Prenatal diagnosis of enzyme defects—an update

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The trends in prenatal diagnosis of enzyme defects predicted last year, namely an increase in diagnosis in the first trimester by chorionic villus sampling and early amniocentesis and greater exploitation of DNA analysis and the diagnosis of genetic errors in preimplantation embryos, have been fulfilled. These developments were also reflected in the papers presented at the 5th International Congress on Early Fetal Diagnosis, held in Prague in July 1990.

Chorionic villus sampling

The move towards demonstrating a deficiency of enzymic activity directly in a biopsy specimen of chorionic villi has continued. In our own laboratory over 50% of all prenatal diagnoses of enzyme defects are now made by direct assay of chorionic villi. The proportion is even higher (75–80%) in other centres, although measurement of the concentration of metabolites in amniotic fluid is sometimes used to support a diagnosis (WJ Kleijer, personal communication). More assay procedures previously used for cultured amniocytes have been shown to be valid for chorionic villi. Interestingly, several assays considered to be tissue specific and thought not to be expressed in chorionic villi can, in fact, be detected by the use of more sensitive radioisotopic assays. For example, the five activities of the urea cycle have been assayed in chorionic villi and prenatal diagnosis of deficiencies of three of the enzymes has been made. The experience of several centres with prenatal diagnosis of enzyme defects by chorionic villus sampling has been reported recently.

Two large surveys in North America suggest that the risk of fetal loss is not significantly greater for transcervical chorionic villus sampling than for amniocentesis when carried out in experienced centres. Another survey came to the conclusion that transcervical and transabdominal chorionic villus sampling are equally safe procedures in experienced hands. These conclusions should allay the fear that chorionic villus sampling is a more risky procedure than amniocentesis.

Although the usually accepted limit for chorionic villus sampling is 9–12 weeks' gestation, it is possible to obtain adequate samples as early as 6–7 weeks by transabdominal needling under ultrasound guidance. This would allow diagnosis to be made by direct assay on chorionic villi before 8 weeks and subsequent termination of a pregnancy with antiprogestins and prostaglandins rather than surgical methods. The safety of this procedure is being assessed, especially with regard to fetal abnormalities. It will be necessary to establish the levels of enzyme activities at this stage of development.

In a few women, sampling by the transcervical and transabdominal routes is difficult or impossible for anatomical reasons. However, it is possible to obtain chorionic villi by transvaginal needle aspiration under the guidance of an intravaginal ultrasound probe. Although it is a more lengthy procedure, accurate sampling is achieved and it could be very useful in these difficult cases. Sampling may be carried out as early as 6–8 weeks.

It seems inevitable that, with wider experience of sampling, technical improvements in assay procedures and the perception that chorionic villus sampling is no more dangerous than amniocentesis, more enzymic defects will be diagnosed in the first trimester by chorionic villus analysis.

Early amniocentesis

Many enzyme defects and metabolic disorders have been diagnosed by the detection of specific metabolites in amniotic fluid obtained at 16 weeks of gestation. Recently it has been shown that it is possible to detect some of these metabolites in amniotic fluid obtained at 11–12 weeks' gestation, thereby permitting diagnosis and termination in the first trimester. Propionic acidemia, tyrosinaemia type I, and argininosuccinic aciduria have all been diagnosed at 11–12 weeks' gestation by measurement of the specific metabolite in amniotic fluid by stable isotope dilution gas chromatography—mass spectrometry with selected ion monitoring. The organicacidurias and aminoacidurias that can be diagnosed by quantitative measurement of metabolites in amniotic fluid obtained in the first and second trimester have been reviewed. By obtaining a sample of amniotic fluid at the same time as transabdominal sampling for chorionic villi it will be possible to confirm a direct enzymic diagnosis by measurement of metabolites in amniotic fluid.

Culture of cells from amniotic fluid taken at 7–11 weeks' gestation is proving to be more difficult than from fluid sampled in the second trimester. Methods are being developed and...
modified to increase the success rate of these cultures and provided enzyme activities are expressed in these amniocytes, it should be possible to make an earlier diagnosis of those enzymic defects which cannot be made directly on chorionic villi but require culture of cells. It has also been possible to make a diagnosis of I cell disease in a fetus by the demonstration of an increase in lysosomal enzymic activities in amniotic fluid taken at 10–12 weeks’ gestation. Work is currently in progress to see if the concentrations and patterns of glycosaminoglycans in early amniotic fluid can be used to diagnose the mucopolysaccharidoses.

DNA analysis

It is possible to investigate the molecular basis of over 250 different genetic diseases using recombinant DNA techniques. The application of these techniques to prenatal diagnosis is advancing rapidly. This is illustrated very clearly by the progress made on the prenat al diagnosis of cystic fibrosis in the period of just over a year since the defective gene was first discovered. Among enzyme defects DNA technology has found greatest application so far for phenylketonuria and closely related disorders. This is because of the relatively high incidence of the disease in white people and the previous lack of a reliable method for prenatal diagnosis. At the same time as providing a reliable prenat al diagnosis for the first time for some cases, however, DNA methods have confirmed the great heterogeneity in this disorder. At least 18 mutations have been reported in the phenylalanine hydroxylase gene (the enzyme affected in phenylketonuria). Several restriction fragment length polymorphisms (RFLPs) have been found in the region of the phenylalanine hydroxylase gene. Currently haplotypes are defined by the pattern of alleles at eight sites produced with seven restriction endonucleases. There are appreciable ethnic differences in the prevalence of these haplotypes. Although certain mutations have been found to occur predominantly in particular haplotypes, as yet no mutation has been shown to be exclusively linked to a specific haplotype. These polymorphisms may be informative within a family with phenylketonuria, however, and prenat al diagnosis has been carried out on this basis.

It is now possible to detect known mutations using allele specific oligonucleotides. Alternatively the polymerase chain reaction can be used to amplify the sequence in the genomic DNA containing the mutations, which can be detected by sequencing, restriction enzyme cleavage, chemical mismatch analysis, or selective hybridisation with oligonucleotides. Thus in a family with phenylketonuria if the mutation in the affected child has been established, it should be possible to monitor a subsequent pregnancy by analysis of DNA from chorionic villi for the specific mutation. If the mutation has not been established, at least two options are available. Known mutations could be screened for in fetal DNA, taking into account ethnic prevalences but there is a risk of not detecting a mutation because it is uncommon or new. Alternatively, RFLP analysis of DNA from the fetus, affected child, and parents may be informative. It has been predicted that the latter approach can be applied to 90% of white families with phenylketonuria.

Another disorder in which DNA analysis has been used for prenat al diagnosis is Tay–Sachs disease, which results from a deficiency of hexosaminidase A and has an occurrence of 1/3600 in the unscreened population of Ashkenazi Jews. Three mutations in the gene encoding the α subunit of hexosaminidase account for over 90% of the mutant alleles in this group. Application of this information was possible when the parents of an affected child were both found to carry the same one of these mutations, a four base pair insertion. This mutation can be readily detected if the sequence containing it is amplified by the polymerase chain reaction and then digested with a restriction enzyme because one of the resultant fragments is four bases longer from the mutant allele than from the normal allele. A subsequent pregnancy was tested for this mutation by analysis of DNA from chorionic villi. The fetus was found to be heterozygous and this diagnosis was confirmed by enzyme assay both prenatally and postnatally.

DNA analysis may be useful, even when a disorder can be diagnosed by direct enzymic analysis, if only a very small sample is available for analysis. Identification of a particular mutation in an affected fetus may have prognostic value for other affected members of the family. About 20 lysosomal enzymes have now been cloned and molecular genetic analysis of cases of the different lysosomal storage diseases has revealed tremendous heterogeneity. Therefore it seems that each kindred or subpopulation will require specific DNA tests for a single small number of mutations. It will be interesting to see what ingenious strategies are devised to cope with this problem.

Fetal cells in maternal circulation

Another source of fetal material for analysis of enzymic defects may be fetal cells in the maternal circulation. It has been claimed that lymphocytes from a male fetus can be detected in the maternal circulation using flow cytometry and antibodies against paternal antigens not present in the mother and that fetal metaphases can be detected in maternal peripheral blood. However, other groups have not been able to confirm these observations. Recently the polymerase chain reaction has been used to demonstrate Y chromosome specific sequences in the blood of women carrying male fetuses. This technique, which requires stringent control of conditions to avoid contamination, is now being evaluated in other laboratories.

If the detection or even isolation of fetal cells from the maternal circulation can be established reliably, it will provide a non-invasive method for prenat al diagnosis of genetic defects with essentially no risk to the fetus. The sex of the fetus could be investigated using Y chromosome specific probes and the polymerase chain reac-
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tion or perhaps in situ hybridisation for X linked disorders. Theoretically, it would be possible to detect specific mutations in an enzyme gene using the polymerase chain reaction or to measure enzymic activity directly in a very small number of isolated fetal cells.

Of related interest is the observed increased activity of some lysosomal enzymes in the serum of pregnant women compared with the activity in the same women when not pregnant—for example, iduronate sulphate sulphatase (the enzyme defective in Hunter’s disease). If a fetus is affected with Hunter’s disease, however, no increase in this enzyme is found in the maternal serum, whereas with a normal or heterozygous fetus the rise is observed. The origin of this increased activity needs further investigation as does the whole subject of fetal material in the maternal circulation.

Testing of preimplantation embryos

Another way of avoiding the development of a fetus carrying a genetic defect is to diagnose the defect in preimplantation embryos and only transfer unaffected embryos to the uterus.79 Recently important ethical and practical steps have been taken towards making this a feasible proposition for some genetic defects. The acceptance by the British parliament of a clause in the Human Fertilization and Embryology Bill permitting carefully regulated research on embryos up to 14 days from conception has removed the legal constraint. It is now clear that it is technically possible to remove one or two cells for analysis from a human embryo obtained by in vitro fertilisation or uterine lavage without affecting the development of the fetus.30 31 Therefore if the microanalytical techniques developed for other small fetal samples can be applied to this material it will be possible to detect genetic defects at the levels of the chromosome, DNA, or gene product—that is, enzyme or specific protein in preimplantation embryos. To date this strategy has been used successfully for X linked disorders by sexing embryos using Y specific DNA amplification and only transferring female embryos.32 Two couples at risk for adrenoleukodystrophy and X linked mental retardation have given birth to female dizygotic twins after sexing of in vitro fertilised preimplantation embryos and transfer of female embryos, although one baby was stillborn. At least three other pregnancies have been monitored after this form of prenatal diagnosis.

The X linked enzymeopathy, Lesch-Nyhan syndrome, resulting from a deficiency of hypoxanthine/guanine phosphoribosyl transferase, is a candidate for this approach to prenatal diagnosis. The activity of this enzyme can be measured in cultures derived from a single blastomere from a mouse preimplantation embryo by a very sensitive assay using a radio-labelled substrate.33 Unfortunately the enzyme does not appear to be expressed at a sufficiently high level in corresponding cultures of human blastomeres to distinguish carried over maternal activity from fetal activity, thereby excluding prenatal diagnosis by enzyme assay on preimplantation embryos. The levels of several other enzymes of clinical relevance, for example, hexosaminidase have also been found to be low at this stage of development.

DNA analysis may be possible for specific human mutations after amplification of the DNA in the blastomere (approximately 6 pg of DNA); β thalassaemia can be diagnosed by amplification of DNA from a single blastomere from a mouse preimplantation embryo.34

An exciting new extension of this procedure to unfertilised human oocytes and to the first polar bodies isolated from them offers the possibility of detection of defects before fertilisation, with the benefit of not testing the embryo or fetus at any stage.35 Preliminary results have shown that it is possible to amplify the DNA sequence containing the sickle cell mutation from human oocytes and polar bodies. Further work is necessary to show whether both sequences are amplified in a heterozygote.

Fetal treatment of enzymic defects

For a small number of enzyme defects treatment in utero is an alternative to prenatal diagnosis and the option of termination.36 This may take the form of vitamin supplementation for disorders responsive to additional cofactor, for example, cobalamin E disease, dietary control as in phenylketonuria, or drug administration to suppress a harmful secondary metabolic activity resulting from the primary enzymic defect, for example, the suppression by gluco-corticoid of adrenal production of adrenocorticosteroids in adrenal hyperplasia (due to a deficiency of steroid 21-hydroxylase). In theory the earlier in pregnancy the diagnosis is made and treatment started, the more effective it should be. The reliable diagnosis of adrenal hyperplasia in the first trimester by DNA analysis permits initiation of treatment at about 9–10 weeks before masculinisation of the genitalia begins. A recent survey of 15 female infants with congenital adrenal hyperplasia who had been treated prenatally from the first trimester, however, shows that only five had normal genitalia.37 Many factors affect the efficacy of the administered hormone in the fetus and close monitoring of the pregnancy is necessary to avoid complications. These observations emphasise the need for more detailed knowledge of the biochemical events in fetal development to formulate rational chemical strategies for fetal treatment.

Addendum to list of enzyme defects that have been diagnosed prenatally

Considerable progress has been made in clarifying the enzymological disorders and in developing methods for their prenatal diagnosis in the first trimester.38 39 Prenatal diagnosis has been carried out for non-ketotic hyperglycinaemia (McKusick No 23830) in chorionic villi by enzymatic analysis of the glycine cleavage system38 and for Canavan’s disease (McKusick No 27190) (R Matalon, personal communication). A pregnancy at risk for the newborn disease of methyloneuraminidase due to a deficiency of aromatic L-amino acid decarboxylase (EC 4.1.1.28)41 has been monitored by analysis
of fetal liver. Two disorders where the methodology for prenatal diagnosis would appear to be available are the defect of mitochondrial fatty acid β-oxidation due to a deficiency of long chain 3-hydroxyacyl-CoA dehydrogenase 43 and arypical phenylketonuria due to a deficiency of glutamyl transpeptidase cyclohydrolase I (Mckusick No 23391). 44