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Genetics

Linkage of $\alpha^{G\text{-Philadelphia}}$ to α-thalassemia in African-Americans*

(α-chain variant/restriction endonuclease mapping)

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ABSTRACT **We have studied the inheritance of the α-chain hemoglobin variant Hb G-Philadelphia ($\alpha_2^{68\,Asn\rightarrow Lys}\beta_2$) in two African-American families. Expression of the α-globin loci was monitored by the percentage of Hb G in these individuals. The variant represented approximately 33% of the total adult hemoglobin in some and 50% in others. α-Globin gene fragments were analyzed by using restriction endonucleases that cleave outside (*Eco*RI), within (*Hin*dIII), and between (*Bgl* II) the normal duplicated α-globin loci ($\alpha\alpha/\alpha\alpha$). Individuals having 33% variant lack one functioning α gene ($\alpha^G/\alpha\alpha$); those with 50% variant lack two genes, one missing on each chromosome (α^G/α). Inheritance of α^G was therefore linked to that of a chromosome with only one functional α-globin gene locus. This locus is probably the result of a nonhomologous crossover. Our results also suggest equal expression of the α-globin loci in humans because the percentages of the variant could be explained solely on the basis of the total number of α genes present. The percentages of Hb G as well as other hematologic data all were consistent with the number of α-globin genes identified by restriction endonuclease mapping. Gene mapping yields a more precise determination of the number of α-globin genes than does study of globin synthesis.**

The existence of two α-globin genes per haploid genome in humans is now well established (1, 2). Solution hybridization (3–6) and restriction endonuclease analyses of DNA (7–11) have shown that the most common cause of α-thalassemia is deletion of structural gene material, although nondeletion types also have been reported (12, 13). The four classical disorders due to α-thalassemia (silent carrier, α-thalassemia trait, Hb H disease, and hydrops fetalis) are most frequently caused by deletion of one to four α-globin genes.

In Asians, deletion of two α-globin genes on the same chromosome or of only one gene is common, whereas in Africans, deletion of only a single α-globin locus per chromosome is the predominant form (9, 10). The α-thalassemia syndromes in Asians commonly arise by gene deletion of either the 5′ α-globin locus or both 5′ and 3′ α-globin loci; in Africans and Mediterraneans, as well as some Asians, generation of a single α-globin locus on a chromosome is due to a nonhomologous crossover (12, 14).

The most common α-chain variant in African-Americans is $\alpha^{G\text{-Philadelphia}}$ ($\alpha^{68\,Asn\rightarrow Lsy}$; α^G), which has been reported to represent 20%, 30%, or 40% of the total number of α-chains present (15). It has been suggested that this distribution may be due to the linkage of the variant α-globin gene to α-thalassemia (16, 17).

We have investigated the expression of α-thalassemia in two African-American families with α^G by using hematologic data, globin synthesis, and analyses of α-globin genes in restriction endonuclease digests of DNA.

MATERIALS AND METHODS

Hematologic Studies. Nine individuals from two African-American families were studied. In addition to α^G, family 2 also had Hb S and β^+-thalassemia genotypes (see Table 1). Hematologic studies were performed with a model S Coulter Counter. The hemoglobins were studied by electrophoresis on cellulose acetate, agar gel, and starch gel as described (18). The identity of α^G was confirmed by analysis of tryptic peptides. Hb G was quantitated by densitometric scanning after electrophoresis on cellulose acetate. When β^S was also present, α^G was measured as the percentage of total α-globin by spectrophotometry after separation of globin chains on CM-cellulose. Hb A_2 levels were measured by spectrophotometry after elution from cellulose acetate strips. In our laboratory the normal range for Hb A_2 is 1.9–3.4%. Globin synthesis studies were performed after incubation of peripheral blood for 2 hr at 37°C with [^{14}C]leucine (339.0 mCi/mmol; New England Nuclear; 1 Ci = 3.7×10^{10} becquerels) (19). Normal values in our laboratory are $\alpha/\beta = 1.01 \pm 0.05$.

DNA Isolation, Restriction Endonuclease Digestions, and Southern Blot Analysis. DNA was isolated by using $NaDodSO_4$ and proteinase K digestion (20), from heparinized peripheral blood (13). Restriction endonuclease digestions of 12–15 μg of DNA per lane were performed as described (7) using conditions defined by the suppliers. The digests were subjected to electrophoresis for 20 hr at 30 V and 12 mA (constant current) on vertical 0.7% agarose gels with a recirculating Tris acetate/EDTA/NaCl buffer system (21). DNA was transferred to nitrocellulose paper and baked as described (22). Filters were preannealed by shaking for 4 hr at 68°C in 20 ml of 0.9 M NaCl/0.09 M sodium citrate containing 0.02% each of Ficoll, bovine serum albumin, and polyvinylpyrolidone and 1% glycine per filter in heat-sealed bags and then hybridized with heat-denatured radioactive probe for 70 hr at 68°C with shaking in 10 ml of annealing solution (preannealing solution plus 0.5% $NaDodSO_4$ but no glycine) per filter. Either sonicated heat-denatured calf thymus DNA or *Escherichia coli* DNA at 500 μg/ml was included in both preannealing and annealing solutions to reduce background adsorption of the mixed (α- + β-globin) cDNA or cloned α-globin gene insert radioactive probes, respectively. Filters were washed three times for 1 hr each with 75 ml of 0.3 M NaCl/0.03 M sodium citrate/0.5% $NaDodSO_4$ at 68°C with shaking and once with 75 ml of 0.3 M NaCl/0.03 M sodium citrate at room temperature with shaking. When necessary, more stringent washing conditions were used (23). Radioactive bands were located by autoradiography using

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Abbreviations: α^G, $\alpha^{G\text{-Philadelphia}}$ ($\alpha^{68\,Asn\rightarrow Lys}$); Hb G, Hb G-Philadelphia ($\alpha_2^{68\,Asn\rightarrow Lys}\beta_2$); kb, kilobase(s).

* This work was presented in preliminary form at the Fourth Cooley's Anemia Symposium, May 1979, New York City.

Table 1. Hematological findings in members of families 1 and 2

	Age, yr	Sex	Hb, g/dl	MCV,* fl	MCH,† pg	β-Globins	α-Globins	% Hb G	% Hb A_2	α/β synthesis
Family 1:										
I-1	42	M	—	—	—	A	A	—	2.9	—
I-2	40	F	11.0	73	24.0	A	A, G^{Phila}	34	2.2	—
II-1	18	F	12.4	68	23.0	A	A, G^{Phila}	46	1.8	0.71
II-2	17	M	14.5	77	25.2	A	A, G^{Phila}	48	1.8	0.86
II-3	16	M	15.3	94	32.4	A	A	—	3.4	1.05
II-4	15	F	12.1	81	27.1	A	A, G^{Phila}	32	2.4	0.97
II-5	9	F	11.9	73	24.8	A	A	—	2.9	—
Family 2:										
I-1	26	M	14.4	71	23.5	A, S	A, G^{Phila}	47‡	1.9	0.92
I-2	24	F	11.8	68	23.2	S, A(β^+thal)	A	—	5.6	1.43
II-1	5	F	10.5	66	22.0	S, A(β^+thal)	A, G^{Phila}	32‡	—	1.39

*Mean corpuscular volume.
† Mean corpuscular hemoglobin.
‡ Measured as percentage of α^G in total α-globin.

preflashed film (24). "End-labeled" *Hin*dIII and *Eco*RI digests of λ phage DNA were used as molecular weight standards on the autoradiograph to determine sizes of the globin gene bands.

Radioactive Probes. Radioactive cDNA was prepared from total adult globin mRNA as described (7). Plasmid JW101 containing double-stranded cDNA for human α-globin (kindly provided by B. Forget) was propagated as described (25) under P-3 conditions as outlined in the National Institutes of Health *Guidelines for Recombinant DNA Research*. Plasmid DNA was isolated (26, 27) and digested with *Mbo* II to produce a number of fragments in addition to a fragment of about 1.8 kilobases (kb) containing the entire α-globin gene sequences. This fragment was isolated (28) from a Tris borate/EDTA/3.5% acrylamide preparative gel (29) and radiolabeled to a specific activity of 10^8–10^9 cpm/μg with [^{32}P]dCTP (2000–3000 Ci/mmol; Amersham) by using *E. coli* DNA polymerase I and calf thymus DNA site-specific primers (30).

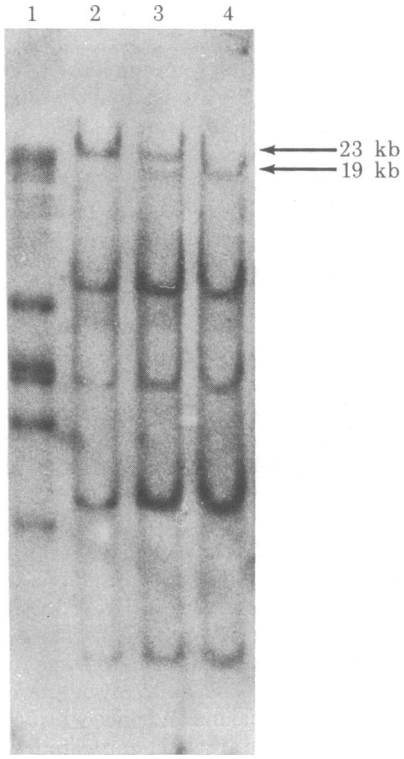

FIG. 1. Autoradiograph of globin gene fragments after *Eco*RI digestion of human DNA and hybridization to a mixed cDNA probe. Three distinct patterns of the α-globin gene fragments are shown: lane 2 (family 1, II-3), 23 kb only; lane 3 (family 1, II-4), 23 and 19 kb; lane 4 (family 1, II-1), 19 kb only. Lane 1 contains "end-labeled" *Eco*RI λ phage fragments used as size markers. Other bands in lanes 2, 3, and 4 contain DNA fragments from β-, δ-, and γ-globin genes.

RESULTS

Hematologic Findings. Four persons in family 1 (I-2, II-1, II-2, II-5) and all members of family 2 had low MCV and MCH without iron deficiency anemia (Table 1). Six persons had α^G and two of them (I-1 and II-1 in family 2) also had β^S. When present, the α^G-containing hemoglobins (Hb G, Hb G/S) constituted approximately one-third (32%, 32%, or 34%) or one-half (46%, 47%, or 48%) of the total hemoglobins. Those with about 50% also had the lowest Hb A_2 levels (1.8% and 1.9%), whereas those with 33% Hb G had Hb A_2 levels of 2.2% and 2.4%. In family 2, the mother (I-2) had 77% β^S and the child had only 67% β^S although they both had Hb S-β^+-thalassemia (see Fig. 2*B*). The lower level of β^S in the child is probably due to the decreased availability of α chains to complex with the β^S (31) because she had only three α-globin genes (see below).

Detection of α-Globin Gene Deletions. The enzyme *Eco*RI, which cleaves outside the duplicated α-globin loci in normal human DNA, yields a fragment approximately 23 kb long.§ A shorter *Eco*RI fragment, approximately 19 kb long, is found when one of the two α-globin loci is deleted (9, 10). The inheritance of the α^G variant is linked to that of the 19-kb *Eco*RI fragment in the two families studied (Figs. 1 and 2). Of the six persons who had α^G, three (II-1 and II-2 in family 1 and I-1 in family 2) had only the 19-kb fragment; the others (I-2 and II-4 in family 1 and II-1 in family 2) had both the 19- and 23-kb fragments.

*Hin*dIII digestion of normal DNA generates 17.0-, 3.7-, and 4.5-kb α-globin gene fragments (9, 33). The 3.7-kb fragment normally connecting the centers of the two α-globin loci is deleted on the chromosome with only a single α-globin locus (9). All three persons in our study homozygous for the 19-kb *Eco*RI fragment lacked the 3.7-kb *Hin*dIII fragment (Fig. 3), consistent with homozygosity for a single α-globin gene on each chromosome. Further analysis of this DNA was performed with *Bgl* II. This enzyme cleaves normal DNA between the dupli-

§ The 23- and 19-kb fragments were estimated to be 20.5 and 18 kb, respectively, in our earlier work (32).

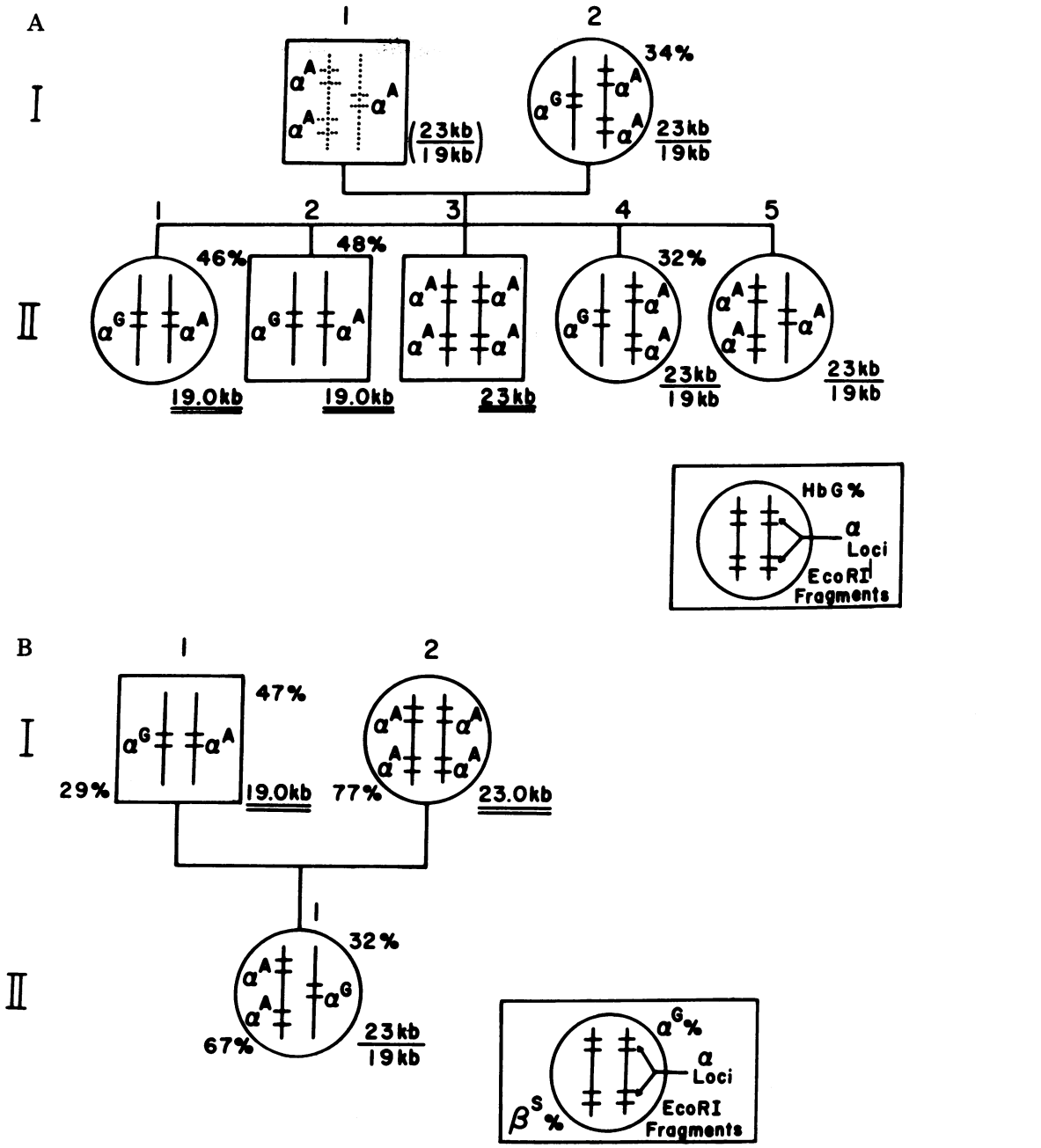

FIG. 2. Pedigrees of the two families studied. (*A*) Family 1. The sizes of α-globin fragments from I-1 were deduced from the pedigree and his hemoglobin phenotype because he was not available for DNA analysis. (*B*) Family 2.

cated α-globin loci to produce two fragments of approximately 12.5 and 7 kb (34). A single *Bgl* II fragment (15.8 kb) is found on a chromosome with a single fused α-globin locus due to an unequal crossover event (14). All subjects who had only the 19-kb *Eco*RI pattern had the new α-globin gene-containing *Bgl* II fragment (approximately 16 kb long) (Fig. 4). In a recent study (11) the generation of a new *Bgl* II α-globin gene fragment also was found in association with a chromosome containing a single α-globin gene in an Algerian family with Hb H disease.

Globin Synthesis Studies. The presence of α-thalassemia due to deletion of structural genes is clearly reflected in decreased erythrocyte indices in the affected family members (Table 1; Fig. 2). The results of globin synthesis studies do not clearly reflect the decreased number of α-globin genes. The α/β ratio in persons with only two α-globin genes was 0.71, 0.86, and 0.92 (II-1 and II-2 in family 1 and I-1 in family 2); in II-4 with three genes the ratio was 0.97 and in II-2 with four genes the ratio was 1.05. Although the decrease in number of genes was paralleled by decreasing α/β ratios in this small sample, the values of 0.92 and 0.97 are clearly within the normal range, despite the presence of only two and three α-globin genes, respectively. As expected, in the two persons in family 2 with Hb S-β^+-thalassemia, the α/β ratios were high, 1.43 in I-2 with four α-globin genes and 1.39 in II-1 with three genes.

DISCUSSION

Previous genetic studies suggested that the α^G mutation in people of African descent occurred on a chromosome with only a single α-globin gene (16). Our analysis of α-globin genes in these two families and the data in a recent abstract (35) confirm this suggestion and explain the clustering of Hb G percentages around 46–48% and 32–34%. The three individuals with 46–

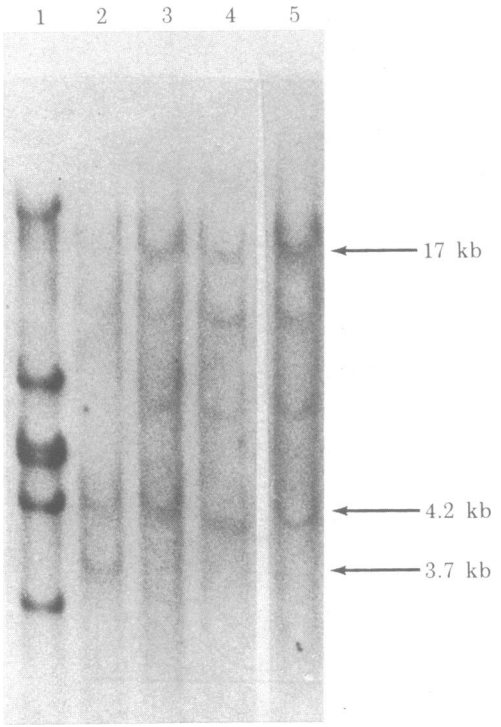

FIG. 3. Autoradiograph of α-globin gene fragments after *Hin*dIII digestion of DNA and hybridization with $\hat{\alpha}$-globin-specific probe. Lanes: 1, marker lane; 2, family 1, II-3; 3, family 1, II-1; 4, family 1, II-2; 5, family 2, I-1. The DNA in lane 2 had only a 23-kb *Eco*RI fragment; the DNA in lanes 3–5 had only a 19-kb fragment (Fig. 1). The 3.7-kb fragment present in lane 2 is absent in lanes 3–5. The normal 17- and 4.2-kb fragments are present in each of these lanes. Additional bands are probably embryonic and other α-globin-like DNA fragments detected by the high-specific-activity probe.

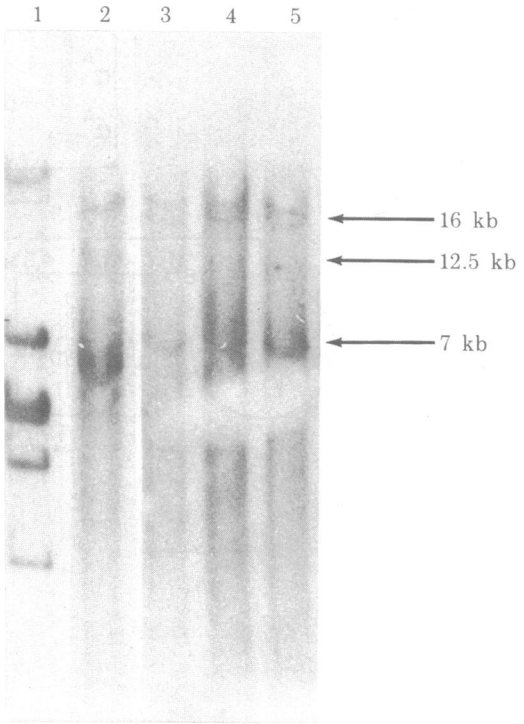

FIG. 4. Autoradiograph of α-globin gene fragments after *Bgl* II digestion and hybridization with α-globin-specific probe. Lanes: 1, markers; 2, family 1, II-3; 3, family 1, II-1; 4, family 1, II-2; 5, family 2, I-1. The DNA in lane 2 had only a 23-kb *Eco*RI fragment; the DNA in lanes 3–5 had only a 19-kb fragment (Fig. 1). Note the doublet in lanes 3–5; the lower band is 16 kb and is not present in lane 2. This 16-kb band is consistent with a nonhomologous crossover causing the short *Eco*RI fragment. The 12.5- and 7-kb fragments contain other normal α-globin DNA or crosshybridizing α-globin DNA-like material, perhaps embryonic. The upper band of the doublet (19 kb) may be due to incomplete digestion.

48% Hb G had microcytic, hypochromic erythrocytes and low Hb A_2 and were homozygous for the single fusion type α-globin gene (Fig. 2). They had two α-globin genes, one being α^G (α^G/α). The individuals with 33% Hb G had three α-globin genes, two normal loci on one chromosome and one fusion α-globin locus containing the α^G mutation ($\alpha^G/\alpha\alpha$) on the other. Globin synthesis studies only differentiated two of the three persons with two α-globin genes from normals and did not distinguish between the two persons with three α-globin genes and comparable controls. The scatter of globin synthesis values has been previously noted in both α- and β-thalassemia in African-Americans and Jamaicans (36, 37); it is uncommon in other ethnic groups with thalassemia.

This study demonstrates a direct correlation between the percentages of the protein variant and the total number of functional α-globin genes, and furthermore it suggests the existence of independently functioning transcriptional units for the α-globin loci in humans. The relative output of the normal non-α-globin genes (G_γ, A_γ, δ, and β) does not correspond directly to the number of loci, suggesting that mechanisms other than simple gene dosage are important. However, even the percentages of an α-chain variant may not always correlate with the number of gene loci present (38).

In the families reported in this study, α^G is linked to α-thalassemia. Although the level of Hb G in heterozygotes is most frequently reported to be approximately 30% or 40%, Baine *et al.* (15) reported two families with about 20% of this variant. This lower level would tend to suggest that the α^G was linked to a normal α-globin gene ($\alpha^G\alpha/\alpha\alpha$). Recently, another α-chain variant which represented 27% of the total α-chains was reported to be linked to a normal α-globin gene (39). Alternatively, if α^G is a mutation that originally occurred on a single fused gene resulting from unequal crossover, then the recent discovery of a chromosome with three α-globin loci may provide an explanation for the uncommon occurrence of persons with 20% of this variant (34). The inheritance of three normal α-globin loci on one chromosome and a single α^G locus on the other ($\alpha^G/\alpha\alpha\alpha$) would result in a variant percentage of about 25%. The frequency of the triplicated α-globin loci resulting from nonhomologous crossover is rare in African-Americans (34) and could therefore explain why so few families with 20–25% Hb G have been reported.

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