CORRESPONDENCE

A Novel β^+-Thalassemia Mutation (Codon 10 GCC → GCA) and a Rare Transcriptional Mutation (−28A → G) in Indians

To the Editor:

Characterization of β-thalassemia mutations in different populations has shown 180 mutant alleles.[1] So far, 25 mutations have been reported among Indians, 5 of which comprise more than 80% of the mutant alleles.[2-4]

We report two interesting Indian families showing a novel β^+-thalassemia mutation and a rare transcriptional mutation. They had come to us for second trimester prenatal diagnosis by globin biosynthesis.

The β-thalassemia mutations were characterized by denaturing gradient gel electrophoresis (DGGE) analysis.[5] Both mutations were detected in fragment B of the β-globin gene spanning from the upstream −64 nucleotide to IVS-I-nt61 containing the promoter boxes and exon-1. DNA Sequencing was performed by the dideoxy method using Sequenase version 2.0 to identify the mutation.[6]

Family I was from Madhya Pradesh in central India. Both parents (I-1) and (I-2) had classical β-thalassemia trait (Fig 1). Their 3-year-old son (II-1) with severe homozygous β-thalassemia had been diagnosed at 8 months of age and had been transfused every month. This child was not available for investigation. Fetal diagnosis at 18 weeks of gestation in the next pregnancy showed that the β/α biosynthetic ratio was 0.021, indicating that the fetus had homozygous β^+-thalassemia (normal β/α ratio, >0.03).

DGGE analysis showed that the mother (I-2) had the IVS I-5 G → C mutation, whereas the father (I-1) had an anomalous DGGE pattern in fragment B.

Sequencing of this region using the forward primer showed a novel mutation at codon 10 GCC → GCA on the coding strand. Both the normal codon (GCC) and the mutant codon (GCA) code for alanine. This C → A substitution creates the sequence CAGTTA in the mutated region. A catalogue of the sequences found at functional splice sites has identified the 5′ consensus sequence C/A AGG TG/AA. The C → A change in codon 10 produces a sequence that has homology to 5 of 6 nucleotides of the normal splice site at the exon 1-intron-I boundary. It has been reported earlier that this homologous sequence in the exon causes alternative splicing at the site giving a β^+-phenotype.[2,7]

Family II was from Karnataka in south India. Both parents (I-1 and I-2) had classical β-thalassemia trait (Fig 2A). Their 6-year-old daughter had a homozygous β-thalassemic picture but had never been transfused. Globin biosynthesis in the next pregnancy showed that the 18-week-old fetus was normal.

DGGE analysis showed the IVS I-5 G → C mutation in the mother (I-2), whereas the father (I-1) had another anomalous DGGE pattern in fragment B. The DGGE pattern in the homozygous child (II-1) was different from that seen in the parents.

Sequencing of this β-globin gene region using the forward primer did not show any mutation. Sequencing with the reverse primer showed a T → C change on the noncoding strand (Fig 2B). This mutation was found in the daughter (II-1) as well, who also showed the presence of the IVS-I-5 G → C mutation. This A → G change in the upstream ATA box at position (−28) is a transcriptional mutant reported among Chinese.[8] Nevertheless, it has not been reported among Indians.

These two new rare mutations could be added to the 25 different β-thalassemic mutations that have been reported among Indians so far.
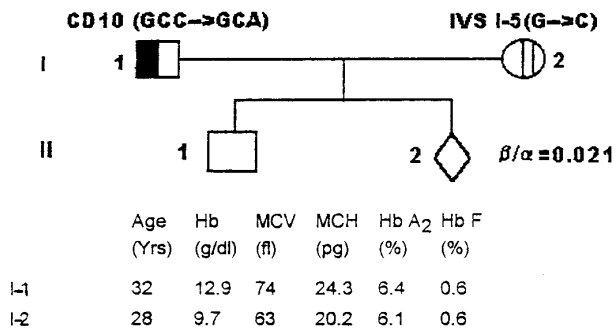
Fig 1. Family I. The hematologic profile and the β-thalassemia mutations characterized in the parents (I-1 and I-2).

	Age (Yrs)	Hb (g/dl)	MCV (fl)	MCH (pg)	Hb A$_2$ (%)	Hb F (%)
I-1	32	12.9	74	24.3	6.4	0.6
I-2	28	9.7	63	20.2	6.1	0.6

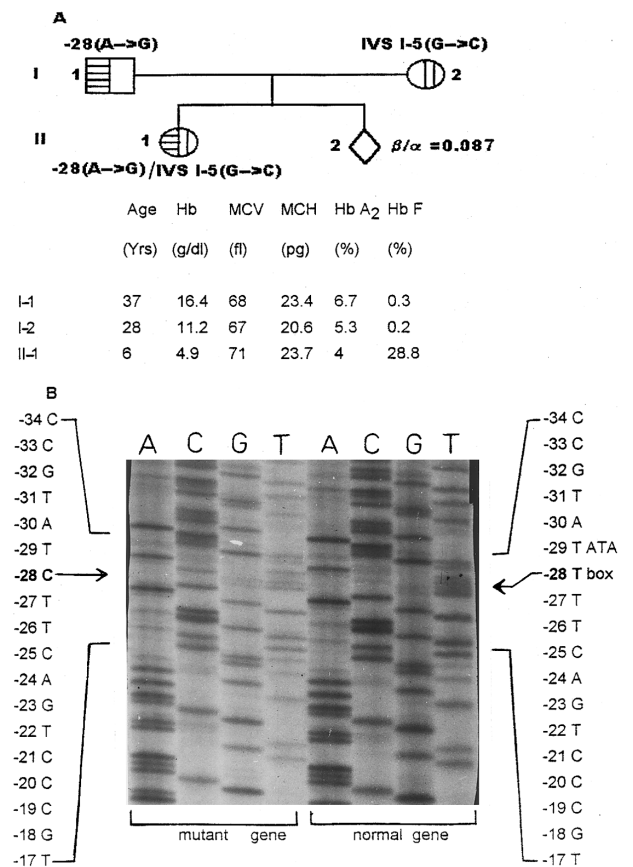|	Age (Yrs)	Hb (g/dl)	MCV (fl)	MCH (pg)	Hb A$_2$ (%)	Hb F (%)
I-1	37	16.4	68	23.4	6.7	0.3
I-2	28	11.2	67	20.6	5.3	0.2
II-1	6	4.9	71	23.7	4	28.8

Fig 2. Family II. (A) The hematologic profile and the β-thalassemia mutations characterized in the parents (I-1 and I-2) and their 6-year-old daughter (II-1). (B) Part of a sequencing gel showing the rare T → C mutation on the noncoding strand at position (−28) of the upstream ATA box in the father (I-1).

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Relationship Between BCR/ABL Fusion Proteins and Leukemia Phenotype

To the Editor:

We read with interest the recent Editorial in *Blood* by Melo[1] concerning the relationship between BCR/ABL variant transcripts and the leukemia phenotype determination. Regarding the chronic myeloid leukemia (CML), a strict correlation was suggested between at least three distinct clinico-hematologic entities and the type of BCR/ABL fusion protein produced by different BCR breakpoints (p210 CML, p190 CML, and p230 CNL). The last BCR/ABL rearrangement variant, with a c3a2 junction, was recently described in five patients with particular clinico-hematologic features proposed as neutrophilic-chronic myeloid leukemia (CML-N).[2]

By revision of 10 cases from the literature, Melo[1] emphasized a correlation between the BCR/ABL transcript b3a2 and the CML with thrombocythemic onset (or mimicking essential thrombocythemia [ET]). We wish to make some comments about this particular form of CML not extensively discussed in the editorial. Recently, by a careful revision of the literature, we identified 29 cases of CML with thrombocythemic onset[3-6] and found 3 further cases (1 with b3a2, 1 with b2a2, and 1 with b3a2-b2a2) of 82 consecutive patients observed in our institution. Taken together, the data showed that BCR/ABL transcripts may be of b3a2, b2a2, or b3a2-b2a2 types, without a strong evidence of one specific transcript variant associated with the Ph⁺ ET phenotype. Although b3a2 is the most frequent among all variants detected in CML cases with thrombocythemic onset, it should be noted that this variant is also the most frequent variant in CML cases with classical features. Moreover, another fusion transcript has been identified in 2 cases of CML with thrombocythemic onset, designated c3a2, not mentioned by Melo.[7,8] Interestingly, 3 of the 5 patients with c3a2 junction recently described as CML-N also showed a high platelet count at onset (1,020, 870, and 1,240 × 10⁹/L, respectively).[2]

In short, we wish to emphasize that at least four different BCR/ABL junction variants (b3a2, b2a2, b3a2-b2a2, and c3a2) may be detected in thrombocythemic CML, although with various frequencies. We feel that the determination of a particular phenotype of CML, namely CML with thrombocythemic onset, might be related to additional genetic changes other than BCR/ABL fusion protein. We agree with Melo[1] that much progress has been made in drawing ''a map linking the DNA, RNA, and protein defects to the specific cells affected and to the clinical features in the patient'' with CML. However, we think that much more caution is needed before assessing a close relationship between one specific molecular defect and a distinct clinico-hematologic manifestation of the disease, at least in CML patients with thrombocythemic features at onset. Further studies on larger series of cases are warranted to clarify whether c3a2 transcript variant is distinctive of a peculiar CML phenotype, ie, either CML-N or CML with thrombocythemic onset or both.

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