The α-Globin Gene Adjacent to the Gene for HbQ-α 74 Asp→His is Deleted, But Not That Adjacent to the Gene for HbG-α 30 Glu→Gln; Three-Fourths of the α-Globin Genes Are Deleted in HbQ-α-Thalassemia

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Two Chinese patients with HbQ-α2 74 Asp→His ββ-α-thalassemia, one HbQ-α2 74 or 75 Asp→His ββ carrier, and one HbG-α2 30 Glu→Gln ββ carrier were studied to determine the number of α-globin genes in their chromosomes. DNA was isolated from white blood cells and bone marrow cells and studied by liquid hybridization and by hybridization of DNA fragments obtained by restriction enzyme endonuclease digestion (Eco RI and Hpa I) after agarose gel electrophoresis and transfer to nitrocellulose filters. The liquid hybridization analysis showed that in HbQ-α2 74 Asp→His ββ-α-thalassemia, as in HbH disease, only one-fourth of the usual number of α-globin genes is present. Hybridization patterns of DNA restriction enzyme fragments showed that in HbQ-α2 74 Asp→His ββ-α-thalassemia one chromosome has both α-globin genes deleted and the other chromosome, which carries the α-mutant gene, has one α-globin gene deleted. Our results show that the HbQ-α 74 Asp→His structural gene is located adjacent to a deleted α-globin gene, whereas the α-globin gene adjacent to HbG-α 30 Glu→Gln gene is not deleted.

DELETION OF α-GLOBIN GENES has recently been shown1,2 to be the cause of homozygous α-thalassemia (Hb Bart’s hydrops fetalis). In HbH disease only one-fourth of the normal number of α-globin genes is present.4 This would indicate that HbH disease is the result of the inheritance of a severe type of α-thalassemia in which two α-globin genes are deleted and a mild type of α-thalassemia in which one of the two α-globin genes is deleted and that the α-globin gene is duplicated, as suggested by Lehmann and Carrell in 1968.5

That the α-globin gene is duplicated in certain populations has earlier been demonstrated from hemoglobin analysis in a Hungarian who carried α-chain mutants Hb Buda and Hb Pest, each at a level of 25% and HbA at a level of about 50%6 and in Malaysians, homozygous for the α-chain mutant Hb Constant Spring (HbCoSp), who carried HbCoSp and normal HbA simultaneously.7 Also, the finding in two Indians with Hb-Rampa-α and Hb-Koya Dora-α in addition to HbA8 indicates that the α-globin gene is duplicated. On the other hand, the finding...
that HbA and HbA2 are absent in Melanesians homozygous for the α-chain mutant HbJ-α Tongariki,9 in all Chinese patients with HbQ-α-thalassemia,10-14 and in blacks with HbG-α Philadelphia-α-thalassemia,15,16 raises the question whether the α-globin gene is duplicated in some individuals and not in others. Lorkin et al.14 suggested that the gene for HbQ-α is linked to an α-thalassemia gene and, in combination with severe α-thalassemia, leads to HbQ-α-thalassemia in which normal α-chains are therefore not synthesized. To see whether this is the case, we have quantitated the number of α-globin genes in two Chinese patients who have HbQ-α-thalassemia and in a Chinese with HbQ-α trait. We also studied a Chinese who was a carrier of Hbg-α Chinese, identical to Hbg Singapore or Hbg Honolulu.17

MATERIALS AND METHODS

Two Chinese patients earlier diagnosed as having HbQ-α-thalassemia (HbQ-H disease) and two Chinese earlier diagnosed as HbQ trait carriers were examined. One HbQ-α-thalassemia patient and one HbQ trait carrier, unrelated to the patient, were from Kuala Lumpur. The other HbQ carrier and the patient who had HbQ and severe α-thalassemia were from Hong Kong.

Methods

Hematologic examinations were done according to standard methods.

Hemoglobin structural studies. Hemoglobin components were purified by DEAE-cellulose column chromatography14 using TRIS-HCL buffer or by the inverted bottle method.19 Starch gel electrophoresis, agar gel electrophoresis, separation of globin chains on CM-cellulose column chromatography, tryptic digestion, fingerprinting, and isolation of abnormal peptides were performed by methods outlined in Lehmann and Huntsman.20 Thermolysin digestion and analysis of abnormal peptides isolated from fingerprints and amino acid sequencing were done as described by Lorkin et al.14

Preparation of DNA. DNA was prepared from peripheral white blood cells and from bone marrow cells (sent frozen from Kuala Lumpur and Hong Kong) as described.12 Various DNA controls were prepared from normal individuals, from patients with hydrops fetalis and hemoglobin-H disease, and from α-thal, and α-thal, trait carriers.

Preparation of globin mRNA and cDNA. Human globin mRNA was prepared from reticulocyte-rich blood, and 32P-labeled cDNA was prepared from mRNA with reverse transcriptase as previously described.1 For liquid hybridization, α-rich cDNA was prepared from gel-purified α-globin mRNA,22 which contains ±85% α-globin genes. For identification of globin genes in agarose gel, mixed α- and β-globin cDNA and cDNA prepared from total globin mRNA of a newborn containing α, β, and γ mRNA were used.

Hybridization Studies

For liquid hybridization, cellular DNA was sheared by limited depurination by boiling in 0.1 N sodium hydroxide for 20 min, followed by neutralization with hydrochloric acid, and precipitated in 70% ethanol. Hybridization was carried out in 10-μl volumes with 1000 cpmp (3pg) of α-globin cDNA and 50 μg cellular DNA incubated at 78°C for 58 hr as previously described.1 For α-thal carriers, unsheared cellular DNA was digested with the restriction endonuclease Eco RI or Hpa I and the digested DNA was electrophoresed in 0.8% agarose gel as previously described.23 The DNA was transferred to a nitrocellulose filter and hybridized with cDNA according to the method of Southern.24 The filter was further processed and autoradiographed.23

RESULTS

Clinical and hematologic findings of the two patients with HbQ-α-thalassemia and the two HbQ trait carriers were similar to those described earlier.11-14,25 The patients had enlarged spleens and microcytic anemia with typical HbH inclusion bodies in the erythrocytes of the peripheral blood. Their hemoglobin pattern
showed the presence of HbQ, Hb Bart's, HbH, and HbQ2. No HbA or HbA2 was detected in either patient. The HbQ trait carriers had no clinical symptoms and had normal hematologic indices. Their hemoglobin pattern showed the presence of HbA, HbQ, HbA2, and HbQ2. The HbQ level was around 27% of the total amount of hemoglobin. Globin chain synthesis study of the patient from Hong Kong showed that she had an α/β synthesis ratio of 0.39.

**Hemoglobin Structural Studies**

Analysis of the HbQ from the two patients with HbQ-α-thalassemia and the HbQ carrier from Hong Kong showed an abnormal peptide in the tryptic digest fingerprint, which replaced the normal tryptic peptide 9. Further studies of chymotryptic digest of the abnormal peptide 9 of the HbQ from the two patients with HbQ-H disease and sequencing of the chymotryptic peptide revealed that the mutation in the abnormal α-chain is at position 74, in which aspartic acid is replaced by histidine. It is therefore identical to HbQ Thai,14 Hb-Mahidol,26 and HbG Taichung.27 In the HbQ carrier from Hong Kong, no further studies of the abnormal peptide 9 could be carried out due to lack of blood.

Study of the HbQ from the carrier from Kuala Lumpur showed that the abnormal component, which moved like HbQ or HbG on electrophoresis at alkaline pH, moved slightly faster than HbQ (αβ 74 Asp→His β2) on agar gel electrophoresis at pH 6.2. A mixture of the purified abnormal component and purified HbQ-αβ 74 Asp→His β2 run on agar gel electrophoresis at pH 6.2 showed a separation of the two abnormal components. Study of the tryptic digest fingerprint of the α-chain of the abnormal hemoglobin from this carrier at pH 6.4 showed that the normal peptide 4, usually seen in the anodic region, was missing and was replaced by an abnormal peptide in the neutral region. Further study of the chymotryptic digest of this abnormal peptide 4 by fingerprinting and amino acid sequencing showed that in this peptide of the α-chain, glutamic acid was replaced by glutamine at position 30. It is therefore identical with HbG Chinese, HbG Singapore, HbG Honolulu, and HbG Hong Kong.16,17,28

Liquid hybridization showed that the percentage annealing in the two patients with HbQ-α-thalassemia is about one-fourth of the value obtained for normal adults, indicating that only one α-globin gene is present and three are absent (Fig. 1). Similar findings were obtained for our control HbH disease patients as have also been obtained previously.4 Result of liquid hybridization in the HbQ trait carrier indicates that one α-globin gene is deleted. Unfortunately, liquid hybridization data are not available for the HbG trait carrier. However, the diagnosis of deletion of one-fourth of the total α-globin genes by liquid hybridization is generally not very discriminative and cannot be relied upon.

**Hybridization Patterns of DNA Restriction Enzyme Fragments**

Eco RI endonuclease recognizes the nucleic acid base arrangement GAATTC and does not cut the coding sequences of the α-globin genes.29–34 Both α-globin genes, located between two restriction points, are left intact on the same fragment of DNA. When nonthalassemic human cellular DNA was digested with Eco RI and hybridized with α-globin and β-globin cDNA, five major fragments were seen. When hybridized with globin cDNA prepared from total globin mRNA from a
newborn, at least seven fragments are observed (Fig. 2). It has been shown that the slowest fragment, about 23.0 kilobases (kb) in length, contained both α-globin structural loci. In homozygous α-thalassemia (hydrops fetalis), where all α-globin genes are deleted, this α-gene fragment is absent (Figs. 2 and 3). In the α-thal trait carrier, both α-globin genes are deleted on one chromosome, but on the other chromosome, both α-globin genes are present. The decrease to half the normal amount of α-globin genes because of deletion of both α-globin genes on one chromosome is not clearly reflected in a decrease of intensity of the 23.0-kb fragment. This method for diagnosis of the α-thal trait carrier is therefore not reliable.
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Fig. 3. Autoradiogram of Eco RI restriction endonuclease digestion patterns of human DNA. Total globin cDNA is used as probe. Lane 1: normal; 2: homozygous α-thalassemia hydrops fetalis; 3: hereditary persistence of fetal hemoglobin (HPFH); 4: HbH disease; 5: α-thal-; 6: HbQ-H disease; 7: HbQ-α 74 Asp→His carrier; 8: HbG-α 30 Glu→Gln carrier.

In the mild α-thal-2 carrier, one chromosome has both α-globin genes intact, but the DNA fragment carrying α-globin genes on the other chromosome is shortened because of a deletion, which includes one of the two α-globin genes. The shortened DNA fragment, which contains the single α-globin gene, migrates faster in agarose gel electrophoresis; therefore, in the α-thal-2 trait carrier, two DNA fragments are observed carrying α-globin genes, one in the usual place at about 23.0 kb in length, the other slightly more anodic at about 19 kb in length.

In our two patients with HbQ-α-thalassemia, the usual fragment containing two α-globin genes is absent. However, a slightly faster moving fragment at 19 kb length, similar to the one in α-thal-1 trait carriers, is present (Fig. 2). The HbQ-α 74 Asp→His trait carrier from Hong Kong shows two fragments in this region: the usual α-globin gene fragment at 23.0 kb in length, seen in normal individuals, and the slightly faster moving gene fragment at 19 kb in length, seen in α-thal-1 trait carriers (Fig. 3). We therefore conclude that in our HbQ-α-thalassemia patients, one chromosome has both α-globin genes deleted and the other chromosome carries a single α-globin gene. In HbQ-α 74 Asp→His trait, one chromosome has both α-globin genes intact and the other chromosome has only one α-globin gene. In the carrier of Hbg-α2 30 Glu→Gln from Kuala Lumpur, only one α-globin gene-carrying fragment is seen at 23.0 kb in length; the DNA α-globin restriction enzyme pattern looks normal. There is no shortening of the α-globin gene-carrying DNA fragment. Apparently, on both chromosomes, the two α-globin genes are present.

Hpa I endonuclease, which recognizes the nucleic acid base GTTAAC arrangement,36 does not cut the coding sequences of α-globin genes32 but cuts DNA at a point between the two α-globin genes. As a result, there are two α-globin gene fragments, one at 14.5 kb in length and the other at 4.2 kb in length. In normal individuals, both fragments appear in the pattern when hybridized with an α-cDNA probe. In homozygous α-thalassemia hydrops fetalis both fragments are absent. In α-thal-1 or α-thal-2 trait carriers, the absence of one or both α-globin genes in one chromosome is masked by the presence of both α-globin genes on the other
chromosome and therefore not clearly detectable by this method. In HbH disease, however, the absence of both α-globin genes in one chromosome and the absence of one α-globin gene in the other chromosome shows the faster migrating DNA fragment at about 4.2 kb in length to be absent (Fig. 4). A similar pattern was seen in our cases of HbQ-α-thalassemia. This shows again that in this condition, one chromosome has both α-globin genes deleted, and the other has one α-globin gene deleted. In the carrier of Hb-α2 30 Glu→Gln from Kuala Lumpur, both α-globin gene-carrying fragments are present.

**DISCUSSION**

Our structural studies reveal that the HbQ in the two patients from Kuala Lumpur and Hong Kong is Hb-α2 74 aspartic acid→histidine β2, identical to HbQ Thai'4 and Hb-Mahidol26 found in Thais and HbG Taichung found in Chinese from Taiwan.27 Although the HbQ in the trait carrier from Hong Kong has not been fully identified, it is most probable that it is Hb-α2 74 Asp→His β2 because aspartic acid at position 74 or 75 is replaced by histidine in the α-chain tryptic peptide 9 and is from a Chinese (Hb-α2 75 Asp→His β2 is found in Iran14). The HbQ (or G) of the carrier from Kuala Lumpur is structurally different; it has a mutation in α-chain where at position no. 30 glutamic acid is replaced by glutamine.

The result of the liquid hybridization study clearly shows that both patients with HbQ-α2 74 Asp→His β2 mutant and α-thalassemia have only one-fourth the total α-globin gene material normally present, indicating they have inherited the severe type of α-thalassemia with both α-globin genes deleted from one parent and that the gene for HbQ-α inherited from the other parent neighbors a deleted α-globin gene. This condition resembles HbH disease except that in the case of HbQ-α-thalassemia, the single nondeleted α-globin gene carries a mutation. However, the diagnosis of deletion of one-fourth of the total α-genes by the liquid hybridization study is not very discriminative and cannot be relied upon.

Our conclusion is further supported by the findings obtained from the restriction enzyme analysis by hybridization of DNA fragments in agarose gel after electro-
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...phoresis and transfer to nitrocellulose filter. The results with the Eco RI (Figs. 2 and 3) as well as with the Hpa I (Fig. 4) endonuclease restriction enzyme analysis agree with the conclusion that the gene for HbQ-α 74 Asp→His neighbors a deleted α-globin gene and that three-fourths of the α-globin gene material is deleted in patients with HbQ-α thalassemia. One may argue that the shortened DNA fragment at ± 19-kb length obtained with Eco RI digestion may be caused by a deletion of a nonglobin gene somewhere on the same DNA fragment not involving the α-globin gene. However, the absence of an α-globin gene on the 4.2-kb length fragment obtained with Hpa I digestion clearly shows that the α-globin gene is deleted on that DNA fragment. Unfortunately, no data on liquid hybridization in the HbG trait carrier are available. However, the diagnosis of one-fourth gene deletion by the liquid hybridization is not very reliable. The restriction enzyme analysis, on the other hand, clearly shows that the gene for HbG-α 30 Glu→Gln does not neighbor a deleted α-globin gene since the DNA fragment carrying the α-globin genes obtained from Eco RI digestion is not shortened. If the α-globin gene adjacent to the gene for the HbG were deleted, one would expect the presence of a slightly faster Eco RI DNA fragment at 19-kb length due to shortening of that fragment. This was not seen in the case of the HbG trait carrier (Fig. 2, lane 8).

The proposed genotypes in the different cases are shown in Table 1.

One may also argue that the one α-globin gene per chromosome in these Chinese is a normal condition in which the α-globin gene is not duplicated, rather than the result of a deletion of one of two α-globin genes normally present. However, an α-thal, trait carrier with both α-globin genes deleted on one chromosome is not clinically ill and shows only mild hematologic abnormalities, whereas a person with HbH-α-thalassemia or HbQ-α-thalassemia is definitely clinically ill with anemia and splenomegaly and shows marked hematologic abnormalities. If one nonduplicated α-globin gene per chromosome was a normal condition, one would not expect the absence of one α-globin gene to contribute significantly to aggravate the disease condition in HbH disease, in which on one chromosome the α-globin genes are absent but on the other chromosome the nonduplicated α-gene is supposed to be normal.

Findings on other structural α-chain mutants have been thought to indicate that there is only one single α-globin gene per haploid genome. On the island of Karkar in Melanesia, homozygotes for HbJ-α Tongariki do not have HbA, and heterozy-

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<td>HbQ-α^{74}Glu–Gln–α-thalassemia</td>
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<td>HbG-α^{30Glu}</td>
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αα, Normal duplicated α-globin genes.
−−, One α-globin gene deleted.
−−/−−, Both α-globin genes deleted.
−−/−−, αα^{74}Glu–Gln–α-thalassemia gene adjacent to a deleted α-globin gene.
αα^{30Glu} = Gα^{30Glu} globin gene adjacent to a normal α-globin gene.
gotes have about 50% HbJ-α Tongariki, which led to the belief that in these Melanesians the α-globin gene is not duplicated. However, Old et al. found through hybridization studies of homozygous HbJ-α Tongariki mRNA to purified cDNAα and cDNAβ that there was a marked deficiency of αmRNA compared to βmRNA. This marked deficiency of αmRNA was also found in HbJ-α Tongariki trait carriers and in Karkar islanders with only HbA. They concluded that perhaps many individuals in this population are homozygous for an α-thal gene and thus have only one functional α-gene on each chromosome and that HbJ-α Tongariki genotype arose by a mutation at this single α-chain locus. If their assumption is correct, hybridization of α-globin gene carrying Eco RI DNA restriction enzyme fragments after electrophoresis on agar gel would show an α-globin gene carrying a DNA fragment shorter than the usual fragment at 23.0 kb in length seen in conditions in which the α-globin gene is duplicated.

Based on the variable expression of the genes for HbG-α Philadelphia in blacks and HbJ-α Mexico in Algerians, it was thought that both the one and the two α-globin structural genes per chromosome exist in these populations. In HbG-α Philadelphia-α-thalassemia no HbA and HbA2 are detected, which indicates that in this disease, the α-globin genes on one chromosome are deleted and on the other chromosome only one α-globin gene, which carries a mutation, is present. The finding of a trimodal distribution of the level of HbG-α Philadelphia and HbJ-α Mexico led to the conclusion that both the one and the two α-globin genes per haploid genome occur in these populations. However, Tolstoshev et al. showed that the variable level of HbJ-α Mexico trait carriers is not due to the presence of different numbers of α-globin genes. They found two α-globin genes per haploid genome in normals as well as in HbJ-α Mexico trait carriers with varying levels of the abnormal hemoglobin. Apparently, varying expression of the structural mutants cannot be taken to indicate the presence of varying numbers of α-globin genes.

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The alpha-globin gene adjacent to the gene for HbQ-alpha 74 Asp replaced by His is deleted, but not that adjacent to the gene for HbG- alpha 30 Glu replaced by Gln; three-fourths of the alpha-globin genes are deleted in HbQ-alpha-thalassemia

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