MOLECULAR BASIS OF THE δ THALASSEMIA IN CIS TO HEMOGLOBIN KNOSSOS VARIANT

To the Editor:

Hemoglobin (Hb) Knossos (α, β, 27 Ala → Ser) is a silent β+ thalassemia variant, which was first described in a Greek family and later was detected in other families of Mediterranean origin. If heterozygotes for Hb Knossos from this area showed normal-borderline red blood cell indices and low HbA₂ levels. HbA₂ was absent in homozygotes for this variant. These data suggested the presence of a δ⁺-thalassemia gene in cis with the Hb Knossos gene. In contrast, in a family from the French West Indies, heterozygotes for Hb Knossos had the high HbA₂ level, typical of a classic β-thalassemia carrier.

Here we describe the molecular characterization of the δ-globin gene in cis to the Hb Knossos gene in three members of the family.

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Fig 1. (A) Pedigree and relevant hematologic features of the Greek family studied: (■), β-thalassemia; (●), Hb Knossos; (□), normal, (○), nonexamined. (B) (Top) Direct sequencing on amplified DNA of the δ-globin gene of the subject III-1. The deletion in codon 59 (AAG → AG), indicated within the box, produces a frameshift and thus several ambiguities are present in the reading of the sequences. The frameshift causes a stop codon at position 60 as shown in the figure. (Bottom) Dot-blot analysis of amplified DNA from a normal (N) subject and three family members (III-1, III-2, and III-3), heterozygotes for the Hb Knossos variant. The amplified δ-globin gene region spans from position -146 5' to the CAP site to position 82 of the IVS2. The sequence of the two oligonucleotides complementary to the mutated and normal region are: δmut: 5' GCAACCCTAGGTAAGG 3'; δnorm: 5' CCTCACCTTAGGTTG 3'.
first described by Fessas et al1 (Fig 1). By using the polymerase chain reaction (PCR) technique and several pairs of primers as previously described, we amplified several DNA regions covering the entire δ and β-globin genes. Direct sequencing on amplified DNA detected in the patient, with the clinical picture of Thalassemia Intermedia from this family (III-1), the compound heterozygous state for the β + 110 and the Hb Knossos mutations. In addition, in one of the δ-globin genes from this patient we found a single nucleotide deletion at codon 59 (AAG → AG). This mutation produces a frameshift that results in the production of a stop codon at position 60 (Fig 1). By sequencing analysis the frameshift at codon 59 was also detected in two carriers of Hb Knossos (Fig 1, II-3, III-2) from the same family. We confirmed this mutation by the allele specific oligonucleotide (ASO) probe's hybridization (Fig 1). These results suggest that the δβ-thalassemia gene segregates together with the Hb Knossos gene and is responsible for the low HbA2 levels in the heterozygotes for Hb Knossos. The presence of two mutations in the same β-globin gene cluster was already described. In the Sardinian δβ-thalassemia the β'39 non-sense mutation is associated with the −196 Ay, C → T substitution, which increases the HbF levels. The δ + 27 G → T substitution and the β + IVS2 nt 745 G → C mutation were detected on the same chromosome in a family of Greek origin, whereas a 7.2-kb deletion that starts from a region 3’ of the δβ-gene and ends within the second intron of the δ-globin gene (δthal) was described in cis to the β + IVS1 nucleotide 5 G → A mutation (Corfu δβ-thalassemia).10 These mutations of the Ay and δβ-globin genes have been found as an isolated lesion on the β-globin gene cluster.4,11-13 Family studies and haplotype analysis supported the hypothesis that a crossing over event between two chromosomes carrying the single mutation may have produced a new chromosome carrying both defects. In the case described herein a frameshift mutation at codon 59 of the δ-globin gene has not been described thus far as an isolated defect, indicating perhaps that this mutation occurred on a chromosome carrying the Hb Knossos defect.

ACKNOWLEDGMENT

Sponsored by the World Health Organization. Support for this study was provided by grants from: Assessorato di Igiene e Sanita Regione Sardegna (progetto ricerca sanitaria finalizzata: “Malattie Genetiche di notevole rilevanza in Sardegna”) and legge Regionale no. 11, April 30 1990, progetto Stategico per il Mezzogiorno, CNR (89.00307.75), and 40% AC90 Istituto di Ricerca sulle Talassemie ed Anemie Mediterranean CNR-Cagliari. G.L. is the recipient of a Cooley’s Anemia Foundation fellowship.

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