A Rapid Method for the Detection of Alpha^{I65} 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 α spectrin domain. Sp α ^{I65} is manifested by an abnormal cleavage of the spectrin α 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 1, with respect to htX^{bal} , PvuII and MspI polymorphisms) [6, 7].

We have used the polymerase chain reaction (PCR) as a rapid means of detecting HE due to spectrin α ^{I65}. 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' GCCTCCCACTCCTCTGTCCC. 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 α ^{I65} 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 α 1/65 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 α 1/65 in Italy. Furthermore, we propose this method for rapid detection of sp α 1/65 in those geographic areas with high frequency of this gene [8]. This molecular approach to α 1/65 HE is simple, does not require radioisotopes and offers advantages over standard biochemical analysis in terms of rapidity of results.

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Acknowledgements

We are grateful to Prof. Jean Delaunay for his help with this article. This work was partially supported by AIRC and 'Progetto Fina-lizzato Sanità' Regione Campania. A.I. is a member of the Italian Pediatric Cancer Research Group.

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