CURRENT TOPIC

Prenatal diagnosis of enzyme defects

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About 250 enzyme deficiencies have been demonstrated in the 750 or so recognised human recessive disorders.¹ Approximately 100 of these enzymopathies can be diagnosed prenatally. Undoubtedly this number will increase as more methods are developed for the detection of enzymic defects in fetal samples (particularly in chorionic villus samples obtained in the first trimester). There are two main limitations to the application of the enzyme assay procedures used in postnatal diagnosis to fetal samples. Firstly the amount of material available for investigation is very small. Therefore the enzymic assay procedure has to be very sensitive, which may necessitate modification of postnatal methods. Secondly the enzyme under investigation may not be expressed in fetal samples that are readily accessible, for example, chorionic villi or cultured amniotic cells, because it is tissue specific. Although sampling of some specialised tissues is possible, the enzyme may still not be expressed at a sufficiently early stage of gestation to allow diagnosis and termination. These problems can, in principle, be overcome by the use of recombinant DNA technology, which detects or tracks mutations in genomic DNA. As this does not require expression of the enzyme protein, it can be applied to any nucleated cell. Currently, however, DNA probes are being used for the prenatal diagnosis of a very small number of enzymic defects (see appendix) and modifications of conventional assay procedures are used predominantly. The expansion of DNA analysis depends upon progress in cloning genes encoding enzymes and in elucidation of the molecular genetic basis of enzymopathies. This article will review the range of enzymic defects that can be detected prenatally and discuss how the method of detection depends upon the molecular basis of the defect, the tissue and subcellular site of action of the enzyme, and the nature of the fetal biopsy available.

Indications for prenatal diagnosis of an enzyme defect

Prenatal diagnosis of an enzymic defect is generally only carried out for pregnancies known to be at high risk for a severe disorder for which there is no effective treatment. Most requests for prenatal monitoring of a pregnancy will be from parents who have previously had a child with an enzyme deficiency and for whom termination of the pregnancy is acceptable. In

the case of X linked disorders, a woman who has an affected brother or whose sister has given birth to an affected child may also request testing. The reliability of the test is crucial and these tests should only be carried out in experienced laboratories. The precise diagnosis of the enzymic defect in the index case is a prerequisite for undertaking such investigations. Because it is selective, prenatal diagnosis of enzymic defects is a highly effective preventative measure. Experience from our laboratory (unpublished observations) and others,²⁻⁴ shows that 18-25% of the fetuses tested are affected with the suspected defect, very close to the expected 25%. If a pregnancy is terminated as a result of testing, it is important to confirm the diagnosis on the aborted fetus to assess the efficiency of the diagnostic procedure and to reassure the parents. If possible a cell culture should be established and specimens preserved from the fetus for future reference. Similarly prediction of an unaffected fetus can be confirmed postnatally by biochemical tests but this is usually only carried out now at the parents' request or if there is any concern over the health of the baby.

Molecular basis and heterogeneity of enzymic defects

A complex sequence of events takes place before an enzyme can function correctly *in vivo*. The gene encoding the enzyme has to be transcribed in a controlled manner and the RNA transcript edited before translation into a precursor enzymic protein. Subsequently most newly synthesised proteins undergo some form of post-translational modification or maturation before or during transport to a specific intracellular or extracellular site of action. The mature enzymic protein may require a cofactor or association with one or more proteins, encoded by other genes, to be fully active. The complexity of the biosynthesis and intracellular localisation of an enzyme affords many opportunities for genetic errors. Accordingly an enzyme deficiency leading to a characteristic pathological phenotype may be due to several different underlying molecular defects. In many disorders there is residual enzymic activity, the amount of which may be related to the age of onset or severity of the disease. It is generally possible to differentiate prenatally among the variant forms of a disease due to different mutations if the molecular basis of the defect has been clearly

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established in the index case. However this will often involve a more elaborate detection procedure than a simple assay of enzymic activity in a crude tissue homogenate.

Fetal sampling

The demonstration of an enzymic defect requires removal of fetal material for biochemical analysis. This is carried out under the guidance of ultrasonography or by fetoscopy.

CHORIONIC VILLUS SAMPLING

Chorionic villus sampling without ultrasound was first used in China in 1975 for diagnostic fetal sexing,⁵ but the first measurement of enzymic activities in chorionic villi was in 1982.⁶ Currently chorionic villus sampling is carried out with the assistance of ultrasonography by transcervical aspiration⁷ or biopsy with forceps⁸ between eight and 12 weeks' gestation but optimally between nine and 10 weeks. Transabdominal aspiration is an alternative that decreases the risk of infection and permits sampling between 12 and 14 weeks obviating waiting for amniocentesis at 16 weeks or later. The risk to pregnancy of chorionic villus sampling is 2–4%, about 2% higher than that for spontaneous abortion and about twice that for amniocentesis.^{9–11} As more centres gain experience of chorionic villus sampling this risk may decrease. Over the last three years chorionic villus sampling has become more widespread and popular because of the advantage of early diagnosis. This has stimulated the development of suitable enzyme assay procedures. A major problem is the contamination of the chorionic villi biopsy specimen with maternal material. The sample should be examined under a low power dissecting microscope and maternal material, mainly decidua, removed. A typical sample of 10–40 mg wet weight will provide enough material for the assay of the enzyme under investigation and a reference enzyme and for the measurement of protein. All the enzymic defects that have been detected in cultured amniotic fluid cells can be detected in chorionic villi cells. Most enzymes can be assayed directly in a homogenate of the biopsy specimen enabling a diagnosis to be made within one to three days of sampling. For a few enzymes, whose activity is very low in normal chorionic villi, it is necessary to obtain a culture of cells from the sample,^{2 3 12} thereby delaying the result for two to three weeks. In contrast the detection of a deficiency of arylsulphatase A or B for metachromatic leucodystrophy and Maroteaux-Lamy disease respectively is difficult in chorionic villi because of the very high activity of a competing enzyme arylsulphatase C in trophoblasts.¹³ Until recently the secondary multiple lysosomal enzyme deficiency in mucopolipidosis II (I-cell) and mucopolipidosis III (pseudo-Hurler dystrophy) could only be demonstrated in cultured chorionic villi cells. Ben Yoseph *et al* have shown that it is possible to detect the primary defect of N-acetylglucosamine 1-phosphotransferase directly in chorionic villi obtained at nine weeks.¹⁴

Fresh intact chorionic villi can also be used to study enzymic activities by the uptake and utilisation of radioactively labelled metabolic precursors and for the extraction of DNA for diagnosis using recombinant DNA techniques (see below). For pregnancies at high risk chorionic villus sampling has become the method of choice because most physicians and parents consider the advantage of early diagnosis to compensate for the increased risk. Transabdominal removal of chorionic villi from the placenta has been successfully carried out in the second and third trimester for diagnosis of genetic abnormalities by direct karyotyping without cell culture.¹⁵ Such samples could also be used for enzymic assays.

AMNIOCENTESIS

Enzymic defects can be investigated both in amniotic fluid by detection of specific metabolites and in cultured amniotic fluid cells by enzyme assay or incorporation studies. Amniocentesis under the guidance of ultrasonography is a well established and reliable procedure with a risk of miscarriage of about 0.5% in centres with experienced staff.¹⁶ A sample of 10–20 ml of amniotic fluid is removed by transabdominal puncture at 15–16 weeks' gestation, the optimal time with regard to cell number and viability. The amniotic fluid and cells are separated and a culture initiated of the cells. Amniotic cells cannot be used directly because many are non-viable and in various states of decay. It can take up to six weeks to obtain sufficient viable cells for some enzyme determinations but three to four weeks is more usual. Experience of cell culture, especially of amniotic cells, is necessary to assess the state of readiness of the cultures for biochemical analysis.¹⁷ Appropriate controls must always be available and analysed at the same time. A wide range of enzymic defects has been diagnosed by analysis of amniotic material. Although most of these disorders can now be diagnosed on chorionic villus samples, amniocentesis will still be widely used until more centres gain experience of chorionic villus sampling, for late presentations and because of the lower risk associated. In some situations a diagnosis made in the first trimester by chorionic villus sampling, particularly of an unaffected fetus, is confirmed by amniocentesis. DNA can be extracted from cultured amniotic fluid cells for analysis.

FETAL BLOOD AND TISSUES

Some enzymopathies cannot be diagnosed in chorionic villi or cultured cells from chorionic villi or amniotic fluid because the affected enzyme is not expressed in these cells. Examples are the liver-specific enzymes of the urea cycle. Some specific fetal tissues can be obtained for biochemical analysis but considerable experience and expertise are needed for these procedures.¹⁸

Fetal blood may be obtained by puncture of vessels in the fetal heart, umbilical cord, or chorionic plate under the guidance of ultrasonography or by fetoscopy. Currently cordocentesis guided by ultrasound at 18–20 weeks' gestation

is recommended.^{19 20} Immunodeficiencies due to enzyme defects have been diagnosed by measurement of enzymes in fetal erythrocytes and/or metabolites in blood.²¹ Adenosine deaminase, the enzyme involved in about 20% of cases of severe combined immunodeficiency, can also be measured in chorionic villi.²² Sometimes relatively high residual activity is found and the diagnosis has to be confirmed on fetal blood. A deficiency of tetrahydrobiopterin has been diagnosed by assaying the enzyme 6 pyruvoyl tetrahydropterin synthase in fetal erythrocytes, together with demonstration of pterins in amniotic fluid.²³ White blood cells are used extensively for the postnatal diagnosis of enzyme defects and fetal white blood cells could be used for the same tests. Very little material is required and the procedure would be rapid because cell culture is not necessary. The risk and lateness for an elective abortion preclude this approach except under special circumstances, such as a failed amniocentesis or a very late request for prenatal testing of a high risk pregnancy.

Fetal liver biopsies taken after 19 weeks' gestation by aspiration under fetoscopic or ultrasound guidance have been used for the prenatal diagnosis of several liver specific enzyme defects: ornithine carbamoyltransferase,²⁴ carbamoyl phosphate synthase I,²⁵ glucose-6-phosphatase type Ia (von Gierke's disease),²⁶ and the peroxisomal disorder, hyperoxaluria type I due to alanine:glyoxylate aminotransferase deficiency.²⁷ Ornithine carbamoyltransferase deficiency can now be diagnosed in chorionic villus samples using a gene specific DNA probe.²⁸ It is predicted that the technique of liver biopsy for enzymic defects will become obsolete as more liver enzyme probes become available.

Fetal skin biopsies may be obtained by fetoscopy under real time ultrasound guidance at 18–21 weeks' gestation.²⁹ Most skin disorders are diagnosed prenatally by histological and ultrastructural studies of the fetal skin biopsy specimens because the underlying molecular defect is unknown. Where an enzyme is involved, as in Ehlers-Danlos syndrome type VI, Fabry's disease, Refsum's disease, or X linked ichthyosis diagnosis can be made on chorionic villi or cultured amniotic fluid cells.

Methods of detecting an enzymic deficiency DIRECT ASSAY

The simplest and most direct way of demonstrating an enzyme defect is to assay the enzymic activity in a crude tissue homogenate using a specific substrate under optimal conditions. For many enzymes it is possible to use synthetic substrates, which combine ease of measurement with the greater sensitivity necessary for fetal samples—for example, the fluorogenic 4-methylumbelliferyl conjugates widely used for the assay of lysosomal hydrolases. For other enzymes it is essential to use a natural substrate because of the strict specificity of the enzyme or because it discriminates among closely related activities.³⁰ In some assays the product is measured indirectly by a coupled

enzymic reaction—for example, the release of glucose by debranching enzyme in amniocytes.³¹ All soluble enzymes can be assayed directly in tissue homogenates but membrane-associated enzymes have to be released by a detergent, which may also act to solubilise a lipophilic substrate.

The amount of enzymic activity is related either to soluble protein or to the activity of a comparable unaffected enzyme with the same subcellular location (or more rarely to a number of cells). A complete deficiency of the enzyme is usually obvious but it is essential to assay material from the index case and a control at the same time. The reference range for normal controls should be established before offering the test. If the activity is well below the normal range but greater than that in the index case it is important to exclude certain situations before concluding that the fetus is a carrier of a recessive trait and therefore unaffected. The low residual activity could be due to a mutant enzyme or to a genetically determined pseudo deficiency, both of which can generally be recognised by characterisation of the index case and family. Another possibility is that a related but unaffected enzyme also acts on the same substrate. In Tay-Sachs disease, which is due to a deficiency of hexosaminidase A, there is considerable residual hexosaminidase activity in chorionic villi or cultured amniotic cells due to the isoenzyme hexosaminidase B. The true deficiency of hexosaminidase A can be measured by a differential assay based on a difference in thermal stability of the two isoenzymes,³² by the use of a specific substrate,³³ or by chromatographic or electrophoretic separation of the two isoenzymes.³⁴ In the AB variant of GM₂ gangliosidosis there is normal hexosaminidase A and B activity towards synthetic substrates but gangliosides are not catabolised *in vivo* because of the deficiency of an activator protein. This situation can be diagnosed prenatally but emphasises the need for clear diagnosis of the index case.³⁵

The diagnosis of primary hyperoxaluria type I by the assay of the peroxisomal enzyme, alanine:glyoxylate aminotransferase in fetal liver homogenates, is complicated by the presence of the cytosolic enzyme, glutamate:glyoxylate aminotransferase, which has activity towards the same substrate.²⁷ As there is not enough material in such a biopsy specimen to measure both enzymes, a correction based on the activity of the competing enzyme in control fetal livers is made. Subcellular fractionation is an alternative solution to the problem of isoenzymes in different subcellular compartments. The maintenance of the integrity of an organelle is necessary for the detection of some enzymic defects. The activity of some enzymes is regulated *in vivo* by transport of substrates or products across a membrane. A defect in the transport process will only be seen in intact organelles and not a crude tissue homogenate, where the barrier between enzyme and substrate is destroyed. The distinction between glycogen storage disease Ia (GSD Ia von Gierke), in which the enzyme glucose 6-phosphatase is defective and GSD Ib and Ic, in which transport

proteins are defective, could be made by assaying the glucose 6-phosphatase in homogenates and microsomes of fetal liver.²⁶ The activity of a non-specific phosphatase also has to be taken into account in diagnosing these disorders.

In Zellweger's syndrome there is an absence or deficiency of peroxisomes and the marker peroxisomal enzyme, catalase, is localised in the cytosol. This observation can be exploited in several ways for prenatal diagnosis of Zellweger's syndrome by establishing whether (i) catalase is sedimented with organelles after centrifugation of amniocytes or remains in the cytosolic fraction,³⁶ (ii) the latency of catalase in digitonin-permeabilised, cultured chorionic villi fibroblasts is the same as that for a cytosolic enzyme or for a peroxisomal enzyme,³⁷ and (iii) peroxisomes can be visualised directly in intact chorionic villi by a cytochemical stain for catalase.³⁸ These techniques also indicate whether there is a general defect of peroxisomes or a defect in a specific peroxisomal component. Visual discrimination between normal villi and villi from a fetus with Tay-Sachs disease has also been shown by fluorescent cytochemical technique for hexosaminidase A.³³ These approaches could be used to investigate other organelle-specific enzyme dysfunctions.

IMMUNODETECTION

The specificity of antibodies can be used in two ways to investigate enzymic defects. The amount of a particular enzyme activity can be measured selectively by precipitation with monospecific antiserum in the presence of other enzymes acting on the same substrate. Diagnosis of Pompe's disease (glycogenosis II) has been made in chorionic villi by measuring the true acid maltase with antibodies in the presence of other α -glucosidases.³⁹ Alternatively the presence or absence of an enzyme protein as opposed to its activity can be monitored by immunoblotting (western) or immunocytochemistry. Two enzymes involved in peroxisomal β -oxidation were shown by immunoblotting to be absent from chorionic villi from fetuses with Zellweger's syndrome.⁴⁰ A deficiency of immunoreactive alanine:glyoxylate aminotransferase protein was demonstrated by immunoblotting in extracts and subcellular fractions of liver from a fetus with primary hyperoxaluria type I and in peroxisomes directly by protein A-gold immunoelectronmicroscopy.²⁷ The ultrastructural technique can detect disorders due to the incorrect subcellular localisation of an enzyme, which could not be detected by immunoblotting or assay of a total tissue extract. All immunochemical techniques depend crucially on having monospecific or monoclonal antibodies.

PRECURSOR UTILISATION

The activity of an enzyme may be measured indirectly by the utilisation or incorporation of a radioactively labelled precursor in living chorionic villi or cultured amniotic cells. This procedure has been useful for the diagnosis of several organic acidurias, aminoacidopathies,

and defects of nucleic acid metabolism. The incorporation of the precursor, 1-¹⁴C citrulline, for the measurement of arginosuccinate lyase in argininosuccinicaciduria is related to incorporation of ³H leucine into protein.⁴¹ For other defects the release of ¹⁴C carbon dioxide from the precursor, for example, 1-¹⁴C octanoate, for medium chain acyl coenzyme A dehydrogenase is related to protein.⁴² However discrepancies have been observed between enzyme activities measured directly and metabolically and confirmation is often required by the identification of accumulating metabolite in the cells or amniotic fluid.

METABOLITE ACCUMULATION

Advances in high performance liquid chromatography, gas chromatography combined with mass spectrometry, electrophoresis, and high performance thin layer chromatography have enhanced the identification and quantitative measurement of specific metabolites in body fluids and cell extracts. Stable isotope analysis with gas chromatography-mass spectrometry detection is a sensitive and selective method, very suitable for prenatal diagnosis. The identification by various methods of a specific metabolite or group of compounds in amniotic fluid obtained at 16–20 weeks has been used extensively to diagnose organic acidurias,⁴³ aminoacidopathies,⁴⁴ and mucopolysaccharidoses.⁴⁵ Most of these disorders can now be diagnosed by direct assay of the relevant enzyme in chorionic villi but metabolite identification in amniotic fluid is often necessary when the first trimester diagnosis is based on uptake of labelled precursor by intact chorionic villi.

RECOMBINANT DNA TECHNIQUES

Much of the pioneering work on the application of recombinant DNA technology to prenatal diagnosis was carried out on the haemoglobinopathies and thalassaemias. The techniques developed in these studies are now being applied to enzyme defects as the genes encoding the affected enzymes are cloned.^{46–47} Currently the genes for about 40 enzymes involved in metabolic disorders have been cloned,⁴⁸ but only a few of those clones have been used for prenatal diagnosis. Cloned or chemically synthesised DNA sequences encoding all or part of an enzyme gene, gene specific probes, are used to detect or track mutations in genomic DNA associated with the enzymic defect. Differences between the normal and mutant DNA are manifested as different patterns of restriction fragments of DNA in Southern blots analysed with a radioactively labelled gene specific probe. The patterns are different because a mutation in the gene or in a sequence closely linked to the gene has created or obliterated a site recognised by a restriction endonuclease. The theory behind this approach and the methodology are described in detail elsewhere.^{46–49}

The essential requirements for prenatal diagnosis of enzymic defects by DNA analysis are careful family studies in cooperation with a clinical geneticist, the probes, DNA from a fetal

sample, and a laboratory experienced in the use and interpretation of these techniques. A minimum of 10 µg of DNA is required for analysis. This can be extracted from chorionic villi (≈10 µg of DNA/10 mg wet weight) or cultured cells from the chorionic villi or amniotic fluid (≈6 µg/10⁶ cells). Gene specific probes can be used to detect gross abnormalities in genes, such as deletions, insertions, and rearrangements, or, point mutations due to changes in one or a small number of nucleotides.

Detection of gross alterations in a gene

Gross abnormalities are the simplest to detect using probes. They can be useful in correlating a section of a gene with a particular function of the enzyme and perhaps consequent phenotypes. Deletions are uncommon in single copy genes but several have been found in the genes encoding enzymes responsible for genetic disorders⁴⁸ and could be used diagnostically within families. A deletion on the short arm of chromosome X was shown to be responsible for a deficiency of glycerol kinase in an aborted fetus.⁵⁰

Detection of a disease-causing mutation

A single base change can affect the transcription and translation of a gene or produce an enzyme protein with a single amino acid change that affects the function of the enzyme. If this base change creates or destroys a restriction endonuclease site, it might be possible to detect it directly by Southern blotting with a gene specific probe. Fewer than about 10% of point mutations can be detected in this way, however, because of the specific structure of restriction endonuclease sites. A mutation in the α-fucosidase gene that obliterates an EcoRI restriction site appears to be the underlying molecular defect in fucosidosis in one group of patients.⁵¹ It is being used to monitor pregnancies at risk for fucosidosis.

Allele-specific oligonucleotides

An alternative method of detecting point mutations can be used if the mutation is known precisely. Two oligonucleotides of about 20 bases are synthesised: one exactly complementary to the normal gene sequence and the other to the mutant gene sequence. By choosing hybridisation conditions where only the 'normal' oligonucleotide will bind to the normal gene sequence and only the 'mutant' oligonucleotide to the mutant gene sequence—that is, complete complementarity is required—it is possible to detect the presence of the two alleles specifically. Allele specific oligonucleotides will be very useful for prenatal diagnosis within families where the mutation has been established. A four base pair insertion in exon 11 of the α-chain of β-hexosaminidase, which has been shown to be the major defect (70% of carriers) in Ashkenazi Jews with Tay-Sachs disease can be detected using two oligonucleotide probes corresponding to the normal and mutant sequences.⁵² By constructing a panel of allele specific oligonucleotides for known mutations in an enzyme gene, new cases of known defects and new but unknown mutations could be recognised pre-

nately. Analysis with oligonucleotide probes can be carried out with 1 µg of DNA, theoretically eliminating the need to culture amniocytes.

A recent development that will be particularly useful for prenatal diagnosis because the amount of DNA is very limited and speed is important, is amplification of a specific DNA sequence by the polymerase chain reaction.⁵³ A 10⁶-fold amplification can be achieved in a few hours using automated equipment. The combination of allele specific probes and polymerase chain reaction of chorionic villi DNA offers a fast, specific, and early diagnosis of known enzymic mutations.

Tracking mutations by linkage to a restriction fragment length polymorphism

It is also possible to detect or track a mutant gene without knowing the nature of the molecular defect by linkage analysis based on DNA polymorphisms. Normal variation occurs frequently in the human genome. This can generate alleles, which give rise to different lengths of DNA when digested with a restriction endonuclease and analysed by Southern blotting. Such restriction fragment length polymorphisms can be used to track a mutant gene if a polymorphism is in the chromosome with the mutant gene but not in the normal gene or vice versa. As these polymorphisms are common there are usually several within a gene or very close to it—that is, they are tightly linked to it and will be detected by Southern blot analysis using the gene specific probe. It should be emphasised that the polymorphism is not the mutation causing the enzyme defect but it can be used to track or identify the mutant gene. Several enzymic defects have been diagnosed prenatally using restriction fragment length polymorphisms in DNA from chorionic villi or cultured amniocytes (see appendix). DNA from the index case, parents and some other members of the family must be analysed to establish the phase between a polymorphism and the mutant gene—that is, whether the polymorphism is on the normal or mutant chromosome. The parents must be heterozygous and the true parents.

Ten independent restriction fragment length polymorphisms have been found linked to the phenylalanine hydroxylase locus. Appropriate haplotypes, that is, a combination of polymorphism and mutant gene, have been found for most families with a phenylketonuria mutation, permitting detection of carriers and diagnosis of affected individuals. It has been found that most cases can be diagnosed prenatally using three restriction enzymes (polymorphisms).⁵⁴ Prenatal diagnosis by DNA analysis of phenylketonuria and the X linked ornithine carbamoyl transferase deficiency⁵⁵ is very valuable because these enzymes are only expressed in tissues not easily accessible to fetal sampling.

For many common genetic disorders the underlying gene defect is unknown but DNA polymorphisms can still be used to track the mutant gene if tight linkage can be shown between the disorder and a polymorphism detected with an adjacent marker DNA probe. This approach can be used for an enzyme defect where the enzyme gene has not been cloned but

it is known to be closely linked to well defined markers that have been cloned. The enzyme, steroid 21-hydroxylase, a deficiency of which is the most common cause of congenital adrenal hyperplasia, is situated within the major histocompatibility complex. This disorder can now be diagnosed in the first trimester using HLA-DNA probes and linkage analysis together with 17-hydroxyprogesterone determination.⁵⁶ Previously prenatal diagnosis was based on HLA serotyping of cultured amniocytes and measurement of 17-hydroxyprogesterone in amniotic fluid.

Many of the new developments in DNA diagnostics will be applied to prenatal diagnosis. They include the more widespread use of non-radioactive probes exploiting fluorescence, chemiluminescence, or the high affinity between biotin and streptavidin or avidin linked to an indicator enzyme, full automation of DNA extraction, and analysis and detection of mutations in solution without immobilisation of nucleic acid.⁵⁷

Future developments

The greater availability and utilisation of chorionic villus sampling will increase the

number of enzyme defects that are diagnosed in the first trimester. This will offer families the advantages of early diagnosis and more safe and reliable termination of a pregnancy. DNA analysis will make possible the early diagnosis of many of those disorders previously only able to be diagnosed in the second trimester. The possibility of diagnosis of genetic errors, including enzymopathies, in preimplantation embryos is being investigated.⁵⁸⁻⁵⁹ Diagnosis of Lesch-Nyhan syndrome by enzyme assays and the sexing of embryos using Y specific DNA probes are technically feasible. The low rate of implantation and other embryological factors present considerable problems, however, apart from ethical considerations. Another situation with ethical implications and technical complications is prenatal diagnosis in multiple pregnancies and the possibility of selective termination. This has been carried out after amniocentesis and chorionic villus sampling.⁶⁰ Recently a twin pregnancy at risk for sialic acid storage disease was investigated in our laboratory. On the basis of a direct biochemical assay for sialic acid and morphological examination of chorionic villi, selective fetocide was carried out.⁶¹

The list of enzyme defects that can be diagnosed prenatally grows inexorably.⁶² Most of these are included in the following appendix.

Appendix

Enzyme defects that have been diagnosed prenatally

Within each section the names of the diseases are in alphabetical order. Unless otherwise indicated diagnosis is based on measurement of enzyme activity or metabolites in chorionic villi, amniotic fluid, or cultured amniotic cells. (*Fetal blood or liver; †newly recognised enzyme defects for which methodology is available; ‡DNA analysis possible.)

Disorder	McKusick No	Defect
AMINO ACID AND ORGANIC ACID METABOLISM		
Canavan†	27190	Aspartoacylase
Cystinosis	21980	Cystine transport protein
Glutaric aciduria type I	23167	Glutaryl-CoA-dehydrogenase
Glutaric aciduria type IIA	30595	Multiple acyl-CoA dehydrogenases
Glutaric aciduria type IIB	23168	Multiple acyl-CoA dehydrogenases
Gyrate atrophy‡	25887	Ornithine aminotransferase
Homocystinuria	23620	Cystathionine β -synthase
3-Hydroxy-3-methylglutaryl CoA lyase deficiency	24645	
Maple syrup urine	24860	Branched chain ketoacid decarboxylase
Methyl malonic aciduria:		
Vitamin B12 non-responsive	25100	Methylmalonyl-CoA mutase
Vitamin B12 responsive	25110	Adenosylcobalamin synthesis
Mevalonic aciduria	25117	Mevalonate kinase
Methylene tetrahydrofolate reductase deficiency	23625	
Multiple carboxylase deficiency	25327	Holocarboxylase synthase
Oculocutaneous albinism	20310	Tyrosinase
Phenylketonuria (PKU I)‡	26160	Phenylalanine hydroxylase
Phenylketonuria (PKU II)‡	26163	Dihydropteridine reductase
Phenylketonuria (PKU III)*	26164	Tetrahydrobiopterin synthesis, 6-pyruvoyl tetrahydropterin synthase

Propionic acidaemia	23200	Propionic CoA carboxylase
Pyroglutamic aciduria	26613	Glutathione synthase
Tyrosinaemia I	27670	Fumarylacetoacetate hydrolase
UREA CYCLE ENZYMES		
Argininosuccinic aciduria	20790	Argininosuccinate lyase
Carbamoyl phosphate synthase deficiency	23730	
Citrullinaemia	21570	Argininosuccinate synthase
Ornithine carbamoyltransferase deficiency*‡	31125	
CARBOHYDRATE METABOLISM		
Fructose-1:6 diphosphatase deficiency	22970	
Galactosaemia	23040	Galactose-1-phosphate uridyltransferase
Galactosaemia	23020	Galactokinase
Glycerol kinase deficiency‡	30703	
Glycogenoses		
Type Ia von Gierke*	23220	Glucose 6-phosphatase
Type II Pompe	23230	Acid α -glucosidase (acid maltase)
Type III Cori	23240	Amylo-1,6-glucosidase (debrancher enzyme)
Type IV	23250	α -1, 4-Glucan: α -1,4 glucan 6-glucosyltransferase (brancher enzyme)
Pyruvate carboxylase deficiency	26615	
STEROID METABOLISM		
X linked ichthyosis‡	30810	Steroid sulphatase
Congenital adrenal hyperplasiaIII‡	20191	Steroid 21-hydroxylase
Congenital adrenal hyperplasia IV	20201	Steroid 11- β -hydroxylase
PEROXISOMAL AND MITOCHONDRIAL DISORDERS		
Zellweger	21410	Multiple enzyme deficiencies
Infantile Refsum	26651	
Neonatal adrenoleucodystrophy	20237	
Rhizomelic chondrodysplasia punctata	21510	Acyl-CoA: dihydroxyacetone phosphate acyltransferase and alkyl dihydroxyacetone phosphate synthase
X linked adrenoleucodystrophy‡	30010	Very long chain acyl-CoA synthase
Other defects in peroxisomal β -oxidation including Pseudo-Zellweger		
Zellweger	26151	3-Oxoacyl-CoA thiolase
Primary hyperoxaluria type I*	25990	Alanine: glyoxylate aminotransferase
Refsum (classical)	26650	Phytanic acid α -hydroxylase
Cytochrome C oxidase deficiency	22011	
Medium chain acyl-CoA dehydrogenase deficiency	20145	
LYSOSOMAL STORAGE DISEASES		
<i>Sphingolipidoses and neutral lipidoses</i>		
Fabry‡	30150	α -Galactosidase A
Farber	22800	Ceramidase
Gaucher:		
Type I	23080	Glucocerebrosidase
Type II	23090	
Type III	23100	
G _{M1} Gangliosidosis:		
Type I (Infantile)	23050	β -Galactosidase
Type II (Juvenile)	23060	
G _{M2} Gangliosidosis:		
Tay-Sachs	27280	β -Hexosaminidase A
Sandhoff	26880	β -Hexosaminidase A and B
AB variant	27275	G _{M2} -ganglioside activator protein
Krabbe	24520	Galactosylceramide β -galactosidase
Metachromatic leucodystrophy	25010	Arylsulphatase A or activator protein
Mucopolipidosis type II (I-cell)	25250	N-Acetylglucosamine 1-phosphotransferase
Mucopolipidosis type III (pseudo-Hurler polydystrophy)	25260	
Mucopolipidosis IV	25265	Ganglioside neuraminidase
Sulphatidosis	27220	Multiple sulphatases

Niemann-Pick:		
Type A, B	25720	Sphingomyelinase
Type C	25722	Cholesterol esterification
Cholesterol ester storage	21500	Acid lipase
Wolman	27800	
<i>Glycoprotein metabolism</i>		
Aspartylglucosaminuria	20840	N-aspartyl- β glucosaminidase
Fucosidosis	23000	α -Fucosidase
Galactosialidosis	25654	β -galactosidase and α -neuraminidase protective protein
α -Mannosidosis	24850	α -Mannosidase
β -Mannosidosis†	24851	β -Mannosidase
Mucopolipidosis I (Sialidosis)	25655	α -Neuraminidase
α -N-acetylgalactosaminidase deficiency†	10417	
Salla	26874	Lysosomal transport of sialic acid
Sialic acid storage	26992	
<i>Mucopolysaccharidoses</i>		
Hurler/Scheie (MPS I)	25280	α -Iduronidase
Hunter (MPS II)	30990	Iduronate sulphate sulphatase
Sanfilippo:		
A (MPS IIIA)	25290	Heparan sulphamidase
B (MPS IIIB)	25292	N-acetyl α -glucosaminidase
C (MPS IIIC)	25293	Acetyl-CoA: α -glucosaminide N-acetyltransferase
D (MPS IIID)	25294	N-acetylglucosamine-6-sulphate sulphatase
Morquio		
A (MPS IVA)	25300	N-acetylgalactosamine 6-sulphate sulphatase
B (MPS IVB)	25301	β -Galactosidase
Maroteaux-Lamy (MPS VI)	25320	Arylsulphatase B (N-acetylgalactosamine 4-sulphate sulphatase)
Sly (MPS VII)	25322	β -Glucuronidase
PHOSPHATASES		
Acid phosphatase deficiency	20095	Lysosomal phosphatase
Hypophosphatasia	24150	Bone/liver alkaline phosphatase
NUCLEIC ACID METABOLISM		
Combined immunodeficiency	10270	Adenosine deaminase
Lesch-Nyhan‡	30800	Hypoxanthine guanine phosphoribosyltransferase
T cell immunodeficiency	16405	Purine nucleoside phosphorylase
Xanthine/sulphite oxidase	25215	Molybdenum cofactor
Xeroderma pigmentosum	27872	DNA excision and repair
HAEMATOLOGICAL DISORDERS		
Acute intermittent porphyria	17600	Porphobilinogen deaminase
Congenital haemolytic anaemia	17240	Glucose phosphate isomerase
Congenital erythropoietic porphyria	26370	Uroporphyrinogen III synthase
Methaemoglobinemia type II	25080	NADH-diaphorase (NADH cytochrome B5 reductase)
Triose phosphate isomerase deficiency	19045	

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