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ONE BASE SUBSTITUTION IN IVS-2 CAUSES A β^+-THALASSEMIA PHENOTYPE IN A CHINESE PATIENT

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SUMMARY Two β-globin genes derived from a Chinese patient with a β^+-thalassemia phenotype have been cloned and sequenced. A four-nucleotide deletion in codon 41 and 42 was found in one of the clones and a C to T substitution at position 654 of IVS-2 was detected in the other clone. The former change, which causes premature termination at codon 59, was found previously in another Chinese patient with homozygous β^0-thalassemia. The latter change, which has not been reported so far, creates a sequence much like the consensus sequence of the 5' splice site. These results suggest that the new base change found in IVS-2 causes aberrant splicing of the β^+-globin mRNA precursor. Thus, this patient is doubly heterozygous for β^0- and β^+-thalassemia.

β-Thalassemias are a group of inherited disorders characterized by deficiency of the β-globin chain of adult human hemoglobin A (1). There are two types of β-thalassemia, called β^0- and β^+-thalassemia. Patients with homozygous β^0-thalassemia have no β-globin chains in their erythrocytes, whereas patients with β^+-thalassemia have a reduced amount of normal β-globin chain.

A number of molecular defects have been identified in the β-globin gene and its flanking region isolated from patients with β-thalassemia. These mutations cause impaired transcription , defects in RNA processing or premature termination of the β-globin message (2). Of these mutations, those that cause aberrant splicing have prompted speculation on the mechanism of splicing, which is one of the most interesting events in eukaryotic gene expression (3-10).

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Here we report a new nucleotide change in the second or large intervening sequence (IVS-2) of the β-globin gene isolated from a Chinese patient, which could result in aberrant splicing of IVS-2 of the β-globin precursor mRNA.

MATERIALS AND METHODS

The patient --- The patient whose DNA was analyzed in this study was a 3-year-old Chinese resident of Taiwan. The main hematological values found in this patient were as follows; Hb 6.0g/dl, HbA 15.7%, HbA2 trace, HbF 84.3%.

Molecular cloning --- High molecular DNA was isolated from peripheral leukocytes of the patient by the method described before (11). One clone of the β-globin gene was isolated as a λ phage Charon28 with a 7.8kb HindIII fragment. A λ phage Charon28 carrying a DNA fragment partially digested by EcoRI was also isolated. These two were identified to contain the β-globin gene by plaque hybridization (12) with Pstβ probe provided by Dr. Maniatis (13). For facilitation of subsequent analyses, a DNA fragment that included the structural gene and the immediate flanking regions of the β-globin gene (13) was subcloned as a 5.0 kb BglII fragment in pBR322 from each phage clone.

DNA sequencing analysis --- The DNA nucleotide sequences of the two β-globin genes were determined by a modification of the method of Maxam and Gilbert (14) for 3′ or 5′ ^{32}P-labeled DNA fragments. The E. coli polymerase I, large fragment (15) and polynucleotide kinase (16) were used for 3′ and 5′ end labeling, respectively.

RESULTS

Two β-globin gene clones, clone 1 and 2, were isolated from the patient using lambda phage vector. The 5.0 kb Bgl11 fragments subcloned in pBR322 were each subjected to nucleotide sequence analysis. The nucleotide sequence of the gene including a 150-nucleotide 5′-flanking region and 50-nucleotide 3′-flanking region of the β-globin gene of each clone was determined. A four-nucleotide deletion in codon 41 and 42 was detected in the β-globin gene of clone 1 (Fig. 1). This deletion shifts the reading frame, resulting in an in-phase termination (TGA) at codon 59 (Fig. 1). Thus, this mutation results in β°-thalassemia phenotype in homozygote. We found the same change previously in a homozygous β°-thalassemia patient in Taiwan (17). In her β-thalassemic gene there are some base substitutions considered to be polymorphisms; A to G at position 41 of IVS-1, G to T at position 74 in IVS-2 and G to A at codon 106. However, these substitutions were not detected in the β-globin gene of clone 1.

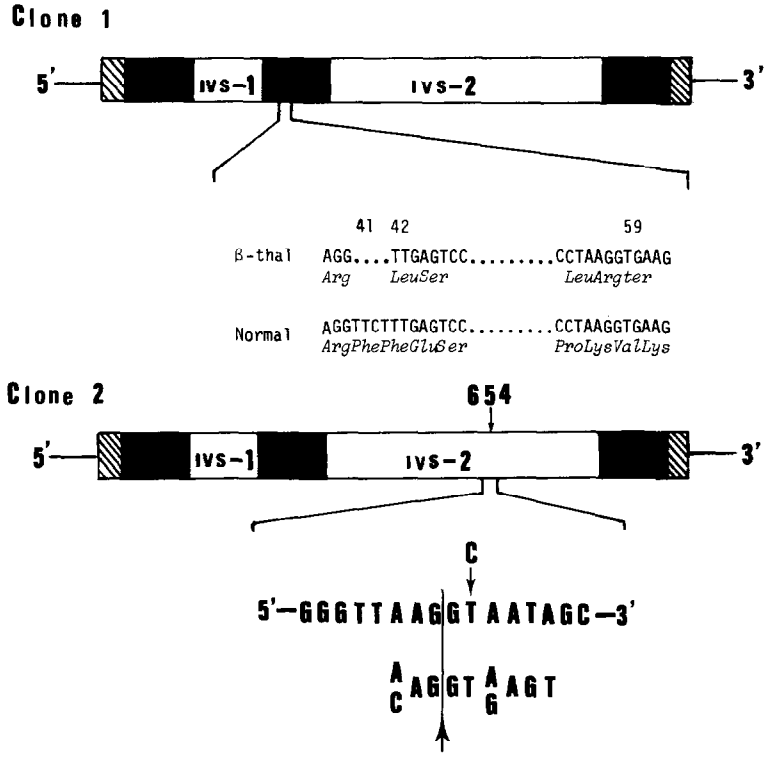

Figure 1. Mutations in two human β-globin genes (clone 1 and clone 2). In clone 1 a four-nucleotide deletion was found in codon 41 and 42 generating a termination codon at codon 59. In clone 2, a C to T substitution was found at position 654 in IVS-2. The homology to the 5' splice consensus sequence (18) and the position of the splice junctions are indicated below the DNA sequence.

In the β-globin gene of clone 2, the only notable change was a base substitution of C to T at position 654 in IVS-2 (Figs. 1 and 2). This mutation, which has not been found in normal individuals so far, creates a sequence identical to the consensus sequence at a 5' splice site at seven of nine nucleotides (Fig. 1).

DISCUSSION

This paper reports the characterization of the β-globin genes isolated from a Chinese individual with a β^{+}-thalassemia phenotype. The β-globin gene of this patient contains a C to T substitution at position 654 in IVS-2 and its counterpart carries a four-nucleotide deletion at codon 41 and 42. The latter mutation causes premature termination of translation generating a β^{0}-thalassemia phenotype in the homozygote as described before

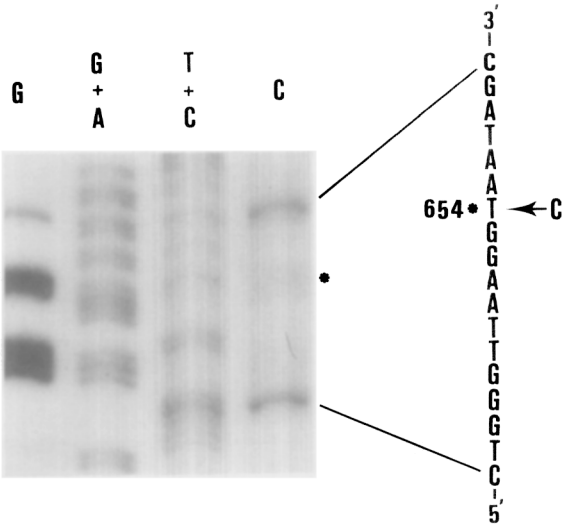

Figure 2. Sequencing gel of the β-thalassemic gene showing the region of the mutation of clone 2. The DNA fragment containing IVS-2 was labeled at the 5' end with ^{32}P by polynucleotide kinase and sequenced .

(17). The former substitution, C to T at position 654 in IVS-2, creates a sequence A-A-G-G-T-A-A-T-A identical to the consensus sequence, A/C-A-G-G-T-A/G-A-G-T at the 5' splice site at seven of nine nucleotides (Fig. 1, ref. 18). Two nucleotides that differ from the consensus sequence may not be specified strictly at the 5' splice junction, because the sequence of the human ε- and γ-globin genes differs from that of the δ- and β-globin genes at one of these bases in IVS-1(19), and sequence differences were found in these two bases in many other genes (20). Thus, the novel sequence created by the C to T substitution at position 654 could be a new 5' splice site in vivo.

Three types of base substitution have been reported in IVS-2 of β-thalassemic genes. A substitution of G to A at position 1 abolishes the authentic splice site causing a β^{0}-thalassemia phenotype (8,21). Each base substitution , T to G at position 705 (9) and C to G at position 745 (8), creates a new 5' splice site with activation of a cryptic 3' splice site located at position 580, causing a β^{0}-thalassemia and a β^{+}-thalassemia phenotype, respectively. These results suggest that the C to T change at position 654 presented here causes aberrant splicing that leads to

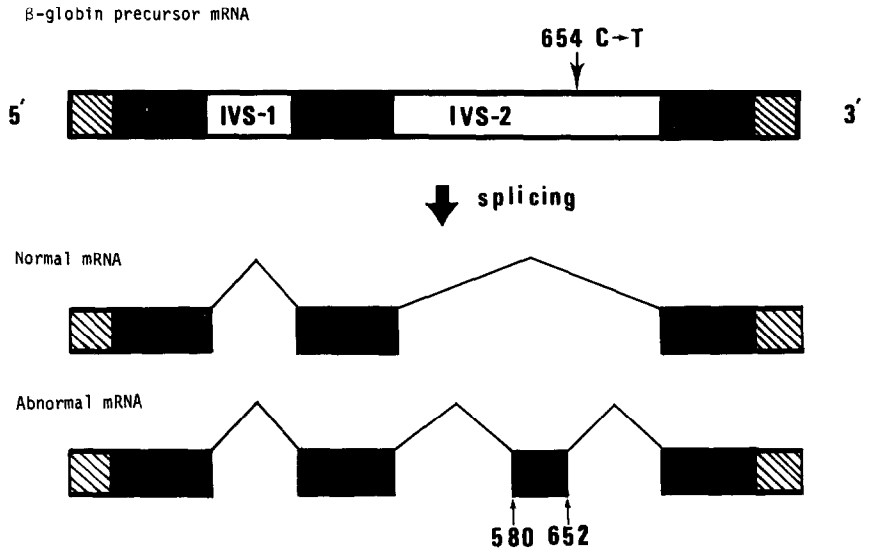

Figure 3. Proposed splicing of the β-globin mRNA precursor in β-thalassemia due to a mutation in IVS-2 of the globin gene. The structure of the precursor mRNA of the β-thalassemic gene is shown with coding regions indicated by solid boxes and IVS regions by open boxes. Flanking regions are shown as boxes with oblique line. A 73-nucleotide region derived from IVS-2 remains in the mature mRNA by aberrant splicing. Normally spliced mRNA is also produced in this patient with a β-thalassemia phenotype.

production of β-globin mRNA containing a region spanning position 580 to position 652 in IVS-2 (Fig. 3). We are now analyzing the total cellular RNA isolated from cos cells transfected with the SV40-β-thalassemic gene hybrid to test this hypothesis.

The mechanism by which the one base substitution in IVS-2 causes the aberrant splicing is not clear. One possibility is that the splicing apparatus could bind to the 3′ side of IVS-2 and then scan the RNA in the 3′- to 5′-direction to choose the first appropriate 5′ splice site. However, studies of splicing of IVS-1 with a single mutation at position 110 (3-6) and splicing of IVS-2 of the γ-globin gene suggested the 5′- to 3′-direction of scanning of the splice site (22). Another possibility is that the aberrant splice site created by the mutation described here is more susceptible than its counterpart to the splicing apparatus because of its more suitable sequence. A third possibility is that the mutation causes the conformation of the precursor mRNA to be spliced in an aberrant way. Further studies on the effect of introducing the mutation in IVS in vitro

to create the splice site and identification of other thalassemic genes causing aberrant splicing could be important in understanding how the base sequence effects the selection of a splice site and the efficiency of splicing.

We conclude that the C to T substitution in IVS-2 causes both normal and aberrant splicing of IVS-2, resulting in a β^{+}-thalassemia phenotype, because a four-nucleotide deletion in the coding region, which was found in another globin gene of this patient is known to create clearly a β^{0}-thalassemia phenotype (17). Dr.Kazazian (personal communication) found the same mutation in a Chinese (23). His group showed by a transient expression study of the cloned gene in HeLa cells and Northern blotting analysis of reticulocyte RNA from the patient that this mutation abolishes completely normal splicing and generates the aberrantly spliced transcripts as proposed above. Thus, they concluded that the C to T substitution in IVS-2 causes a β^{0}-thalassemia phenotype. A possibility which still remains is that there are some differences among patients with the same mutation in IVS-2 of the β-globin gene in terms of factors involved in splicing besides the primary sequence of the splice site.

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