Prenatal Diagnosis of Thalassemias and Hemoglobinopathies

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ABSTRACT

Thalassemia syndromes and hemoglobinopathies are of clinical genetic significance because of the severity of the sequelae associated with particular genetic constitutions in these conditions, their occurrence at high frequencies in certain populations of Mediterranean, African, and Asian origin, and the high frequency of recurrence in pregnancies of at risk families. Application of recently developed techniques of molecular genetics to the antenatal diagnosis of the most deleterious of these conditions (homozygous \(\beta\)-thalassemia [Cooley's anemia], homozygous \(\alpha\)-thalassemia [Barts hydrops fetalis], sickle cell anemia, and related severe hemoglobinopathies) now affords parents the option to interrupt a pregnancy in which the fetus has the genetic constitution causing one of these abnormalities.

The two different analytical strategies utilize fetal cells obtained by amnioncentesis. In one, fetal blood is obtained either by sonographically guided placental aspiration or by aspiration from a placental vein directed by fetoscopy. Globin chain synthesis is monitored by the incorporation of radiolabelled amino acids in the isolated erythrocytes and the determination of the ratio of radioactive label incorporated into the various globin chains separated by column chromatography or electrophoresis. This technique is applicable to the antenatal diagnosis of \(\alpha\)-and \(\beta\)-thalassemia and to selected hemoglobinopathies. However, in the most experienced centers, fetal blood sampling is associated with a greatly increased risk of fetal loss. The other analytical approach utilizes deoxyribonucleic acid (DNA) isolated from fibroblasts to evaluate the presence of quantitatively and/or qualitatively normal DNA sequences, which constitute the structural gene(s) encoding specific globin polypeptide chains. This approach is most generally applicable to the detection of structural gene deletions as in \(\alpha\)-thalassemia; maternal and fetal risk is the same as that for conventional amniocentesis.

Introduction

The inherited anemias of man can be subdivided into three major groups on the basis of the underlying molecular lesion: (1) those affecting the synthesis and/or qualitative properties of the globin chains; (2) those affecting heme metabolism; and (3) those resulting from alterations of enzymes necessary for the maintenance of required erythrocyte met-
abolic functions, e.g., glucose-6-phosphate dehydrogenase, NADH dehydrogenase. Included in the first category are the thalassemia syndromes, defined by the decreased or absent production of specific globin chains, and the hemoglobinopathies, characterized by the occurrence of qualitatively abnormal globin chains. The thalassemias differ from the hemoglobinopathies in that changes in the amino acid sequence of the globin chains do not appear to occur. In thalassemia, the α or β globin is indistinguishable from normal but is synthesized in reduced amounts.¹¹ These two entities are of clinical genetic significance because of the severity of the sequelae associated with specific mutant forms of these diseases, e.g., β-thalassemia in Caucasians; sickle cell anemia, because of the high frequency of occurrence in certain populations of Mediterranean, African, and Asian origin; and the high probability of recurrence in progeny of at risk couples. Recent dramatic technical innovations have made possible the prenatal diagnosis of homozygous α-thalassemia, β-thalassemia, and the more deleterious hemoglobinopathies. The two distinctly different methodological approaches to such diagnoses — the analysis of globin chain synthesis in fetal blood samples and the determination of globin gene DNA sequences in fetal fibroblasts — are the subject of this paper.

Frequencies of Occurrence, Molecular Bases and Prognoses

Hemoglobinopathies

In the American Black population, the frequencies of the major hemoglobinopathies, Hb SS, Hb SC, and Hb S/β-thalassemia, are, respectively, 1.6/1000, 1.2/1000, and 6/10,000.³⁴ In certain parts of Africa the carrier frequency of the Hb S may reach 45 percent.³¹ Sickle cell anemia (Hb SS disease) and most of the other hemoglobinopathies result from single amino acid substitutions in one specific globin chain, e.g., glu → val at residue 6 of the β chain in Hb S.⁴⁶ This occurs as a consequence of a point mutation (single nucleotide substitution) in the structural gene encoding the globin chain. In rare instances, more profound changes involving a number of contiguous amino acid residues may result from other types of mutational changes. Examples of these changes include the frame shift mutation that alters residues beyond α 139 in Hb Wayne,¹ the small multisite mutation which results in the deletion of four amino acids, β 91–95, in Hb Gun Hill¹⁰ and the translocation which appears to fuse portions of the α- and β-globin chains in Hb Lepore.³

These changes in amino acid composition can alter the qualitative properties of the hemoglobin tetramer in such physiologically significant properties as oxygen affinity, stability, and precipitability. However, many of these substitutions affect the properties of the globin chains in only minor ways and have no significant pathophysiological effect upon even the individual who is homozygous for the alteration. In contrast, sickle cell anemia results from the unique attribute of this mutant hemoglobin, the ability to form large tubular fibers which distort the morphology of the erythrocyte.¹⁶ Other sickling allelic combinations include Hb SO⁴ A R A B which is more deleterious than Hb SS disease, Hb SC which is relatively benign, and Hb S/β-thalassemia which is less deleterious than either condition in the homozygous state.

Large gaps exist in our ability to relate the basic molecular and cellular changes in sickle cell anemia to clinical considerations of the origin of sickle cell crises and organ damage. The chronic hemolytic anemia may be accompanied by elevated incidence of infection, retardation of growth and sexual development, organ damage including cerebral vascular accidents, splenic infarction, and bone and
skin abnormalities. Aplastic crises, hemolytic crises, and vaso-occlusive infarctive crises may occur repeatedly. However, there is a great degree of variability in the expression of these complications in individual patients, some being severely affected while others have few complications. This finding raises the question of the desirability of antenatal for this disease.

**Beta Thalassemia**

Beta-thalassemia has a high incidence in Mediterranean populations and as much as 10 to 20 percent of some Greek and Italian populations are heterozygous for this condition. Beta-thalassemia homozygotes (Cooley's anemia), who occur at a frequency of 1/2500 live births in these populations, are well at birth but develop progressively worsening anemia as fetal hemoglobin synthesis falls. Stunting of growth, delayed sexual development, severe skeletal deformities affecting the skull and long bones and greatly increased incidence of infection are typical. Those patients who survive their early years develop hemochromatosis, and death usually results from cardiac damage in the second or third decade. Homozygous beta-thalassemia in Blacks is characterized by chronic anemia but few severe complications. The reason for this difference in phenotypic expression is not well understood.

In homozygotes, two forms of beta-thalassemia are phenotypically demonstrable, beta+ thalassemia in which a relative deficiency of beta globin chains occurs, and beta- thalassemia in which no detectable beta-globin chains are synthesized. In nonthalassemic individuals, the alpha to beta chain synthetic ratio approximates 1.0. In beta heterozygotes, the ratio is about 2.0 as expected for reduced or absent function of one beta globin allele. In beta-thalassemia, the alpha to beta chain ratios vary between 5 and 25. These reductions in beta-globin synthesis do not result from deletion of the beta-globin structural gene; it is grossly intact. Hybridization experiments utilizing DNA isolated from patients' cells and DNA produced from beta-globin messenger ribonucleic acid (RNA) by reverse transcriptase (cDNA) establish the presence of beta-globin gene sequences in beta+ and beta- thalassemic individuals. However, beta-globin messenger RNA is decreased in beta+-thalassemia and absent or abnormal in beta-thalassemia. The fundamental defect, then, appears to lie in nucleotide sequences within the beta-globin structural gene, which are necessary for the normal rate of transcription and/or processing of messenger RNA from this gene. Those beta-chains which are synthesized are indistinguishable from those produced by normal individuals.

**Alpha Thalassemia**

The alpha-thalassemias are found in many of the world's populations but are especially frequent in certain Asian and Middle-Eastern populations, e.g., 20 percent of Thai neonates carried an alpha-thalassemia gene. Both biochemical and genetic data indicate that most normal individuals have four copies of the alpha-globin gene. The most severe form of alpha-thalassemia, hydrops fetalis, is characterized by no synthesis of alpha-globin chains and the accumulation of two unusual hemoglobins, Hb Barts (consisting of four alpha-chains) and Hb H (consisting of four beta-chains). This disease, which is incompatible with life, results from the deletion of all four alpha-globin structural genes. The other clinically significant alpha-thalassemia syndrome is Hb H disease which is associated with mild to moderate hemolytic anemia, reticulocytosis, and splenomegaly. Severity is variable, but in general, Blacks have lower Hb H and less severe anemia than do orientals. The alpha to beta synthetic ratio is about 0.3 to 0.6, and these individuals are believed to possess
only a single gene for globin synthesis.\textsuperscript{5} The loss of two of the $\alpha$ loci results in the $\alpha$-thalassemia trait, and loss of one locus in the silent carrier state. Each of these is characterized clinically by mild hypochromic microcytic anemia or solely by microcytosis.\textsuperscript{43}

Rationale for Antenatal Diagnosis

Several criteria must be considered in deciding upon the validity of the need for prenatal diagnosis of an hereditary disorder. Among the considerations are: (1) the clinical consequences to the affected individual should be of such significance as to warrant potential interruption of the pregnancy; (2) adequate clinical treatment for affected individuals is unavailable; (3) the risk factor should be significant and clearly established; and (4) an unambiguous assessment of the genotype of the fetus can be determined. Certain of the hemoglobinopathies and thalassemias are suitable for such diagnoses because of: (1) their high rate of occurrence in geographic subgroups; (2) the high risk of occurrence and recurrence in pregnancies where both members of a couple are heterozygotes (25 percent); (3) the ease with which the carrier state can be established in each member of a couple; and (4) the severity of the disease process associated with some of these syndromes in the absence of an effective means of clinical therapy. Only those conditions associated with potentially severe sequelae, such as Hb SS disease, homozygous $\beta$-thalassemia in Caucasians, and homozygous $\alpha$-thalassemia, are entities for which antenatal diagnosis is appropriate. Despite the early lethality of hydrops fetalis, prenatal diagnosis is useful in this condition because a significant number of women carrying fetuses with this abnormality develop severe toxemia.\textsuperscript{42} The clinical consequences of all of the heterozygous states of mutant/normal alleles at either of the $\alpha$ or $\beta$ globin loci are insufficient to justify antenatal diagnosis. Certain heteroallelic combinations, such as Hb E/ $\beta$-thalassemia\textsuperscript{12} and Hb S/O\textsuperscript{4RAB, 33} may be at least as deleterious as the homozygous form of the more well known allele. Thus, a detailed knowledge of parental genotypes and phenotypes is obligatory for accurate genetic counseling.

Analysis of Hemoglobin Synthesis in Fetal Blood Cells

The clinical observation which established the potential feasibility of prenatal diagnosis of hereditary hemoglobin abnormalities was the detection of the synthesis of adult hemoglobin in the human fetus at gestational ages of as low as five to eight weeks.\textsuperscript{20, 29} Hb A synthesis constitutes approximately 8 to 14 percent of the total hemoglobin synthesized in isolated erythrocytes at this gestational age.\textsuperscript{20} The fundamental requirements necessary for the application of this knowledge to the prenatal diagnosis of hemoglobinopathies and thalassemias are the following: (1) a method to obtain fetal blood samples with a high rate of success but with a low rate of associated fetal morbidity or mortality; (2) a reliable means of in vitro radiolabelling of globin chains, which achieves radioactivity levels sufficient for accurate quantitative comparison of globin chain synthesis in different fetuses; (3) a simple, reproducible means for separating and identifying different globin chains and their mutant variants; and (4) an estimate of the average synthetic rates of each globin chain and the amount of variability in these rates among normal fetuses. These technical requirements largely have been met, although there still remains a serious problem in the safe sampling of fetal blood.

Fetal Blood Sampling

Blood sampling (usually several specimens of 10 to 100 microliters) is per-
formed using sonographically guided placental aspiration or with the aid of direct visualization of a placental vein using fetoscopy.\(^{19}\) Sampling is usually performed in the 18 to 20th week of gestation to maximize fetal size, amniotic fluid volume, and simplicity of fetal localization. Blood lost into the amniotic cavity in this process often contains a high percentage of fetal cells and, therefore, it may be useful to sample amniotic fluid as well. It is estimated that sampling and typical blood loss account for only a few percent of the fetal/placental blood volume.\(^{2,19}\) Fetoscopy assisted venapuncture of placental blood vessels has been reported to be successful in obtaining fetal blood in at least 90 percent of attempts.\(^{2,7,22}\) Both blind placental aspiration and fetoscopy with aspiration from a placental vein to date have been associated with an undesirable level of risk of trauma to the fetus and to the placenta. A frequency of spontaneous abortion of 5 to 15 percent occurs following this procedure.\(^{2,22,28}\) The success rate of obtaining useful specimens appears to be lower, and the frequency of fetal loss higher when blind placental aspiration is used rather than fetoscopy.

Both methods of fetal blood sampling yield specimens which are more or less contaminated with maternal blood.\(^{2,19,28}\) While in some instances this does not interfere with determination of the fetal globin phenotype, in others, such as the determination of \(\beta\)-thalassemia, obtaining a fetal blood sample of sufficient purity to establish the diagnosis by biosynthetic methods constitutes a significant problem. The ratio of maternal to fetal cells can be determined directly with the Coulter counter since maternal and fetal cells are different sizes. A second useful method is the Kleihauer-Betke staining procedure.\(^{8}\) Since significant globin chain synthesis occurs only in reticulocytes and fetal blood contains up to 20 percent reticulocytes, one approach to reducing contaminating maternal synthesis is to suppress the low frequency of maternal circulating reticulocytes by transfusion.\(^{33}\) Increased maternal and fetal risks from this procedure are those attendant to any blood transfusion during pregnancy. Alternatively, enrichment of fetal cells can be accomplished by selective immunoprecipitation\(^{26}\) or selective hemolysis of adult cells.\(^{21}\) Yet another approach which has been employed is to determine globin chain synthesis ratios in a maternal blood specimen drawn at the same time as fetal blood sampling. The effect of this synthesis on the synthesis values obtained for the fetal blood specimen is then calculated.\(^{13}\)

**Fetal Globin Chain Synthesis**

To identify the synthesis of the several globin chains, an isolated, intact red cell pellet is collected by centrifugation, washed and incubated with a radioactive amino acid (commonly \(^3\)H leucine) for several hours. The cells are then lysed in hypotonic solution, and the newly synthesized radioactive globin chains are fractionated by column chromatography or electrophoresis. A detailed description of the typical procedure is found in the article by Alter et al.\(^2\) Globin chains are extracted in acid-acetone, dried under \(N_2\) and applied to a cation exchange column such as carboxymethyl cellulose, e.g., Whatmann CM52. The column is developed with a gradient of increasing buffer concentration in 8M urea and provides good separation of different globin chain types.\(^{14}\) An example of this separation is shown in figure 1. Control incubations of maternal blood samples should be carried out at the same time. The method is applicable to the direct demonstration of homozygosity for an abnormal hemoglobin type (hemoglobinopathy), for example, Hb SS, if the mutant chain can be well separated from the normal \(\alpha, \beta,\) and \(\gamma\) globin chains. In this example, no normal \(\beta\) chain would be produced (no radioactive-
ity in the region of the gradient where normal β chains elute), and a new major peak of radioactivity would occur (figure 1). Heterozygotes in this example would contain both normal and abnormal β chains and thus possess both peaks. Identity of the peaks is confirmed by the addition of non-radioactive known normal globin chain markers.

This method also is useful in the antenatal diagnosis of the thalassemias (figure 2). Here the ratio of globin chain synthesis, β to γ in the case of β-thalassemia, and β⁺γ to α in the case of α-thalassemia, is the important diagnostic criterion. These results are compared to known normal fetal values. The β to γ (or β to β + γ ratio) ratio in normal fetuses is relatively constant with gestational age until 32 weeks and is greater than 0.07 with a mean of 0.11, whereas β-thalassemia heterozygotes have values in the range of 0.058 and homozygotes 0.03 or below. The majority of β-thalassemia homozygotes have no demonstrable β-globin synthesis. This result is taken as definitive evidence for homozygosity as is the absence of α-globin synthesis for homozygous α-thalassemia.

Using these methods of prenatal diagnosis of disorders of hemoglobin synthesis, approximately 200 at risk pregnancies have been evaluated. Follow-up studies of both full term and interrupted pregnancies have confirmed the diagnosis in nearly all cases, although a few false negative results (affected child at birth when diagnosed antenatally as heterozygote) have been reported. Thus, the major limitation in the application of this approach to the antenatal diagnosis of these conditions is the technical obstetrical skill necessary to acquire the fetal blood sample and the greatly increased risk as compared to standard amniocentesis.

**Hemoglobin-Specific Antibodies**

Antibody methods also have been employed for the direct detection of different types of hemoglobins in blood cells. For samples of blood involving populations of erythrocytes, the component hemoglobin types can be measured by radial immunodiffusion or by fluorescent-labelled antibody. The latter approach is suitable for detection of the hemoglobin phenotype of single red blood cells. The advantages of rapidity of results, the ease of exportation of the technique to the average clinical laboratory, and the possible application of the method to amniotic fluid specimens, thus obviating the need for fetal blood sampling, make this approach attractive. The major technical shortcoming of the method is its dependence on antibodies type specific for each specific hemoglobin...
Antenatal Diagnosis by DNA Analysis: Molecular Hybridization and Restriction Endonuclease Mapping

From the foregoing discussion, it would be valuable to have a diagnostic method not dependent upon fetal blood sampling. A major limitation in the use of cultured amniotic fluid fibroblasts for prenatal diagnosis of inherited defects is their incapacity to express phenotypically a variety of gene functions of interest, e.g., the synthesis of hemoglobin. Since it is estimated that the haploid human genome contains 10,000 to 100,000 genes encoding proteins, it is not surprising that the fibroblast expresses only a fraction of the total human genetic repertoire. How, then, can genetic abnormalities be assessed in a cell type which intrinsically does not synthesize the desired product but does contain the genes to do so?

All cell types carry all genes. One powerful approach to define mutants resulting from deletion of a structural gene is the method of RNA-DNA or DNA-DNA hy-

Figure 2. Fractionation by carboxymethylcellulose column chromatography of globin chains synthesized in blood cells from a fetus with homozygous \( \beta^+ \) -thalassemia. Idealized profile for the distribution of globin chains radiolabelled with \(^3\)H leucine.

Figure 3. Idealized RNA-DNA hybridization saturation curve. Purified radiolabelled globin gene specific mRNA in increasing amounts is incubated with a fixed amount of DNA under conditions allowing reannealing of complementary sequences. When all DNA sequences which can react have done so with the mRNA probe, saturation is reached and no further RNA-DNA hybrid forms with addition of more mRNA. Identical amounts of DNA and RNA are used from the fibroblast lines with or without the deletion of the gene.
bridization. Classically, different amounts of radiolabelled, purified, messenger RNA (mRNA) specific for the gene, e.g., globin mRNA, are incubated in solution with a fixed quantity of denatured (single stranded) DNA isolated from normal cells or mutant cells (figure 3). Since mRNA has a nucleotide sequence complementary to the DNA strand of the gene from which it is transcribed, DNA containing this gene will reanneal to form a double-stranded mRNA-DNA hybrid. A comparison is made between the quantity of hybrid formed under standard conditions of incubation for normal DNA and DNA isolated from cells of the known or suspected mutant. If a deletion of the structural gene has occurred, no hybridization will occur. Partial deletions may give intermediate results. In this way, it was possible to establish that β globin gene sequences are present in individuals with homozygous β°-thalassemia.41

A technically superior variation on this theme is to prepare radioactive DNA complementary to the purified mRNA by in vitro synthesis using the mRNA as template for the enzyme, reverse transcriptase. This copy DNA (cDNA) has a very high specific radioactivity and can be hybridized under controlled conditions to single stranded DNA isolated from cells of normal individuals and putative mutants. The cDNA reanneals with the opposite DNA strand from that hybridizing with mRNA. Again, the amount of hybrid formed can be quantitated from the radioactivity of the hybrid and the presence or absence of the structural gene determined. If more than one copy of each structural gene is present per haploid genome (as is the case for the α-globin gene), then the number of copies present per DNA equivalent can be estimated.

Pioneer work in the antenatal diagnosis of α-thalassemia using these methods was begun by Kan and his associates.24,30,47 Molecular hybridization of globin cDNA with DNA isolated from fibroblasts of normal and thalassemic individuals has clearly established that most normal individuals contain four copies of the α globin structural gene whereas the α-thalassemia silent carrier state contains three copies; heterozygous α-thalassemia, two copies; Hb H disease, one copy; and homozygous α-thalassemia, no copies of this gene (figure 4).24 This technique has been employed for antenatal diagnosis of a small number of pregnancies at risk for Hb H disease30 and homozygous α-thalassemia.15,24,47 In each case, the diagnosis made by this method has been confirmed by hemoglobin analysis at birth or at termination of the pregnancy.

![Figure 4. Percentage of α-cDNA annealed (hybridized) to the DNAs of patients with α-thalassemia syndromes and normal controls (solid dots). The means and one standard deviation are shown. The Δ denotes amniotic fluid fibroblast DNA from a pregnancy at risk for homozygous α-thalassemia, and the □ the same for a pregnancy not at risk for α-thalassemia. Clearly the at risk fetus did not have either hydrops fetalis or HB H disease. Figure reprinted from Kan, Golbus and Dozy24 with permission.](image-url)
There are several technical limitations of this method which deserve mention. Hybrid formation requires relatively large amounts of fibroblast DNA. The estimate of hybrid formation is quite sensitive to contamination of the specific globin gene cDNA with variable amounts of other globin gene cDNA originating because of mRNA impurity. This accounts for the background level of hybrid formed in the homozygous α-thalassemia (figure 4). Finally, although deletion of each of the α-genes results in an incremental loss of hybrid formation, rigorous and simultaneous comparison of the specimen fibroblast DNA sample to known positive and negative controls is obligatory. DNA template saturation studies of this kind have practical application only to the detection of large deletion mutants. They have no value for the detection of more subtle changes in the nucleotide sequence of genes for globin synthesis.

**Restriction Endonuclease Mapping**

During the past seven years, a fundamentally new technology making possible the isolation and sequencing of specific DNA fragments has revolutionized molecular genetics. The basis of this technological breakthrough has been the identification, characterization, and commercial availability of cleavage site-specific restriction enzymes, bacterial endonucleases that can recognize specific nucleotide sequences in double-stranded DNA and catalyze in vitro double stranded cleavages in the region of a specific nucleotide sequence. A variety of these restriction endonucleases with different nucleotide sequence specificities are available to fragment DNA into different pieces of specific size classes. In principle, these methods can be applied to the detection and demonstration of both deletion mutations and nucleotide substitution mutations, since in the latter case an altered nucleotide sequence might generate different cleavage sites in the mutant gene from that found in the normal gene.

This approach very recently has been applied to the antenatal diagnosis of both the thalassemias\textsuperscript{15,36} and sickle cell anemia.\textsuperscript{23} Orkin et al\textsuperscript{36} have mapped the globin genes within specific molecular weight fragments of fibroblast cellular DNA separated by electrophoresis on agarose. After electrophoresis of DNA fragmented by specific restriction endonucleases Eco RI and Hind III, the distribution of specific globin gene sequences is determined by molecular hybridization with a specific radioactive globin gene cDNA probe. The presence of globin
genes, determined simply as the presence or absence of the radioactive probe associated with specific fragments generated by the different restriction enzymes, is established by autoradiography. A schematic outline of the method and a representative result are shown in figures 5 and 6, respectively.

In control fetal fibroblasts from clinically established cases of α-thalassemia, no DNA fragments specific for α globin cDNA have been detected. This result confirms the complete deletion of all four α globin genes suggested by the DNA-DNA molecular hybridization saturation studies. One pregnancy at risk for homozygous α-thalassemia has been correctly diagnosed by this method, and the diagnosis confirmed by the presence of Hb Barts in the fetal blood at termination of the pregnancy. Analysis of amniotic fluid cell DNA from a fetus at risk for δ β-thalassemia showed the deletion of particular β globin nucleotide sequences and demonstrated the feasibility of this sensitive method for the antenatal diagnosis of the gene deletion causing δ β-thalassemia.

In theory, any mutation arising by nucleotide substitution could also be recognized if a restriction endonuclease with suitable nucleotide specificity was available. For example, the amino acid substitution in Hb SS is known to arise from a change in the DNA sequence from GAG-GAG* to GTGAG. A restriction endonuclease recognizing GAGG* would produce a fragment with a breakpoint at this position in the DNA sequence from normal individuals but not that from Hb SS homozygotes. Such an enzyme, Mnl I, has recently been isolated and has great potential for prenatal detection of the homozygous sickle cell condition.

Linkage of susceptible cleavages sites outside the globin structural gene can also be used to establish the likely genetic constitution of the fetus. For example, Kan and Dozy have used polymorphism of a restriction endonuclease Hpa I cleavage site to establish the prenatal diagnosis of sickle cell disease. DNA from people of African origin with Hb S genotype yield two DNA fragments (13.0 kilobases and about 7 kilobases) in 97 percent of the cases examined, whereas normal individuals produced fragments of only seven kilobases. In a family where the sickle gene was contained in the variant 13 kilobase fragment, i.e., the Hb S allele and

* Guanine adenine guanine guanine adenine guanine
† Guanine thymine guanine guanine adenine guanine
‡ Guanine adenine guanine guanine
the variant cleavage site are on the same chromosome, DNA from amniotic cells produced both sizes of β globin gene fragments. This finding indicated the fetus carried the sickle cell trait and was confirmed by globin chain synthesis in a fetal blood sample. The method is so sensitive that the fibroblast yield from 15 ml of fetal blood sample. The method is so sensitive that the fibroblast yield from 15 ml of amniotic cells produced both sizes of /3 globin gene fragments. This finding indicated the fetus carried the sickle cell trait and was confirmed by globin chain synthesis in a fetal blood sample. The method is so sensitive that the fibroblast yield from 15 ml of amniotic fluid can be used directly without subculture to provide sufficient DNA quantities for the analysis.

Conclusion

Antenatal diagnosis of hemoglobinopathies and thalassemias can be accomplished either by means of analysis of globin chain biosynthesis in fetal reticulocytes or by means of analysis of globin gene DNA isolated from amniotic fluid fibroblasts. The major impediment to application of the first procedure is the high risk of fetal loss associated with the in utero blood sampling procedure. Although only three antenatal diagnoses of defects in hemoglobin synthesis have been attempted using restriction endonuclease mapping of globin gene sequences, the sensitivity, versatility, and safety of these methods make them the favored choice for future development and application. These techniques can in principal be applied to any inherited disease in which a gene probe (gene-specific mRNA or cDNA) can be obtained.

References


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