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Structure of cloned δ-globin genes from a normal subject and a patient with δ-thalassemia; sequence polymorphisms found in the δ-globin gene region of Japanese individuals

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ABSTRACT

The δ-globin genes of a normal Japanese and a Japanese patient with homozygous δ-thalassemia were cloned, and the nucleotide sequence of a region including the gene was determined. Comparison of the nucleotide sequences of these two individuals with that of pHδ1, δ-globin clone from the gene library constructed by Maniatis et al., showed differences in the large intervening sequence (IVS 2), at positions 137, 151, 186, 188, 291, 292 and 540 as one base substitutions, at 339 and 823 as one base additions, at 548 as a one base deletion, and a 9 bp duplication between positions 651 and 659, and differences in the 3'-flanking sequence at 51 and 98 nucleotides 3' to the AATAAA sequence. However, in the region studied, no differences was observed in the nucleotide sequences of the normal subject and the patient with δ-thalassemia. Therefore, these differences may represent polymorphisms of the δ-globin gene present in Japanese individuals. These data suggest that IVS 2 is more divergent than other regions, and that a DNA region(s) other than the globin gene may affect expression of the gene.

INTRODUCTION

Previous studies on molecular defects present in patients with thalassemia, a heterogeneous group of inherited diseases characterized by reduced synthesis of globin chains, have mainly been concerned with α- or β-thalassemia (1).

δ-Globin is a subunit of HbA2 ($\alpha_2\beta_2$), a minor component of human adult type hemoglobins, and its gene is located about 5 kilobase pairs (kb) away from the β-globin gene towards the 5' end (2). So far, ten patients with homozygous δ-thalassemia who have no HbA2 and do not suffer from anemia have been reported in Japan (3, 4). However, the molecular defects in δ-thalassemia have not been investigated.

In this work, we analyzed δ-globin genes prepared from a normal Japanese subject and a patient with δ-thalassemia by cloning techniques. We found no difference in the nucleotide sequence of the gene or its flanking regions in the two DNA samples studied, but observed many sequence polymorphisms.

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MATERIALS AND METHODS

Source of DNA DNAs of large molecular weight were isolated from leukocytes of a normal Japanese subject and from cultured lymphocytes of a patient who was a homozygote for δ-thalassemia, as described by Blin and Stafford (5). Hematological data on this patient (Iz) were reported previously (3, 4).

Restriction enzyme analysis of DNAs DNAs were digested completely with EcoRI, HindIII, BglII, PstI and BamHI (Takara Shuzo Co., Ltd, Japan) at 37°C in the buffer recommended by the manufacturer, and fractionated in 0.6 % agarose gel by electrophoresis. Tnen DNA fragments were transferred onto nitrocellulose filters and hybridized to various DNA probes labelled with ^{32}P by nick translation as described by Southern (6). The DNA probe pγ3.4, which was used as a probe to detect the γ globin gene, was constructed by subcloning the HindIII 3.4 kb fragment into pBR322 from γ-globin gene-cloning HγG5 lambda phage (2), kindly given by Dr. T. Maniatis. Pstδ (pHδ 1) (7), Pstβ , pε0.7 (8) and RIH (7) subclones were also provided from Dr. T. Maniatis.

Isolation of the δ-globin gene Three 10 μg portions of each DNA sample were partially digested with 40 units of EcoRI for 3, 5 and 7 minutes, respectively. The three digests were combined and 10 to 17 kb DNA fragments were collected from the mixture by sucrose density gradient centrifugation and cloned into λ phage vector to prepare a gene library by the method described by Maniatis et al. (9), except that λCh28 was used instead of λCh4A. Recombinant phages with large cellular DNA inserts including the δ-globin gene were isolated directly from the gene library without amplification by the plaque hybridization technique of Maniatis et al. (9). Furthermore, a PstI fragment of 2.3 kb, which contains the δ-globin gene and its short flanking regions, was obtained from the phages and subcloned into pBR322 (see RESULTS AND DISCUSSION).

Experiments using recombinant DNAs were conducted with a P3-EK2 containment system, in accordance with the guidelines of the Ministry of Education, Science and Culture of Japan.

DNA sequencing DNA fragments hydrolyzed by suitable restriction endonucleases and fractionated in an appropriate (0.6 - 2.0 %) preparative agarose gel by electrophoresis were labelled with ^{32}P at the 5' end with polynucleotide kinase (10) or at the 3' side with the Klenow fragment (11) of E. coli DNA polymeraseI. γ-^{32}P-dATP, α-^{32}P-dATP and dCTP (∿3,000 Ci/m nol) used for labelling were purchased from Amersham. The DNA sequence was determined by chemical degradation procedures as described by Maxam and Gilbert (12).

RESULTS AND DISCUSSION

Restriction endonuclease mapping Restriction enzyme digests of DNAs isolated from a normal Japanese and from patient with δ-thalassemia were fractionated by electrophoresis and hybridized to probes containing the DNA region of the γδβ gene complex (Table I, Fig. 1). All fragments detected by hybridization were approximately the same size as those reported previously (7). In addition, no difference was found in the restriction maps of the two DNA samples. These findings suggested that this homozygous patient has a δ-globin gene of the normal size. However, the possibility that non-α-globin genes are missing completely from one chromosome of the patient and that molecular changes responsible for the phenotype of thalassemia exist in the residual δ-globin gene located on the another chromosome cannot be excluded.

A similar conclusion was reached by Wilson et al. (13), who made a restriction map of another patient with homozygous δ-thalassemia. They reached this conclusion by supposing that a 4.4 kb PstI fragment hybridizable to the Pstβ probe was the fragment containing the δ-globin gene. However, we found that a 2.3 kb PstI fragment gave a strong hybridization band with Pstδ probe, whereas a 4.4 kb PstI fragment gave only a faint band. Our findings

Table I. Restriction fragments identified in the region of the non α globin genes

	probe				
	pε0.7	pγ3.4	RIH	Pstδ	Pstβ
EcoRI	3.8	7.6, 2.7, 1.6	7.2	2.3, 1.7	5.2, 3.6
BamHI	0.8	15.5, 5.1, 2.7	15.5	15.5, 4.4	8.3, 1.8, 0.7
HindIII	8.3	8.2, 3.4 (7.5, 2.7, 0.7)*	17.4	17.4	7.8
BglII	2.1	13.0	2.7	7.7	5.2
PstI	ND	5.1, 4.3, 0.9	4.8	2.3	4.4
XbaI	ND	7.5, 5.1, 3.4	1.2	10.8, 2.9	10.8
SacI	ND	22.0, 5.3, 2.7	22.0	22.0	9.2

DNAs from a normal Japanese and from a patient with homozygous δ-thalassemia were digested with various restriction endonucleases. The sizes of DNA fragments identified by blot-hybridization using the indicated probes are shown in kilobases. The HindIII digest of normal DNA was found to contain the fragments marked by an asterisk, due to the presence of two HindIII polymorphic sites in the γ genes, in addition to fragments from DNA lacking these sites. These polymorphic sites were not found in DNA from the patient with δ-thalassemia. ND means "not determined".

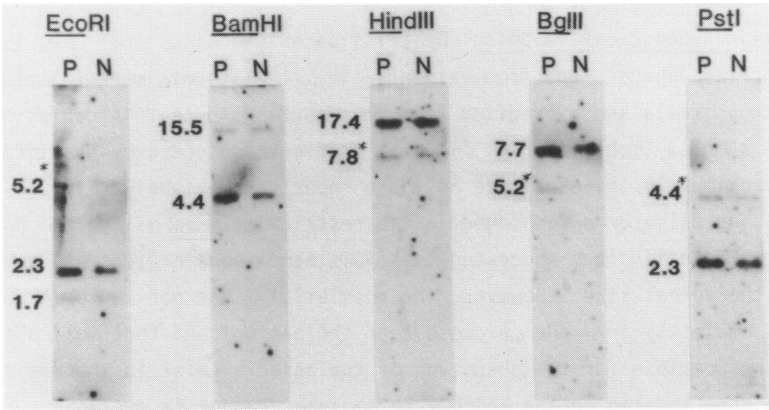

Figure 1. Restriction fragments identified by blot-hybridization with Pstδ probe. The sizes of DNA fragments are indicated in kilobases. Restriction fragments containing the β-globin gene marked by asterisks are generated by cross-hybridization with Pstδ probe. N; normal Japanese. P; the patient with homozygous δ-thalassemia.

indicate that the δ globin gene is located on this 2.3 kb PstI fragment, while the β globin gene is located on the 4.4 kb fragment. So the data described here provided a firmer basis for this conclusion.

Isolation of the δ-globin gene To compare the δ globin genes of two DNA samples more exactly, we next attempted amplification of the genes. The recombinant lambda phages N1 and N2 containing the large region covering the δ-globin gene were isolated from the normal subject, while Iz1 and Iz2 were isolated from the patient with δ-thalassemia. These clones were found to contain the DNA region, as shown in Figure 2.

From each clone, a 2.3 kb PstI fragment containing the complete δ-globin gene together with short flanking regions was subcloned into pBR322. pSP3

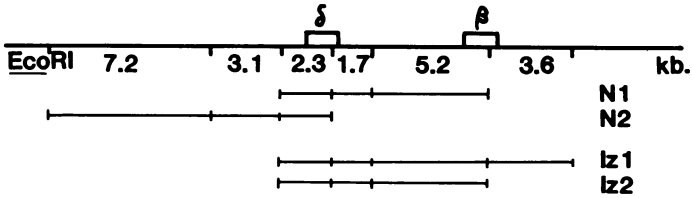

Figure 2. The structural δ and β genes are shown by the rectangles on the top line and DNA inserts in respective recombinant phage clones are indicated. N1 and N2 are from the normal Japanese, and Iz1 and Iz2, from the patient with δ-thalassemia.

and pIP3, isolated from Nl and Izl, respectively, were observed to have identical insertion orientations with that of pHδl (14). Then they were digested with AluI, AvaII, DdeI, HaeIII, HinfI, HphI, MboII, Sau3A and Sau96-I, and fractionated on 5 % polyacrylamide gel by electrophoresis (Fig. 3). It was found that the endonuclease digestion patterns of the two subclones were identical with that of pHδl.

Primary structure of the δ-globin gene from Japanese individuals We determined the complete nucleotide sequence of a region of about 2,000 bp of pSP3 and pIP3 in a region extending from 120 nucleotides 5' to the susceptible mRNA capping site to 130 nucleotides 3' to AATAAA sequence of the δ globin gene. Comparison of our data with those on pHδ 1 (14), showed no difference in the 5' flanking region (about 120 nucleotides), the 5' untranslated region, and a small intervening sequence or coding region.

On the other hand, many changes were observed in the large intervening sequence as shown in Figure 4, and summarized in Figure 5. The one base substitutions were T to A, at positions 137 and 540, A to T at 151, C to T

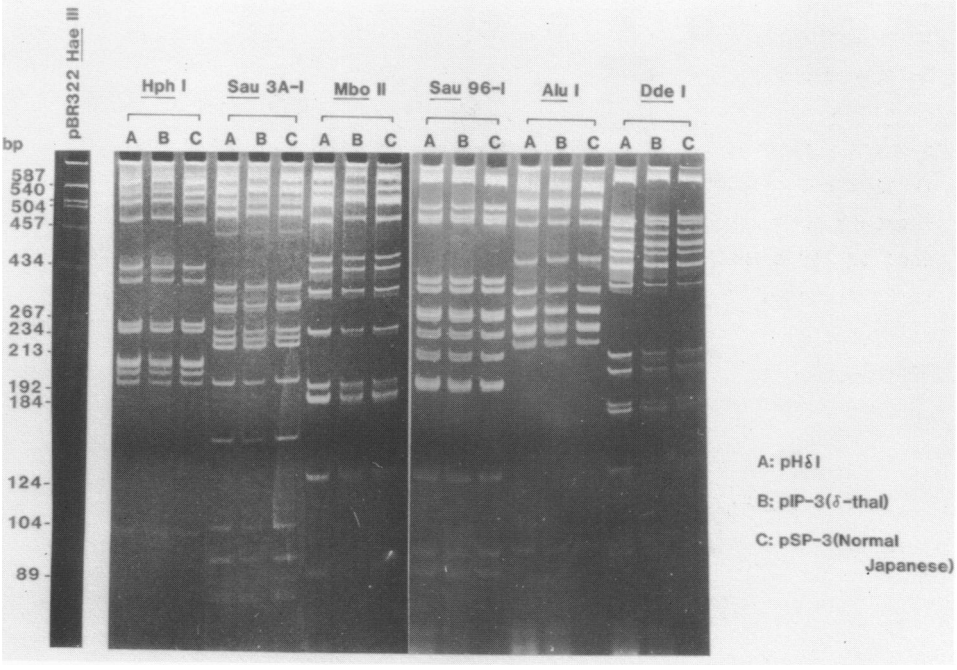

Figure 3. DNAs of pHδ 1, pIP3 and pSP3 were digested with the various restriction endonucleases indicated at the top, and subjected to electrophoresis in 5 % polyacrylamide gel.

at 186, 188 and 292, and T to C at 291. There were 2 one base additions of C to CC and TT to TTT at 339 and 823, respectively. One nucleotide at 548 was deleted. There was a 9 nucleotide (AGCATATAT) duplication between 651 and 659, which was probably generated by unequal crossing over.

There were also changes in the 3' flanking sequence at 51 and 98 nucleotides 3' to the AATAAA sequence, these changes were CC to C at 51 and C to G at 98.

Since all these changes were found in all subclones of phage lambda clones from both the normal subject and the patient with δ thalassemia, they seem to be sequence polymorphisms present in Japanese individuals. There have been some reports about sequence polymorphisms in the coding sequence, intervening sequence and 3' untranslated region of γ (15) and β (16) globin genes. Though Lawn et al.(17) reported a polymorphic PstI site in the IVS 2 of δ globin gene, it was surprising to notice the many changes described above. Our data are consistent with the findings of Slightom et al.(15),

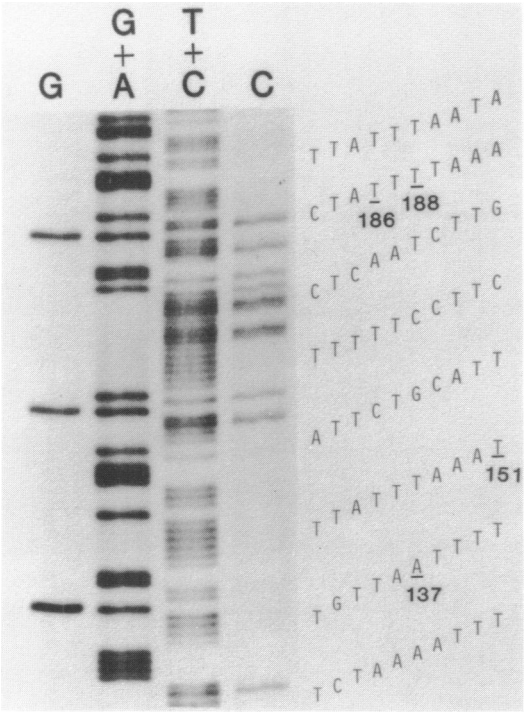

Figure 4. Nucleotide sequence of the coding strand in the region of IVS 2 at positions including 137, 151, 186 and 188. One base substitutions are underlined.

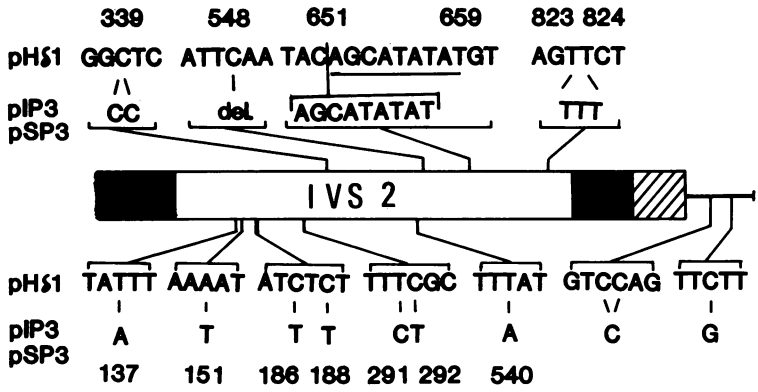

Figure 5. Sequence polymorphisms found in the δ-globin gene of Japanese individuals: The gene is indicated as protein coding (filled boxes), 3' untranslated sequence (hatched box) or large intervening sequence (IVS 2) (open box). Nucleotide changes as deletion or insertion (upper) and as base substitutions (lower) are shown in comparison with the sequence of pHδ1. The positions of nucleotides from the 5' end of IVS 2 are indicated.

that in the γ globin gene, IVS 2 and the 3' untranslated region are more divergent, and 110 bp of IVS 2 adjoining the 5' splice point and 80 bp of IVS 2 adjoining the 3' splice point are markedly conserved.

In the patient with δ-thalassemia, no molecular defects were found in the δ-globin gene or its flanking regions. Thus we thought that a more upstream region might affect expression of the δ globin gene, as observed in the case of the histone gene of the sea urchin (18). So we also determined the nucleotide sequence of normal and δ-thalassemia clones up to 300 nucleotide 5' to the capping site, as shown in Figure 6. However, so far as determined, no difference was found between these clones.

Possibly a "regulatory sequence(s)" is located far apart from the struc-

-300

GCCCGATCATTCCACTATATTAGTCCAAACACTCTACGAAATAGAACTAAGGAGGATATT

TTTAGAAACAACTGCTGAAAGAGATGCGGTGGGGAGATATGCAGAGGAGAACAGGGTTTC

TGAGTCAAGACACACATGACAGAACAGCCAATCTCAGGGCAAGTTAAGGGAATAGTGGAA

TGAAGGTTCATTTTTCATTCTCACAAACTAATGAAACCCTGCTTATCTTAAACCAACCTG

CTCACTGGAGCAGGGAGGACAGGACCAGCATAAAGGCAGGGCAGAGTCGACTGTTGCTTA

*

Figure 6. Nucleotide sequence of the 5' flanking region of the δ globin gene up to 300 nucleotides 5' to the susceptible mRNA capping site marked by an asterisk. The CCAAT and TATA boxes are underlined.

ture gene, because Van der Ploeg et al. (19) reported that the β globin gene was not expressed in a patient with γ-β- thalassemia who has a deletion only in a region between the ε and δ globin gene. Therefore, though we could not detect any structural changes around the γδβ globin gene complex by the blot-hybridization technique, we think that this patient with homozygous δ-thalassemia may have a small deletion or addition affecting the normal "regulatory sequences" in some part other than the region of the sequence determined.

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