Diversity of Human γ-Globin Gene Loci Including a Quadruplicated Arrangement

By Shunichi Shimasaki and Iwao Iuchi

A quadruplicated γ-globin gene as a (5'γ-γ-γ-γ-3') was detected in an adult Chinese during a survey designed to detect 5′γ- and 3′γ-globin genes in Japanese. Five triplyclicated (5'γ-γ-5'γ-) and two single (5'γ-) haplotypes were also detected in 103 healthy adult Japanese. All of the unusual

chromosomes appeared to reflect an unequal but homologous crossover between 5′γ- and 3′γ-globin genes. A new Bgl II polymorphic site located around the 3' terminal region of the 5′γ-globin gene was also discovered.

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THE DUPLICATED human fetal globin genes encode different forms of γ-chains designated as 6γ and 4γ. The two genes originate by duplication of five kilobases (kb) of DNA, either through a simple unequal recombination of two small preexisting repetitive elements or through a more complex series of events not requiring any preexisting repeats.1

Based on a comparison of the DNA sequences of the two γ-globin genes, the occurrence of interchromosomally unequal but homologous crossovers between 6γ- and 4γ-globin genes (gene conversion) has been strongly suggested.1,2 An unusual arrangement of the γ-globin gene locus in a chromosome has also been reported in some racial groups,3 as expressed in a haploidy formula of γ-γ-γ/ or γ/.

We examined the arrangement of γ-globin genes to learn the frequency of intergenic crossover in Japanese. The results of that survey are reported, with special emphasis on the discovery of a quadruplicated γ-globin gene haplotype (four γ-globin gene loci on one chromosome).

MATERIALS AND METHODS

Routine hematologic studies were carried out by standard methods.3 Conventional methods were used for hemolysate preparation and fetal hemoglobin (Hb F) determination.4 Blot hybridization studies were performed with DNAs of 104 healthy unrelated adults (103 Japanese and one Chinese).

A DNA sample of high mol wt was isolated from leukocytes collected from 10 mL of venous blood by standard methods.3 Four micrograms of each sample were digested with 20 U of Bgl II for five hours under conditions recommended by the manufacturer (Takara Shuzo Co, Ltd., Kyoto, Japan). Cleaved DNA fragments were precipitated with ethanol and redissolved in 30 μL of TE (10 mmol/L of Tris-HCl, 1 mmol/L of EDTA, pH 8.0) buffer. The DNA digest was electrophoresed on a 0.6% agarose gel (pH 8.0) and transferred onto a nylon membrane (Zeta-Probe, Bio-Rad, Richmond, Calif) by Southern blotting method.6

The DNA fragments on the membrane were hybridized to a mixture of three radioactive probes: (a) a 3.4-kb Hind III fragment containing the 5′γ-globin gene; (b) a 2.3-kb Pst I fragment containing the δ-globin gene; and (c) a 3.8-kb Pst I 1-Bgl II fragment containing the β-globin gene. Each of the DNA probes was derived separately from recombinant plasmids pr γ 3.4,3 Pst β (pHb)3 or Pst β14 isolated individually from transformed E coli HB 101 under P2-EK1 containment conditions in accordance with the guidelines of the Ministry of Education, Science and Culture of Japan. The probe fragments were nick-translated11 in the presence of α-32P dCTP to convert them into a radioactive form. Their specific activity was in a range of 1 to 3 × 106 dpm/μg. After hybridization, the nylon membranes were washed stringently, dried, and exposed to x-ray film at −70 °C for 15 hours according to the protocols described by Goossens and Kan.12

DNA samples that showed an abnormal pattern on autoradiograms were further digested by other restriction endonucleases including Bcl I, Xmn I, Pst I, Xba I, Bam HI, and Eco RI, followed by the same analyses except for hybridization to an individual probe.

RESULTS

Bgl II cleaves human DNA to produce the linked γ-globin gene (6γ and 4γ) loci and each of the δ-globin and β-globin gene loci. Autoradiograms obtained by a mixture probe demonstrated clear bands certifying complete DNA digestion with Bgl II and providing an accurate estimation of the DNA fragment sizes (Fig 1).

The autoradiograms of 104 individuals obtained by Bgl II digestion and use of the mixture of probes revealed 95 instances carrying the DNA bands of 13, 8.2, and 5.0 kb with approximately equal intensity on scanning. They could be accordingly classified into the most common genotype (group N in Fig 1). The other nine DNA samples, however, showed a clear but fainter 13-kb band than did those of 8.2 and 5.0 kb (groups A, B, C, and D in Fig 1). In addition, seven of these nine samples showed an extra restriction fragment. DNA samples from these nine individuals were accordingly classified into four groups: A (5 cases), B (one case), C (2 cases), and D (one case) based on the band pattern.

In order to identify these extra bands, hybridization to each probe was carried out individually; only the γ-globin specific probe hybridized to each of the extra bands (Fig 2). Moreover, additional extra bands (8.1 kb) were observed in the samples of group C which had not been recognized as containing an extra band when hybridized to the mixture of probe.

The sizes of the extra bands were estimated to be 18, 23, and 8.1 kb in groups A, B, and C, respectively, and 10 and 3.2 kb in group D. It was evident that the 8.1-kb band in group C was superimposed on the 8.2-kb Bgl II band derived from the δ-globin gene by hybridization to the mixture of probes.

The nine DNA samples were digested with Bcl I. Autoradiograms detected the 20-kb fragment commonly seen due to digestion outside the linked γ-globin gene loci in each and an extra fragment of varied size in all samples except for the

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The DNA samples were then digested with Pst I, which cleaves at the site of the codon corresponding to the 136th amino acid of the Aγ-globin chain (third exon) but not at the same position in the Gγ-globin gene. The autoradiograms obtained with a γ probe revealed the three normal bands (Fig 4). The relative intensities of the intergenic 4.9-kb fragment between and Aγ-globin gene was compared to the two bands of 4.0 kb and 2.8 kb derived from the upstream region of the Gγ-gene and the downstream region of Aγ-gene, respectively. The ratios were higher in groups A and B and lower in group C than were those of the common group. Namely, the relative intensities of the 4.9-kb band to 4.0-kb band were 1.4, 1.8, 2.5, 0.8, and 1.4 for groups N, A, B, C, and D, respectively.

Xba I, Bam HI, and Eco RI were also used to characterize these unusual DNAs. All of the intergenic DNA fragments shown with arrows in Table 2 demonstrated the same tendency as did Pst I digestion. For example, the Xba I digestion showed the relative intensities of the intergenic 4.9-7.5-Kb fragment as 1.9, 2.5, 3.3, 1.4, and 1.9 for groups N, A, B, C, and D, respectively. Restriction endonuclease maps around the fetal globin genes of these unusual chromosomes were established by these findings, as in Fig 5.

Two different-sized fragments of group B observed by the Bgl II, Bcl I, and Xmn I digestion were thought to come from the heterozygosity of the γ-globin gene cluster. The individual in group B was therefore considered to be heterozygous for the quadruplicated γ-globin gene rather than homozygous for a chromosome with three γ-globin genes. The individuals of groups A and C were heterozygous for triplicated and single γ-globin genes, respectively. The frequen-
cies of the triplicated γ-globin haplotype and the single one in this survey were 0.024 and 0.010, respectively.

The nine individuals of groups A, B, C, and D showed no hematologic abnormalities or evidence of hemolysis (RBC, PCV, Hb, MCV, MCHC, MCH, reticulocyte count, and serum bilirubin), and their Hb F of 0.2% to 0.4% were within the normal range.

**DISCUSSION**

A high degree of homology between the \(^\gamma\)- and \(^\gamma\)-γ-globin genes extends from about 500 bp 5' to the Cap site to a region of simple base sequence within the large intervening sequence (IVS-2). This simple base sequence region seems the most likely site for an interchromosomally unequal but homologous crossover. The crossover mechanism between the \(^\gamma\)-γ and the \(^\gamma\)-γ-globin gene may be shown as in Fig 6, based on a general model for genetic recombination proposed by Meselson and Radding. When a branch migration from a cross-bridge site is short, and the strand cleavage occurs within the gene, a new single hybrid gene represented as \(^\gamma\)-γ is generated. When the branch migration extends upstream beyond the gene, the single γ-globin gene is an intact γ-gene. In the case of the triplicated γ-globin gene chromosome, on the other hand, the central gene generated by the crossover may be \(^\gamma\)-γ or \(^\gamma\)-γ depending on subsequent DNA replication or mismatch repair in the heteroduplex region. This haplotype is therefore expressed as \(5\gamma\), without defining the central γ-globin gene.

The region of the crossover on the eight unusual chromosomes we studied was inferred from the \(Pst\) I and \(Xmn\) I analysis. The \(Pst\) I sites, specific for \(^\gamma\)-γ genes, were not present in all of the inner genes of the triplicated and the quadruplicated γ-globin gene chromosomes, but analyses of the single γ-globin gene chromosomes clearly showed this cleavage site. These results suggest that all the crossover events occurred upstream from the \(Pst\) I site in the 3rd exon, of the γ-gene.

In the \(Xmn\) I analysis, the appearance of the extra fragments of 13 and 18 kb in groups A and B, respectively, suggests that the crossover occurred within the normal 8.2-kb \(Xmn\) I fragment. The left \(Xmn\) I site defining the 8.2-kb fragment in Fig 3 is at 1.3 Kb upstream from the Cap site of the \(^\gamma\)-γ-globin gene. The crossover region of groups A and B, therefore, occurred between the \(Xmn\) I site 1.3 kb 5' to

**Table 1.** Size (in Kilobases) of the Fragments Digested with \(Xmn\) I

<table>
<thead>
<tr>
<th>No. of Case</th>
<th>Group</th>
<th>N</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.2</td>
<td>13.82</td>
<td>8.2</td>
<td>8.2</td>
<td>3.3</td>
<td>8.2</td>
</tr>
<tr>
<td>2</td>
<td>8.2, 7.0, 1.2</td>
<td>13.82</td>
<td>—</td>
<td>7.0, 2.1, 1.2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>7.0, 1.2</td>
<td>13.82</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>—</td>
<td>13.82</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>—</td>
<td>13.82</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

**Table 2.** Size (in Kilobases) of Restriction Fragments in the Region of a Fetal Globin Gene

<table>
<thead>
<tr>
<th>Group</th>
<th>Xba I</th>
<th>Ban I</th>
<th>Hpa I</th>
<th>Eco RI</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7.5, 4.9, 3.7</td>
<td>15.5, 4.9, 2.6</td>
<td>7.2, 2.6, 1.7</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>7.5, 4.9, 3.7</td>
<td>15.5, 4.9, 2.6</td>
<td>7.2, 2.6, 1.7</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>7.5, 4.9, 3.7</td>
<td>15.5, 4.9, 2.6</td>
<td>7.2, 2.6, 1.7</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>7.5, 4.9, 3.7</td>
<td>15.5, 4.9, 2.6</td>
<td>7.2, 2.6, 1.7</td>
<td></td>
</tr>
</tbody>
</table>

\(\wedge\): Increased intensity; \(\wedge\): decreased intensity.
the Cap site of the $\gamma$-globin gene and the $Pst$ I site in the 3rd exon of the $\gamma$-globin gene.

Formation of these hybrid chromosomes by interchromosomal crossovers occurs within the region proposed by Shen et al. The quadruplicated $\gamma$-globin gene haplotype may have arisen through an unequal but homologous crossover between the triplicated and the usual duplicated haplotypes or between two triplicated haplotypes. It is accordingly represented as $5'\gamma-\gamma-\gamma-\gamma-3'$.

The process of unequal but homologous crossover produces an equal number of chromosomes with a triplicated $\gamma$-globin gene locus and a single one. However, the frequency of the triplicated $\gamma$-globin gene chromosome was twice as great as that of the single $\gamma$-globin gene chromosome in this survey. It is unlikely to suppose that a single $\gamma$-globin gene haplotype confers a serious selective disadvantage; two newborns who were homozygous for a single $\gamma$-globin gene have been reported without severe $\gamma$-thalassemia. The number of $\gamma$-globin gene loci does not affect Hb F production in adults since the Hb F levels were within normal limits.

This article provides a description of a $Bgl$ II polymorphic restriction site near the globin genes. One individual in group D demonstrated a site that split the usual 13-kb fragment containing the $\alpha$-$\gamma$ and $\alpha$-$\gamma$-globin genes into 10-kb and 3.2-kb fragments. The probe used in this hybridization was an $\alpha$-$\gamma$ Hind III 3.4-kb fragment which could also hybridize to the homologous $\delta$-$\gamma$-gene sequence. If the new polymorphic $Bgl$ II site is located 3.2 kb 3' to the leftward $Bgl$ II site of the 13-kb fragment shown in Fig 1, it would be observed on the autoradiogram. On the other hand, if the 3.2-kb fragment derived from the opposite end of the 13-kb fragment, it would not be expected to hybridize to the $\gamma$ probe. The observed 3.2-kb fragment was, therefore, formed by a new $Bgl$ II site near the 3' terminal region of the $\delta$-$\gamma$-globin gene.

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REFERENCES

2. Slightom JL, Blechl AE, Smithies O: Human fetal $\delta$- and $\alpha$-globin genes: Complete nucleotide sequences suggest that DNA can be exchanged between these duplicated genes. Cell 21:627, 1980
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