THREE-BASE DELETION IN EXON 3 OF THE $\beta$-GLOBIN GENE PRODUCED A NOVEL VARIANT ($\beta$GUNMA) WITH A THALASSEMIA-LIKE PHENOTYPE

To the Editor:

Most of the molecular defects producing a $\beta$-thalassemia phenotype are linked to a pretranslational process of $\beta$-globin chain synthesis. Only a few mutations create a $\beta$-thalassemia phenotype, posttranslationally.\(^1\) We have now identified a novel structural mutant caused by the three-base deletion in exon 3 of the $\beta$-globin gene that creates a $\beta$-thalassemia phenotype in a Japanese individual. The patient was a 36-year-old man who presented to the hospital with a mild anemia; hemoglobin (Hb), 11.3 g/dL; hematocrit (Hct), 38.3%; mean corpuscular volume (MCV), 64.2 fL; mean corpuscular hemoglobin (MCH), 19.8 pg; and mean corpuscular hemoglobin concentration (MCHC), 30.8 g/dL. His HbF and HbA2 levels were within normal ranges. The serum iron and total iron binding capacity were 122 and 225 pg/dL, respectively. Blood film examination showed a slight anisopoikilocytosis and no inclusion body was observed on staining with brilliant cresyl blue. The Hb electrophoresis, the heat and isopropanol stability tests showed no abnormal Hb. The globin chain synthesis ratios were 0.12 and 0.45 for $\gamma/\alpha$ and $\beta/\alpha$, respectively. Both $\beta$-globin genes of the patient were cloned as 7.8-kb Hind111 fragments in bacteriophage Charon 28.\(^2\) The 4.9-kb BgII fragments from the recombinant phage clones were inserted into the plasmid vector, pUC 13, and the $\beta$-globin gene sequences from both alleles were determined.\(^3\) DNA sequence analysis demonstrated that one clone (clone B) has the normal sequence, and in another clone (clone A), a 3-bp (AGG) deletion within codons 127 and 128 in exon 3 of the $\beta$-globin gene (Fig 1A). This mutation results in the deletion of two amino acid residues, glutamine and alanine at residues 127 and 128, and an insertion of a new proline at residue 127, as depicted in Fig 1A. Therefore, the $\beta$-globin chain synthesized from this mutant allele would consist of 145 instead of 146 amino acid residues. We further confirmed this mutation by dot blot hybridization of the amplified genomic DNA with specific oligonucleotide probes using procedures described previously.\(^4\) Figure 1B demonstrated that the patient was heterozygous for this mutation. Haplotype analysis in the $\beta$-globin gene cluster\(^5\) demonstrated that the haplotype (+ - - - - - +) was linked to this mutant allele. Analysis of the synthetic globin chains by pulse-labeling using carboxymethyl-cellulose column chromatography failed to demonstrate abnormal protein, suggesting that this $\beta$-globin variant is highly unstable.

In the $\beta$-globin chain, codons 127 (Gln) and 128 (Ala), located within the H-helix of the globin chain, are essential for the $\alpha_1\beta_1$ subunit interaction.\(^6\) Replacement of the Gln-Ala dipeptide at these positions by a Pro residue in the $\beta$-globin chain variant disrupts the H-helix of this $\beta$-globin chain and therefore interferes with the $\alpha_1\beta_1$ dimeric formation. The uncombined $\beta$-globin chain synthesized from the mutant allele would be rapidly removed by proteolysis and a $\beta$-thalassemia phenotype would ensue. This is also the case with other $\beta$-chain variants, such as Hb Showa-Yakushiji,\(^7\) Hb Indiana- pois,\(^7\) Hb Houston,\(^7\) and the recently described Hb Galicia,\(^7\) which are associated with a $\beta$-thalassemia phenotype. The novel $\beta$ globin chain mutant described here was named Hb Gunma.
Fig. 1.  (A) DNA sequencing gel representing sequences in the vicinity of codons 127 and 128 where three bases, AGG (TCC of the antisense strand), are deleted from the mutant allele of the patient. Ladders represent the nucleotide sequence of the antisense strand (anti). Clone A and clone B derived from the mutant and normal alleles, respectively. The nucleotide sequence of the sense strand (sense), their corresponding amino acids, and codon numbers are also shown. Deletion of AGG in codons 127 and 128 shown in a shaded box leads to the elimination of Gln-Ala dipeptide and insertion of new Pro at codon 127. (B) Dot blot analysis of the amplified DNA samples using allele specific oligonucleotide probes for the AGG deletions in codons 127 and 128. The sequence of the oligonucleotide for normal probe is 5'-ACCAGTGCAAGGTGCCTAT-3' and is 5'-ACCAGTGCCCTGCCTATCAG-3' for its mutant counterpart. N and P indicate the normal individual and the patient, respectively.
often cause a dominant form of thalassemia and show no predilection for malaria in endemic regions of the world. Am J Hum Genet 45:A242, 1989 (abstr)
Three-base deletion in exon 3 of the beta-globin gene produced a novel variant (beta gunma) with a thalassemia-like phenotype [letter]

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