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A Novel Deletion in δβ-Thalassemia Found in Japan

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High molecular weight DNA from a Japanese individual homozygous for δβ-thalassemia was analyzed by the blot hybridization technique of Southern. Results indicated a large deletion of the non-α-globin gene cluster, starting in the vicinity of 3' to the A_γ-globin gene and extending through the 3' side of the β-globin gene. Persistent expression of the γ-globin gene in adult life has been supposed to be caused by loss of a region located about 3-4 kb 5' to the δ-globin gene from comparison of the extents of deletions in several different forms of δβ-thalassemia and HPFH (hereditary persistence of fetal hemoglobin). But the novel deletion found in the present case of δβ-thalassemia suggests that the above putative regulatory region does not have this effect on expression of the γ-globin gene. Some explanations of expression of fetal type globin genes in this δβ-thalassemia are discussed.

The γ-globin gene is mainly expressed in fetal life and its expression is replaced by those of the δ- and β-globin genes after birth (1). However, in the δβ-thalassemia and HPFH, the γ-globin gene is expressed in adult life (2). In HPFH, synthesis of the γ-globin chain is well balanced by that of the α-globin chain and there are no symptoms characteristic of thalassemia. Expression of the γ-globin gene is insufficient in δβ-thalassemia and the consequent mild imbalance in synthesis of the γ- and the α-globin chains results in slight anemia. Various forms of these two disorders provide good systems for studies on the molecular mechanisms involved in the switch of non-α-globin gene expression (γ→δ,β).

Previous analyses of the gene structure of the non- α-globin gene cluster in the two disorders suggested that the γ-globin gene regulatory region, deletion of which results in persistent expression of the γ-globin gene in adult life (3-5), is located about 3-4 kb 5' to the δ-globin gene, because

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this region is absent in all deletion types of HPFH, but not in δβ-thalassemias reported (3-13). This hypothesis on the regulatory region provides an interesting model of the regulation of the globin gene expression during development. To examine this model and elucidate the factors that affect the globin gene expression, we studied a Japanese case of δβ-thalassemia, which was expected to differ from the cases of δβ-thalassemia studied previously in terms of mutation of the non- α-globin gene cluster, because of its different ethnic origin.

Here, we report that this case showed extensive deletion of the non- α-globin gene cluster, including the putative regulatory region.

MATERIALS AND METHODS

Genomic DNA preparation. Total cellular DNAs were isolated from peripheral leukocytes of a Japanese individual homozygous for δβ-thalassemia and from a normal subject (14). The patient with δβ-thalassemia was icteric and showed slight splenomegaly. Hematological data on this patient were as follows: Hb 12.0 g/dl, HbA 0%, HbA_2 0%, HbF 100% (G_γ/A_γ =0.65/0.35), with morphological abnormalities of erythrocytes.

Restriction endonuclease mapping of DNA. Total cellular DNAs isolated from the patient and the normal subject were digested completely with several restriction enzymes and then fractionated on 0.6-0.8% agarose gel by electrophoresis. The digests were transferred to nitrocellulose filters and hybridized to appropriate probes labeled with ^{32}P by nick translation (15,16). The probes employed in this study were as follows: Pstβ and Pstδ are PstI fragments containing the β- and the δ-globin genes, respectively (17). The RIH probe contains a sequence of about 0.5 kb intergenic DNA 4 kb to the 5' side of the δ-globin gene. Eco2.3 is a 2.3 kb EcoRI fragment derived from the 3' flanking region of the A_γ-globin gene (18). Hinγ is a 3.4 kb HindIII fragment including the A_γ-globin gene subcloned from λHγG5 (18). Bglψβ is a 4.0 kb BglII fragment containing the ψβ-globin gene. The extents of the regions included in these probes are shown in Fig.1.

RESULTS

Deletion of the non-α-globin gene cluster. Genomic DNA from the patient was digested with EcoRI, BamHI and BglII restriction enzymes, fractionated on agarose gels and transferred to nitrocellulose filters (16). The filters were hybridized to Pstβ ,Pstδ ,RIH and Bglψβ probes. No specific bands were obtained from the δβ-thalassemia DNA (Fig.2), indicating that the DNA region ranging from the β-globin gene to the ψβ-globin gene is entirely deleted on both chromosomes. The deleted region contains the regulatory sequence of the γ-globin gene proposed previously (3-5). The location of this sequence is

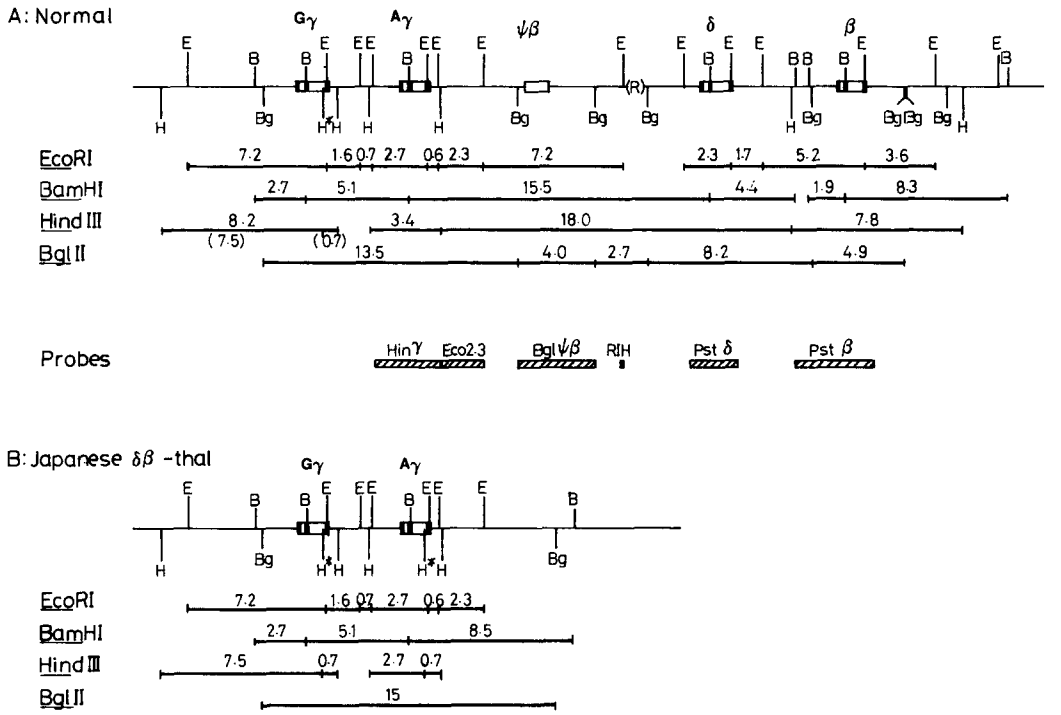

Fig. 1. Maps of restriction endonuclease cleavage sites within the non-α-globin gene cluster in DNAs of normal subject (A)and the Japanese thalassemia patient (B) with probes used in this study. Abbreviations: H; HindIII, E; EcoRI, B; BamHI, Bg; BglII, H*; HindIII cleavage site polymorphism in the γ-globin genes. (R); the putative regulatory region. The size of the fragments detected by probes are shown in kilobases. The normal map is derived from references 4,6,18 and 19.

shown by (R) in Fig.1. The 3' end point of the deletion could not be defined precisely. However, it is clear that the deletion involved loss of the entire β-globin gene from the fact that the BamHI digest hybridized to the Pstβ probe did not generate the 8.3 kb fragment observed with normal DNA (Fig.2).

Extent of the deletion. For determination of the extent of the 5' end point of the deletion in this δβ-thalassemia, the restricted genomic DNA from the patient was hybridized to the Hinγ probe. EcoRI digests contained 7.2, 2.7, 1.6, 0.7 and 0.6 kb fragments that were identical to those obtained from normal DNA, indicating that the G_γ- and the A_γ-globin genes are present in the δβ-thalassemia DNA. The faint band of 3.7 kb was formed by cross hybridization of the ε-globin gene with the Hinγ probe. Digests with HindIII hybridized to the same probe gave fragments of 7.5, 2.7 and 0.7 kb instead of

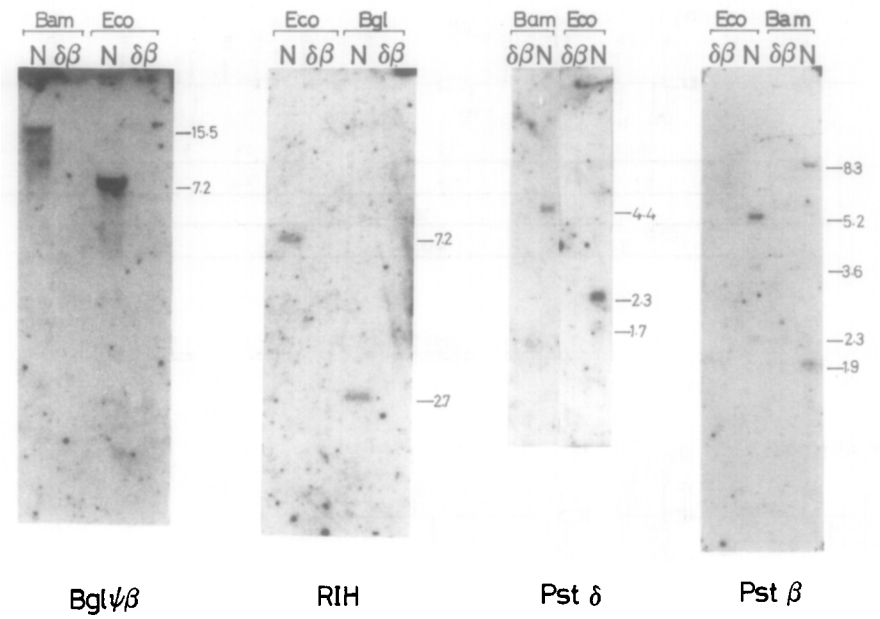

Fig. 2. Hybridizations of DNAs from a normal subject and the Japanese δβ-thalassemia patient to Pstβ, Pstδ, RIH and Bglψβ probes. The DNA of the patient gave no bands that could be observed with the DNA of the normal subject. N: a normal subject, δβ: a Japanese with homozygous δβ-thalassemia.

the 8.2 and 3.4 kb fragments obtained with normal DNA (Fig.3). This suggests that two HindIII restriction site polymorphisms in both of the γ-globin genes (20) are present on each chromosome in this patient. This observation is consistent with the fact that the entire $G\gamma$- and $A\gamma$-globin genes are present in this patient and that the patient is homozygous for δβ-thalassemia genotypically. The following abnormal fragments were detected in digests with BamHl and BglII hybridized to the Hinγ probe. In BamHl digest, the 15.5 kb fragment found in the normal subject was replaced by a 8.5 kb fragment (Fig.3). This finding is compatible with the extensive deletion of the intergenic region between the $A\gamma$- and δ-globin genes (Fig.2), which contains the putative γ-globin gene regulatory region located about 3-4 kb upstream from the δ-globin gene. The BglII digest of thalassemia DNA did not show the normal 13.5 kb fragment containing both the $G\gamma$- and $A\gamma$-globin genes, but a 15 kb fragment. This observation is consistent with a fact that the Bgllll fragment containing the ψβ-globin gene was deleted from this DNA. Thus the

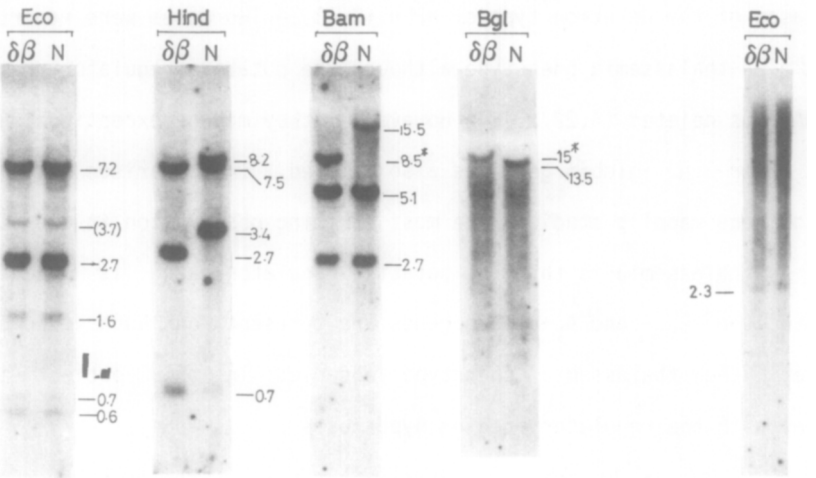

Fig. 3. Hybridizations of normal and Japanese δβ-thalassemia DNAs to Hinγ and Eco2.3 probes. The digests with EcoRI and HindIII gave normal bands after hybridization with Eco2.3 and Hinγ, but the digests with BamHI and BglII hybridized to Hinγ gave abnormal bands. The size of the band hybridized with the ε-globin gene is given in parenthesis. Asterisks indicate abnormal bands.

BglII site at the 3' end of the normal 13.5 kb fragment is missing in DNA of this δβ-thalassemia (Fig.2). When Eco2.3 was used as a probe, EcoRI digests of the DNAs from the patient and a normal individual, both gave a 2.3 kb band and a smear of hybridization, because Eco 2.3 is known to contain a moderately repetitive sequence (21). These results indicate that the 5' end point of the deletion in this δβ-thalassemia is located in the region between the EcoRI site 2.7 kb 3' to the A_γ-globin gene and the BglII site 0.5 kb 5' to the ψβ-globin gene (Fig.1).

DISCUSSION

There are several reports on deletion of the non-α-globin gene cluster in various types of δβ-thalassemia and HPFH (3-13,22,23). It is suggested that the γ-globin gene regulatory region, whose deletion results in persistent expression of the γ-globin gene in adult life, is located between a pair of Alu repeat sequences about 3-4 kb upstream from the δ-globin gene (5), because this region was present in various forms of δβ-thalassemia but absent

in all cases of the deletion type of HPFH (3-13). Two cases were reported to manifest a δβ-thalassemia phenotype although the putative regulatory region in their DNAs was deleted (4,22,23). However, they may be exceptional cases because their A_γ -globin gene was also deleted. In comparison with these results of gene mapping studies, the most striking observation in the Japanese case of δβ-thalassemia is that the putative regulatory area is absent, but that both the G_γ - and A_γ-globin genes are present on both chromosomes. Therefore, the thalassemia phenotype observed in this patient is not consistent with the regulatory region hypothesis.

There are several possible explanations of the insufficient expression of γ-globin gene in this δβ-thalassemia. First, the regulatory sequence might not be present in the region described before, but might be located somewhere beyond the 3' side of the β-globin gene. Tuan et al. demonstrated that the 3' end points of the deletion in Sicilian and Turkish cases of δβ-thalassemia were located much closer to the 3' side of the β-globin gene than those of the deletion in HPFH-1 (USA) and HPFH (Ghana) (24). Therefore, some sequence 3' to the β-globin gene present in δβ-thalassemia but not in HPFH could have some role in γ-globin gene expression. Alternatively, in HPFH, the 3' region that was brought into the vicinity of the γ-globin gene by the deletion might contain some sequence enhancing expression of the γ-globin gene (24).

A second possibility is that there is some molecular defect within the γ-globin gene that was not detected by the restriction mapping, but that reduces synthesis of the γ-globin chain.

A third possibility is that one of the two boundaries, which are assumed to form the chromosomal domain essential for expression of the globin genes (9,25), is deleted from the fetal type globin gene domain in this δβ-thalassemia, because of the extensive deletion extending to near A_γ -globin gene.

Some of them could be verified by determination of the nucleotide sequence around the end point of the deletion and the γ-globin gene in DNA of this δβ-thalassemia. Such studies are now in progress. Precise analysis of the

structure of DNA in δβ-thalassemia and HPFH should provide some clues for understanding the mechanism of gene regulation during development.

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