CONCISE REPORT

A Novel Mutation in the TATA Box in a Japanese Patient With β+ -Thalassemia

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A single base substitution (A-G) at position –31 within the highly conserved proximal promoter element, the TATA box, was identified in the β-globin gene cloned from a Japanese woman with β+ -thalassemia. It appears that she is homozygous for this specific allele, as determined by haplotype analysis using seven different polymorphic sites in the β-globin gene cluster. Transient haplotype analysis using seven different polymorphic sites homozygous for this specific allele, as determined by Southern blot analysis with appropriate probes.

RESULTS

DNA polymorphism haplotype of the β-globin gene cluster. To determine whether the patient was homozygous for a thalassemia allele, we analyzed her DNA with respect to common DNA polymorphisms within the β-globin gene cluster. This patient was homozygous at seven different polymorphic restriction sites, as shown in Fig 1. Homozygosity for one haplotype and the consanguineous marriage of her parents strongly indicated that the patient was homozygous for a particular type of the β-globin gene mutation. Thus we used a single cloned β-globin gene for further analysis.

DNA sequence of the β-thalassemia gene. We determined the DNA sequence of the thalassemia gene region.

MATERIALS AND METHODS

Patient. The patient studied was a Japanese woman with transfusion-independent β-thalassemia intermedia. Spleenomegaly and jaundice were noted. Hematocrit data were Hb 7.2 g/dL, hematocrit 32%, RBC 430 × 10^6/μL, mean corpuscular volume (MCV) 74.4 μm^3, mean corpuscular hemoglobin (MCH) 16.7 pg, mean corpuscular hemoglobin concentration (MCHC) 22.5 g/dL, and reticulocyte count 3.6%. There was poikilocytosis with target cells and basophilic stippling on the peripheral blood smear. Bone marrow examination revealed erythroid hyperplasia; the granuloid-to-erythroid cell ratio was 0.21. Hemoglobin electrophoresis on cellulose acetate revealed HbA at 80.1% and HbA2 at 5.9%. The HbF level was estimated to be 14.0% by the alkali denaturation method.4

Probe excess-S1 nuclease mapping of the β-globin gene region was subcloned as a 4.9-kb fragment with the origin of DNA replication and tandemly repeated sequences. Twenty micrograms of the β-globin gene recombinant and 2 μg of an α-globin gene recombinant (an internal reference) were added as calcium phosphate precipitates to each 10-cm culture dish of subconfluent COS cells. Total cellular RNA was prepared from the transfected cells after incubation for 48 hours.

β-thalassemia gene cloning and DNA sequencing. The β-thalassemia gene was cloned in bacteriophage Chamor 28 as a 7.8-kilobase (kb) HindIII fragment. The β-globin gene region was subcloned as a 4.9-kb BglII fragment in pBR322 and sequenced by the method of Maxam and Gilbert.

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from 200 bases 5' to the Cap site to 200 bases 3' to the polyadenylation site of the cloned $\beta$-globin gene. Comparison of the nucleotide sequence of the thalassemia $\beta$-globin gene to that of a human $\beta$-globin gene revealed two base changes, an A-G change at position -31 5' to the Cap site and a G-T change at position 74 in IVS2 (Fig 3). The A-G substitution is located within the TATA box, also called the Goldberg-Hogness box (CATATAAA to CGTAAAA). The TATA box is known as a conserved sequence that is required for efficient and accurate transcription of many eukaryotic protein-coding genes. Thus the A-G transition found in the globin gene of the patient could cause a thalassemia phenotype by decreasing the level of the transcription of the gene. The G-T substitution in IVS2 has been reported to be a DNA polymorphism also found in normal globin genes.

Functional analysis of the $\beta$-thalassemia gene. To investigate the possibility that the A-G substitution at position -31 was responsible for the reduction in $\beta$-globin chain production, we examined the function of the gene in a transient expression assay in COS cells. Plasmid recombinants containing the normal and thalassemia $\beta$-globin genes were transfected into COS cells with an $\alpha$-globin gene recombinant as an internal standard. Total cellular RNA was extracted from COS cells after incubation for 48 hours and then analyzed by S1 nuclease mapping after hybridization with the $\beta$- and $\alpha$-globin gene probes (Fig 4). The major S1 nuclease-resistant band derived from $\beta$-globin RNA was two nucleotides longer than expected (Fig 4) because the two 3' terminal nucleotides of exon 1 and IVS1 are identical. The major band generated by $\alpha$-globin RNA and the minor band by $\beta$-globin RNA were due to the partial failure of S1 nuclease to remove the GC-rich segment at the 3' end of IVS1 as observed by Busslinger et al. The mutant globin gene directed the synthesis of the $\beta$-globin RNA to a lesser extent than normal (Fig 4). Direct scanning of the autoradiogram demonstrated a 45% reduction of $\beta$-globin RNA relative to normal. When we performed the S1 nuclease mapping experiment with the 5' part of the $\beta$-globin probe (a 344-nucleotide-long HindIII fragment), no differences between the mutant and the normal genes were observed at the initiation site of transcription (data not shown).

DISCUSSION

We observed that the one base substitution (A-G) at position -31 (the second base of the TATA box) in the $\beta$-globin gene isolated from a Japanese woman with $\beta^+$-thalassemia reduced the efficiency of transcription by a factor of 2. Three other types of mutation in the TATA box of the $\beta$-thalassemia globin genes have been described. The first detection was in a Kurdish Jew with an A-C change at position -28. This mutant gene directed the production of a two- to threefold decrease in transient expression in Hela cells and a fourfold reduction in vitro transcription assays. The second type of mutation was an A-G substitution at the same position detected in a Chinese patient. A three- to fivefold decrease in transcriptional efficiency of this TATA box mutant was seen using transient expression in Hela cells. In RNA prepared from the erythroid cells of the patient, $\beta$-globin RNA was one-tenth as abundant, relative to $\alpha$-globin RNA, than normal. An A-G transition at position -29 was recently detected in an American black. This mutant gene produced $\beta$-globin RNA at 25% the normal level both in heterologous and erythroid cells. These results suggest that expression of the $\beta$-globin gene with a one-base
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7. The two mutations at positions -87 and -88, which lie in the sequence ACACCC, proposed as a distal promoter element, also have a quantitatively similar effect on the transcriptional efficiency in $\beta\text{-thalassemia}$ patients.
8. The TATA box is important not only for the efficiency of transcription but also for accuracy in the transcription start site. However, the three types of TATA box mutations of $\beta\text{-globin}$ genes mentioned (A-G at position -28, A-G at position -29, and A-G at position -31) have only a quantitative effect on gene expression. Dierks et al observed little or no heterogeneity in the initiation site of the transcript when the TATA box of the rabbit $\beta\text{-globin}$ gene was deleted but a longer stretch of sequence preceding the Cap site was preserved. These observations suggest that the transcription initiation site of the $\beta\text{-globin}$ gene are not affected by TATA box mutations as shown in thalassemic genes because the sequence from the Cap site to the TATA box was preserved.
9. The TATA box mutation studied here most likely arose primarily in a Japanese person, since the ancestors of this patient are Japanese and this same type of mutation has not been found among other ethnic groups. This is the first report of the molecular basis of $\beta\text{-thalassemia}$ in a Japanese person.

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