CONCISE REPORT

Spectrin Tunis (α1^78): A New α1 Variant That Causes Asymptomatic Hereditary Elliptocytosis in the Heterozygous State

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Spectrin Tunis (α1^78) was found in the heterozygous state in a young white North-African man and his mother. Both of them presented with mild elliptocytosis. Using onedimensional electrophoresis, a sharp 78 kd fragment was present with a reciprocal decrease of the α1 80 kd domain. Kinetic analysis unambiguously confirmed that the 78 kd fragment developed at the expense of the α1 80 domain. The α1 74 kd peptide was not flanked with a peptide lacking a 2 kd fragment. From this fact, it could be inferred that the site for additional proteolysis is located upstream from arginyl residue 39 and, more precisely, should lie 10 to 20 amino-acid residues (−2 kd) from the α-chain N-terminus. The percentage of spectrin dimers in 4°C extracts was high (over 40%), contrasting with the absence of clinical symptoms related to elliptocytosis. This is the first mutation responsible for elliptocytosis found in Tunisia.

The propositus is a 24-year-old Tunisian man who complained of asthenia, headache, dyspnea, and tachycardia. Clinical examination, however, was negative. In particular, icterus and spleen enlargement were absent. Elliptocytosis was discovered fortuitously. It consisted of bulky elliptocytes with no tendency to bud (Fig. 1). Red cell indices were normal (RBC, 5.24 × 10^12/L; reticulocytes, 47 × 10^9/L; Hb, 163 g/L; Ht, 0.48; MCV, 85 fL). The concentrations of bilirubin and of haptoglobin, 5 mg/L and 0.84 g/L (controls, 1.28 ± 0.25 g/L), respectively, indicate that there must be very little hemolysis, if any. Elliptocytosis has been transmitted by the mother, who also appeared free of clinical symptoms. Her elliptocytes had a similar aspect and her red cell indices were normal. The father, who is nonconsanguineous with respect to the mother, is clinically and hematologically normal. The propositus has one sister and five broth-

THE RED CELL skeleton includes a protein network that laminates the inner surface of the plasma membrane and governs erythrocyte shape and deformability. It comprises three major proteins: spectrin, protein 4.1, and actin (for review, see reference 1). Spectrin is usually referred to as a fibrillar αβ dimer. Two dimers associate head-to-head to form a heterotetramer. Limited trypsin digestion allowed the division of the α-chain into five domains, termed α1 to α5, the α1 domain contributing to the head of spectrin and containing the α-chain N-terminus (for review, see reference 2). Of the four domains forming the β-chain, the β1 (C-terminus-containing) domain is facing the α1 domain. Many cases of hereditary elliptocytosis (HE) result from mutations affecting spectrin or protein 4.1 (for review, see reference 3). Most spectrin variants involve the cephalic region (α1 and β1 domains). Alpha-1 alterations have usually been identified by studying changes of the α1 trypsin digestion patterns. It is not certain, however, whether all recorded α1 alterations result from mutations that do lie in the α1 domain itself. In Spa174 HE, the α1 80 kd peptide is reduced and the normally existing α1 74 kd peptide is reciprocally accentuated. New peptides develop at 46 kd (or 50 kd) and at 65 kd in Spa146 (or Spa1^98) HE and Spa1^195) HE, respectively. Recently, a unique elliptocytogenic α1 variant has been described with additional peptides at 43 and 42 kd. All defects of the head of spectrin result in reduced dimer self-association. We herein report on spectrin Tunis (α1^78), that is defined as a new abnormality of the α1 domain digestion pattern and is associated with mild elliptocytosis in the heterozygous state.

CASE REPORT

The propositus is a 24-year-old Tunisian man who complained of asthenia, headache, dyspnea, and tachycardia. Clinical examination, however, was negative. In particular, icterus and spleen enlargement were absent. Elliptocytosis was discovered fortuitously. It consisted of bulky elliptocytes with no tendency to bud (Fig 1). Red cell indices were normal (RBC, 5.24 × 10^12/L; reticulocytes, 47 × 10^9/L; Hb, 163 g/L; Ht, 0.48; MCV, 85 fL). The concentrations of bilirubin and of haptoglobin, 5 mg/L and 0.84 g/L (controls, 1.28 ± 0.25 g/L), respectively, indicate that there must be very little hemolysis, if any. Elliptocytosis has been transmitted by the mother, who also appeared free of clinical symptoms. Her elliptocytes had a similar aspect and her red cell indices were normal. The father, who is nonconsanguineous with respect to the mother, is clinically and hematologically normal. The propositus has one sister and five broth-

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Blood smears. F, father; M, mother; P, propositus.

Fig 1.
Fig 2. One-dimensional analysis of spectrin limit digests. Spectrin digests were analyzed on 7% to 15% linear acrylamide gels. P. propositus; F. father; M. mother. The densitometric records are presented in the 80 to 74 kd domain.

METHODS

The methods used have been described or referred to in detail by Pothier et al. They include (a) the preparation of erythrocyte ghosts and crude spectrin extract, (b) sodium dodecylsulfate (SDS)-polyacrylamide gel electrophoresis (homogeneous 7% acrylamide concentration) according to Laemmli, (c) the study of spectrin dimer self-association with separation of spectrin dimer and tetramer in nondenaturing gels, and (d) the one- and two-dimensional electrophoretic analysis of spectrin limit digests. All tests (except for spectrin dimer self-association) were repeated on two separate samples within a 10-week interval in the propositus, his father, and his mother. Kinetic analysis of spectrin digestion was carried out twice in the propositus.

RESULTS

Spectrin dimer self-association was deficient. In the propositus, the mother, and the father, the spectrin dimer percentages in the 4°C extracts were 43.5%, 41.9%, and 8.55%, respectively (controls, 7.7 ± 2% [n = 19]). The association constants (Lmol⁻¹) were (in the same order): 1.75 × 10², 1.97 × 10², and 4.94 × 10² (controls, 4.59 ± 0.8 ± 2σ [n = 27]). SDS-polyacrylamide gel electrophoresis of total

Fig 3. Kinetic analysis of limited tryptic digestion. Limited spectrin digestion was carried out for different times. In order to improve separation of the 80 kd and the 78 kd bands, gels with 7% to 15% exponential concentration were used (one-dimensional analysis). Abscissa, digestion times in hours. Ordinates, peak ratio. a. (80 + "78")/80 + "78" × 100, in controls (n = 3), in whom "78" represents a normally existing faint band (see text). b. (80 + "78")/80 + "78" × 100, in the propositus. c. (80/80 + 78 + 74) × 100, in the propositus. d. (78/80 + 78 + 74) × 100, in the propositus. The 78 kd fragment develops in proportion as the 80 kd fragment diminishes. The small divergence of curves (a) and (b) reflects the small accentuation of the 74 kd fragment in the propositus (see text).
membrane proteins was normal in all members of the family (not shown). Densitometric determination showed neither quantitative alteration nor a duplication of either the α or the β chain (using long migration times for the search of a duplication). In addition, the amount of band α was strictly normal. It is remarkable that the 4.1α/4.1β ratio itself was unchanged: 3.22, 3.07, 2.79 in the propositus, the mother, and the father, respectively (controls, 2.71 ± 0.38 [n = 5]). This ratio is higher than that recorded in previous studies due to methodological changes; only this part of the gel extending from band 3 to band 4.2 was scanned (instead of the entire gel). This finding further suggests that there must be very little accompanying hemolysis, if any.

One-dimensional analysis of spectrin limit digests (20 hours) revealed a unique abnormality in the propositus and his mother (Fig 2). It consisted of a sharp band of 78 kd. It is to be noted that a faint band (0.8%) exists at the same level under normal conditions; however, its nature has not been identified thus far. Careful densitometric studies showed that the new 78 kd band compensated exactly the decrease of the αl 80 kd band (Table 1). The ratio of the 78 kd peak to the sum of this peak and of the 80 kd peak (78/ (80 + 78) ratio) was 0.30 (20 hours digestion). On two-dimensional analysis, the 78 kd spot (pl, 5.05) could not be detected unless small amounts of protein were applied (not shown). In order to demonstrate further that the 78 kd peptide is derived from the αl domain, we carried out a kinetic analysis (Fig 3). It appeared unambiguously that the 78 kd peptide developed at the expense of the αl 80 kd fragment. There was a lag between the appearance of the αl 80 kd fragment and that of the 78 kd fragment. The sum of the 80 kd and of the 78 kd peaks remained nearly equivalent (see below) to the 80 kd peak of the controls at the corresponding times. Taken together, these data indicate that the 78 kd fragment is a product of the 80 kd fragment. It will be referred to as the αl 78 kd fragment.

The αl 74 kd fragment was slightly but significantly increased (Table 1). This was also clearly visible in the kinetic analysis (Fig 3). It is critical that it was not flanked with a shorter band that could have been considered as deriving from the αl 78 kd fragment (Fig 2). It appears, therefore, that the cleavage at arginyl residue 39, that generates the αl 74 kd fragment from the αl 80 kd fragment, removes the sites where the abnormal proteolysis (80 kd → 78 kd) takes place.

### DISCUSSION

To our knowledge, the present abnormality of spectrin digestion has not been described before. It is manifested by the presence of a sharp 78 kd fragment in limited tryptic digests of spectrin. Densitometric analysis of one-dimensional gels and a kinetic analysis of limited spectrin digestion in the presence of trypsin demonstrated that the 78 kd fragment is derived from the αl domain. As a result, the αl78 abnormality belongs to the family of abnormalities designated αl74, αl186 (or αl179), αl145, 6,7 and αl143-42,8 all of which are able to generate elliptocytosis.

The altered spectrin must carry a novel, yet unknown, mutation. The small accentuation of the αl74 kd peptide may be due to an instability of this normal cleavage site similar to that we noted in spectrin Nice (β229/216)9 and that culminates in Spo178 HE.4 Most significant is the fact that the 74 kd fragment is not itself accompanied by a shorter fragment, indicating that the ectopic cleavage point must take place upstream from the arginyl residue 39 and that the proteolysis site occurs probably no farther than 10 to 20 amino acids from the N-terminus, owing to the 2 kd lacking fragment. This situation is at variance with that observed in αl174, αl186, and αl143-42 abnormalities, in which the ectopic cleavage points are downstream from arginyl residue 39. If the mutation does not exactly coincide with the ectopic site of digestion at least we can assume that it is located in its neighborhood.

We noticed the high proportion of spectrin dimer in the 4°C extract, indicating that the abnormal dimers are totally unable to self-associate. Such high figures are usually associated with clinical symptoms, whereas the present carriers display no HE-related manifestations (even the 4.1α/4.1β ratio was normal). We have no explanation for this discrepancy.

The Spo178 abnormality is the first mutation found in association with elliptocytosis in Tunisia. Further studies will be necessary to assess whether it corresponds to an exceptional mutation or whether it is sporadic in this country. Waiting the elucidation of the ultimate molecular change, we suggest that the present abnormal spectrin be designated spectrin Tunis (αl78).

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