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Workshop

DNA Polymorphism and Detection of Genetic and Infectious Diseases

DNA-Diagnosis of Hemoglobinopathias and Thalassemias

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Hemoglobinopathias and thalassemias are the two major types of inherited disorders of hemoglobin in man. While the hemoglobinopathias exhibit qualitative changes of the globin molecule, the thalassemias result from an imbalance in α- and non-α-globin chain production. In recent years the structural features of the normal human globin genes as well as the molecular lesions in several hemoglobinopathias and many forms of thalassemias have been determined by the application of recombinant DNA technology. While globin gene deletions are the predominant underlying molecular defects in α-thalassemia syndroms, the majority of hemoglobinopathias and β-thalassemias are due to point mutations within the respective globin gene regions.

For diagnostic purposes the identification of mutant genes in cellular DNA is theoretically possible because of the direct or indirect specificity of restriction enzymes. A direct identification of the defective gene can be made if the mutation changed an enzyme's cleavage site and thus changes the normal DNA restriction pattern. For example, the direct detection of the sickle cell gene with restriction enzyme Mst II and the hemoglobin (Hb) M Milwaukee gene with Sst I have recently been described (10,9,2,3,7). An indirect identification of chromosomes that carry a mutant gene relies on the presence of inherited DNA sequence polymorphisms within the cellular genome, giving rise to variations in restriction sites. Examples of this indirect diagnostic procedure are the identification of defective β-globin genes, causing hemoglobinopathias (e.g. Hb Freiburg, Hb Köln, Hb Presbyterian (8,4,5) or β-thalassemias (1,8)).

A third possibility to identify chromosomes carrying point mutations or small deletions relies on oligonucleotide mapping procedures that have successfully been applied for diagnosis of some hemoglobinopathias and thalassemias. 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 or the mutated gene sequence. These experimental conditions can also be used in diseases with an autosomal dominant inheritance pattern as in the Hb Freiburg disorder, where normal homozygotes can be differentiated from Hb Freiburg patients (Horst et al. unpublished).

All these methods have been applied for pre- and postnatal diagnostic purposes. In genetic counselling they have been used together with chorion biopsy or amniocentesis to provide prenatal diagnosis in families at risk. In the case of α-thalassemias prenatal diagnosis might only be applied to permit a mother with a fetus with hydrops fetalis to choose whether to carry the fetus through the full 9 month of pregnancy. However, together with hematological and family studies DNA-analysis data are especially useful to differentiate between α-thalassemia-1 and α-thalassemia-2 patients and thus to determine the exact diagnosis (6).

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Molecular genetics of the X-linked muscular dystrophies

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The mutations for Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) have been localised to the same region of the short arm of the human X chromosome at Xp21 by linkage analysis to bridging DNA markers (1,2,3). Linkage studies show that the frequency of recombination between markers in this region in the families segregating for these disorders is high (4,5,6). One marker in particular is deleted in both a patient suffering from DMD, chronic granulomatous disease and retinitis pigmentosa (7) and in a patient suffering from DMD and glycerol kinase deficiency (8). The former has a visible cytogenetic deletion. This marker is linked at approximately 10cM from the DMD locus (5,6). An additional marker on the opposite side of the DMD and BMD loci also within Xp21 is linked at a similar genetic distance (9). Although these two markers together can now be used for antenatal diagnosis (10), only a few families can be helped. More closely linked are being identified.

Strategies are now being developed to isolate additional sequences localised within these deletions (11). These approaches should eventually lead to the identification of the molecular basis of DMD and BMD and permit the investigation of the observed high mutation rate and the degree of heterogeneity of the mutations at the DNA level.

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