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The Survey of the Frequency of Single and Triplicated α-Globin Gene Loci and Their Imbalance in Japanese

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ABSTRACT. A survey of an interchromosomally unequal but homologous crossover in human α-globin gene cluster was performed with the DNA of the healthy Japanese adults. Of 200 haplotypes, 4 were found to be unusual α-globin gene clusters ; two were the triplicated [$-\alpha_2-\alpha_2-\alpha_1-$] generated by leftward crossover and the other two were the single [$-\alpha_2\alpha_1$(or α_1)$-$] created by rightward crossover. However, these identified haplotypes of single and triplicated α gene were not correspond in each to a reciprocal counterpart, produced by the same crossing over mechanism. Individuals with these unusual α-globin gene haplotypes showed neither clinical nor hematologic abnormalities.

Key words : Human α globin gene — Unequal but homologous crossover — Single and triplicated α gene haplotype — α-Globin gene mapping

The human α-like globin genes are organized as 5'-ζ-$\varphi\zeta$-$\varphi\alpha_1$-α_2-α_1-3' on chromosome 16.[1] Heteroduplex analysis[2] and partial base sequencing[3] of the DNA within the α-globin gene cluster have identified three homologous units in which unequal but homologous crossover may be facilitated.[4]

Two types of crossover event called as leftward and rightward crossover have been suggested to elucidate the unusual single α gene arrangement by Embury *et al.*[5] One goes leftward deletion with a loss of 4.2 Kb fragment and the other goes rightward deletion with a loss of 3.7 Kb fragment. Lie-Injo *et al.*[6] also reported the two types of triplicated α-globin loci which seemed to be the reciprocal counterparts for each of the leftward and rightward deletion of single α genotypes.

This paper aims to describe the frequency of the unequal crossover within the α-globin gene cluster in Japanese and the detection of the two single and the two triplicated α-globin gene haplotypes which were not, however, the counterparts of each other generated by the same crossover mechanism.

MATERIALS AND METHODS

Blot hybridization studies were performed with the DNAs of 100 healthy and consanguineously unrelated Japanese adults. Routine hematologic examinations were carried out by the standard method.[7] DNA was prepared from white blood cells collected from 10 ml of venous blood by a standard method.[8] Four micrograms of the sample were then digested completely with restriction

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endonucleases under the conditions recommended by the manufacturer (Takara Shuzo Co., Ltd., Kyoto, Japan). The endonucleases used for analysis were Bam HI, Bgl II, Xba I and Hind III. DNA fragments thus obtained were electrophoresed on a 0.6% agarose gel and transferred onto a nylon membrane (Zeta-Probe™, Bio-Rad, Richmond, Ca.) according to Southern's method.[9] The DNA fragments on the membrane were then hybridized to a ^{32}P-labeled α-globin gene probe prepared by nick translation as described by Maniatis *et al.*[10] An α-globin gene probe was a 0.95 Kb Hind III-Xba I fragment containing a region from the 3' portion of the second exon to the 3' flanking region of α_1-globin gene which was derived from pBRα_1.[2] The specific activity of the probe was in a range of $1\sim3\times10^8$ dpm/μg. After hybridization, the nylon membranes were washed stringently, dried and exposed to X-ray film at -80°C for about 15 hrs according to the protocols described by Goosens and Kan.[11]

RESULTS

Blotting pictures of the endonuclease digest of the DNA gave the following results.

Bam HI

Bam HI cleaves usual human DNA outside the linked α_2- and α_1-globin genes leaving a 14.5 Kb band as shown in group U of Figure 1-a. Four of the 100 DNA samples, however, showed an extra band together with the usual 14.5 Kb band. They were classified into two groups of A and B according to the extra band pattern. The sizes of the extra bands were estimated to be 19 Kb in group A and 11.0 Kb in group B, respectively (Fig. 1-a).

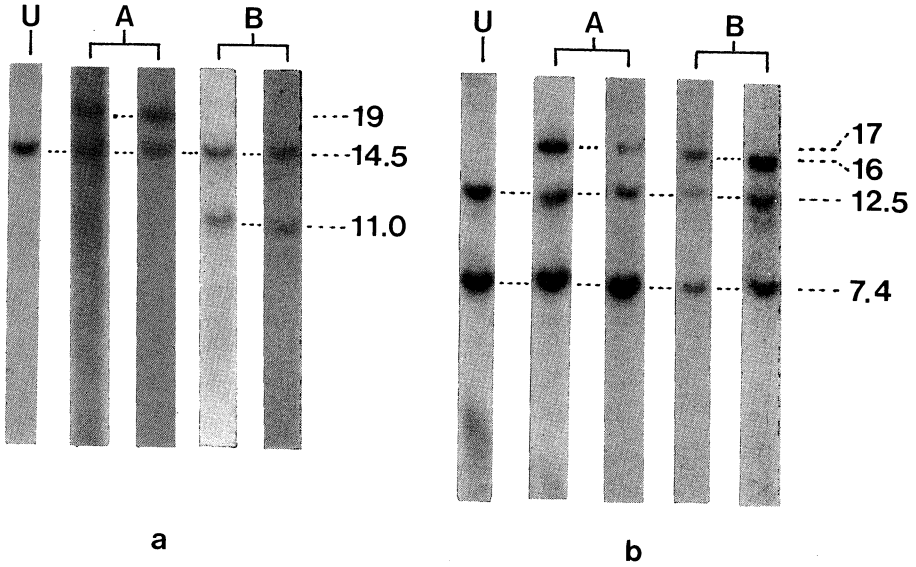

Fig. 1. DNA fragments obtained by Bam HI and Bgl II. U : usual 96 individuals, A and B : group with an unusual fragment pattern. (a) Bam HI digest, (b) Bgl II digest. The numbers indicate fragment sizes in Kb.

Bgl II

Bgl II cleaves usual human DNA outside and between the two α-globin genes producing two DNA bands of 12.5 and 7.4 Kb shown as group U in Figure 1-b. The group A and B also revealed an extra band of 17 and 16 Kb, respectively together with the presence of the common 12.5 and 7.4 Kb bands in both groups (Fig. 1-b).

Xba I

Xba I cleaves usual human DNA outside the linked α_2- and α_1-globin genes affording a 16 Kb band shown as group U in Figure 2-a. Four unusual DNAs showed an extra band of 20 in group A and 12 Kb in group B, respectively.

Hind III

Hind III split usual human DNA within the coding sequences of the two α-globin genes yielding three DNA fragments carrying α-globin genes. The sizes of the fragments are 17, 3.7 and 4.5 Kb in order from 5' to 3' of the DNA. The 17 Kb band located at the upstream of the Hind III site were, however, not detected because the probe in this study is 0.95 Kb Hind III-Xba I DNA fragment derived from the downstream portion of the Hind III site of an α_1-globin gene. The bands observed on the autoradiogram were, therefore, 3.7 and 4.5 Kb fragments in group U (Fig. 2-b). The DNAs of group A showed an extra band of 4.2 Kb together with the presence of the usual 4.5 and 3.7 Kb bands. On the other hand the DNAs of group B showed the two usual sizes of the α-globin gene but the 3.7 Kb band was weaker than those of group U or group A in the scanning intensity (Fig. 2-b).

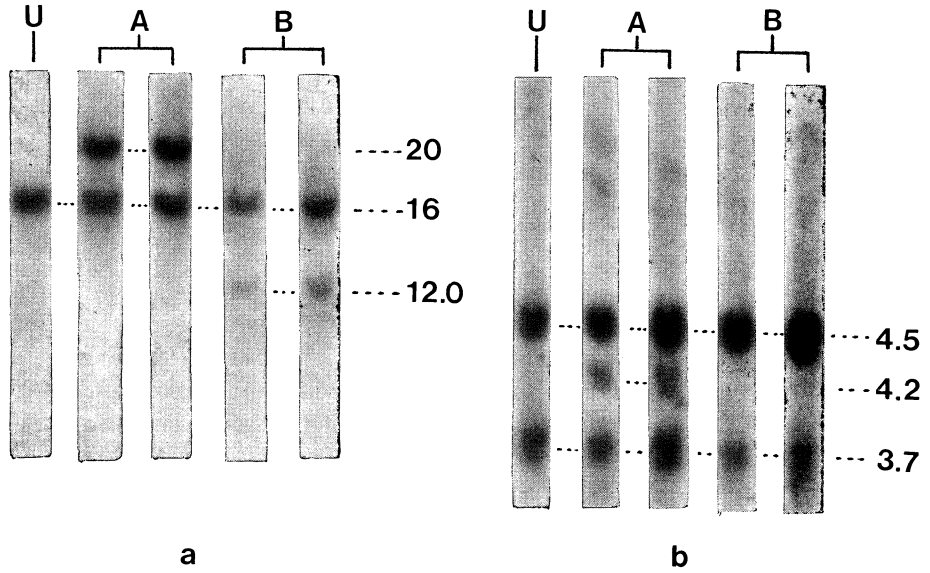

Fig. 2. DNA fragments obtained by Xba I and Hind III. U : usual 96 individuals, A and B : group with an unusual fragment pattern. (a) Xba I digest, (b) Hind III digest. The numbers indicate fragment sizes in Kb.

Hematologic examination

All of the 4 individuals of group A and B did not show any hematologic abnormalities or hemolytic tendencies (RBC, PCV, Hb, MCV, MCHC, MCH, reticulocyte count and serum bilirubin) indicating the product of an unequal but homologous crossover seemed to be harmless to the carriers.

DISCUSSION

Lauer *et al.* demonstrated for the first time the DNA sequence homology within and flanking the α_2- and α_1-globin genes by comparing the results of the restriction endonuclease sites and the heteroduplex studies.[2] More precise homologous regions were determined by the subsequent analyses of the DNA sequences of α_1-, α_2- and $\varphi\alpha_1$-globin genes[3,4,12] as shown in Figure 3.

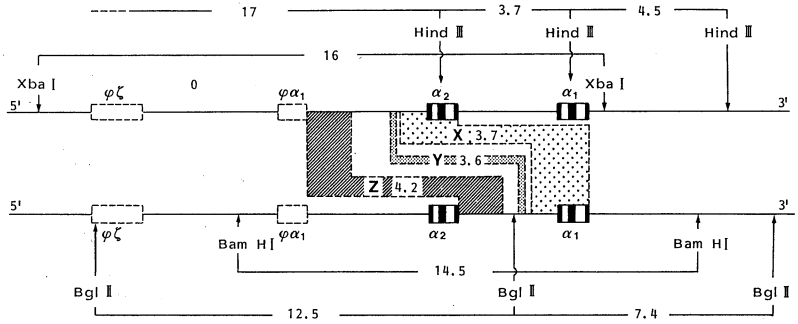

Fig. 3. Homologous regions with the segment of α-globin gene cluster around the $\varphi\zeta$, $\varphi\alpha_1$-, α_2-, and α_1-globin genes and the cleavage site by restriction endonucleases. The rectangular blocks represent the respective genes and pseudogenes. The shaded region marked X, Y, Z denote the homologous regions. The numbers indicate fragment sizes in Kb.

When the crossover to produce single or triple α genes in a haploid was occurred within the leftward homologous region of Z, the hybrid chromosomes generated by this so called leftward crossover would give 4.2 Kb shorter or 4.2 Kb longer bands than usual in the digest of enzymes which cleave the DNA outside the α-globin gene such as Bam HI and Xba I. When the crossover to produce the same haplotypes was occurred within the rightward homologous region of Y or X, the resultant hybrid fragment created by so called rightward crossover would give the bands differed by 3.6 or 3.7 Kb from the usual fragment of the digests of Bam HI and Xba I.

The DNA sizes of the extra bands observed in the digests by four enzymes in group A and B are summarized in Table 1 together with their theoretically expected sizes yielded by unequal crossing over phenomena. These results would conclude evidently that the unusual chromosomes of group A are the triplicated α-globin gene haplotypes [$-\alpha_2$-α_2-α_1-] generated by the leftward crossover and those of group B are the single α-globin gene haplotypes [$-\alpha_2$ α_1 (or α_1)$-$] generated by the rightward crossover. The unusual chromosomes of group A and group B are, therefore, unlikely to be the counterparts created by the crossover events in the same region. It seems reasonable to suppose

TABLE 1. Extra fragment size of group A and B and the expected restriction fragment sizes of single and triplicated α gene haplotype generated by leftward and rightward crossover. The numeral in the parentheses are common with those of usual haplotype.

	Bam HI	Bgl II	Xba I	Hind III
Observed :				
group A	19	17	20	4.2
group B	11	16	12	—
Expected :				
rightward crossover :				
single α	10.8	16.2	11.8	(4.5)
triplicated α	18.2	3.7 (7.4, 12.5)	19.2	3.7 (3.7, 4.5)
leftward crossover :				
single α	10.3	8.3 (7.4)	11.3	(4.5)
triplicated α	18.7	16.7	19.7	4.2 (3.7, 4.5)
usual duplicated α	14.5	7.4, 12.5	16.0	3.7, 4.5

(Kb)

that the process of unequal crossover would produce equal numbers of chromosomes with a triplicated α-globin gene locus and a single one. However the single α-globin gene haplotypes [$-\alpha_1-$] generated by leftward crossover and the triplicated α-globin gene haplotypes [$-\alpha_2-\alpha_1\alpha_2$(or α_2)$-\alpha_1-$] generated by rightward crossover were not detected in this study. Although much additional data accumulations were required for the interpretation of this discrepant gene frequency, the putative selective advantage for the single α-globin gene genotype given by the malaria infection[13] is less likely for the Japanese.

It is evident that all the individuals of group A and B were the heterozygotes for triplicated or single α-globin gene because they showed the DNA fragment with both usual and extra bands sizes. The frequencies of the triplicated α-globin haplotype and the single one in this survey were 0.01 although they were not the counterparts each other for the haplotypes generated by the same crossover region.

It seems likely that heterozygotes for the triplicated α-globin gene or single one show no clinical or hematological abnormalities. This finding is consistent with previous publications.[5,6,13]

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