To the Editor:

In members of a family of southern Sardinian descent, we have recently detected a 7.2 kb deletion in the β-globin gene cluster that starts in the ψβ-δ intergenic region and ends in the IVS-2 of the δ-globin gene. Heterozygotes for this deletion showed reduced hemoglobin (Hb)-A₂, while double heterozygotes for this mutation and the codon 39 non-sense mutation had the phenotype of normal HbA₂ β-thalassemia.¹ We report here the sequence analysis of the breakpoints of this 7.2 kb deletion and describe another family from the Ferrara region in which several members were found to carry the same mutation.

Hematologic analysis, Southern blot, DNA amplification, dot blot analysis with allele-specific oligonucleotide probes, and DNA se-
quencing on amplified DNA were done according to standard methods.

The deletion breakpoints were defined on the anticodon strand of the amplified DNA from the propositus of the Sardinian family. DNA amplification was carried out by using two oligonucleotide primers: one was complementary to a sequence at position 280 ± 2 bp from the supposed 5' breakpoint on the coding strand, and the other was complementary to a sequence at position 149 ± 2 bp from the supposed breakpoint on the anticoding strand. Using this approach, we amplified specifically a fragment of DNA of 419 nucleotides (nt) belonging to the chromosome in which the 7.2 kb deletion resides. Co-amplification of other DNA sequences were not observed. Direct sequencing on amplified DNA was carried out using an internal oligonucleotide primer complementary to a sequence at position 98 ± 2 nt from the 3' breakpoint on the coding strand (Fig 1a). The sequence analysis showed that the 5' and 3' breakpoints are located −5896 ± 2 nt from the δ-globin gene cap site and 820 ± 2 nt in the IVS-2 of the δ-globin gene, respectively, separated from each other by 7201 bp. The chromosome containing the 7.2 kb deletion had the following haplotype: +−−−−−.

The propositus of the family originating in the Ferrara region has low mean corpuscular volume (MCV; 65 fl) and mean corpuscular hemoglobin (MCH; 20.5 pg), normal HbA₂ (2.1%), and increased α/γ-hemoglobin chain synthesis (α/γ ratio, 2.1), and was thus classified as a normal HbA₂ β-thalassemia heterozygote. His spouse has normal red blood cell indices and normal HbA₂ levels. One of their children has the hematologic characteristics of a typical β-thalassemia heterozygote, while the other has normal red blood cell indices, a normal HbA₂ level, and a balanced α/γ globin chain synthesis ratio. These data suggest that the propositus is double heterozygous for δ̲ and β-thalassemia. Dot blot analysis with allele-specific oligonucleotide probes complementary to the most common mutations in the Mediterranean area, including the IVS-1 nt 5, detected the codon 39 non-sense mutation in both the high HbA₂ and the normal HbA₂, β-thalassemia heterozygote of this family. In order to define the postulated δ-thalassemia mutation, we tested the patient DNA with a series of oligonucleotide probes complementary to the known δ-thalassemia mutations, including the 7.2 kb deletion detected in the Sardinian family. Dot blot analysis on amplified DNA and hybridization with these oligonucleotide probes detected a positive signal with the oligonucleotide probe complementary to the 7.2 kb deletion breakpoints in the DNA from the propositus and his child with a normal HbA₂ level (Fig 1b), indicating that both were carriers of the 7.2 kb deletion. These results were confirmed by Southern blot analysis.

The 7.2 kb deletion characterized in this study silences the δ-globin gene and may thus be characterized as deletion δ̲-thalassemia. This deletion δ-thalassemia shows identical breakpoints to a 7.2 kb deletion associated in cis to a β-thalassemia mutation (G→A substitution at position 5) previously reported as Corfu δ̲β̲-thalassemia, because the homozygous state resulted in the phenotype of δ̲β̲-thalassemia. This finding led to the suggestion that DNA sequences in the ψβ region regulate the expression of the “in cis” γ̲ and β-globin genes. This suggestion is obviously excluded by our study, which has demonstrated that the 7.2 kb deletion is associated with a normal function of the “in cis” β̲ and γ̲-globin gene. Accordingly, re-examination of the original patient by more sensitive techniques detected, in fact, a small amount of HbA (Kattamins, personal communication, April 1989). The Sardinian δ̲-thalassemia chromosome shared the same 5' chromosomal subhaplotype but not the 3' subhaplotype with the Corfu δ̲β̲-thalassemia, most probably indicating a single origin of the 7.2 kb deletion with subsequent spread to different chromosomes by homologous recombination.

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REFERENCES


Deletion delta-thalassemia: the 7.2 kb deletion of Corfu delta beta-thalassemia in a non-beta-thalassemia chromosome [letter]

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