A Rapid Method for the Detection of AlphaI65 Hereditary Elliptocytosis

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Hereditary elliptocytosis (HE) designates a group of hemolytic disorders with elliptical erythrocytes due to a weakened horizontal stress-supporting protein network in the membrane skeleton [1]. Most defects leading to spectrin self-association alteration involve the αI spectrin domain. Sp αI/65 is manifested by an abnormal cleavage of the spectrin αI domain upon limited tryptic digestion: the α 65-kD fragment becomes prominent instead of the usual 80-kD fragment [2]. This change resulted in all subjects studied so far from a cleavage following Arg 137 resulting, in turn, from the insertion of a leucyl residue at position 154 of the α-spectrin chain [3]. This relatively common mutation has been found in Africans and Americans Blacks, in North Africans [4] and recently in white Italian people [5]. In all cases investigated, it was associated with the same haplotype (-1 l·, with respect to ihXbal,Pvull and Mspl polymorphisms) [6, 7].

We have used the polymerase chain reaction (PCR) as a rapid means of detecting HE due to spectrin αI/65. Eight individuals from 3 different kindreds referred to us for HE were studied. Biochemical diagnoses were performed after determination of spectrin self-association and partial tryptic digestion as described elsewhere [7]. Dot blot hybridization of amplified DNA confirmed a TTG insertion between codon 153 and 154 [7]. Two primers were selected to allow the amplification of the fourth exon of the α-spectrin gene: sense 5’ TCCCTGCTCCCAGTGTCTGT and anti-sense 5’ GCCCTCCC.ACTCCTCTGTCCC. For amplification we used TaqI polymerase with 30 cycles of denaturation (95 °C for 5 min for the first, and 90 °C for 30 s for the following cycles), annealing at 55 °C for 30 s and extension at 55 °C for 90 s. After amplification, the 242-bp PCR product was digested with the restriction enzyme AlwNI (Biolabs, England).

Fig. 1. AlwNI restricted PCR-amplified DNA product of exon 4 of human α-spectrin gene. Lane 2 = Amplified fragment before digestion (242 bp); lane 1 = fragments obtained after AlwNI digestion in an αI/65 heterozygous and in a normal subject (lane 3).

In normal chromosomes, bands corresponding to 10, 107 and 125 bp were produced after digestion. In HE chromosomes 10- and 232-bp bands appeared because the insertion of a TTG
codon abolishes one normally occurring AlwNI site. All HE αI/65 heterozygous subjects showed the expected pattern (232-, 125-, 107- and 10-bp bands).

On the basis of this rapid technology, we could confirm the homogenous molecular basis of HE αI/65 in Italy. Furthermore, we propose this method for rapid detection of sp αI/65 in those geographic areas with high frequency of this gene [8]. This molecular approach to αI/65 HE is simple, does not require radioisotopes and offers advantages over standard biochemical analysis in terms of rapidity of results.

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