CONCISE REPORT

A Shortened Variant of Red Cell Membrane Protein 4.1

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In a healthy 32-yr-old woman with normal red cell morph-ology, a shortened variant of cytoskeletal membrane protein 4.1 is described at the heterozygous state. One haploid set of protein 4.1 migrates below protein 4.2 and displays a reduction in mass of approximately 8500 with regard to the normal haploid set. The shortening corresponds to a deletion of about 75 amino acids and concerns both subcomponents a and b of protein 4.1. It seems to involve some phosphorylation site(s). It was transmitted to the proposita’s son (who inherited elliptocytosis with band 4.1 deficiency from his father). To our knowledge, the present abnormality is the first unequivocal variant of erythrocyte membrane protein 4.1 recognized up to now.

Along with spectrin (bands 1 and 2)* and actin (band 5), protein 4.1 is a major protein of the red cell membrane skeleton. Actin and protein 4.1 interact with spectrin at the junction of spectrin heterotetramers. The resulting spectrin–actin–protein-4.1 complex plays a critical role in erythrocyte shape and deformability. Recently, partial or total lack of band 4.1 has been described in patients with various forms of hereditary elliptocytosis. It was postulated that this alteration could result from a deficient attachment of protein 4.1 onto the membrane. In one person whom we examined, however, the red cells with partial absence of band 4.1 were morphologically normal. In addition, an ectopic phosphorylation at the level of band 4.2 suggested that protein 4.1 could still be present in the membrane. Using electrophoresis on a polyacrylamide gel gradient, we have shown that one haploid set of protein 4.1 is shortened by approximately 75 amino acids. The shortening concerns both subcomponents a and b of protein 4.1. It seems to involve some phosphorylation site(s), and it is genetically transmitted.

CASE REPORT AND METHODS

Band 4.1 partial deficiency was detected in a 32-yr-old woman following the discovery of an elliptocytosis and a complete lack of protein 4.1 in her son. The child inherited elliptocytosis and part of his band 4.1 deficiency from his father (in whom band 4.1 is also reduced). It is important to note that the proposita is devoid of any clinical symptom and that her red cell count and morphology are normal (Fig. 1). Technical details of this study are indicated in the legends of Fig. 2 and 3.

RESULTS AND DISCUSSION

In controls, electrophoresis on a polyacrylamide gel gradient permitted the splitting of band 4.1 into its subcomponents a and b, the molecular weights of which are 82,500 and 81,500, respectively (Fig. 2). The proposita displays bands 4.1a and 4.1b in correct positions, although with reduced intensity. The use of polyacrylamide gel gradient also permitted the displacement, from behind band 4.2, of a doublet that now appears below this band. This doublet will be referred to as bands 4.1a’ and 4.1b’, with molecular weights of 74,000 and 73,000, respectively. Subcomponents 4.1a’ and 4.1b’ differ by the same mass (8500, e.g., approximately 75 amino acids) from subcomponents 4.1a and 4.1b respectively. As a result, they also differ from each other by the same mass as bands 4.1a and 4.1b do (1000, e.g., 8–9 amino acids).

The relationship between subcomponents 4.1a and 4.1b is uncertain. As pointed out by Sauberman et al., their relative intensity may vary. In the control, subcomponent 4.1a appears to be more intense than subcomponent 4.1b (scanning of the 4.1a–4.1b doublet was not feasible due to the close proximity of the two bands). In the proposita, bands 4.1a and 4.1b, or 4.1a’ and 4.1b’, have approximately the same intensity. One may conceive that subcomponent 4.1b derives from subcomponent 4.1a by a proteolytic process. They would be controlled by a single genetic locus. The parallel conversions (4.1-4.1a’-4.1b’) support this view: it is most unlikely that independent mutation involving separate genetic units would lead to such identical variations.

The shortening may result from a genic deletion or from an anticipated nonsense codon on the corresponding mRNA. It may also result from a change providing
an additional proteolytic site. In any event, one must assume that the alteration occurs opposite to the site at which the cleavage responsible for the 4.1a→4.1b conversion would take place. Otherwise, both subcomponents would be reduced to a single band. On radioautograms, components 4.1a' and/or b' appear less labeled than components 4.1a and/or b (Fig. 3), suggesting that the missing portion may contain some phosphorylation site(s).

It is important that subcomponents 4.1a' and b' are not the products of artifactual proteolysis, since antiprotease agents were not used. Genetic data practically rule out this possibility. The proposita's husband (with 100% elliptocytes and 2% reticulocytes) also displays reduced amounts of subcomponents 4.1a and b, but is strictly devoid of subcomponents 4.1a' and b' (Fig. 2). As noted by Mueller and Morrison in various patients with hereditary elliptocytosis, subcomponent 4.1a intensity is decreased relative to that of subcomponent 4.1b. According to Sauberman et al., this pattern would merely result from the rejuvenation of the red cell population, even though the reticulocyte count is only slightly elevated. The proposita's son (with 100% elliptocytes and 2% reticulocytes) displays the sum of the parental alterations: (1) he lacks subcomponents 4.1a and 4.1b (although it is possible that trace amounts of subcomponent 4.1b are present) and (2) he displays the 4.1a' and b' doublet (Fig. 2). Minute quantities of subcomponent 4.1b would account for some residual phosphorylation at the level of protein 4.1 in the proposita's son (Fig. 3). The genetic picture of this family, therefore, strongly suggests the direct genetic origin of subcomponents 4.1a' and b'.

In some cases, partial or total lack of band 4.1 probably reflects a genuine absence of protein 4.1 subcomponents from the membrane. In the proposita's husband, or in the patients described by Féo et al. and Tchernia et al., the absence of band 4.1 may result from a defective attachment of band 4.1 subcomponents onto the membrane. Elliptocytosis was present in all these patients, whether they are heterozygous or homozygous. The possible persistence of trace amounts of subcomponent 4.1b would suggest that at least the latter has retained minimal binding capacity. The corresponding mutation is not necessarily carried by

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**Fig. 1.** Erythrocyte morphology and parameters. The morphology was normal. The red cell parameters were: erythrocytes, $4.46 \times 10^{12}$/liter; Hb, 13.8g/100 ml; PCV, 39.5%; MCV, 89 fl; MHC, 31.1pg; MCHC, 35.1%; reticulocytes, 0.8%. (Photography: Dr. D. Treille.)

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**Fig. 2.** Red cell membrane protein electrophoretic profiles. Ghosts were prepared according to Dodge et al. Electrophoresis was carried out using a SDS-containing 5%-15% polyacrylamide gel gradient. Coomassie blue bands were named after Fairbanks et al. (a) Control in whom band 4.1 clearly splits into subcomponents a and b. (b) Proposita who displays reduced subcomponents 4.1a and 4.1b and additional subcomponents 4.1a' and 4.1b'. (c) Proposita's husband who is elliptocytic and partially lacks subcomponents 4.1a and 4.1b (subcomponents 4.1a' and 4.1b' are absent). (d) Proposita's son, who is also elliptocytic, completely lacks subcomponents 4.1a, possibly retains trace amounts of subcomponent 4.1b, and presents subcomponents 4.1a' and 4.1b'.
Fig. 3. Radioautograms of red cell membrane protein following in vitro phosphorylation. Phosphorylation was performed according to Roses and Appel, except that the incubation time was 10 min. Major phosphorylated bands were named after Fairbanks et al. Phospholipids. (a, b, c, and d: same as in Fig. 2). In b and d subcomponents 4.1a and/or b appear slightly phosphorylated; in d, the labeling in the band 4.1 region is decreased, but not abolished, supporting the presence of residual amounts of protein 4.1b. (–) No cAMP; (+) 5 μM cAMP.

protein 4.1 itself and may involve any protein that contributes to the attachment of protein 4.1. In other cases, partial lack of band 4.1 does not reflect a genuine absence of protein 4.1 subcomponents from the membrane. The total stock of protein 4.1 is present, but part of it displays an ectopic electrophoretic migration. The proposita of this study illustrates this hitherto undescribed situation. Under these circumstances, the mutation almost certainly involves protein 4.1 itself and not an interacting protein. It is remarkable that a deletion of approximately 75 amino acids, interestingly a major cytoskeletal protein, leaves the red cell morphology unaltered—at least at the heterozygous state. What the result of homozygosity would be is unknown at the present time. It may never have been observed because it is lethal. The shortened variant of protein 4.1 described in this communication represents a clinically and morphologically recessive trait, the frequency of which has now to be determined. Our results, therefore, call for more stringent screening of the band 4.1–4.2 region in the future.

REFERENCES

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