Prenatal diagnosis of ornithine carbamoyl transferase deficiency using a gene specific probe

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SUMMARY A gene specific DNA probe has been used to predict the genotype of two fetuses in families at risk for ornithine carbamoyl transferase deficiency. Although the probe does not detect the mutation directly, prediction was possible by examining restriction fragment length polymorphisms of the parents and sibs to identify the X chromosome carrying the mutation. It is suggested that in all pregnancies, regardless of the predicted outcome, the biochemical status of carrier mothers should be monitored because hyperammonaemia and arginine deficiency may have a deleterious effect on the fetus.

Ornithine carbamoyl transferase deficiency (OCTD), the commonest of the inherited disorders of the urea cycle, is an X linked disorder. Most boys have no detectable enzyme activity and develop severe hyperammonaemia in the neonatal period. Despite intensive treatment most die during the first year of life. In contrast, the clinical manifestation in heterozygous females is very varied, the variation arising because of the random inactivation of X chromosomes during fetal life. Thus, a small proportion of females become hyperammonaemic in early childhood and require careful management to control the biochemical abnormalities. Other heterozygotes have few or no symptoms so that loading tests are needed to identify them as carriers. The most sensitive method is the measurement of orotic acid in the urine after protein or alanine loading.¹ While this will detect the majority of carriers, occasionally no biochemical abnormality can be detected in an obligate heterozygote² so a negative result does not exclude heterozygosity. The difficulty for families has been compounded by the fact that until recently no simple method of prenatal diagnosis was available that could be offered to relatives who had only a low probability of being carriers. OTC activity is not present in amniocytes and prenatal diagnosis required fetal liver biopsy.³ Because the OTC activity of the fetal liver only develops during the second trimester, prenatal diagnosis had to be delayed until 18 to 20 weeks' gestation.

The cloning of the OTC (OCT*) gene^{4 5} has enabled the development of a simple test in the first trimester. It has also enabled 'gene tracking' to be used for heterozygote detection and exclusion.⁶ The identification of carriers is potentially important for other reasons as it has been suggested that the biochemical abnormalities in the mother may adversely affect the developing fetal brain.⁷ Thus, it may be important to monitor the pregnancies of carrier mothers even when it is known that the fetus is not affected, and a heterozygous fetus may be at particular risk.

We describe the prenatal exclusion of OCTD in a male and the first trimester diagnosis of a heterozygous female. In the second family, the mother was known to be intolerant of protein. During the pregnancy her biochemical status was monitored and her diet supplemented with arginine.

Methods

In family A, amniocytes were grown in Ham's F10 medium, supplemented with 20% fetal calf serum, until sufficient were available for DNA extraction. In family M, chorion villus sampling (CVS) was performed using the transcervical ultrasound guided silver Down's cannula.⁸ Approximately 20 mg of villi were obtained which was sufficient for cytogenetic and DNA analysis. Part of the sample was cultured for karyotyping.⁹

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*The IUB recommended name for this enzyme is ornithine carbamoyl transferase (EC 2.1.3.3.-OCT) superseding the previous name ornithine transcarbamylase (OTC) by which the gene is still known.

Chorionic villi and amniocytes were processed as described previously.¹⁰ For family A, 30 µg of DNA was harvested from the amniocytes. For family M, the chorionic villus sample yielded 29 µg of DNA. The extracted DNA was digested with *MspI* and the fragments blotted onto nitrocellulose after separation in a 0.8% agarose gel.¹¹

The OTC probe was a cDNA clone isolated from a liver cDNA library.⁵ This sequence is absent in a patient suffering from OTC deficiency and possessing a visible cytogenetic deletion.¹²

This particular OTC probe detects an *MspI* restriction fragment length polymorphism (RFLP) (figure) and an additional *BamHI* RFLP.¹³

Case reports and results

FAMILY A

The proband (II.1 in the figure) in this family was born at term after a normal pregnancy and during the early weeks of life tended to vomit excessively. At the age of 11 months she became lethargic and the vomiting more frequent. There was some deterioration in her developmental skills. At the age of 14 months she became more irritable and following sedation lapsed into coma. She had marked hyperammonaemia (plasma ammonia 366 µmol/l) and the plasma amino acids showed an excess of glutamine and alanine. Urine orotic acid excretion was grossly raised (up to 3520 µmol/mmol creatinine; normal <5). Her mother developed orotic aciduria after protein loading, confirming her carrier status. She was therefore advised that she had a 1 in 2 risk that her sons would have severe disease and of the need to assess any daughter carefully to make sure that she did not have, like her first child, disease that required treatment.

The mother (I.2) presented to us 11 weeks into her second pregnancy seeking fetal sexing and prenatal diagnosis if the fetus was male. A fetal liver biopsy was scheduled for 20 weeks' gestation, but blood samples were also obtained from the proband and her parents to determine if the mother was heterozygous for the *MspI* RFLP, thereby permitting prenatal diagnosis by gene tracking with the OTC probe. In the event, the family was 'informative' and so DNA was prepared from amniocytes obtained by amniocentesis at 16 weeks' gestation. Amniocyte chromosome analysis at 18 weeks revealed a normal male karyotype, 46,XY. The results of DNA analysis (figure) on cultured amniocytes were available four weeks after amniocentesis, the day before the scheduled liver biopsy.

The father (I.1) had the 6.5 kb band, the mother (I.2) had both a 6.5 and 5.8 kb band, and the

FIGURE Above. Pedigree of family A and family M indicating the segregation of the 6.5 and 5.8 kb bands that represent the *MspI* restriction fragment length polymorphism with the OTC probe. Below. The autoradiographs of the blots from each family with the variable bands (6.5 and 5.8 kb) arrowed.

proband (II.1) only a 6.5 kb band, indicating she had inherited the 6.5 allele along with the OTC deficiency mutation from her mother. DNA from the fetus revealed the 5.8 kb band indicating that this male had received the X chromosome carrying the normal OTC gene.

Fetal liver biopsy was no longer considered justified, and the pregnancy continued to term. The baby boy, born in February 1985, is fit and well.

FAMILY M

This couple first presented to us five years ago after their son (II.1) died from hyperammonaemia aged 10 days. There was no detectable OTC activity in his liver. The mother (I.2) has moderate protein intolerance, naturally adopting an average daily pro-

tein intake of only 30 g (normal > 60 g). Increased urinary excretion of orotic acid after a protein load confirmed that she was heterozygous for OCTD.

As indicated in the figure, there then followed a further five unsuccessful pregnancies: a spontaneous abortion in the first trimester of a female fetus, the abortion of two male fetuses at 19 to 20 weeks' gestation after prenatal diagnosis of OTC deficiency by liver biopsy, an abortion of a trisomy 13 fetus diagnosed after first trimester chorionic villus sample karyotyping to determine sex, and then a missed abortion detected at seven weeks.

This unfortunate woman presented again six weeks into her seventh pregnancy seeking prenatal diagnosis on any male fetus. Blood for DNA analysis was obtained from her and her husband (I.1), and, fortunately, freshly frozen postmortem liver tissue from which DNA could be obtained was available from the two previously affected male fetuses.

The results of DNA analysis (figure) showed that I.2 was heterozygous for *MspI* RFLP, and so chorionic villus sampling was performed at nine weeks' gestation with the object of analysing the fetal karyotype (maternal age 40 years) and for prenatal diagnosis of OTC deficiency by gene tracking with the OTC gene probe. The chorionic villus sample karyotype and results of DNA analysis were available 10 days later. The fetus had a normal female chromosome complement, 46,XX, and had inherited the same X chromosome from her mother as the two previously affected males; she was therefore carrying the OTC deficiency mutation. The father (I.1) had the 6.5 kb allele, I.2 had both the 6.5 and 5.8 kb alleles, and a previously affected male (II.4) and the fetus (II.7) had just the 6.5 kb band.

While delighted to be carrying a girl, the mother appreciated that there was a small chance that the baby could have significant protein intolerance, but the couple decided that since this risk was small they would continue the pregnancy. However, it was also important to avoid biochemical abnormalities caused by the mother's carrier status. Plasma ammonia and amino acids were monitored throughout her pregnancy. The plasma ammonia remained normal (less than 40 $\mu\text{mol/l}$) throughout. However, her plasma arginine concentration was low (15 $\mu\text{mol/l}$ at 15 weeks' gestation). At 20 weeks she was started on a small supplement of arginine (2 g daily) and her plasma arginine concentrations rose to the lower end of the normal range (25 to 30 $\mu\text{mol/l}$). This dose continued throughout the pregnancy and the puerperium when the mother wanted to establish breast feeding. The baby is currently well.

Discussion

The way in which the options available to family M changed in the four years after the birth of their first affected boy illustrates the remarkable progress that has been made in genetic prediction for OCTD. When first seen in the genetic clinic, all that could be offered to the couple was fetal sexing after amniocentesis and termination of all males at 18 to 20 weeks' gestation. The introduction of fetal liver biopsy for prenatal diagnosis in males³ offered them the chance to try for a healthy son and limit abortion to only those males who were affected, although this was still relatively late in pregnancy. Chorionic villus sampling permitted fetal sexing in the first trimester and exploitation of the OTC gene specific probe for genetic prediction, once a common RFLP was discovered. The term 'gene tracking' has been applied to the use of RFLP linkage for genetic prediction in these two pregnancies, because the actual mutation itself is not detected. Rozen *et al.*,⁶ using the OTC gene probe cloned by Horwich *et al.*,⁴ found one affected male out of 15 had a deletion of the 3' part of the OTC gene, but the others gave restriction patterns indistinguishable from normal. These workers also described two different RFLPs with the enzyme *MspI* and found that 69% of 35 normal women were heterozygous for one or both RFLP. Our cloned gene probe detects one of these *MspI* RFLPs and an additional *BamHI* polymorphism.¹³ Therefore, prenatal diagnosis by DNA analysis is possible in approximately 80% of women at present. Fetal liver biopsy will always provide a valuable backup where the mother is not heterozygous for a RFLP, or the samples available from the family are insufficient to establish the linkage phase. Mutation can also complicate linkage phase assignment. If freshly frozen tissue had not been available from an affected fetus in family M, linkage phase could not have been established unless a protein load test had shown conclusively that the maternal grandmother (mother of I.2) was a carrier of the OTC deficiency mutation. Only in that situation can we say that I.2 does not represent a new mutation and therefore can establish linkage phase by examining DNA from her father. It happens in the present two families that the female offspring (II.1 in family A and II.7 in family M) are homozygous for the 6.5 allele, and therefore the paternal allele can be inferred automatically. However, if the female offspring were heterozygous for the RFLP like their mother, knowledge of the father's allele would have been critical to linkage phase assignment, and paternity testing would be essential. These practical aspects of gene tracking in X linked disorders have recently been discussed in relation to haemophilia A.¹⁴

Carrier status has implications for pregnancies beyond the need for prenatal diagnosis. Some of the women have protein intolerance and readily discernible biochemical abnormalities including hyperammonaemia. This may harm the fetus, particularly if the female fetus is heterozygous for OCTD with a compromised ability to metabolise ammonia. In parents with urea cycle disorders because of the metabolic block, arginine becomes an essential or semi-essential amino acid: supplements are needed to prevent hyperammonaemia and to maintain normal growth.¹⁵ On the low protein diet the plasma arginine concentrations of the mother in family M were low and were therefore supplemented to prevent deficiency. We recommend that during pregnancy the diet should be assessed and plasma amino acids and ammonia monitored.

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