A Second Allele of Spectrin \(\alpha\)-Gene Associated With the \(\alpha^{1/35}\) Phenotype (Allele \(\alpha^{Ponte de Sõr}\))

To the Editor:

Many mutations of spectrin \(\alpha\)-gene result in hereditary elliptocytosis (HE) and are manifested by various peptide map abnormalities. A given alteration may derive from several mutations located close to one another. In an opposite way, the so-called \(\alpha^{1/35}\) abnormality has long been found to be associated only with the duplication of TTG codon 154 (Leu),

\[
\text{GGT} \rightarrow \text{GAT}\]

defining allele \(\alpha^\text{Dup154}\), as hereafter designated. This allele has a mild expression and appears in people from Black Africa, Northern Africa, and Southern Italy. We describe here two unrelated kindreds with a symptomless \(\alpha^{1/35}\) change. Unexpectedly, the underlying mutation was a distinct and novel change: 151 GGT \(\rightarrow\) GAT; Gly \(\rightarrow\) Asp. The mutated allele was termed allele \(\alpha^{\text{Ponte de Sõr}}\).

Unrelated families SV and RN were Portuguese and French, respectively (Fig 1). All members were symptomless and disclosed normal red blood cell indices. Elliptocytosis was observed in individuals SV II.1 (40%) and RN I.2 (20%). The methods used in protein chemistry have been described or referred to before.3,4 Segments of genomic DNA encompassing exon 2, 3, and 4 were amplified by polymerase chain reaction and sequenced directly or after subcloning in plasmid vector PGEM3Z. In some experiments, amplified DNA was digested using BstEII or Fok I.

The percentage of spectrin dimers in crude Sp extracts (4°C) was normal in three members of family SV (it was not investigated in family RN). In individuals SV I.2 and SV II.1, two-dimensional maps of spectrin disclosed the presence of a spot defining the \(\alpha^{1/35}\) phenotype (data not shown). Partial amino acid sequencing showed that this fragment arose from an abnormal cleavage after Arg 137, as is observed in the presence of the \(\alpha^{\text{Dup154}}\) allele, and disclosed the 151 Glu \(\rightarrow\) Asp substitution. Nucleotide sequencing (individual SV II.1 and RN I.2) displayed the 151 GGT \(\rightarrow\) GAT change and ruled out the duplication of TTG codon 154 (Fig 2). Whenever they were looked for, a BstEII site was abolished and a Fok I site was created due to the a151 mutation (data not shown). Nucleotide sequences of exons 2 and 3 (individual II.1) did not show any additional alteration. Quantification of the a(1/35)FokI fragment and assessment of exon 40 mutation established heterozygosity for allele \(\alpha^{EELY}\), a low expression allele, in four individuals (Fig 1).

Taken together, two unrelated kindreds displaying the \(\alpha^{1/35}\) phenotype failed to carry the common \(\alpha^{\text{Dup154}}\) mutation but bore a novel mutation at position 151. The latter created the same abnormal tryptic peptide from the spectrin \(\alpha\)-chain resulting from Arg 137. A limited elliptocytosis accompanied the \(\alpha^{EELY}_{\text{Ponte de Sõr}}\) diploidy, but not the \(\alpha^{\text{Dup154}}_{\text{Ponte de Sõr}}\alpha\) diploidy. The modulation by allele \(\alpha^{EELY}\) assigned the primary alteration to spectrin \(\alpha\)-gene. The normal sequence of exons 2 and 3 ruled out the possibility of any other mutation in the same conformational unit (helices 2 or 3) or in helix 1 of repeat a2. Finally, the resemblance of the picture yielded by alleles \(\alpha^{\text{Ponte de Sõr}}\) and \(\alpha^{\text{Dup154}}\) and the fact that the latter is held responsible for elliptocytosis led us to consider that the former yields elliptocytosis in a like fashion.

ACKNOWLEDGMENT

Supported in part by the "Association Française contre les Myopathies," the "Conseil Scientifique de l’UFR Xavier-Bichat, Université Paris VII," the "Centre National de la Recherche Scientifique," and the "Institut Pasteur de Lyon." We thank Dr L. Denoroy (CNRS URA 1195, Lyon, France and Service Central d’Analyse, CNRS, Vernaison, France) for having performed partial amino acid sequencing.

REFERENCES

Fig 2. Nucleotide sequencing of part of exon 4 (individual SV II.1).
A second allele of spectrin alpha-gene associated with the alpha I/65 phenotype (allele alpha Ponte de Sor) [letter]

L Boulanger, D Dhermy, M Garbarz, C Silva, J Randon, R Wilmotte and J Delaunay