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A newly identified deletion at the α-globin locus removing the promoter region of the α_1 gene

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In four related patients we have identified an up to now unknown α-globin allele that is characterized by a 970-bp deletion encompassing the entire α_1-promoter region. From this newly identified (α–$\alpha^{\Delta 970}$) allele, most likely only the α_2 gene is expressed.

Keywords: α-thalassemia, PCR; promotor region, deletion

The most common causes of α-thalassemia are deletions that remove a part, one or both of the functional α-globin genes. These deletions cause diminished expression of the α-globin protein, which may result in relatively low Hb and/or MCV values. We here report the identification of a new deletion in the α-globin gene that affects the α_1 regulatory sequences.
A 25-year-old pregnant woman presented with slightly decreased MCV value (76 fl) and normal erythrocyte value (4.0 x 10^{12}/l). Iron deficiency was excluded. HPLC analysis indicated no Hb variants, and the presence of normal HbA_2 concentration (3.0%) excluding β-thalassemias.

Materials and Methods

PCR based detection of α-thalassemias: Genomic DNA was isolated from 80-200 µl EDTA blood using GENTRA Capture column kit according to manufacturer's instructions. Described PCR methods were used for detection of α_1 and α_2 genes (1-3), MED (1), SEA (4), 20.5 (1,4), 3.7 and 4.2 (1-3,5) deletions. With regard to the combined α_1/–3.7 PCR, 13.5 µl of mix contained 1.3 M betaine, 1.3% DMSO, 200 mM dNTP, 1.75 mM $MgCl_2$, 0.5 µM forward primer P55: 5'- GTC CAC CCC TTC CTT CCT CA (Genbank GI:14523048 position 32757-32776 and 36311-36330) and reverse primer 37RM: 5'- GGG GGG AGG CCC AAG GGG CAA GAA (position 38297-38320), DNA template (12 ng) and 1 U of Expand Taq Polymerase (Roche). Amplification conditions were: 92°C, 4:30; 4 cycles of 98°C, 0:30; 75°C down to 64.5°C at 0.2°C/s, 64.5°C, 1:00; 72°C, 1:30; 31 cycles of 98°C, 0:20; 64.5°C, 1:00; 72°C, 1:30, followed by final extension at 72°C for 10 min (MJResearch PTC 200 thermocycler). PCR products were analyzed on a 1.5% agarose gel in 1 x TBE.
Sequence analysis: Primer P55 and a 5' 4 nucleotides shortened 37RM, both 5' extended with a M13 sequence were used for a PCR under conditions as described above with annealing temperature of 60°C instead of 64.5°C. After agarose gel electrophoresis the 1 kb band was cut out, purified (Qiaquick gel extraction kit; Qiagen) and diluted 1000 times. Two µl was used as a template for a nested PCR with M13 primers. PCR conditions were: 92°C, 5:00; 30 times 98°C, 0:30; 50°C, 0:30, 72°C 1:30 and final extension at 72°C for 10 min. PCR products were sequenced commercially (BaseClear Labservices Leiden).

Results and discussion

Analysis of genomic DNA from the index patient by PCR-based methods showed that α_1 and α_2 genes were present and that MED, SEA, 20.5, 3.7 and 4.2 deletions were absent. The presence of an additional band of 1 kb in the combined α_1/–3.7 PCR (figure 1A) suggested a genomic polymorphism. In the presence of a –3.7 deletion and an intact α_1 gene, the combined α_1/–3.7 PCR generates 2 products: a wildtype α_1 band of 2010 bp (primer P55 at primer binding site (PBS) II and primer 37RM) and a 1867 bp –3.7 band (primer P55 at PBS I and primer 37RM; figure 1B).
Sequencing of the 1 kb PCR product revealed an up to now unknown α-globin allele (which we nominated α-$\alpha^{\Delta 970}$) characterized by a 970 bp deletion (36599 until 37568; sequence GI:14523048), together with a 3 bp insertion (Fig. 1C). Thus, in the α_1/–3.7 PCR, amplification of the region between primer P55 PBS II and 37RM PBS had yielded the aberrant 1 kb band.
To investigate whether the deletion was germline or somatically acquired, DNA from both parents was analyzed in the combined α_1/–3.7 PCR. Figure 1A shows the presence of the 1 kb band in the father's, and not in the mother's lane, strongly suggesting a germline transmission of the affected allele from the index patient's father.
The deleted region encompasses the entire promoter region of the α_1 gene, including the 'CAAT' and 'ATA' box, a number of putative SP1 sites (6), and 26 bp encoding the 5' end of the mRNA. Thus the

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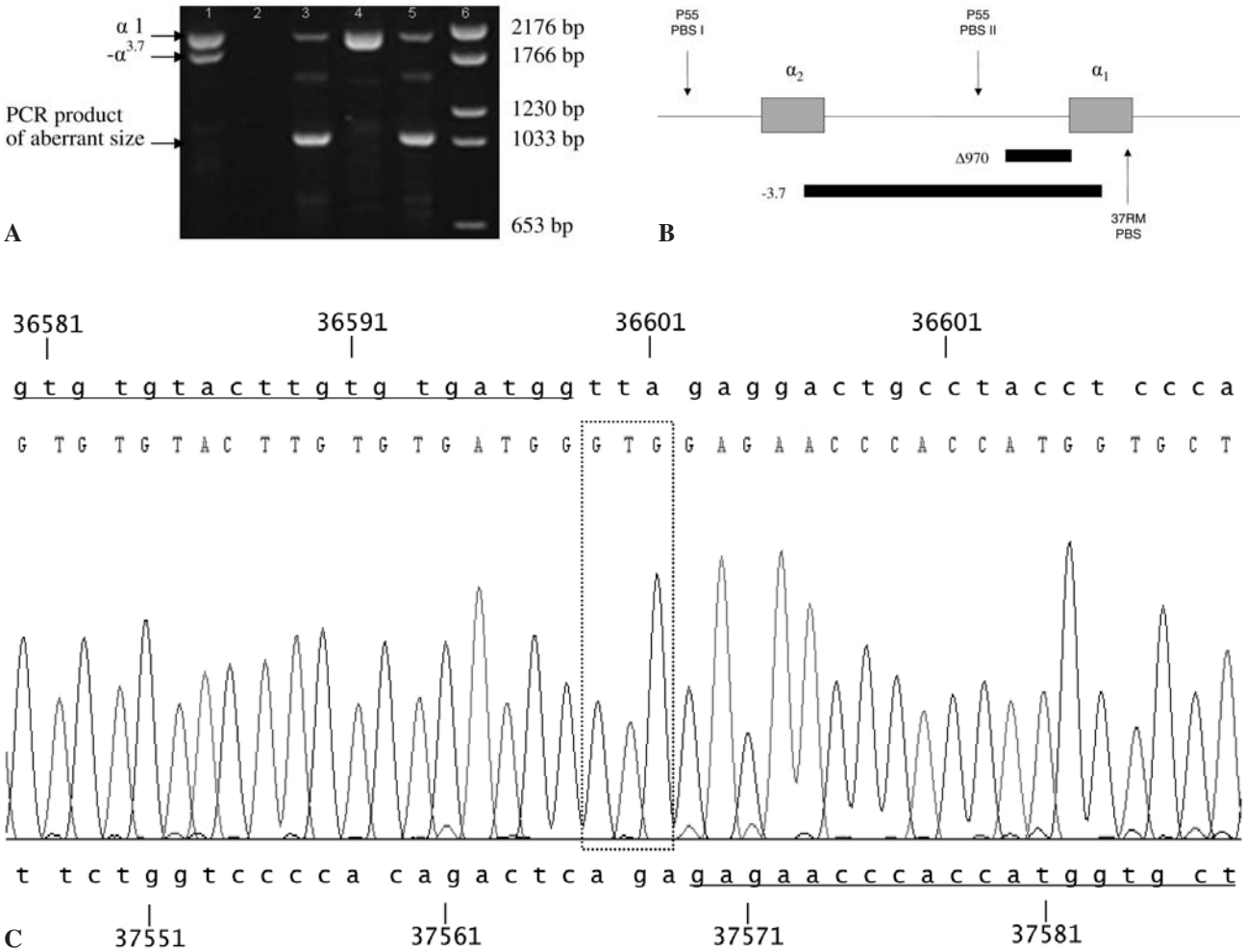

Figure 1. (A) PCR products generated in the combined α1/–3.7 PCR on agarose gel after electrophoresis. $-\alpha^{3.7}/\alpha\alpha$ control DNA (lane 1), H_2O (lane 2), patient (lane 3), patient's mother (lane 4), patient's father (lane 5) and molecular weight marker (lane 6). (B) Schematic representation of the α globin locus. Black bars indicate deleted regions. Arrows indicate primer binding sites (PBS). (C) DNA-sequence chromatogram of the critical region. Square region indicates the three inserted nucleotides. The underlined sequences show alignments with sequence GI: 14523048

affected α_1 globin gene is very unlikely to be expressed. Theoretically, this newly identified $\alpha-\alpha^{\Delta 970}$ allele resembles both the $-\alpha^{3.7}$ and $-\alpha^{4.2}$ alleles in that in all three cases just one functional α-globin gene remains. However, the three alleles may differ with regard to transcription and translation because of different 5' and/or 3' untranslated regions (7).

So far, we have identified four patients that carry the $\alpha\text{-}\alpha^{\Delta 970}/\alpha\alpha$ genotype: the index patient, her father, a brother and a sister. All of them exhibited MCV-values in the range of 73-78 fl. The Δ970 deletion may well be responsible for these slightly lowered MCV values.

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