Hemoglobin Köln: Direct Analysis of the Gene Mutation by Synthetic DNA Probes

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The molecular defect leading to Hb Köln has been analyzed by synthetic oligonucleotides. Thus, DNA of 19 nucleotides, in length corresponding to the normal and mutant β-globin gene sequences, were used to develop a direct assay for the β*-gene that codes for this most common form of the unstable hemoglobins. The use of synthetic oligonucleotides established that the Hb Köln mutation is due to a G-to-A transition. The conditions described here should result in the determination of all Hb Köln genotypes with a high level of confidence.

EMOLOGOBIN (Hb) Köln disease belongs to the group of nonphosphorhetic hemolytic anemias with a dominant inheritance pattern in which anemia is attributable to instability of the Hb molecule. The molecular pathology of this most common of the unstable Hbs was found to be the result of replacement of valine with methionine at β 98 (FG 5), causing increased oxygen affinity and instability, possibly because of hemedepletion.1,2 The clinical features of patients with Hb Köln are those associated with hemolytic anemia. They may vary individually from mild to severe, and transfusion therapy might have to be started in childhood.

The underlying molecular defect in the chromosomal DNA leading to the amino acid substitution in Hb Köln has not directly been analyzed thus far, but it can be explained by a G-to-A transition caused by a point mutation. A direct detection of the mutation by virtue of the specificity of restriction enzymes was not possible because of the lack of suitable endonucleases. However, identification of the chromosomes that have passed the mutant gene on for three generations was possible by analyzing linked polymorphisms for DNA restriction sites within the -globin gene cluster.3 Although the results indicated the possible use of this method for prenatal diagnosis from DNA to amniotic fluid cells or chorion biopsy material, a diagnosis could only be expected in those cases where the haplotype constellations were informative. In all other cases prenatal diagnosis of the Hb Köln mutation has thus far been precluded because current Hb analysis is not available for identification of the β-anomaly from fetal blood with absolute certainty.

Here we report the detection of the Hb Köln hemoglobinopathy by direct analysis of the G-to-A transition mutation on the chromosomal DNA via synthetic oligonucleotide probes, thus establishing a method available for prenatal diagnosis of the disease.

MATERIAL AND METHODS

Oligonucleotides were labeled with adenosine 5'-γ-32P-triphosphates (γ-32P-ATP) (NEN, Boston; >7000 Ci/mmol) by a kinase reaction using T4 polynucleotide kinase (Boehringer, Mannheim). Labeled oligonucleotides were separated from unreacted γ-32P-ATP by chromatography on Whatman (Maidstone, Kent, England) DE 52 cellulose.5

DNA samples of peripheral blood from normal and affected individuals were prepared as described.6 Ten to 12 μg DNA were digested overnight with EcoRI (Bethesda Research Laboratories, Bethesda, Md) and additionally with HpaI (Boehringer, Mannheim, Germany) in the buffer recommended by the commercial suppliers at 37°C. The samples of the digested DNA were electrophoresed in 1% agarose. The gels were incubated overnight at room temperature in methanol (Epplen, personal communication) and dried for direct hybridization as described by Pirastu et al.7

After three and a half hours' hybridization the gels were washed in 6× SSC (1) four times for 30 minutes at room temperature, (2) overnight at 37°C, and (3) finally for a short time at hybridization temperature (β*-probes: Hβ19A, 55°C and at a 10°C higher temperature respectively (β*-probe: Hβ19k, 63°C as specified in figure legends and exposed to x-ray film (Kodak, XAR, Rochester, NY) with intensifying screens at ~80°C.

RESULTS

Mutational sequence and synthetic probes. The basis of the Hb Köln disease is the substitution of the valine residue at position 98 of the normal β*-peptide chain by a methionine residue in the β*-chain.1 Sequencing analysis of the human β*-globin gene has demonstrated that the codon for the valine residue at position 98 of the β*-chain is GTG.8 Since there is only one possible codon for methionine (ATG), it is very likely that the Hb Köln hemoglobinopathy is caused by a point mutation involving a G-to-A transition at the corresponding position in the gene (Fig 1A).

Recent investigations have shown that synthetic oligonucleotides exhibit an extremely specific hybridization behavior15,16 and can therefore be used to detect single base-pair changes in genomic DNA.15-18 The specific oligonucleotides complementary to the β*- and β*-genotypes of the β-globin gene (Fig 1B) were taken to establish a molecular method for distinction between the normal and the mutated genes.

Restriction fragments and genotype identification. Since it has been shown that restriction fragments one to three kilobases (kb) in size are optimal for the detection of single point mutations, combined digestions of the endonucleases EcoRI and HpaI were selected from the several possibilities for the generation of 2.2-kb restriction frag-
gene (data not shown) as well as to chromosomal DNA of normal and Hb Köln-affected individuals (Fig 2A and B) of a German family.  

With the \(\beta^4\) synthetic oligonucleotide probe, neither the lanes containing the serially diluted cloned \(\beta^A\)-type/\(\beta\)-globin gene sequence (data not shown) nor the DNA from \(\beta^A\beta^4\) genotype individuals showed hybridization signals (Fig 2B, lanes 4 to 6). Only DNA from the individuals with Hb Köln yielded hybridization signals in the 2.2-kb position. Thus, the synthetic \(\beta^4\)-oligonucleotide probe is capable of distinguishing \(\beta^A\beta^A\) homozygotes from \(\beta^A\beta^4\) heterozygotes.

**DISCUSSION**

We have adopted the recently described oligonucleotide mapping procedure for the analysis of single point mutations to detect the Hb Köln mutations in chromosomal DNA of affected individuals. By means of synthetic \(\beta^A\)- and \(\beta^4\)-oligonucleotide probes it was possible to follow the segregation of the \(\beta^4\)-gene mutation through three generations of a family with Hb Köln-affected members.

The oligonucleotide analysis procedure has successfully been applied for diagnosis of some disorders like sickle cell anemia, \(\beta\)-thalassemias, and \(\alpha\)-antitrypsin deficiency.  

Here genotype analysis relies on the detection of normal homozygotes, heterozygotes, and defective homozygotes exhibiting the respective three sets of intense (+ + + ), intermediate (+ - ), and missing (- - ) band signals upon hybridization with oligonucleotides complementary to the normal (N) or the mutated (M) gene sequence: N + + + - - - /M - - - + + + .
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In diseases with an autosomal dominant inheritance pattern, as in the Hb Köln disorder, normal homozygotes are differentiated from Hb Köln-affected ones by the first two sets of signals. Under controlled washing and hybridization conditions it can be seen that the probe specific for the mutated gene sequence yielded significant hybridization signals only with chromosomal DNAs from Hb Köln genotypes. The use of the synthetic oligonucleotide probes described here together with the enzymatic digests should therefore result in the determination of all Hb Köln genotypes with a high level of confidence. With regard to genetic counseling, this method could be used together with chorion biopsy or amniocentesis to provide prenatal diagnosis in families at risk.

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