RECOMBINANT DNA TECHNOLOGY IN THE DIAGNOSIS OF HUMAN INHERITED DISEASE

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Over 3,000 Mendelian traits implicated in the pathology of human inherited disease, have been catalogued to date (1). Only a very small proportion can be diagnosed either antenatally or preclinically by conventional protein analysis. Moreover, many diseases are not amenable to antenatal genetic analysis since their diagnostic proteins are not present in the accessible foetal tissues. The advent of recombinant DNA technology has promised to circumvent these problems since direct investigation of the genetic material obviates the need for specific tissue samples. In addition, antenatal diagnosis and carrier detection of many genetic defects should be possible without the prerequisite of needing to identify either the primary gene product or the biochemical mechanism of the disease. Differing approaches to disease diagnosis will be described and the extent of their application to disease diagnosis to date, presented.

DIRECT ANALYSIS OF GENETIC DISEASE USING GENE PROBES

In order for the base-pair change or deletion event responsible for the disease phenotype to be detectable using restriction enzymes, two conditions must be met. First, the nature of the disease must be sufficiently well understood for the locus responsible to be identified, isolated, and used as a hybridization probe to examine the disease-associated allele(s). Second, for the aberration to be detected, a restriction site must be introduced or removed in the case of a point mutation, or the length of DNA between sites must be altered by sequence additions, or rearrangements.

Although the application of direct analytical technique to disease diagnosis is still in its infancy, Table 1a illustrates the extent to which it has already been applied. Coverage of the thalassaemias and haemoglobinopathies is by no means exhaustive here due to the disproportionate number of globin gene mutations detected to date. This is well reviewed (2).

Direct analysis of intragenic defects may also be accomplished using chemically-synthesized oligonucleotide probes thereby obviating the requirement for the mutation to lead to an altered restriction site. Discrimination between genotypes relies upon a base-pair mismatch between
the oligonucleotide and a given allele being sufficient to abolish
hybridization under the conditions used. This approach has been used for
analysis of α1-antitrypsin deficiency, sickle-cell anaemia, and the
thalassaemias (Table 1a). However, its application to the diagnosis of
diseases known to be molecularly heterogeneous requires extensive
investigation of each specific mutation.

The analysis of various diseases characterized by severe chromosomal
lesions has also been attempted using a variety of cloned probes. These are
listed in Table 1b.

A veritable armoury of gene probes is at present available which are
potentially useful in the direct analysis of disease loci (3).

INDIRECT ANALYSIS OF GENETIC DISEASE USING RFLPS DETECTED BY GENE PROBES

If a gene defect is not a gross deletion, then it may often go
undetected due to the lack of a suitable restriction enzyme (Table 2). One
alternative is to use DNA polymorphisms flanking the locus of interest as
genetic markers. RFLPs are neutral base-pair changes which introduce or
remove a restriction site, or sequence deletions, additions or
rearrangements which affect the length of DNA between sites. RFLPs are not
rare, occurring in the human genome approximately every 200 to 300 base-
pairs (4, 5), a frequency which demonstrates the extensive variation still
to be exploited in clinical medicine. Inheritance of a disease allele can
thus be monitored over the generations by following the inheritance of
readily detectable RFLPs linked to the gene in question. A list of diseases
to which this approach has been applied is given in Table 3.

INDIRECT ANALYSIS OF GENETIC DISEASE USING RFLPS ASSOCIATED WITH LINKED DNA
SEGMENTS

When for a particular disease, the relevant gene is not available as a
probe, direct analysis is impossible. Instead, linkage between a cloned DNA
segment and the locus of interest can be established and the inheritance of
RFLPs associated with the linked DNA segment investigated. The use of
linked RFLPs is merely an extension of classical linkage analysis; it
provides the indirect means to detect the presence, and follow the
inheritance of, genetic lesions and base-pair substitutions at a particular
locus without needing to possess a cloned copy of the locus itself. The
tighter the linkage between the DNA segment and the locus of interest, the
smaller will be the number of recombinants as both loci tend to segregate
together. A list of genetic diseases for which linkage with polymorphic DNA
segments has been established is given in Table 4.

Most of the DNA polymorphisms identified to date are due to the
presence or absence of particular restriction sites, while deletions,
insertions, and copy number variation seem to occur less frequently.
Although RFLPs seem to be abundant in the genome, most exhibit a low allele
frequency. Clearly much effort must be indirected toward the further
identification and localization of clinically useful RFLPs before such
analysis becomes a routine procedure in medical genetics.

Disease diagnosis is an essential basis for causal therapy and
presymptomatic diagnosis is in many instances a prerequisite for successful
treatment or prevention. The ability to detect presymptomatically a disease
which is at present not amenable to therapy may however present serious
ethical problems. Furthermore, the relative simplicity of the methodology
and the low cost incurred in prenatal diagnosis may well divert both