Testing for cystic fibrosis

A start has at last been made on the development of a prenatal test for cystic fibrosis. At first, it will only be useful in some families.

A BATCH of papers published in this issue of *Nature*, together with a paper in the current issue of *Science*, signal the beginning of the end of the hunt for the cystic fibrosis gene, defects of which are responsible for the disease. Although the gene remains to be identified, at least it is now certain that it resides in the middle of the long arm of chromosome 7, within a region that comprises no more than about a million base pairs. And the new data offer immediate prospects for the prenatal testing for cystic fibrosis.

Approximately one in twenty Caucasians carry a defective cystic fibrosis gene, and about one child in 2,000 inherits such a gene from both parents and consequently dies of the disease, usually by the age of 30. Despite the frequency of the disease, nothing has been learnt of the gene (or genes) in which the defects arise and no gene product has been convincingly demonstrated. It has not therefore been possible as yet to devise a prenatal screening test for cystic fibrosis relying on the detection of the defective gene or its product. An alternative approach, laid out in detail by Botstein *et al.* (*Am. J. hum. Genet.* 32, 314; 1980), is to look for and find a restriction-fragment linked polymorphism (RFLP) close enough to the cystic fibrosis gene to act as a linkage marker.

An RFLP is no more than a variation in DNA sequence that generates or abolishes a recognition site for a restriction enzyme. Because these enzymes cut through double-stranded DNA, the positions of their recognition sites on a stretch of DNA determine the sizes of the "restriction fragments" of DNA they generate. The chromosomes of any homologous pair are bound to differ by many RFLPs, which can therefore, in principle, be used to tell which of the pair of chromosomes carries, for example, a defective cystic fibrosis gene. If the RFLP is located closely enough on the chromosome to the cystic fibrosis gene not to become separated from it by the normal process of genetic recombination, it will also serve as a linkage marker for the defective gene. Because the RFLP is only a linkage marker, rather than a direct assay of the defective gene, it cannot be used for population screening but only predictively, in family studies.

It has been calculated that if RFLPs are more or less evenly distributed along and among the chromosomes and are

"informative", no more than 150 would be sufficient to provide one closely linked with any gene. So far, however, although several hundred useful RFLPs have been found and many have been localized, they are not evenly distributed and most are informative for only a minority of families. In searching for a linkage gene that could be on any chromosome, it therefore makes sense to begin with the chromosomes that are most saturated with RFLPs and conventional markers (such as different forms of serum enzymes). In this way, by the middle of 1985, several research groups had excluded about 40 per cent of the human genome as the site of the cystic fibrosis gene.

The first sign of success came at the Eighth International Gene Mapping Workshop in Helsinki in August where Hans Eiberg, of the University Institute of Medical Genetics in Copenhagen, reported a tentative link between cystic fibrosis and a form of the serum enzyme paraoxonase (an aryl esterase). Eiberg calculated that the paraoxonase gene and the cystic fibrosis gene lie within ten million base pairs of each other. However, this linkage — now published by Eiberg and others in *Clinical Genetics* 28, 265; 1985 — was less useful than it might have been because paraoxonase is poorly characterized and the chromosomal localization of its gene is unknown. Rather than dampening the enthusiasm of those who had failed to find an RFLP linkage, Eiberg's result revived their flagging energy.

Among those searching for an RFLP were Lap-Chee Tsui and colleagues at the Hospital for Sick Children in Toronto. In July of this year they came to an agreement to test some of the 200 or so useful RFLPs generated by Collaborative Research Inc. Much to their joy, and purely by chance, a cystic fibrosis-linked RFLP turned up among the first half a dozen RFLPs that Tsui tested. The result was reported at the American Society of Human Genetics meeting in October, and has just been published in *Science* (230, 1054; 1985).

What was not reported at the meeting was the chromosomal localization of the RFLP, information that would immediately have enabled others to concentrate exclusively on any RFLPs that they knew to be associated with the chromosome in question. Members of both the Toronto and Collaborative

Research teams say that they had only preliminary evidence at the time and were not prepared to risk presenting a faulty localization in public. Nevertheless, rumour emerged that their RFLP was on chromosome 7, as they confirm on page 380, although other rumours pointed towards different chromosomes.

Spurred on by the rumours, two other research groups have now found RFLPs that are linked to cystic fibrosis and report them on pages 322 and 384. One of the groups is centred on Bob Williamson's laboratory at St Mary's Hospital Medical School in London. Its RFLP probe is an "anonymous" DNA probe on the long arm of chromosome 7. Williamson's group also reports linkage between the cystic fibrosis gene and two other chromosome 7 genes — that for the T-cell receptor beta chain (p.384) and a collagen gene (*Lancet* 29 November p.1241). The linkage reported by the other group, centred on Ray White's laboratory at the Howard Hughes Medical Institute in Salt Lake City, is to an RFLP that is in the locus of the *met* gene, which is a human oncogene on the long arm of chromosome 7 (see p.285). The overlap between the various probes locates the cystic fibrosis gene in the middle of the long arm of chromosome 7.

Both Williamson's and White's RFLPs are much more tightly linked with the cystic fibrosis gene than is Tsui's. His RFLP is probably no closer than 15 million base pairs (0.5 per cent of the human genome) to the gene. Williamson's and White's are each within about a million base pairs of it, conceivably very much closer. The greater the distance between the RFLP and the gene, the greater the chance that they will be separated by recombination during meiosis, confounding attempts to use the RFLP as a prenatal diagnostic marker of the defective gene. In these terms, Tsui's RFLP is not diagnostically useful, whereas the other two are. What is likely to emerge, when the dust settles, is a set of markers which will make the reliable prenatal diagnosis of cystic fibrosis a reality assuming that all cases are due to a mutation of the same gene.

Both to develop a screening test that avoids the need of family studies and to discover the fundamental basis of cystic fibrosis, vigorous attempts will now be made to identify the gene itself and to find out what it produces. **Peter Newmark**