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# A Case of Erythrocyte Membrane Protein 4.2 Deficiency with Coombs Negative Hemolytic Anemia

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**Abstract** A patient with mild hemolytic anemia and osmotically fragile, stomatocytic erythrocytes was studied. Analysis of the erythrocyte membrane proteins by SDS-PAGE revealed a partial deficiency of protein 4.2. All the other membrane proteins in the erythrocytes were normal. Enzyme activities in the erythrocytes were normal or increased. Deficiency of protein 4.2 is likely to be associated with the hemolytic anemia and stomatocytosis in this patient.

*Key words*: Hemolytic anemia, Erythrocyte, Protein 4.2, Stomatocytosis

## INTRODUCTION

Since the initial report by Lock et al in 1961,[1] a number of cases of hemolytic anemia with stomatocytosis have been reported. Stomatocytosis with osmotically fragile erythrocytes, which results in chronic hemolytic anemia, has been described by Miller and Meadow,[2,3] while stomatocytosis with normal osmotic fragility of erythrocytes has been documented in Mediterranean migrants in Australia.[4~6] Although stomatocytes are not always associated with reduced erythrocyte survival, the abnormality in membrane permeability often results in chronic hemolysis. The severity of chronic hemolysis and the degree of osmotic fragility of erythrocytes vary among affected patients, suggesting that hereditary stomatocytosis is a heterogeneous group of disorders. Recently, there have been a number of reports of erythrocyte membrane protein 4.2 deficiency associated with stomatocytosis.[7~9,16~20] The protein 4.2 deficiency is regarded as one form of hereditary stomatocytosis. Hemolytic anemia in patients with the protein 4.2 deficiency does not always respond completely to splenectomy, in contrast to hereditary spherocytosis.

In this report, we describe a patient with chronic hemolytic anemia associated with a reduction in major erythrocyte membrane protein 4.2. The membrane protein 4.2 deficiency appears to play a key role in erythrocyte membrane stabilization and deformability in this patient.

## CASE REPORT

A 16-year-old Japanese male visited our outpatient clinic for investigation of anemia. He had required phototherapy for neonatal hyperbilirubinemia (total bilirubin; 22 mg/dL) and shortly thereafter developed chronic

anemia and splenomegaly. During infancy and childhood, his hemoglobin ranged from 7.5 to 12.5 g/dL and the reticulocyte count varied markedly from 1.5% to 18.4%. Hemolytic attacks were usually associated with infections. However, blood transfusions have not been required to date.

At age 16, when first seen in our department, his general condition was fair, and he appeared well nourished. Physical examination findings were essentially normal except for splenomegaly. The spleen was palpable 2FB below the costal margin. Hemoglobin was 13.6 g/dL, hematocrit 39.1%, red cell count 4.25 x $10^6/\mu$L, MCV 92 fL, MCH 32 pg, MCHC 34.8 g/dL, and the reticulocyte count 3.2%. Serum haptoglobin was less than 10 mg/dL. A bone marrow aspirate showed erythroid hyperplasia. Iron was 100 $\mu$g/dL and total iron-binding capacity was 270 $\mu$g/dL. Concentration of hemoglobin F was 0.6%. The osmotic fragility test by Parpart's procedure was positive, while both the sugar- water and the Ham tests were negative. Both direct and indirect Coombs test were also negative.

## MATERIALS and METHODS

Red cell morphology was examined by differential interference microscopy after fixation with 1.0% glutaraldehyde in phosphate-buffered saline, at pH 7.4. Enzymes in the erythrocytes of the patient were assayed as previously described.[10,11] Membrane protein analysis was performed as follows. Heparinized blood was washed three times with 0.154M NaCl, and the leukocytes were removed by aspiration. Erythrocyte membranes were prepared by hypotonic lysis in 5.0 mM sodium phosphate pH 8.0, as described by Dodge et al.[12] They were washed five or six times with the lysis buffer, and kept frozen at −80℃ until analyzed. SDS- PAGE was performed as described by Laemmli et al[13] with a 3% acrylamide stacking gel and a 12% acrylamide separating gel. Samples were prepared for electrophoresis by mixing 1 vol of membranes with 2 vol of SDS sample buffer (3 g/dL sodium dodecylsulfate, 0.38 M dithiothreitol, 20% v/v glycerol, 0.19 M Tris-HCl, pH 6.8, and 50 mg/dL bromophenolblue), followed by boiling for 3 min. Fifty micrograms of membrane protein as measured by the protein dye technique was applied to each sample well after denaturation. The gels were stained with Coomassie Brilliant Blue. Erythrocyte membranes were prepared from a healthy donor, for a normal control, the patient and his parents.

## RESULTS

*Erythrocyte morphology*

Fig.1 shows the differential interference microscopic features of erythrocytes from the patient. A characteristic erythrocyte shape, the stomatocyte, was noted. No microspherocytes, which are typical of hereditary spherocytosis or elliptocytes were detected. Erythrocytes from the patient's parents were also examined and no stomatocyte was found.

*Erythrocyte enzyme activities*

As seen in Table 1, all enzyme activities assayed were normal or increased, reflecting a young, reticulocyte-rich population of red blood cells in this patient.

*Protein composition of erythrocyte membrane*

Analysis of erythrocyte membrane proteins by SDS-PAGE revealed partial deficiency of protein 4.2 in the patient. The analyses of membrane proteins of erythrocytes from the patient's parents were also

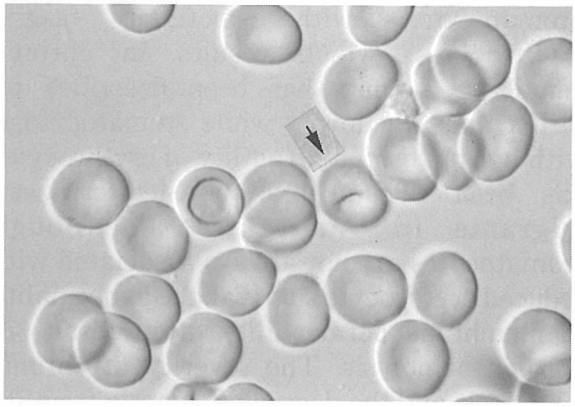

Fig.1 The feature of erythrocyte from the patient examined by differential interference microscope. (The arrowhead shows stomatocyte.)

Table 1. Enzyme Activity in Erythrocyte

| Enzyme | Normal range (unit/g-Hb) | Patient (unit/g-Hb) |
|---|---|---|
| Hexokinase(HK) | 0.78~1.06 | 1.70 |
| Glucosephosphate isomerase (GPI) | 51.1~60.6 | 61.5 |
| Phosphofructokinase(PFK) | 7.38~11.73 | 22.01 |
| Aldolase (ALD) | 0.56~2.37 | 5.57 |
| Triosephosphate isomerase(TPI) | 1156~1556 | 980 |
| Glyceraldehyde 3-phosphate -dehydrogenase(GA-3PD) | 117~185 | 229 |
| Phosphoglycerate kinase(PGK) | 261~357 | 314 |
| Monophosphoglyceromutase (MPGM) | 16.0~211.9 | 26.2 |
| Enolase(ENL) | 3.70~5.59 | 6.60 |
| Pyruvate kinase(PK) | 10.8~14.9 | 20.6 |
| Lactate dehydrogenase(LDH) | 139~185 | 214 |
| Glucose 6-phosphate -dehydrogenase(G6PD) | 9.30~11.39 | 11.64 |
| 6-Phosphogluconic dehydrogenase (6PGD) | 7.17~8.31 | 11.51 |
| Glutathion reductase(GR) | 3.53~5.15 | 7.84 |
| Glutathion peroxidase(GSH-Px) | 18.3~29.8 | 18.9 |
| Adenylate kinase(AK) | 171~247 | 229 |
| Adenosine deaminase(AD) | 0.89~1.32 | 1.14 |
| Acetylcholinesterase (Ch-E) | 24.0~33.3 | 28.9 |

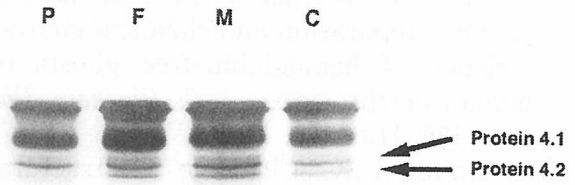


Fig.2 SDS-PAGE of erythrocytic membrane proteins.
P; Patient, F; Father, M; Mother, C; Normal Control

performed, and their erythrocytic band 4.2 appeared normal (Fig.2).

## DISCUSSION

In early reports, most cases of erythrocyte membrane protein 4.2 deficiency were found in Japanese and this disease was considered to be unique to Japanese. However, inherited hemolytic anemia with protein 4.2 deficiency and similar clinical symptoms were later reported in the United States and Tunisia[7,16] indicating that this erythrocyte membrane protein disorder is not specific for Japanese.

Yawata et al described two types of protein 4.2 deficiency, as follows; (i) Complete protein 4.2 deficiency with severe clinical symptoms, (ii) Partial protein 4.2 deficiency with the presence of a 68 kD or 74 kD protein, both of which are immunoreactive with anti- protein 4.2 IgG.[18,19] Rybicki et al also reported a decreased amount of an immunoreactive protein 4.2 analog (68 kD and 74 kD) by immunoblotting with purified anti-4.2 antibody in a patient, a Japanese-American female, with chronic hemolytic anemia.[9] In the present study, we detected a partial deficiency of protein 4.2 and this corresponds to the type (ii) described above.

Very little is known about the functions of the protein 4.2. It is known to bind to the cytoplasmic pole of band 3.[14] It has also been shown to be associated with ankyrin as well as band 3 in the erythrocyte membrane.[15] The fact that erythrocytes from patients with

protein 4.2 deficiencies were less deformable, as shown by using an osmotic gradient ektacytometric scanner, suggests that protein 4.2 plays a key role in erythrocyte membrane stabilization and deformability.[9]

The efficacy of splenectomy for protein 4.2 deficiency is doubtful. Rybicki et al reported that erythrocyte morphology, osmotic fragility and cell deformability were improved by splenectomy[16], whereas erythrocyte phenotype did not improve in response to splenectomy in other reports.[9,18,21] In our patient, the clinical manifestations are relatively mild and we are currently following him without medical intervention.

The hereditary nature of the protein 4.2 deficiency is unclear. In the present study, we also checked all hematological parameters of the patient's parents and analyzed their erythrocyte membrane proteins, but found no significant abnormalities. Therefore, the patient can be considered to have a idiopathic disorder and dominant inheritance can be ruled out. We are currently studying other members of his family to determine the inheritance pattern of this disease.

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