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INVITED COMMENTARY: CURRENT ISSUES IN OBSTETRICS AND GENETICS

Prenatal Diagnosis of Ornithine Transcarbamylase Deficiency

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Ornithine transcarbamylase deficiency (OTCD) is an X-linked metabolic disorder typically producing severe symptoms in affected males and a much milder phenotype in carrier females. Ornithine transcarbamylase (OTC) is a mitochondrial matrix enzyme, expressed mainly in the liver, that catalyses the biosynthesis of citrulline from ornithine and carbamyl phosphate, and initiates detoxification of nitrogenous waste through the urea cycle. OTCD is the most common urea cycle defect, with an estimated frequency of 1 in 70 000 (Brusilow and Horwich, 1995).

There is a spectrum of severity of the OTCD phenotype in males and females (Brusilow and Horwich, 1995). Males with little or no OTC activity present with severe, intractable hyperammonaemia beginning several days after birth and proceeding rapidly to coma and death. The absence of metabolic compromise during fetal life is explained by the placental circulation dialysing the affected fetus, and the delay in development of symptoms after birth is related to minimal protein ingestion in the perinatal period. With an increasing protein load and failure to incorporate nitrogen into urea, several amino acids accumulate, notably glutamine, and eventually hyperammonaemia ensues. Partial OTCD in males (due to mutations which do not entirely inactivate the OTC gene product) may produce chronic mild hyperammonaemia, associated with variable degrees of developmental delay, or may become manifest only during periods of illness or prolonged fasting that lead to a catabolic state. Most female carriers are not severely affected, and the severe neonatal course is not seen in female infants (Maestri *et al.*, 1998). Many females have no symptoms or report only nausea and malaise following high protein meals. Occasional carriers with no prior medical history suggesting OTCD develop lethal hyperammonaemia in response to severe metabolic stress, such as surgery. Rare females have chronic metabolic instability and develop hyperammonaemia early in childhood with a phenotype similar to partial deficiency in males. In females, the proportion of active normal X chromosomes versus active chromosomes bearing the OTC mutation is the major determinant of residual activity. Hence, severity in one heterozygous female family member does not predict severity in other female carriers of the same kindred.

A presumptive diagnosis of OTCD can be made in a patient with elevated plasma ammonia and glutamine and reduced citrulline in the absence of acidosis or ketosis. Orotic aciduria is usual in OTCD because carbamyl phosphate is converted to orotate instead of citrulline in the absence of OTC. Asymptomatic female carriers can usually be detected by measuring urine orotate following a dose of allopurinol, which inhibits conversion of orotate to uridine. In the past, the diagnosis of OTCD was often confirmed by measuring enzyme activity in a liver biopsy. DNA-based testing for mutation in the OTC gene is becoming a more common means of confirmation. OTC maps to chromosome Xp21.1 proximal to the genes for Duchenne muscular dystrophy and chronic granulomatous disease. 10 exons spread over about 80 kb of DNA are transcribed into a 1.5 kb message. The majority of identified genetic alterations leading to OTC deficiency are missense mutations, premature stop mutations, or small insertions or deletions within the OTC coding region, or splice site mutations immediately adjacent to exons. 10 per cent of cases are due to gross rearrangements, and most rearrangements are partial deletions of the gene. Deletions of the whole gene and contiguous regions of the X chromosome are uncommon (Francke, 1984). Early reports suggested the possibility of a few hot spots for mutations (Maddalena *et al.*, 1988). Subsequent comprehensive studies, using direct sequencing plus Southern blot analysis to identify all types of genetic alterations, failed to detect a high frequency of recurrent mutations among unrelated patients (Tuchman *et al.*, 1996). Mutations are scattered throughout the gene, and nearly every family has a unique alteration. The great majority of males with neonatal onset of OTCD have mutations in the coding region of the gene or in adjacent splice sites. Direct mutation analysis is less likely to detect mutations in males or females with biochemical studies suggestive of partial OTCD (Glynn *et al.*, 1998). These data may indicate that mutations causing partial deficiency often lie outside the coding region of the gene (e.g. cryptic splice sites in introns or mutations in regulatory regions) or that some patients diagnosed with partial deficiency actually have a genetic defect in a gene other than OTC.

Prenatal diagnosis of OTCD can be performed by direct mutation analysis of a fetal sample consisting of chorionic villi, amniotic fluid cells or cultured amniocytes. Prior to pregnancy, or very early in pregnancy, a mutation should be sought in an affected male family

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member or obligate female carrier, a process that may take several weeks. Direct sequencing of exons and splice sites is becoming the standard method of mutation screening. In female patients who screen negative by these methods, Southern blotting may detect heterozygosity for a deletion of the gene. Once a mutation has been found, testing a fetus should take a few days to a week. Direct mutation analysis of a fetus without prior studies of an obligate gene carrier is suboptimal because of time considerations and because the method is not 100 per cent sensitive. Failure to detect a mutation in a fetus when the family mutation is unknown does not necessarily indicate a normal fetus. Other limitations of direct mutation detection include failure to identify a mutation in some OTCD kindreds and detection of missense alterations of unknown significance. Stop mutations, frameshifts, splice site mutations and deletions are presumed to have functional significance because they all result in gross alterations of the OTC protein. However, novel missense alterations could be normal variants that do not cause disease. Because OTCD is rare, any variant that is reasonably frequent in the general population cannot be disease related. Several investigators have catalogued normal polymorphisms in the gene (Rozen *et al*., 1985; Hata *et al*., 1988; Tuchman, 1993). Any variant that is not a known polymorphism can be assessed for functional significance on the basis of evolutionary conservation. Tuchman *et al*. (1995b) compared the human OTC sequence with 25 homologous genes to identify conserved amino acid residues. Missense alterations at highly conserved sites are likely to be functionally important. Interpretation of other missense variants remains problematic. Functional analysis by *in vitro* expression of a protein bearing the variant is possible but lies outside of the realm of standard clinical diagnosis.

For families in which a firm diagnosis of OTCD has been established, yet no mutation can be found, linkage analysis can be a highly accurate alternative to mutation-specific diagnosis. Linkage analysis is an indirect method of diagnosis that exploits the tendency of genes that lie close together on a chromosome to be transmitted from parent to child with no recombination. There are many polymorphisms in and near the OTC gene. In kindreds with more than one affected family member, linkage analysis can be used to determine which alleles at linked markers co-segregate with the disease. Any fetus bearing the disease-associated allele is extremely likely to be affected and fetuses not bearing the associated allele are very unlikely to be affected. This method is typically 99 per cent accurate. The main limitation of linkage-based diagnosis is its use in kindreds with a single affected individual. One-third of singleton affected offspring should be new mutants provided that the male and female mutation rates are equal and that fitness is zero in males, but segregation analysis suggests that mutation rates are not equal (Bonaiti-Pellie *et al*., 1990). Increasing use of mutation-specific diagnosis is providing direct data addressing the frequency of new mutations, but reports differ in their estimates of the mutation rate. Tuchman *et al*. (1995a) reported that 2 of 28 singleton males and 12 of 15 singleton females were new mutants. Maestri *et al*. (1998) found that 5 of 9 singleton males and 11 of 11 singleton females were new mutants. As it stands, linkage analysis in kindreds with a single affected person can be used with a high degree of accuracy to exclude the possibility that a fetus carries the OTCD gene. If linkage analysis with intragenic or flanking markers shows that the fetus does not carry the same copy of the OTC gene as the affected singleton child, then the fetus is not at risk for OTCD. However, if the fetus and previous affected child have inherited the same copy of the OTC gene, then the study should be viewed as uninformative.

In families with singleton affected offspring with a known mutation, many mothers test negative for the mutation carried by their child. In these cases, the child is declared a new mutant, and the mother is often told that her risk of having a second affected child is minimal. However, at least one case of 'gonadal mosaicism' has been reported in which three affected females were born to a couple in which the mother and father tested negative for a constitutional OTC mutation (Komaki *et al*., 1997). The father was found to be a mosaic for a mutation in the OTC gene that could be detected in spermatozoa. The rate of gonadal mosaicism in OTCD is unknown but is probably low. Nevertheless, couples with a child who is apparently a new mutant should be counselled on the small chance of a second affected child and offered prenatal diagnosis for future pregnancies.

REFERENCES

Bonaiti-Pellie C, Pelet A, Ogier H, Nelson J, Largilliere C, Berthelot J, Saudubray J, Munnich A. 1990. A probable sex difference in mutation rates in ornithine transcarbamylase deficiency. *Hum Genet* **84**: 163–166.

Brusilow SW, Horwich AL. 1995. Urea cycle enzymes. In *The Metabolic and Molecular Basis of Inherited Disease*, Scriver CR, Beaudet AL, Sly WS, Valle D, Stanbury JB, Wyngaarden JB, Frederickson DS (eds). McGraw-Hill, Inc.: New York; 187–1232.

Francke U. 1984. Random X inactivation resulting in mosaic nullisomy of region Xp21.1–p21.3 associated with heterozygosity for ornithine transcarbamylase deficiency and for chronic granulomatous disease. *Cytogenet Cell Genet* **38**: 298–307.

Glynn MW, Brusilow SW, Maestri NE, Bale AE. 1998. Mutation detection in 70 OTC probands: high new mutation rates in singleton males and females. *Am J Hum Genet* **63**: A267.

Hata A, Tsuzuki T, Shimada K, Takiguchi M, Mori M, Matsuda I. 1988. Structure of the human ornithine transcarbamylase gene. *J Biochem* **103**: 302–308.

Komaki S, Matsuura T, Oyanagi K, Hoshide R, Kiwaki K, Endo F, Shimadzu M, Matsuda I. 1997. Familial lethal inheritance of a mutated paternal gene in females causing X-linked ornithine transcarbamylase (OTC) deficiency. *Am J Med Genet* **69**: 177–181.

Maddalena A, Spence JE, O'Brien WE, Nussbaum RL. 1988. Characterization of point mutations in the same arginine codon in three unrelated patients with ornithine transcarbamylase deficiency. *J Clin Invest* **82**: 1353–1358.

Maestri NE, Lord C, Glynn M, Bale A, Brusilow SW. 1998. The phenotype of ostensibly healthy women who are carriers for ornithine transcarbamylase deficiency. *Medicine* **77**: 389–397.

Rozen R, Fox J, Fenton WA, Horwich AL, Rosenberg LE. 1985. Gene deletion and restriction fragment length polymorphisms at the human ornithine transcarbamylase locus. *Nature* **313**: 815–817.

Tuchman M. 1993. Mutations and polymorphisms in the human ornithine transcarbamylase gene. *Hum Mutat* **2**: 174–178.

Tuchman M, Matsuda I, Munnich A, Malcolm S, Strautnieks S. 1995a. Proportions of spontaneous mutations in males and females with ornithine transcarbamylase deficiency. *Am J Med Genet* **55**: 67–70.

Tuchman M, Morizono H, Reish O, Yuan X, Allewell NM. 1995b. The molecular basis of ornithine transcarbamylase deficiency: modelling the human enzyme and the effects of mutation. *J. Med Genet* **32**: 680–688.

Tuchman M, Plante RJ, Garcia-Perez MA, Rubio V. 1996. Relative frequency of mutations causing ornthine transcarbamylase deficiency in 78 families. *Hum Genet* **97**: 274–276.

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