Annotations

Prenatal diagnosis of cystic fibrosis

The usual way of diagnosing a genetic disorder in utero is by measuring the concentration of protein product of the mutant gene in an accessible fetal tissue. This is not yet an option for cystic fibrosis. Though the chromosomal localisation of the cystic fibrosis gene is known, and though a 'candidate' gene has been described, the protein product has yet to be identified. Because most of the clinical manifestations of cystic fibrosis are seen in exocrine glands it seems unlikely that the defective protein or enzyme will be expressed in either amniotic fluid cells or in chorionic tissue.

None the less prenatal detection of cystic fibrosis has been possible for several years. One method depends on monitoring early clinical signs of the disorder by measuring a group of microvillar enzymes in amniotic fluid supernatant taken during the second trimester. The other method exploits the tight linkage of an arbitrary set of deoxyribonucleic acid (DNA) markers to the cystic fibrosis gene, and can be applied either to chorionic villus samples taken in the first or second trimester of pregnancy, or to amniotic fluid cells taken during the second trimester. Each method has its advantages and disadvantages; both will probably become obsolete once the cystic fibrosis gene is cloned and sequenced, and the exact mutation defined.

DNA based methods

Late in 1985 several reports¹⁻³ appeared describing DNA markers linked to the cystic fibrosis gene, which allowed the chromosomal locus to be placed in the region 7q21-34 on the long arm of chromosome 7. A large collaborative study of families collected from Europe and North America⁴ showed that in all informative cases the cystic fibrosis gene was linked to a panel of chromosome 7 markers, and therefore in all probability was coded for by the same genetic locus. More recent information using linkage disequilibrium suggested that most European families with cystic fibrosis have inherited the same mutant allele from their ancestors. If this is confirmed, cystic fibrosis may prove to have a formal similarity to sickle cell anaemia and to be a genetic disorder caused by a limited number of mutational events at the same genetic locus.

The panel of DNA probes currently used for the prenatal diagnosis of cystic fibrosis is shown in the table. Most define two allele polymorphisms with the size of fragment depending on the presence or absence of a restriction enzyme cleavage site. Several of the probes may be used with more than one restriction enzyme (for example, pJ3-11, and MspI,² or PvuII⁵) to generate additional marker systems. In some cases, however, (for example, pJ3-11, and TaqI, or PvuII) the frequency of the major allele is too high for more than a few individuals to be heterozygous, and these markers are therefore of limited value. The various met probes (pmetH,¹ pmetD,⁶ pmet5⁵) are derived from the met proto-oncogene, and others are anonymous DNA fragments. The most suitable probes are pKM-19 and pXV-2c that show few recombinants with the cystic fibrosis gene.⁷ The met probes and pJ3-11 have reported recombination fractions of .004 and .003, respectively.⁴ With p7C22⁸ and p79a⁹ there are likely to be recombinants several times in every 100 meioses, and such probes should be used with caution in prenatal diagnosis. In practice virtually all families are fully informative when a combination of the most tightly linked probes are used.

In using linked markers to track the cystic fibrosis

Table DNA probes used in prenatal diagnosis of cystic fibrosis

Probe	Restriction enzyme	No of alleles	Frequency of major allele	Recombination fraction with cystic fibrosis
p79a	MspI	2	0.6	0.05
	HindIII	2	0.7	
p7C22	EcoRI	2	0.75	0.025
pmetH	MspI	3	0.6	0.004
	TaqI	2	0.7	
pmetD	TaqI	2	0.85	0.004
	BanI	2	0.6	
pmet5	TaqI	2	0.7	0.004
	MspI	2	0.9	
pJ3-11	MspI	2	0.5	0.003
	TaqI	2	0.95	
	PvuII	2	0.85	
pXV-2c	TaqI	2	0.5	Very small
pKM-19	PstI	2	0.7	Very small

gene it is always necessary to examine the family in which the disorder is segregating. Normal practice is to take blood samples from both parents and from an index affected child and to establish the phase relation between markers and the cystic fibrosis gene. This is illustrated in the figure for pJ3-11 and the restriction enzyme *MspI*. Because the index affected child is homozygous for the 1.8 kb DNA fragment on a Southern blot, it can be inferred that this is the fragment in phase with the mutant gene in each of the heterozygous parents. If a chorionic villus biopsy is carried out in the first trimester of a subsequent pregnancy, each of the three possible Southern blot patterns gives unambiguous information on the genotype of the fetus.

The convenient pattern of bands shown on the left hand side of the figure is not usually found with the first marker system tested. One or both parents may be homozygous for the marker, or the index affected child may have the same band pattern as one of the parents. In this case, further marker systems should be tested until either one marker or a combination of markers produces a unique pattern for the index affected child.

It is not usually possible to carry out prenatal diagnosis using linked DNA markers unless there is an index affected child available to establish the phase relations. Unaffected children are unsuitable for typing because it is not possible to tell whether they are homozygous or heterozygous normal. Occasionally it is possible to use an extended family when an affected child on the paternal or maternal side can be used for phase ascertainment.¹⁰⁻¹¹ Such

Figure Schematic representation of use of pJ3-11 and *MspI* in prenatal diagnosis of cystic fibrosis. Band patterns seen on a Southern blot are shown below parents and index affected child in box (left). The index affected child is homozygous for the 1.8 kb fragment, which is therefore in phase with the cystic fibrosis gene in each parent. Predictions for analysis of a chorionic villus sample are shown in box (right): Hz—heterozygous; N—normal homozygote; CF—affected homozygote.

typing 'at a distance' increases the chances of an unrecognised recombinant leading to erroneous phase assignments and should not be used if information has to be acquired from the less tightly linked p7C22 or p79a systems.

Experience of prenatal diagnosis of cystic fibrosis during the first trimester using linked DNA markers has grown rapidly since the first report.¹² Super *et al*¹³ described 30 cases; the total from centres in the United Kingdom by December 1987 was 85 (Dr M Super, personal communication). As might be expected there have been some initial difficulties in acquiring adequate amounts of undegraded DNA from occasional over conservative villus biopsy specimens, and in four of the 85 attempts no diagnosis was possible. In seven pregnancies miscarriage followed villus biopsy. In the remaining cases there were no difficulties in either confirming or excluding the presence of cystic fibrosis in the fetus.

Microvillar enzyme testing

In 1983 it was reported that the activities of two peptidases, γ -glutamyltranspeptidase and aminopeptidase M, were significantly depressed in amniotic fluid supernatant during the second trimester in the presence of a fetus with cystic fibrosis.¹⁴ It was subsequently shown that the activities of amniotic fluid disaccharidases¹⁵ and the intestinal isoenzyme of alkaline phosphatase¹⁶ were also greatly reduced. These enzymes have the common feature of localisation on the luminal surface of microvillar membranes, and are found in high concentrations in fetal meconium. It now seems probable that the passage of meconium into amniotic fluid is impaired in the fetus with cystic fibrosis at a critical stage in the second trimester. The primary evidence for this comes from the depleted activities in amniotic fluid of enzymes such as intestinal alkaline phosphatase, which could only originate in the fetal gut. Confirmatory evidence is found in the occasional observations of abnormal ultrasonar scans that suggest a meconium plug in the ileum or jejunum.¹⁷ Pathological examination of the second trimester fetus with cystic fibrosis has now shown clear evidence of extremely abnormal meconium constitution.¹⁸

In contrast to DNA analysis, measurement of microvillar enzyme is simple, quick, and cheap. Chromogenic substrates are available and assay of a set of enzymes can be carried out on 50 μ l of amniotic fluid in less than one hour. Because measurement of microvillar enzyme is monitoring a subtle clinical abnormality in the fetus, however, resolution of normal from abnormal activities in amniotic fluid must be achieved empirically. It must

be expected that some fetuses with cystic fibrosis will have microvillar enzyme activities above any arbitrarily defined cut off point (false negatives), and some normal fetuses will be below the cut off point (false positives). Because mothers with apparently affected fetuses will be reluctant to continue their pregnancies, the validation of this system of diagnosis rests to some extent on being able to confirm the prediction in the aborted fetus.

Inspection of fetuses aborted after abnormal microvillar enzyme activity has been reported shows that in most cases the ileum and jejunum are grossly distended and filled with green or black meconium of sticky, glue like consistency.¹⁹ The albumin concentration in the meconium is usually enormously raised. When it is not, there is often a high activity of trypsin like protease.¹⁸ This led to the suggestion that in some fetuses there may have been a burst of pancreatic activity at some point between amniocentesis and termination of pregnancy that could have degraded the more labile intestinal proteins. This idea is supported by the finding of high concentrations of pancreatic oncofetal antigen in meconium from fetuses with normal albumin concentrations and increased protease activities.²⁰

Some doubts remain about the validity of using biochemical methods to confirm diagnosis in a cystic fibrosis abortus. The advent of DNA markers has provided an independent method of verification, given that there is a living affected child to establish phase relation. Curtis *et al*²¹ showed that adequate amounts of undegraded DNA could be extracted from fetal intestine and other tissues, even after they had been stored frozen for several years. In this way they were able to confirm diagnosis in all eight of the abortuses typed.

It has gradually become apparent that the optimal time for testing for microvillar enzyme activity is at 17 or 18 completed weeks of pregnancy.²² Earlier amniocentesis leads to a significantly greater false negative rate. Working at this stage of gestation, Boué *et al*²³ reported 200 prospective diagnoses on pregnancies with a 1:4 risk of cystic fibrosis referred to their Paris centre. There was one case incorrectly predicted as abnormal among 142 normal infants (admitted false positive rate 0.7%), and two missed cases of cystic fibrosis among 58 where the diagnosis was an affected fetus (admitted false negative rate 3.4%). These are impressive figures. Much depends, however, on whether all of the 53 terminations of pregnancy were indeed for cystic fibrosis. Of the 43 abortuses examined, all had the biochemical or macroscopic appearance of cystic fibrosis discussed earlier. None the less in this series there were apparently 58 of 200 (29.0%) affected cases, more than expected in a Mendelian recessive condition.

A feature of this study was the concordance between different microvillar enzymes in signalling a normal or affected pregnancy. It has been reported that occasionally extremely low values of γ -glutamyltranspeptidase can be associated with normal activities of other microvillar enzymes.²⁴ In seven such pregnancies there were six live born babies with cystic fibrosis, and this pattern of activity must be treated with suspicion. Brock *et al*²⁵ reported a series of 258 pregnancies with a 1:4 risk of cystic fibrosis, in which they found a false negative rate of 4.4% and a false positive rate of 2.3%. Of the pregnancies in their series, however, 32.2% were found or predicted to be abnormal, suggesting a true false positive rate of 8–10%.

If the false negative and false positive rates of tests for microvillar enzyme are estimated conservatively as 5% and 8%, respectively, there are important implications for prenatal diagnosis. For a pregnancy with a 1:4 risk, the chance of a positive test signalling an affected fetus is 80%, and of a negative test signalling a normal fetus 98%. For lower risks, such as when only one partner has a family history, negative tests give extremely high probabilities of normality. Positive tests are ambiguous, however, and in most cases the chance of normality is still greater than the chance of abnormality. For this reason it has been argued that testing for microvillar enzyme activity should be restricted to pregnancies in which the risk is 1:4.²⁶

Conclusions

Two different methods of prenatal diagnosis of cystic fibrosis are currently available. The method of choice is diagnosis during the first trimester on a chorionic villus sample using linked DNA probes. The advantages are the high degree of accuracy of diagnosis and the opportunity for termination of pregnancy during the first trimester when the fetus is affected. A disadvantage is the need for a blood or DNA sample from an index affected child in order to establish linkage phase. An alternative form of diagnosis is that testing for microvillar enzyme activity on amniocentesis samples taken during the second trimester. The advantages are the suitability of such testing for all pregnancies with a 1:4 risk of recurrence, and the speed and ease of analysis. Disadvantages are the comparatively high false positive and false negative rates and the fact that if termination of pregnancy is indicated it must be carried out after 18 weeks of pregnancy.

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