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Case Report

Contiguous Xp11.4 Gene Deletion Leading to Ornithine Transcarbamylase Deficiency Detected by High-density Single-nucleotide Array

Mizuho Ono[1], Junnosuke Tsuda[2], Yoko Mouri[2], Junichi Arai[2], Tadao Arinami[1], and Emiko Noguchi[1]

[1]*Department of Medical Genetics, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Ibaraki, Japan*

[2]*Department of Neonatology, Mito Children's Hospital, Ibaraki, Japan*

Abstract. Ornithine transcarbamylase (OTC) is one of the enzymes involved in the urea cycle. OTC deficiency, which is caused by impaired synthesis of OTC in the liver, is the most common inherited disease of urea cycle disorders. In this paper, we describe the case of an OTC-deficient Japanese boy wherein an analysis based on high-density single-nucleotide polymorphisms (SNPs) revealed the absence of the entire *OTC* locus and nearby genes. We identified a deletion on Xp11.4; the size of the deletion fragment was approximately 1 Mb. The deleted region included genes encoding transmembrane 4 superfamily member 2 (*TSPAN7*), MID1 interacting protein 1 (*MID1IP1*) and part of the retinitis pigmentosa GTPase regulator (*RPGR*) in addition to *OTC*. The results of a high-density SNP assay and PCR confirmed that the mother of the patient was a carrier of the mutation. Previously, determination of breakpoints for large unknown deletions was timeconsuming and laborintensive. However, the use of the widely available DNA chip technology allows for rapid determination of deletion breakpoints; therefore, it will become a standard technique in study of patients with a large genomic deletion of contiguous genes for provision of comprehensive genetic counseling and initiation of clinical management.

Key words: OTC deficiency, high-density SNP assay, RPGR

Introduction

Ornithine transcarbamylase (OTC; OMIM 300461), a mitochondrial matrix enzyme, is one of the enzymes involved in the urea cycle; this enzyme catalyzes the biosynthesis of citrulline from ornithine and carbamyl phosphate. The human *OTC* gene is located on the short arm of the X chromosome (1), and OTC deficiency (OMIM 311250), which is caused by impaired synthesis of OTC in the liver, is the most commonly inherited disease of urea cycle disorders (2). The clinical signs and symptoms of this disease are caused by the toxic effects of hyperammonemia on the brain (3). Complete OTC deficiency in a male infant is usually lethal or results in severe brain damage (4). Heterozygous female carriers of this disorder may show great variation in the number of

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Correspondence: Dr. Emiko Noguchi, Department of Medical Genetics, Graduate School of Comprehensive Human Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8577, Japan
E-mail: enoguchi@md.tsukuba.ac.jp

enzyme-deficient hepatocytes, and hence, the clinical picture may vary from an asymptomatic carrier status to severe disease with hyperammonemic episodes and central nervous system damage (5).

Most of the disease-causing mutations in OTC deficiency are missense/nonsense mutations, and a recent study in which array comparative genomic hybridization (CGH) analysis was performed has shown that 15.7% of the DNA samples obtained from patients without disease-causing point mutations showed loss of a part or all of the *OTC* gene (6). There are some disease-causing genes located close to *OTC*, such as the dystrophin-encoding gene causing muscular dystrophy (7) and retinitis pigmentosa GTPase regulator (RPGR; OMIM 312610) causing retinitis pigmentosa (8); therefore, it is important to detect the deleted region in the genome of patients in order to determine the course of treatment and provide genetic counseling. In this paper, we describe the case of an OTC deficient-Japanese boy wherein high-density single-nucleotide polymorphism (SNP) analysis revealed the absence of the entire *OTC* locus and nearby genes.

Patient Characteristics

A Japanese male infant was delivered at full term via a cesarean section because of pregnancy toxemia. He was the first child in the family, and there was no family history of sudden infant death or inborn error of metabolism. He was well at birth and had a birth weight of 3,345 g, but developed tremors in both arms on day 1. He was admitted to the hospital for further evaluation on day 2. He developed tachypnea, and laboratory tests revealed hyperammonemia (733 μg/dl). He was kept in an incubator and treated with dialysis. His ammonia level elevated up to a maximum of 5,730 μg/dl but remained in the range of 200–300 μg/dl during treatment. The plasma levels of amino acids, which were determined using a blood sample collected on day 3, were as follows: glutamine, 3,739.3 nmol/ml (normal range, 422.1–703.8 nmol/ml); citrulline, trace amounts (normal range, 17.1–42.6 nmol/ml); ornithine, 172.4 nmol/ml (normal range, 31.3–104.7 nmol/ml); lysine, 573.0 nmol/ml (normal range, 108.7–242.2 nmol/ml); arginine, 40.5 nmol/ml (normal range, 53.6–133.6 nmol/ml); and alanine, 772.6 nmol/ml (normal range, 208.7–522.7 nmol/ml). A high level of urinary orotic acid was also observed in the urine sample collected on day 3.

He developed multiple organ failure and died on day 16 despite use of all available treatments.

Methods

Informed consent for genetic analysis was obtained from the patient's parents, and this study was approved by the ethical committee of the University of Tsukuba, Japan.

PCR analysis using a sample of the patient's DNA failed to amplify the exons of *OTC*; this suggests a large deletion in the *OTC* locus. Next, we performed a high-density SNP assay using Human610-Quad (Illumina, San Diego, CA, USA) that contained more than 610,000 rationally selected tag SNPs and markers per sample, according the manufacturer's protocol. The scanned image was imported into BeadStudio (Illumina) for analysis. After identifying the deleted region, we designed primers (DelF, 5'-CCCAAGCTTGACTTCTCTGG-3'; DelR, 5'-AGTTCCTGCAGAGCATGAGA-3') using the Primer 3 software (http://primer3.sourceforge.net/) in order to detect the breakpoints. Subsequently, the PCR product was subjected to direct sequencing using BigDye Terminator v.1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). For carrier testing, a high-density SNP assay and PCR analysis were performed using a DNA sample obtained from the patient's mother, who had not shown any OTC deficiency-related symptoms.

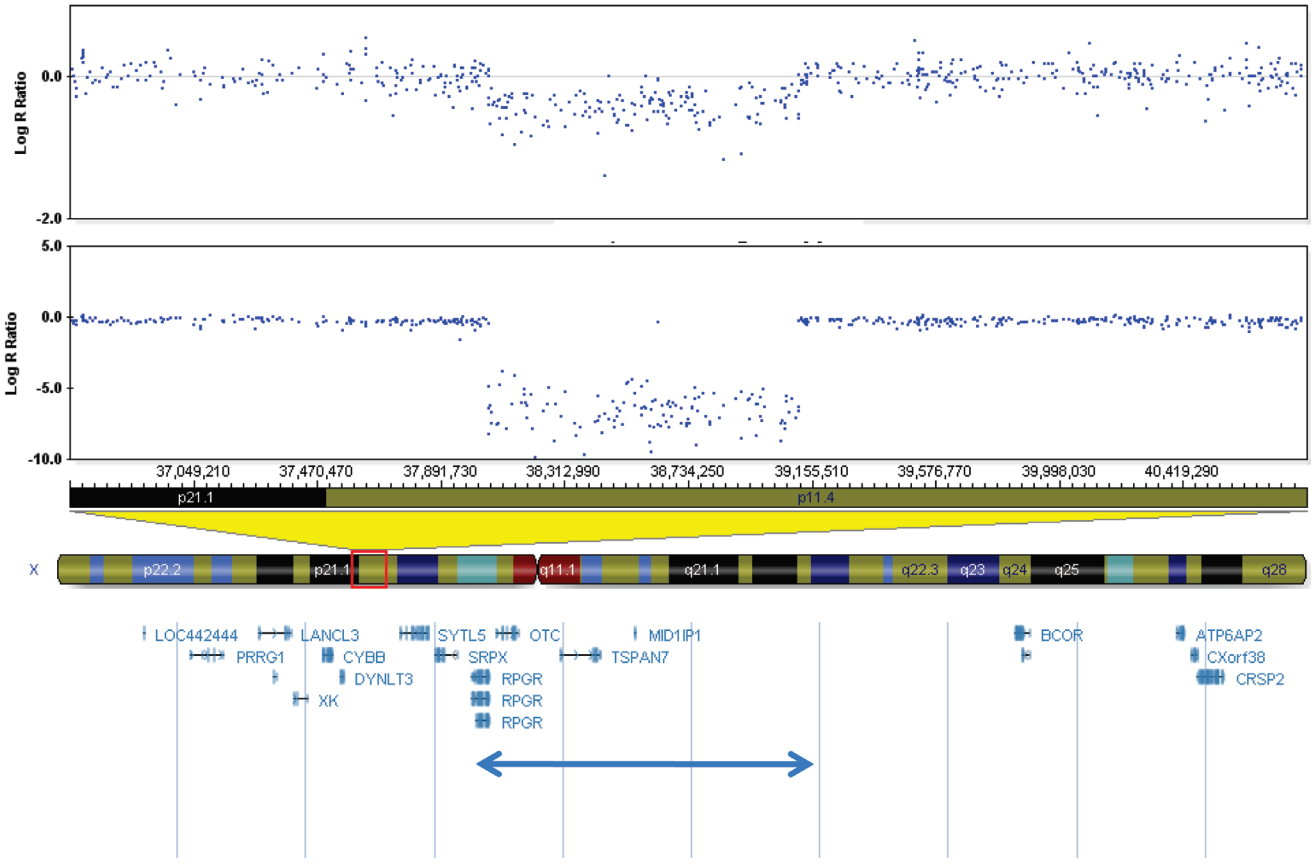

Fig. 1 Deletions detected by high-density SNP array. LogR ratios of the results of SNP copy number analysis for the patient's mother (top) and for the patient (middle). Chromosomal band positions and transcripts are shown at the bottom. The deleted region is indicated by the arrow.

Results and Discussion

The results of the high-density SNP assay are shown in Fig. 1. We detected a deletion on Xp11.4 (fragment size, approximately 1 Mb). The deleted region includes genes encoding transmembrane 4 superfamily member 2 (*TSPAN7*), MID1 interacting protein 1 (*MID1IP1*) and part of *RPGR*, in addition to *OTC*. Analysis of breakpoints using PCR and sequencing revealed a telomeric breakpoint in intron 6 of *RPGR*. The results of the high-density SNP assay and PCR confirmed that the patient's mother was a carrier of the mutation (Figs. 1, 2).

The clinical picture of OTC deficiency depends on the degree of enzyme activity impairment. Complete deletion of *OTC* leads to the absence of OTC enzymatic activity in the patient's liver and this explains why the patient died in the present case. Approximately 20% of female carriers become symptomatic; they have hyperammonemic episodes and exhibit central nervous system damage (9). The patient's mother, a carrier of the mutation, had not displayed any OTC deficiency-related symptoms or dietary preferences such as avoidance of high-protein foods. In females, X inactivation in the liver is much more skewed and correlates well with the OTC activity, and the degree of X inactivation varies considerably, even within the same liver (9, 10). Although the female carrier

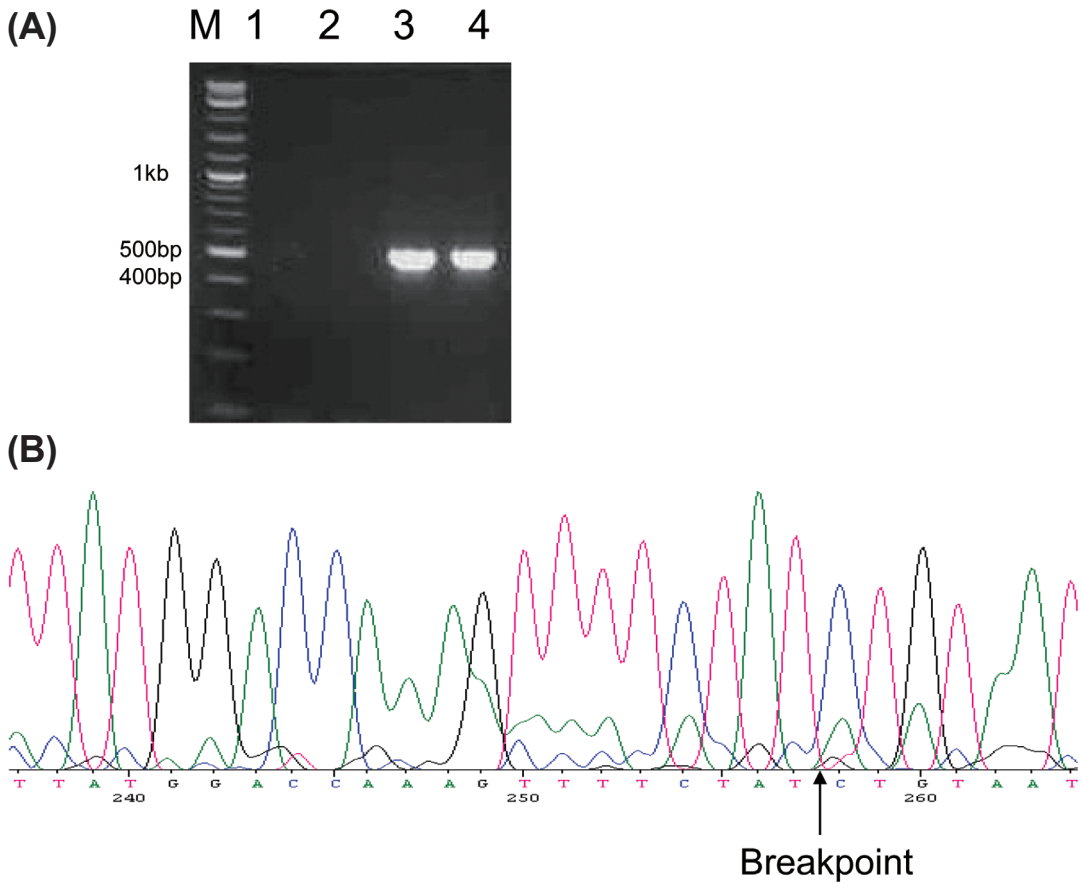

Fig. 2 (A) PCR amplification with deletion-specific primers. M, 100 bp marker; 1, negative control (distilled water); 2, negative control (a healthy male subject); 3, patient; 4, the patient's mother. (B) Sequence of the deletion breakpoint. The arrows indicate 5'- and 3'-breakpoints at positions 1,023,226 bp and 2,077,444 bp, respectively, relative to the published sequence (GenBank accession NT_079573).

is asymptomatic, she should be alerted to the possibility of developing a hyperammonemic crisis (11).

Recent advances in clinical management of OTC deficiency, including liver transplantation, have improved patient prognosis; therefore, detailed genetic analysis is needed for better management of patients. Most mutations causing OTC deficiency consist of single-base substitutions, while a smaller proportion of mutations consist of small deletions or insertions and larger deletions (2). Several studies have reported a large deletion in *OTC* a neighboring gene (12, 13), and the results of a recent study involving array CGH revealed partial or complete deletion of *OTC* in 11 of 70 samples of patients lacking point mutations (6). Previously, breakpoint determination for large unknown deletions was timeconsuming and laborintensive. However, recent DNA chip technology such as array CGH and high-density SNP assays allows us to detect deleted regions within a few days. In this case, the patient lacked the entire *TSPAN7* and *MID1IP1* genes and part of *RPGR* in addition to *OTC*. It has been reported that RPGR is the cause of X-linked retinitis pigmentosa (RP) (8). RP is characterized by night blindness; concentric constriction of visual fields; pigment deposits,

predominantly in the midperiphery of the retina; and attenuation of retinal vessels. In males, X-linked RP is associated with a severe phenotype in terms of the onset and progression of the disease. Females with disease-causing mutations can develop RP; therefore, the patient's mother was recommended to consult an ophthalmologist for a checkup, although she did not suffer from visual impairment. It has been suggested that *TSPAN7* is associated with X-linked mental retardation (14) and autism spectrum disorder (15). The function of MID1IP1 has not yet been elucidated. Analysis of deletion breakpoints did not show the shared breakpoints identified in previous reports (2, 6, 16, 17), suggesting that the deletion is family-specific.

This case has shown that detailed molecular genetic analysis is necessary for patient management and for providing genetic counseling to the family members involved. Use of the widely available high-density SNP array allows for rapid identification of deletion breakpoints; therefore, it will become a standard technique in study of patients with large genomic deletions of contiguous genes for provision of comprehensive genetic counseling and initiation of clinical management.

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