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MUTATION IN BRIEF

Partial Duplication [dup. TCAC (178)] and Novel Point Mutations (T125M, G188R, A209V, and H302L) of the Ornithine Transcarbamylase Gene in Congenital Hyperammonemia

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INTRODUCTION

Human ornithine transcarbamylase (OTC) deficiency, the most prevalent genetic defect of ureagenesis, is inherited as a partially dominant X-linked trait (Brusilow and Horwich, 1989). Affected males often die during the neonatal period with untractable hyperammonemic coma and markedly deficient enzyme activity in the liver. Nevertheless, a substantial proportion of affected males present with a late onset disease, characterized by recurrent episodes of hyperammonemia. Their OTC activity is usually partially deficient or present with abnormal kinetic properties (Briand et al., 1982; Rabier et al., 1989, 1991). Heterozygous females may be symptomatic with variable severity ranging from a mere dislike of protein-containing foods to life-threatening comas (Rowe et al., 1986).

The gene encoding the human OTC has been cloned, sequenced, and mapped to chromosome Xp21.1 (Horwich et al., 1984; Lindgren et al., 1984; Hata et al., 1988). The OTC gene (73 kb) contains 10 exons and encodes a 354 amino acid protein. Hitherto, a number of point mutations, intragenic deletions, and large scale deletions in the OTC gene have been reported (Tuchman, 1993). Here, we report on the first partial duplication and describe four novel point mutations in OTC deficiency.

PATIENTS AND METHODS

DNA was obtained from either peripheral blood cells or liver biopsy of probands. When no material from a deceased proband was available, and the mother was an obligate carrier, we studied the mother's leukocyte DNA. A woman was considered an obligate carrier when she had two sons (or a male relative and a son) with OTC deficiency or when her protein loading test was positive (Ng et al., 1984). Single strand conformation polymorphism (SSCP) analysis was performed according to the method reported by Orita et al. (1989). The 10 exons of the OTC gene were amplified and submitted to SSCP analysis. When a variant pattern of migration was found, 75 healthy controls were studied under the same conditions.

Amplified exons displaying variant patterns of migration were purified by separation on a Nusieve-GTG-agarose gel (FMC Bioproducts) and fusion of the desired band in 1 volume of water at 65°C. Ten microliters of this dilution was mixed with 0.5 μl (5 pmol) of the forward amplification primer and 9.5 μl of terminator mix (PRISM™ READY REACTION Dye Deoxy™ Terminator Cycle Sequencing Kit, Applied Biosystems), cycled according to the manufacturer's instructions, and analyzed using an Applied Biosystems 373A DNA sequencer. When an abnormal sequence was observed, the experience was repeated twice starting from PCR amplification of the genomic DNA.

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TABLE 1. Novel Mutations (Patients 1–5) and Recurrent Mutations in OTC Deficiency (Patients 6–14)

Patient	Sex	Age at onset	OTC liver activity (%)	Outcome	Mutation	
1	Male	Neonatal	?	Fatal: 4 days	dup. TCAC (178)	(exon5)
2	Male	Neonatal	<1	Fatal: 14 days	T125M	(exon4)
3	Male	Neonatal	2	Fatal: 10 days	G188R	(exon6)
4	Male	Neonatal	1	Fatal: 3 days	A209V	(exon6)
5	Female		?	Alive: 3 years	H302L (exon 9) + polymorphism E270R	
6	Male	Late	7	Fatal: 9 years	R40H	(exon2)
7	Male	Late	2,5	Alive: 10 years	R40H	(exon2)
8	Male	Late	2	Alive: 13 years	R40H	(exon2)
9	Male	Late	18	Fatal: 8 years	L88N	(exon3)
10	Female		25	Alive	R92ter	(exon3)
11	Male	Late	45	Fatal: 13 years	R129H	(exon4)
12	Male	Late	2	Alive: 11 years	R129H	(exon4)
13	Male	Late	6	Alive: 2 years	R129H	(exon4)
14	Female		?	Alive	R141ter	(exon5)

RESULTS

A male patient with neonatal onset OTC deficiency was found to carry a 4 bp duplication in exon 5 (TCAC). This duplication at codon 178 (codon176-CTC-AC(T-CAC)-GCT-CCA-G-intron 5) was responsible for a frameshift, downstream the coding sequence (Table 1). In addition, novel point mutations were found in two probands (patients 2 and 5) and two obligate carriers (patients 3 and 4). Patient 2 carried a C to T transition at the second nucleotide of codon 125 (exon 4) resulting in the substitution of a methionine for a threonine (T125M). The mother of patient 3 had a G to C change at codon 188 (exon 6), resulting in the substitution of an arginine for a glycine (G188R). The mother of patient 4 had a C to T change at codon 209 (exon 6) resulting in the substitution of a valine for an alanine in the protein (A209V). Finally, patient 5 had an A to T transition at codon 302 (exon 9) resulting in the substitution of a leucine for a histidine (H302L) (Table 1). These sequence variations were not found in 75 unrelated controls. Patient 5 also carried a polymorphism previously reported in exon 8 (E270R) (Tuchman et al., 1992) (not shown). Nine patients carried the previously reported mutations: R40H (patients 6–8), L88N (patient 9), R92Ter (patient 10), R129H (patients 11–13), and R141Ter (patient 14) (Hata et al., 1989; Grompe et al., 1991; Reish et al., 1993; Matsuura et al., 1994; Tuchman et al., 1994) (Table 1).

DISCUSSION

We report here the first partial duplication of the OTC gene (4 bp) in a boy with neonatal onset hyperammonemia. This duplication located at the 3′ end of exon 5 is expected to alter the splicing of intron 5 and cause a frameshift downstream the coding sequence. The mechanism of this duplication remains unknown as no TCAC tetranucleotide has been found in the vicinity of the duplication.

In addition, we report three novel missense mutations of the OTC gene in males with neonatal onset (T125M, G188R, and A209V) and one in a female with late onset OTC deficiency (H302L). Among them, two mutations altered CpG dinucleotides in conserved domains of the protein (the threonine at codon 125 and the glycine at codon 188 are conserved in human and rat). Similarly, the alanine at codon 209 and the histidine at codon 302 are conserved in human, rat, *Aspergillus*, and *E. coli* (Huygen et al., 1987). The absence of these sequence variations in 75 control chromosomes strongly supports the view that these substitutions are the disease-causing mutations.

The last five mutations have been previously described in OTC deficiency. Hemizygosity for the R40H, L88N, and R129H mutations is reportedly associated with a late onset form of the disease (Reish et al., 1993; Strautnieks and Malcom, 1993; Matsuura et al., 1994; Tuchman et al., 1994) and in our study as well (Table 1).

Hitherto, almost 60 different mutations have been reported in OTC deficiency. Recurrent mutations involved CpG dinucleotides at arginine codons 40 (4 patients: Tuchman et al., 1994; this report), 129 (6 patients: Strautnieks and Malcom, 1993; Matsuura et al., 1994; Tuchman et al., 1994; this report), 141 (7 patients: Maddalena et al., 1988; Hata et al., 1989; Lee and Nussbaum, 1989; Strautnieks et al., 1991; this report), and 277 (7 patients: Finkelstein et al., 1990; Hata et al., 1991; Matsuura et al., 1993; Gilbert-Dussardier et al., 1994; Tuchman et al., 1994). Moreover, half the mutations reported thus far involved

CpG dinucleotides, which are regarded as mutations hot spots in OTC deficiency (Maddalena et al., 1988; Grompe et al., 1989; Hata et al., 1989; Lee and Nussbaum, 1989; Finkelstein et al., 1990; Grompe et al., 1991; Hata et al., 1991; Hentzen et al., 1991; Strautnieks et al., 1991; Matsuura et al., 1993; Srautnieks and Malcom, 1993; Gilbert-Dussardier et al., 1994; Matsuura et al., 1994; Tuchman et al., 1994). While most OTC mutations are "private" mutations, about 60% of them involve exons 4–6 and 8. We suggest therefore giving priority to these exons when screening the OTC gene for mutations in OTC deficiency.

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