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Familial Lethal Inheritance of a Mutated Paternal Gene in Females Causing X-Linked Ornithine Transcarbamylase (OTC) Deficiency

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A Leu148Phe substitution of the ornithine transcarbamylase (OTC) gene was identified in a 2-year-old girl with OTC deficiency (14% of control). Her two elder sisters died in childhood of hyperammonemia, and the patient also died of OTC deficiency. Enzyme activity in Cos1 cells transfected by the mutant cDNA was undetectable, thereby indicating a definite pathogenic mutation. Familial gene analysis showed that the mother had wild-type OTC alleles on both X-chromosomes and the father was a mosaic for the mutant allele in his lymphocytes and spermatozoa. This clinical case shows that a somatic and germline mosaicism for a single-gene disorder led to an unusual pattern of X-linked inheritance in the family, and all three daughters in the family died of OTC deficiency. The possibility that inherited factors will lead to skewed X-inactivation needs to be considered. Am. J. Med. Genet. 69:177–181, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: OTC; somatic mosaicism; germline mosaicism; skewed X-inactivation

INTRODUCTION

Ornithine transcarbamylase (OTC) (EC 2.1.3.3.) deficiency, the most common heritable urea cycle disorder, is an X-linked disease [Nagata et al., 1991; Brusilow and Horwich, 1995]. With the increasing number of cases being identified, it became clear that male patients have variable clinical manifestations, from an early onset associated with an unfavorable prognosis to a late onset with a better prognosis, depending on residual enzyme activity and environmental factors [Hata et al., 1991; Matsuda et al., 1991; Brusilow and Horwich, 1995]. Furthermore, the variable clinical manifestations are applicable, even in females. In most female carriers, conditions are asymptomatic, but in some subjects, symptoms similar to those seen in male patients can be present. When X-inactivation occurs unequally with two alleles in the fetal liver tissue and a normal allele can be inactivated accidentally, a lesser amount of OTC activity will be the result [Hata et al., 1989]. There are reports of two male patients with mosaicism for intragenic deletion of OTC gene [Maddalena et al., 1988; Legius et al., 1990]. We identified a male carrier with mosaicism for a missense mutation (Leu148Phe). The mutant allele was transmitted to a daughter and possibly two elder ones, the result being death with OTC deficiency, even in the presence of wild-type OTC allele.

MATERIALS AND METHODS

Clinical Report

A 17-month-old Japanese girl with hepatomegaly but no other signs or symptoms of a disorder was examined because her two sisters had died of a Reye-like syndrome with hyperammonemia at age 5 years and 2 years, respectively. The mother was apparently healthy, but the father's orotic acid excretion (followed by the allopurinol test) was elevated to 42.6 μg/mg creatinine, and the mother's test results were normal. Laboratory data on the girl documented an elevation of SGOT and SGPT, and urinary orotic acid excretion (50.0 μg/mg creatinine). Following a needle biopsy of the liver, OTC deficiency was diagnosed when she was 2 years old, as 14% of the normal OTC activity was noted. Other urea cycle enzyme activities were normal. Her physical and mental development was normal, under dietary control, until age 5 years when she died of a severe attack of hyperammonemia associated with acute respiratory infection. Her autopsied liver, parental leukocytes, and spermatozoa from the father were analyzed genetically. Karyotypes of the father,

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mother, and patient were 46,XY, 46,XX, and 46,XX respectively.

DNA Analysis of Mutant OTC Gene

Genomic DNA was purified from the liver using standard procedures [Maniatis et al., 1989] and was amplified by PCR in vitro using Taq DNA polymerase and 10 pairs of synthetic oligonucleotide primers of sense and antisense strands, which covered each exon (exons 1–10) and adjacent introns [Matsuura et al., 1993]. Amplified DNA fragments were ligated into the plasmid vector pUC18 with T4 DNA ligase, and several independent clones were sequenced, using T7 Sequenase kits (United States Biochemical Corporation). For gene tracking in the family, amplified 120-bp DNAs covering the mutation, using peripheral blood cells and spermatozoa [Hata et al., 1989], were digested with restriction enzyme *Taq*I.

PCR-Based Skewed X-Inactivation Analysis

Random or nonrandom X-inactivation in females was assessed by determining PCR-based differential methylation patterns between two alleles of the human androgen receptor (HUMARA, GenBank), which contains a highly polymorphic trinucleotide repeat in the first exon, located between Xcen-q22 [Allen et al., 1992; Kishino et al., 1995]. The differential methylation at a *Hpa* II (methylation-senstive enzyme) recognition site near a polymorphic CAG repeat correlates with X-inactivation. *Hpa*II-digested patient's DNA from the liver, and nondigested DNAs were used as a template for PCR amplification in the presence of α ^{32}P-dCTP. After 30 cycles of PCR, the amplified products were resolved by denaturing polyacrylamide gel electrophoresis, and polymorphism patterns were detected by autoradiography.

DNA Sex Determination

Genomic DNA was PCR-amplified using a pair of nucleotide primers encompassing the pseudo autosomal boundary region of the X and Y chromosomes [Nakahori et al., 1991], as described elsewhere [Tanoue et al., 1993].

Expression Study of Mutant OTC cDNA

Transient expression study of *OTC* cDNA was carried out in Cos1 cells, as described [Matsuura et al., 1994; Lee and Nussbaum, 1989], using mutagenesis kits (Transformer site-directed mutagenesis kit, CLONTECH, USA). Full-length normal, mutant Leu148Phe, and Leu304Phe (mutant control) cDNAs were subcloned separately into the in vitro expression vector pCAGGS [Miyazaki et al., 1989]. Transfection of the cDNA in cultured Cos1 cells was performed using Lipofection (GIBCO BRL,USA). Plasmid pCH110 containing the lacZ gene of E coli was cotransfected as an internal standard. Transfection cells on the third day were used for enzyme assay and Western blotting. Transfected cDNA was confirmed by sequencing.

RESULTS

In the liver sample obtained from the patient, a G-to-C substitution at nucleotide 444 was identified in exon 5 of the OTC gene, an event resulting in phenylalanine instead of leucine at codon 148 (Fig. 1). The amplified DNA of 120 bp containing the mutation was digested by *Taq*I into 97 bp and 23 bp, whereas a wild-type *OTC* allele was intact. On the basis of this observation, a PCR-*Taq*I treatment was used for gene tracking in the family, as shown in Fig. 2A,B. The father's and patient's lymphocyte samples showed two different *OTC* alleles, the wild-type and the mutant. Samples of the father's spermatozoa also had two *OTC* alleles (Fig. 2C). PCR products from the mother's genomic DNA of lymphocytes revealed an undigested only (Fig. 2B), indicating that both alleles were wild-type, and ten clones of the mother's *OTC* gene were all normal by DNA sequencing, which suggested that both alleles of the mother's *OTC* gene were intact. Paternal and maternal sexuality was confirmed at the DNA level, as having the products of the X and Y chromosomes in the father and X chromosome in the mother. For the PCR of HUMARA gene, PCR product from the patient's nondigested DNA separated 2 band sets with almost equal intensity (Fig. 3). While in PCR product from the patient's *Hpa*II-digested DNA, the bands derived from the mother (M1 and M1′; M1′, another stable conformation of M1) were much more intense (Fig. 3). These results show that the methylation of X-chromosomes derived from the mother occurred dominantly and paternal X-chromosomes that have mutated *OTC* allele was activated significantly. Undetectable and 34.4% of OTC activity were detected in Cos1 cells transfected with Leu148Phe (the patient) and Leu304Phe mutant control cDNAs, respectively, although galactosidase activity was essentially equivalent. A low signal of OTC protein was detected in Cos1 cells transfected with Leu148Phe compared to normal and mutant control cDNA transfected cells (Fig. 4).

DISCUSSION

In previously reported female patients with OTC deficiency, a mutant allele was inherited from a carrier

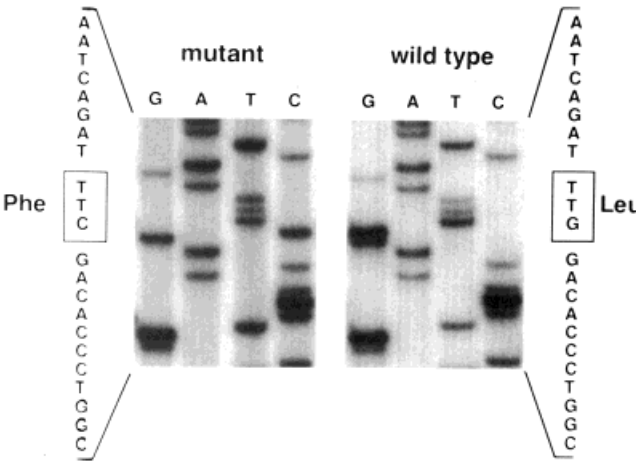

Fig. 1. Sequencing gels showing a C-to-G missense mutation at nucleotide 444, with phenylalanine replacing leucine at codon 148.

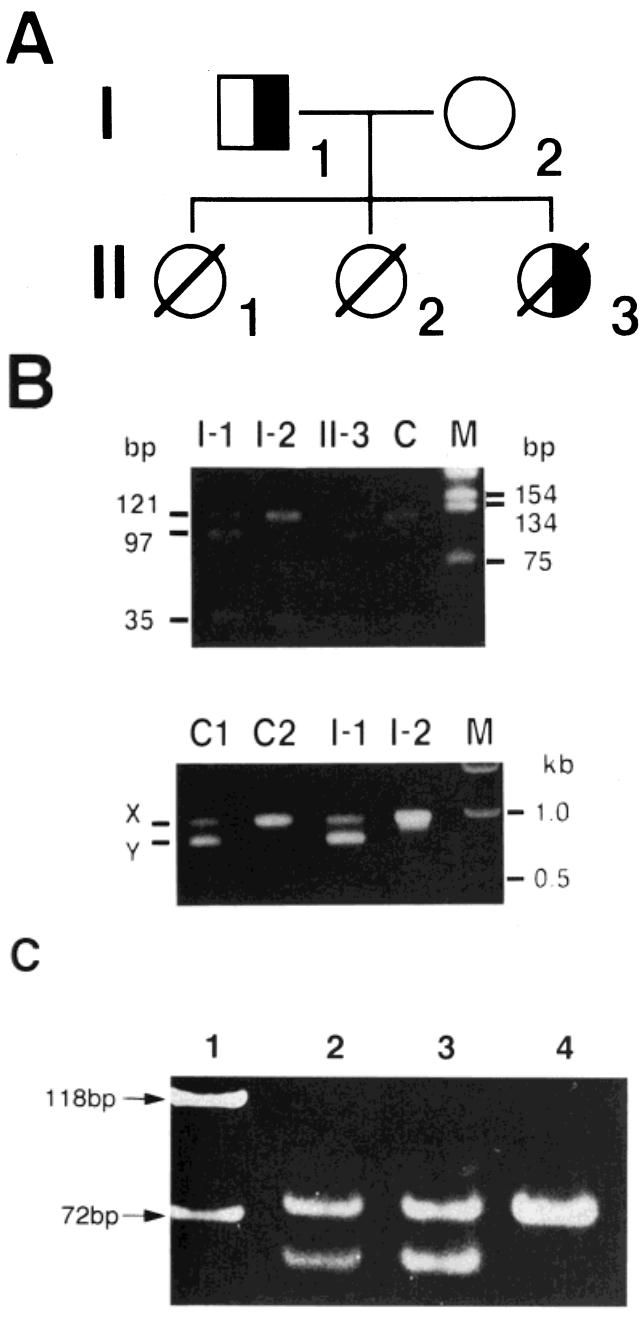

Fig. 2. **A:** Family tree of the patient. Two sisters of the patient died of a Reye-like syndrome with hyperammonemia. **B:** The mutant allele was digested with *Taq*I, and resulted in 97 and 23 bp (invisible) fragments. The intact 120 bp was shown in the wild-type. Father (I-1) and the patient (II-3) had two alleles; wild-type and mutant ones. Mother (I-2) had wild-type alleles alone. The bottom figure indicates sex determination, using PCR by X-Y specific primers. C1 (normal male), C2 (normal female). Samples from father (I-1) and mother (I-2) showed X and Y chromosomes and X chromosome, respectively. **C:** Somatic and germline mosaicism of the father. **Lane 1**, marker; **lane 2**, father's leukocytes; **lane 3**, father's spermatozoa; **lane 4**, mother's leukocytes.

mother, or was a de novo mutation in paternal or maternal germlines [Hata et al., 1989; Nagata et al., 1991; Brusilow and Horwich, 1995]. In our particular family, one daughter died of OTC deficiency and two other

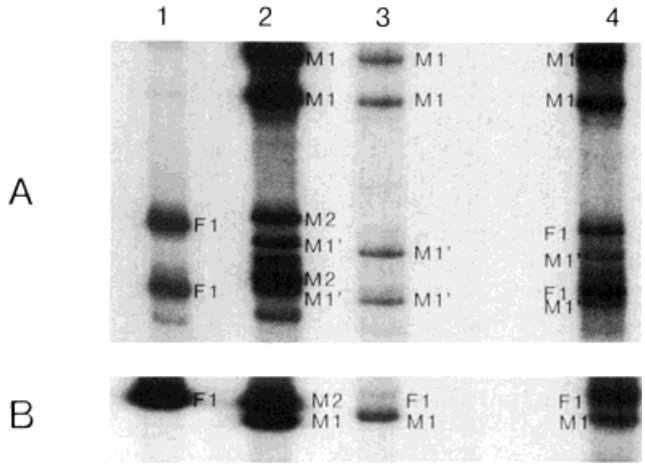

Fig. 3. PCR-based X chromosome-inactivation assay. PCR products from the father's (**lane 1**, F1) and mother's (**lane 2**, M1 and M2) nondigested DNA samples and a pair of DNA samples, digested (**lane 3**, M1 and F1) and nondigested (**lane 4**, M1 and F1) with *Hpa*II from the patient's liver are separated on a denaturing polyacrylamide gel. **A:** Single-strand conformation polymorphism analysis. Each allele is seen as 2 band sets. M1 forms another stable conformation (M1′). **B:** Double-strand DNA conformation.

daughters died of a similar episode with hyperammonemia, the mother was first thought to be an obligate carrier of the OTC deficiency. However, allopurinol test for orotic acid excretion showed that the mother was not a carrier, it was the father who was OTC deficient. The mother's *OTC* genomic DNA analysis showed that she had two wild-type alleles of the OTC gene. Unusually, the mutant allele of the patient was inherited from the asymptomatic father with mosaicism carrying the wild-type and mutant alleles (Leu148Phe), as detected in peripheral leukocytes and spermatozoa. The mutation (Leu148Phe) produced an undetectable OTC activity in the in vitro expression study, thereby indicating that the missense mutation was the actual cause of the disease. Thus, the three daughters may have inherited the mutant allele from the father. Maddalena and colleagues [1988] and Legius and colleagues [1990] reported data on two boys with somatic mosaicism for intragenic deletion of the

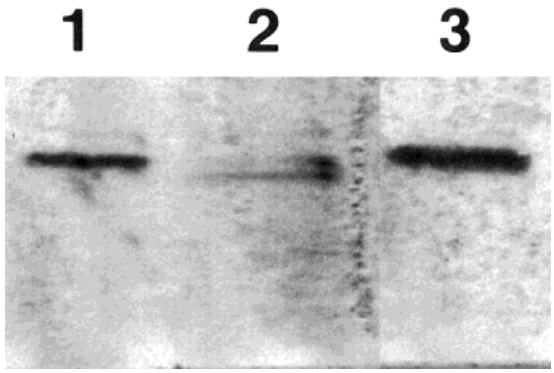

Fig. 4. Western blot analysis of the human *OTC* gene in transfected Cos1 cells. Ten μg protein was used in normal OTC control (**lane 1**), the patient's OTC protein (**lane 2**), and a mutant control (**lane 3**).

OTC gene, as evidenced in peripheral leukocytes. They developed symptoms at age 3.5 years and 6 months, respectively. Thus, this is apparently the first observation of mosaicism for the mutant OTC gene in both somatic and germline tissues. Usually, germ line mosaicism and somatic mosaicism occur independently after germline cells are seperated from somatic ones in very early stages of embryogenesis. Only very early postzygotic mutations (similar to our case) are likely to be represented in both somatic cells and the germline. These rare events occurred in a single patient with type IV Ehlers-Danlos syndrome [Kontusaari et al., 1992], hemophilia A [Brocker-Vriends et al., 1990], osteogenesis imperfecta [Wallis et al., 1990], and Duchenne muscular dystrophy [Bunyan et al., 1994].

The most intriguing question is why all three daughters with heterozygosity of *OTC* alleles died of OTC deficiency, although it was assumed but not confirmed at the gene level. Concerning the manifestation of OTC deficiency in female patients, we consider that: one of the female X chromosomes inactivates at random in early embryogenesis, at about the 32- to 64-cell stage [Lyon, 1972]. Because the number of progenitor cells for individual tissues at this time is presumed to be less, extreme ratios (80:20 or greater) of X-inactivation are not rare (approximately 10% of normal females) [Willard, 1995]. Furthermore, alleles of the OTC gene are subject to random X-inactivation [Ricciuti et al., 1976; Mrozek et al., 1991]. Biases of maternal normal X chromosome for inactivation had apparently occurred. Of other X-linked disorders, multiple affected females have been identified in families with Duchenne muscular dystrophy [Reddy et al., 1984], hemophilia B [Taylor et al., 1991], Lesch-Nyhan disease [Marcus et al., 1992], hemophilia A [Ingerslev et al., 1989], and Fabry disease [Ropers et al., 1977]. Such skewed X-inactivation appears to be similar to events in our patients, thereby suggesting that certain inherited factors may determine the nonrandomness of X-inactivation. The X-inactivation center (*XIC*) and the *XIST* gene may be appropriate candidates for a gene alteration in subjects with nonrandom X-inactivation.

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