CONCISE REPORT

Molecular Basis of Hemoglobin-H Disease in the Mediterranean Population

By Y. W. Kan, A. M. Dozy, G. Stamatoyannopoulos, M. G. Hadjiminas, Z. Zachariades, M. Furbetta, and A. Cao

We investigated the molecular basis of hemoglobin-H disease by hybridization and restriction endonuclease mapping of the DNA in the Mediterranean populations. Of the 12 patients studied from Cyprus and Sardinia, 8 had the typical deletion defect with a single remaining α-globin gene. The nondeletion type of α-thalassemia was found in 3, and a "dysfunctional" gene in one. We conclude that the predominant cause of α-thalassemia in these populations is gene deletion.

The human α-globin gene loci are duplicated1-4 and the two loci are tightly linked5,6 on chromosome 16.7 In a diploid cell, the normal genotype for α-globin can be represented as αα/αα. Recent studies have demonstrated that in both the Asian and the black populations, gene deletion is the common cause of α-thalassemia.8-11 The clinical states of α-thalassemia are in a large part explained by the two genotypes observed: the α-thalassemia-2 gene, due to deletion of a single locus (−α); and the α-thalassemia-1 gene with deletion of both loci (−−). In Asians, both genotypes are common, and different combinations produce the four α-thalassemia syndromes of increasing severity: (1) The silent carrier state (−α/αα); (2) α-thalassemia trait (−−/αα) or (−α/−α); (3) hemoglobin-H disease (−−/−α); and (4) homozygous α-thalassemia-1 (−−/−−). In blacks, the α-thalassemia-2 genotype predominates, while the α-thalassemia-1 genotype is very rare. Hence, in this population, α-thalassemia trait is commonly due to homozygosity of the α-thalassemia-2 gene (−−/−−), hemoglobin-H disease is rare, and the lethal homozygous α-thalassemia has never been described.

In the Mediterranean population, α-thalassemia is not uncommon. Hemoglobin screening programs have shown the incidence of α-thalassemia trait to be 6% in Sardinia and 12% in Cyprus.12-13 Hemoglobin-H disease is also present, but homozygous α-thalassemia is rare, with only one proven case reported14 and two deduced by history15 in the Cypriot population. Recently, a different type of lesion causing α-thalassemia, where both α-globin genes are present, was defined as a cause of hemoglobin-H disease in both the Asian16 and the Mediterranean populations.17 Phenotypically, this nondeletion defect resembles α-thalassemia-1, but both α-globin gene loci are present and the suppression of α-globin synthesis is...
incomplete. Thus, when inherited with the α-thalassemia-1 gene, the nondeletion defect results in hemoglobin-H disease rather than the lethal hydrops fetalis. A high incidence of the nondeletion α-thalassemia lesion in the Mediterranean populations would explain the occurrence of hemoglobin-H disease with virtually no hydrops fetalis. To investigate this, we studied the molecular basis of hemoglobin-H disease in Cyprus and Sardinia using molecular hybridization and gene mapping.

**MATERIALS AND METHODS**

Twelve patients were studied, six each from Cyprus and Sardinia. The diagnosis of hemoglobin-H was established by hemoglobin electrophoresis at both pH 8.6 and pH 7.0, and by brilliant cresyl blue staining of the peripheral blood. The percent of hemoglobin-H varied from 5% to 20%. None of the patients had hemoglobin Constant Spring.

DNA was prepared from the peripheral blood leukocytes by SDS proteinase K digestion and phenol extraction, as previously described. DNA from normal Asian patients with hydrops fetalis, hemoglobin-H disease, α-thalassemia-1, and normal was used as controls. In addition, DNA was prepared from a Chinese patient with the nondeletion type of hemoglobin-H disease and another with hemoglobin-H Constant Spring.

**Hybridization in Solution**

cDNA was reverse-transcribed from globin mRNA enriched in α-globin sequences and 32P-dCTP (specific activity 350 Ci/m mole, Amersham Corp., Piscataway, N.J.) was used as the radioactive precursor. Seventy-five micrograms of sheared cellular DNA was annealed to the 1000 cpm α-globin cDNA under conditions previously described. The percent of the α-globin cDNA annealed was assayed with hydroxylapatite.

**Gene Mapping**

Ten micrograms of cellular DNA was digested with the enzyme Eco RI. The digested DNA was electrophoresed in 0.8% agarose gel, transferred to nitrocellulose filters, and hybridized with nick-translated 32P-labeled α-globin cDNA as previously described.

**RESULTS**

The percentages of α-globin cDNA annealed by the DNA from 12 Mediterranean patients with hemoglobin-H disease varied over a wide range (Fig. 1). In nine patients, the values were within the range obtained for Asian patients with hemoglobin-H disease with a single gene. In three of the Mediterranean patients, the percentages of α-globin cDNA annealed were higher than those of the Asian patients with hemoglobin-H disease and the other nine Mediterranean patients. The values were similar to results from Asian patients with α-thalassemia-1, hemoglobin-H Constant Spring, and the nondeletion type of hemoglobin-H disease, where two α-globin genes were present.

Restriction endonuclease mapping was used to study the arrangement of the α-globin genes in the 12 Mediterranean patients (Fig. 2). In normal DNA digested with Eco RI, the duplicated α-globin loci resided in a DNA fragment 23.0 kb in length. The three Mediterranean patients with two α-globin genes showed the normal 23.0 kb fragment as in DNA from the Asian nondeletion hemoglobin-H disease. Hpa I digestion of the DNA from these three patients confirmed that both α-globin loci were intact (data not shown).

Restriction maps of the nine patients in the group with the lower hybridization levels showed that they all had the shortened 19.0 kb Eco RI band characteristic of
DISCUSSION

In this study, we investigated the molecular mechanism of hemoglobin-H disease in the Mediterranean area to assess the frequency of the nondeletion type of α-thalassemia in Cyprus and Sardinia, where α-thalassemia is common but homozygous α-thalassemia is extremely rare. Hemoglobin-H is a useful model for

---

**Fig. 1.** Percent α-globin cDNA annealed by DNA from Asian and Mediterranean subjects. CS and ND are DNA from hemoglobin-H Constant Spring and the nondeletion type of hemoglobin-H, respectively. In the Mediterranean group, [□] denotes Cypriots and [○] Sardinians.

---

**Fig. 2.** Eco RI restriction endonuclease map of DNA. Lane 1: homozygous α-thalassemia; 2: normal; 3: Asian nondeletion hemoglobin-H disease; 4: Asian hemoglobin-H disease, deletion type; 5: Mediterranean nondeletion hemoglobin-H disease (2 α-globin genes by hybridization) 6,7: Mediterranean hemoglobin-H disease, deletion type. The 23-kb fragment contains two loci (αα) and the 19-kb the single locus (−α). In one patient, an additional 2.6-kb fragment band was seen (lane 7). The arrow indicates the size of these fragments in kilobases. X is an α-like fragment that cross-hybridized with the α-globin probe and is present in all samples including homozygous α-thalassemia.
this type of analysis because the finding of more than a single α-globin gene in hemoglobin-H disease indicates the presence of a nondeletion lesion. Also, in contrast to the difficulties encountered in the clinical diagnosis of α-thalassemia trait, the diagnosis of hemoglobin-H disease can be established unequivocally by demonstrating hemoglobin-H in the erythrocyte.

The genotypes of these Mediterranean patients with hemoglobin-H disease can best be illustrated by the presence of four types of thalassemia genes: α-thalassemia-1 (−−), α-thalassemia-2 (−α), nondeletion α-thalassemia (ααbh), and the 2.6-kb locus (−α2b). Hybridization analysis showed that both α-globin loci were present in the nondeletion type, and gene mapping showed the organization to be apparently indistinguishable from Asian patients with the same lesion.6 The nature of the dysfunctional α-globin gene in the 2.6-kb Eco RI band has not been defined. In the Cypriot patient who had this, as well as the 19.0-kb Eco RI band, quantitative hybridization in solution indicated the presence of a single locus rather than two. This finding differs from that of Orkin and coworkers who detected two loci in their patient with the 19.0-kb and 2.6-kb Eco RI band. Further restriction mapping of the 2.6-kb locus is needed to determine if this locus contains the complete α-globin gene.

We can designate the genotypes seen in these patients as follows: −−/ααbh in three patients, −−/−α in eight, and −α/−α2b in one. Of the 24 chromosomes that bear α-globin genes in these 12 patients, the α-thalassemia-1 gene (−−) was present in 11, α-thalassemia-2 (−α) in 9, nondeletion (ααbh) in 3, and dysfunctional (−α2b) in 1. Thus, the deletion genotype predominates. While the nondeletion α-thalassemia lesion, found in 3 of 24 chromosomes, appears to be more common than in the Asian population, it does not seem frequent enough to account for the lack of homozygous α-thalassemia in these Mediterranean islands. Additional studies of large populations with α-thalassemia to determine the frequency of the α-thalassemia-1 and α-thalassemia-2 genes, and investigation of the role of the ζ-globin gene are necessary to define the molecular basis of α-thalassemia in this population.

ACKNOWLEDGMENT

We thank Dr. B. Forget for the plasmid JW 101, the Office of Program Resources and Logistics (Viral Cancer Program, Viral Oncology, Division of Cancer Cause and Prevention, National Cancer Institute, Bethesda, Md.) for the reverse transcriptase, and J. Gampell for editorial assistance.

REFERENCES


Molecular basis of hemoglobin-H disease in the Mediterranean population

YW Kan, AM Dozy, G Stamatoyannopoulos, MG Hadjiminas, Z Zachariades, M Furbetta and A Cao