erythroid band 3 gene. These mutations are highly heterogeneous and include nonsense, frameshift, and missense mutations scattered throughout the whole coding region of the gene. Excluding the six previously studied kindred with five reported mutations, we detected band 3 mutations in 14 (58%) out of the 24 studied kindred. Considering that (1) the yield of SSCP screening is usually
SPHEROCYTOSIS CAUSED BY BAND 3 GENE MUTATIONS

estimated to range from 70% to 90%, (2) the screening of individual exons would not detect major gene deletions, insertions or rearrangements, and (3) other regions of band 3 gene that were not examined in this study, such as the promoter region and the 3'-untranslated region, may be crucial for the expression of band 3 protein in the plasma membrane, the actual percentage of band 3 deficient HS caused by mutations in the erythroid band 3 gene is most likely considerably higher and, possibly, all cases of band 3 deficient HS may be caused by mutations in the erythroid band 3 gene.

The detected mutations are highly heterogeneous and are located in different regions of the band 3 gene suggesting the absence of a founder effect in which a distinct mutation, or a group of similar mutations, provides a selective survival advantage, leading to a preferential accumulation of the mutations in the population. The point mutations and the Δ117-121 deletion involve amino acid residues and motifs that have been highly conserved during evolution, indicating that these sites are of critical importance for band 3 biosynthesis. The heterogeneity of the mutations is further shown by recent reports of several additional band 3 mutations.

Although the reverse transcription of reticulocyte RNA followed by PCR an restriction digestion is not an accurate quantitative technique, the large number of subjects studied by this approach allows us to conclude that the molecular mechanisms leading to band 3 deficiency are most likely heterogeneous. In the group of nonsense and frameshift mutations, the band 3 deficiency appears to be a direct consequence of the decreased amount of mRNA corresponding to the mutant allele. Although the mechanism leading to the absence or decrease of the mutant mRNA has not been elucidated, it has been well-documented that nonsense mutations in messenger RNA, with the exception of the 3'-ends of the coding regions, cause a reduction in steady-state mRNA levels either by decreasing the production of the mutant mRNA molecules or by promoting their rapid degradation. The situation is analogous to the absence of the mutant ankyrin mRNA alleles that is also associated with spherocytosis as reported by us previously and to the recently reported nonsense mutation in the cytoplasmic domain of band 3 associated with decreased mRNA accumulation.

In contrast to the previous group, the amounts of the mutant and the wild-type band 3 mRNA are similar in the group of HS subjects with point mutations and the five amino acid deletion. The underlying mechanisms leading to band 3 deficiency in this group of HS are presently unknown and may include any step of band 3 biosynthesis from abnormal band 3 processing in the endoplasmic reticulum, defective assembly of band 3 into dimers, tetramers, or higher oligomers leading to premature band 3 degradation in the endoplasmic reticulum or the Golgi compartment, to premature loss of band 3 from the cell membrane. We have previously demonstrated for substitution R760Q in the membrane domain of band 3 that while similar amounts of the mutant and wild type mRNA are detectable in reticulocytes, the mutant protein is completely absent from the red cell membrane.

The molecular pathogenesis of band 3 deficiency may differ for mutations in the membrane domain and for the two mutations in the cytoplasmic domain. These two mutations reside within the region that has been previously shown to be involved in ankyrin binding. It has recently been shown that coexpression of band 3 with ankyrin helps to ferry band 3 from endoplasmic reticulum to Golgi apparatus and may help to facilitate the incorporation of band 3 into the plasma membrane. Mutations in the cytoplasmic domain of band 3 could therefore lead to band 3 deficiency by weakening band 3-ankyrin interaction in the ER thus leading to reduced incorporation of the mutant band 3 into the plasma membrane.

The finding that band 3 mutations represent a common molecular defect in a subset of patients with HS provides new insights into molecular determinants of red cell surface area. Band 3 protein, which traverses the plasma membrane multiple times, is thought to stabilize the membrane by interacting with the adjacent “boundary” lipid molecules.
A proposed decrease in the content of band 3 could lead to formation of protein-free areas of the lipid bilayer lacking the stabilizing protein-lipid and protein-protein interactions. Such areas of the membrane might be unstable and susceptible to a release from the membrane. A release of such band 3-free membrane regions may represent one of the mechanisms leading to surface area deficiency and spherocytosis in the HS deficient subjects. Such a hypothesis is supported by our recent finding of decreased density of intramembrane particles in these subjects. Another hypothetical mechanism of surface area deficiency may involve a weakened self-association of band 3 to tetramers/oligomers, or a weakened binding to ankyrin and thus the underlying skeleton, which may lead to an accelerated loss of band 3 from the cell membranes accompanied by the loss of adjacent lipids.

In contrast to the other subsets of HS in which the spectrin deficiency, often combined with deficiency of ankyrin leads to the assembly of a dysfunctional erythrocyte membrane skeleton, the spectrin-based membrane skeleton may remain intact in the band 3 deficient erythrocytes. This hypothesis is supported by the recent report of relatively intact membrane skeletons in erythrocytes from mice with a complete knockout of band 3. Because the membrane skeleton represents the main supporting structure of the red cell membrane, its more or less normal assembly in the band 3 deficient erythrocytes may underlie the milder clinical phenotype of the band 3 deficient HS compared to the spectrin and/or ankyrin deficient HS.

In conclusion, we report here 13 novel band 3 gene mutations associated with the phenotype of mild, dominantly inherited spherocytosis with deficiency of band 3 protein and with the presence of pinched red cells in the PB smear.

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Characterization of 13 novel band 3 gene defects in hereditary spherocytosis with band 3 deficiency

P Jarolim, JL Murray, HL Rubin, WM Taylor, JT Prchal, SK Ballas, LM Snyder, L Chrobak, WD Melrose, V Brabec and J Palek