Vitamin B₆-Responsive Ornithine Aminotransferase Deficiency with a Novel Mutation G237D

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OHKUBO, Y., UETA, A., ITO, T., SUMI, S., YAMADA, M., OZAWA, K. and TOGARI, H. *Vitamin B₆-Responsive Ornithine Aminotransferase Deficiency with A Novel Mutation G237D*. Tohoku J. Exp. Med., 2005, **205** (4), 335-342 — Ornithine aminotransferase (OAT) deficiency (MIM: 258870) is a rare congenital metabolic disorder characterized by gyrate atrophy of the choroid and retina. Here, we report a 37-year-old male with gyrate atrophy of the choroid and retina who has been treated for 18 years. At the age of 7 years, the patient consulted an ophthalmologist due to progressive loss of vision. A large atrophied area was observed in his retina, and OAT deficiency was suspected. At the age of 19 years, amino acid analysis revealed high serum ornithine levels (1140 nmol/ml), with the normal range being 40-100 nmol/ml. He was treated with vitamin B₆ 300 mg/day for 6 months, which successfully reduced his serum ornithine levels by 20-30%. For 18 years since, his serum ornithine levels have been maintained with vitamin B₆ medication. There was no further impairment of vision or increase in the atrophied area, as judged by ophthalmoscopic examination. OAT activity was undetected in white blood cells of the patient and was 105% and 45% of normal values in his wife and son, respectively. OAT gene analysis revealed a novel mutation of Gly237Asp in exon 7 (710G > A) in both alleles of the patient, while his son was a heterozygote for the mutation. Notably, this novel mutation is associated with a vitamin B₆-responsive phenotype. Therefore, early diagnosis and treatment with vitamin B₆ may prevent loss of vision in some patients with OAT deficiency. ——— ornithine aminotransferase; gyrate atrophy; hyperornithinemia; vitamin B₆

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Gyrate atrophy (GA) is a rare inherited form of chorioretinal degeneration. The initial complaint of decreasing night vision is associated with the appearance of sharply demarcated, circular areas of chorioretinal atrophy in the midperiphery of the fundus. During the first three decades of

life, the atrophic areas enlarge, coalesce, and spread toward the posterior pole. Furthermore, colorless, elongated crystals have been observed overlaying the remaining pigmented areas in older patients. In addition to myopia, posterior subcapsular cataracts, and vitreous opacities also which

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may be present (Takki and Simell 1976).

Ornithine amino transferase (OAT) is a nuclear-encoded pyridoxal phosphate requiring mitochondrial matrix enzyme that catalyzes the interconversion of ornithine, glutamate and proline (Fig. 1). In humans, hereditary deficiency of OAT (MIM: 258870) activity, which has been shown to be autosomal recessive (Takki and Simell 1974), results in a markedly increased levels of plasma ornithine and is associated with loss of vision due to GA (Takki and Simell 1973). The enzyme is present in many mammalian tissues, including liver (Peraino and Pitot 1963), kidney (Herzfeld and Knox 1968), and ocular tissues (Hayasaka et al. 1980; Shiono et al. 1982). The mechanism of GA remains unknown; however, the adverse effects of creatine or pyrroline-5-carboxylate (P5C) deficiency on retinal function are thought to be a causative factor of GA (Valle and Simell 1995).

OAT activity has been assayed by spectrophotometric (Strecker 1965) and radioactive methods (Phang et al. 1973). O'Donnel et al. (1977) has reported a high-performance liquid chromatography (HPLC) assay for OAT. The principle of this assay is the measurement of a dihydroquinoxolinium compound produced by the reaction of *o*-aminobenzaldehyde (OAB) and P5C, which is catalyzed by OAT.

The OAT gene on chromosome 10q26 spans 21 kb of DNA (Mitchell et al. 1988a), encoding a transcript of 2.2 kb in 11 exons (Mitchell et al. 1988b). OAT deficiency patients have been reported worldwide with diverse ethnic and racial backgrounds. More than fifty pathologic mutations have been reported to date and eight of these

have been reported in Japanese patients (Mashima et al. 1996).

Here, we report a novel missense mutation in the OAT gene, which is associated with a vitamin B₆-responsive phenotype of OAT deficiency. To our knowledge, this is the first case report of OAT deficiency showing high serum ornithine levels, very low OAT enzyme activity, genetic mutation, and successful management of an OAT patient.

MATERIALS AND METHODS

Case

A 37-year-old male consulted an ophthalmologist at 7 years of age due to impairment of vision. The retina of his left eye was detached because of an injury during toddlerhood. His right eye had a large atrophied area, which was so severe that the choroid could be seen with an ophthalmoscope (Fig. 2), and he was suspected for having OAT deficiency due to the presence of GA. At the age of 19 years, he consulted our department, and amino acid analysis revealed high levels of serum ornithine (1140 nmol/ml). An aminogram revealed that other amino acids were within the normal ranges (Table 1). He was diagnosed as having OAT deficiency based on ophthalmoscopic observation and amino acid analysis. After 6 months of vitamin B₆ therapy at 300 mg/day (orally, 3 times daily), levels of serum ornithine were again measured. He developed cataract in his right eye during the third decade of his life, and he was operated on at the age of 32 years. Around that time, he had a son with his healthy wife, and when his son was 9 months old, he consulted us to check for OAT deficiency. Ophthalmologic analysis revealed no abnormalities, and serum ornithine levels were normal.

For the present study involving enzymatic and genomic analyses, peripheral blood was extracted from the

Fig. 1. Ornithine metabolism. ASA, Argininosuccinic acid; OAT, Ornithine aminotransferase; P5C, Pyrroline-5-carboxylate; P5CD, P5C dehydrogenase; P5CR, P5C reductase.

TABLE 1. Amino acid levels of OAT deficient patient before and after 18 years of vitamin B₆ treatment

Amino acids	Before treatment (nmol/ml)	After 18 years of treatment (nmol/ml)	Normal range (nmol/ml)
Ornithine	1140.6	921.2	40 - 100
Lysine	135.1	104.3	150 - 260
Histidine	100.5	77.6	75 - 110
Arginine	128.2	104.3	70 - 160
Threonine	166.1	129.0	110 - 240
Serine	166.6	110.5	98 - 210
Glutamine	599.8	526.3	560 - 890
Glycine	224.3	193.1	110 - 310
Alanine	373.9	262.8	280 - 650
Valine	286.1	204.9	180 - 370
Cystine	35.9	40.9	37 - 63
Methionine	31.0	20.6	21 - 40
Isoleucine	86.6	48.4	43 - 110
Leucine	139.4	98.6	88 - 200
Tyrosine	70.7	70.0	48 - 95
Phenylalanine	72.1	60.1	50 - 83
Proline	196.5	128.7	110 - 310
Citrulline	23.1	25.6	22 - 52

patient, his wife and their son after obtaining informed consent.

Plasma amino acid analysis

Plasma amino acid concentrations after overnight fasting were measured with an automated amino acid analyzer (SRL, Tokyo).

OAT activity

Our OAT assay was modified using a previously reported HPLC method (Heinonen et al. 1998). The end product of the OAT reaction, i.e. P5C, was allowed to react with OAB, and the resulting dihydroquinoxolinium compound was separated by HPLC and detected spectrophotometrically. The standard enzyme reaction mixture contained 300 μ l of cell lysate supernatant, 30 μ l of 0.5 M L-ornithine (Fluka, Buchs, Germany), 20 μ l of 0.1 M α -ketoglutarate (Fluka), and 25 μ l of 0.064 mg/ml pyridoxal phosphate (Fluka). After 4 hours of incubation at 37°C, protein was removed by trichloroacetic acid precipitation. P5C was then allowed to react with OAB to obtain a dihydroquinoxolinium derivative. A 50 μ l aliquot of plasma sample or P5C standard (Sigma

Chemical, St. Louis, MO, USA) was incubated with 10 μ l of 7.5 mg/ml OAB (Sigma Chemical) for 60 min at room temperature. A 50 μ l H₂O blank was also incubated with 10 μ l of OAB. The acquired dihydroquinoxolinium compound was separated by HPLC, and its absorbance was measured at 254 nm.

The HPLC system consisted of a CR-7A system (Shimadzu, Tokyo) incorporating an in-line photodiode array absorption detector with an Asahipak GS-320 HQ column (7.6 mm i.d. $\times$ 300 mm; Asahi Seiko Kogyo, Tokyo). Chromatography was performed with a mobile phase of phosphate buffer (50 mM KH₂PO₄; pH 5.0), and at a flow rate of 0.5 ml/min.

DNA analysis

DNA was extracted from 200 μ l of blood using a QIAamp DNA blood mini kit (Qiagen, Tokyo) according to the manufacturer's instructions.

Nine segments of the OAT gene encompassing the entire coding region (exons 3 to 11) were amplified by PCR. OAT genomic DNA (100 ng) was amplified by 2 $\times$ PCR Master Mix (50 units/ml Taq DNA polymerase, 400 μ M dATP, dCTP, dGTP, and dTTP, 3.0 mM MgCl₂;

Promega Corporation, Madison, WI, USA) containing nuclease-free water in a total volume of 50 μ l, with 0.8 μ M concentrations of PCR primers. The PCR primers used in this study are shown in Table 2. Amplification was performed with the Gene Amp PCR system 9700 (Applied Biosystems, Foster City, CA, USA) using an initial denaturation step of 94°C for 10 min followed by 40 cycles of 94°C for 30 s, 53°C for 30 s, and 72°C for 30 s, and a final extension step of 72°C for 5 min. Amplified PCR products were analyzed on a 1% agarose gel with a 50-bp ladder (Invitrogen, Carlsbad, CA, USA) as a molecular weight marker. PCR products were purified with a GeneClean Spin Kit (Qbiogene, Irvine, CA, USA) according to the manufacturer's instructions.

For sequencing, PCR products were purified and an aliquot was used in the cycle sequencing reaction using a DNA sequencing kit (Applied Biosystems), the same primer used in PCR (Table 2) and an autoanalyzer (ABI PRISM 310 Genetic Analyzer; Applied Biosystems), according to the manufacturer's instructions.

A homology search was performed using the

GENETYX-MAC computer software Package (Software Development Co., Tokyo).

Protein secondary structure prediction

Consensus secondary structure predictions were made using software available on the NPS@ website at <http://npsa-pbil.ibcp.fr/> (Combet et al. 2000).

RESULTS

Plasma amino acid analysis and ophthalmoscopic view results

Serum ornithine levels in the patient were 1140.6 nmol/ml (normal range; 40-100 nmol/ml) when he was 19 years old. There were no abnormalities detected in other amino acids (Table 1). High-dose vitamin B₆ administration was performed, and serum ornithine levels fell to 831.5 nmol/ml after 6 months (Fig. 3). After 18 years of medication, his serum ornithine levels were 921.2 nmol/ml. Administration of vitamin B₆ thus

TABLE 2. PCR primers

Exons	Primers (5' to 3')
Exon 3	Forward: CAAGCAATCCTCCACCTCA Reverse: TCAAGAAAAGGGAAAAGACG
Exon 4	Forward: ATTATTTTCCTTTCTTATGT Reverse: ATCTTTGCTTTGTATTCTG
Exon 5	Forward: AAATACCCTCAAAAATAATC Reverse: GAACTCCAGGGCTCAAAGAC
Exon 6	Forward: AATAATCACTGTTTGTTC Reverse: AAAGTAGTTGGCTGGCTGTA
Exon 7	Forward: CGTGCTCTTCAGGATCCAAA Reverse: AGCTCTGCACCAGGCACCAG
Exon 8	Forward: AAAGGAAATAGTTGGTGAGT Reverse: TTCAGGCAATAAGTAAGTGG
Exon 9	Forward: ACTCTGGGGAAAAGGATGCT Reverse: GGAAGCCCACGGAGGAAATC
Exon 10	Forward: TGAAAAATAGCAATGAAAGT Reverse: TTGAACCCAGGAGGCAGAGT
Exon 11	Forward: GTAGAGACGGGTTTCATAAT Reverse: GCACATCAAAACACTTCAAC

reduced serum ornithine levels by 20-30%.

On the first ophthalmoscopic consultation, the atrophic area covered most of the retina. Moreover, the choroid and sclera were partially visible. However, this did not result in total blindness, as only the macula remained in the shape of an island. Although ophthalmoscopic improvement was uncertain, there was no exacerbation nor were there side effects of vitamin B₆ administration.

Serum ornithine levels of the patient's son were 147.4 nmol/ml at age 9 months, which was

Fig. 2. Ophthalmoscopic view of the patient's right eye during the first consultation with an ophthalmologist. Wide atrophied areas can be seen in most of the retina. The choroid and sclera were partially visible.

Fig. 3. Serum ornithine levels of the patient before and after 18 years of treatment with vitamin B₆ (300 mg/day).

A. OAT gene analysis

a. Wild type

b. Homozygote

c. Heterozygote

B. The pedigree of the OAT patient

Fig. 4. A: OAT gene analysis. a, wild type (patient's wife); b, homozygote (patient) showing guanine to adenine change at position 710 (Gly 237Asp); c, heterozygote (patient's son). B: Pedigree of OAT deficiency patient. ■, patient (homozygote); ○, patient's wife (wild type); ▨, patient's son (heterozygote). Enzyme activity of the patient, his wife and his son were < 1%, 105% and 45%, respectively, when compared with normal controls.

within the normal range, and abnormalities were not seen on ophthalmoscopy. At 5 years of age, ornithine levels were 67.5 nmol/ml, which is within the normal range.

The patient's wife had no relevant medical history, and did not show any abnormalities on serum amino acid analysis or ophthalmoscopy.

OAT activity

OAT enzyme activity (as measured by HPLC), in five normal volunteers ranged from 1.09×10^{-7} to 1.53×10^{-7} mmol/h/mg, and the average enzyme activity was $1.31 \times 10^{-7} \pm 0.20 \times 10^{-7}$ mmol/h/mg (mean $\pm$ S.D.) in this group.

OAT enzyme activity was not detected in the patient, and level in the patient's son were 0.59×10^{-7} mmol/h/mg (45% that seen in the control group), while those in the patient's wife were 1.38×10^{-7} mmol/h/mg (105% that seen in the control group) (Fig. 4B).

OAT mutation

Direct sequencing of OAT genomic DNA and all coding exons revealed a guanine to adenine change in the patient and his son at position 710 in exon 7, accompanied by an amino acid substitution Gly237Asp (Fig. 4). This mutation is homozygous in the patient, heterozygous in his son and is not present in his wife.

Moreover, a homozygous cytosine-to-thymine change at position 1134 was detected in exon 10 in the patient and his son, but was not accompanied by an amino acid substitution (Asn378). This single-nucleotide polymorphism (SNP) is not present in the patient's wife.

Protein secondary structure prediction

Secondary structure modeling predicted an increase in the size of an α -helix in the immediate vicinity of the Gly237Asp amino acid substitution.

DISCUSSION

OAT deficiency is characterized by progressive degeneration of the retina and choroid, and has autosomal recessive heredity. Because of the unique ophthalmoscopic observations involving

the gyrus, it is also called gyrate atrophy of the choroid and retina. It manifests mainly as a gradual loss of vision, and exhibits symptoms of night blindness, tunnel vision, and cataract (Kaiser-Kupfer et al. 1983). In the differential diagnosis of OAT deficiency, hyperornithinemia, hyperammonemia, and homocitorullinuria (HHH) syndrome (Salvi et al. 2001) and retinitis pigmentosa (Lee et al. 2003) should be considered. HHH syndrome exhibits hyperornithinemia, hyperammonemia, and homocitorullinuria as its three major manifestations. However, this syndrome has no ophthalmoscopic symptoms, which differentiates it from OAT deficiency. Retinitis pigmentosa has similar optical symptoms, such as progressive loss of vision and night blindness, but without hyperornithinemia. Since Takki and Simell (1973) first reported OAT deficiency, various reports on the ophthalmoscopy, treatment, enzyme activity and genetics have been published (Kaiser-Kupfer et al. 1981; Kuwabara et al. 1981; Vannas-Sulonen et al. 1985; Mashima et al. 1992a, b). Ophthalmologic examination and gene analyses of Japanese cases have been reported (Hayasaka et al. 1981; Mashima et al. 1996), but enzyme activity and familial genomic analysis have not been reported. In the present paper, we reported a familial study of OAT deficiency with a novel mutation that was vitamin B₆-responsive, covering their biochemical results, enzyme activity and gene analysis. We reported a novel mutation that was vitamin B₆-responsive.

For measurement of OAT activity in this study, we used five volunteers without abnormal aminograms as a control group, and enzyme activity was measured. When compared with the enzyme activity in the control group, the enzyme activity of our patient was undetectable (< 1%), while those of his wife and child were 105% and 45%, respectively. Serum ornithine levels were high in the patient but were normal in his wife and son.

Numerous mutations in OAT deficiency have been reported. In Finnish OAT deficiency patients, only two types of mutation are known: Leu402Pro (1205T > C) and Arg180Thr (539G > C) (Mitchell et al. 1989). In contrast, eight variants on the

OAT gene have been reported in Japanese patients. One of these is the deletion of one-half of the allele and the others are point mutations (Mashima et al. 1996). Val332Met (Ramesh et al. 1988) and Ala226Val (Michaud et al. 1995) are reported as vitamin B₆ reactivity mutations. It is intriguing that these mutations are located in a coenzyme joint region (Lys292) or are adjacent to a noncovalent binding site (Gln230, Asp262). Gene analyses in this study of the OAT patient's genomic DNA revealed a 710G > A (Gly237Asp) mutation and a 1134C > T (Asn378Asn) SNP. This is the first report of a 710G > A mutation. The amino acid substitution Gly237Asp caused a change in secondary conformation around codon 237 (from random coil or extended strand to alpha helix). The DNA sequence around codon 237 is conserved among rat, mouse, drosophila, moth-bean, plasmodium and yeast (Yoshida et al. 1997). These facts indicate that this new mutation reduces enzyme activity. The 1134C > T change was previously reported in 9 of 31 Caucasian subjects (Martin et al. 1991).

DNA sequence analysis showed that the patient and his son are homozygous and heterozygous for the 710G > A mutation, respectively. This newly identified mutation causes Gly237Asp substitution adjacent to the coenzyme joint and in a noncovalent bonding site, as described above. In addition, the 1134C > T SNP is homozygous in the patient and his son, while the patient's wife is heterozygous for this SNP.

Indeed treatment with vitamin B₆ (300 mg/day) reduced our patient's serum ornithine by 20-30%. Furthermore, therapy with vitamin B₆ retarded the progress of GA and limited the progress of his visual impairment for 18 years. This new mutation may therefore be designated a vitamin B₆-responsive mutation.

We reported here in a familial study of OAT deficiency. There are few reports on the relationship between enzyme activity and genetic mutations. In this report, using gene diagnosis, we confirmed the proband and autosomal recessive hereditary. Over fifty different mutations have been reported in OAT deficiency (Brody et al. 1992; Michaud et al. 1992). Most of these are

point mutations. It is thought to be advantageous, with respect to time and cost, to first analyze enzyme activity. This analysis also leads to a definitive diagnosis.

With regard to treatment, the collection of gene analysis data may be able to predict responsiveness to vitamin B₆ based on the site of mutation. The analysis in this report could be useful for classifying OAT deficiency as being responsive or nonresponsive to vitamin B₆. However, both enzyme activity and gene analysis are necessary for diagnosis and successful treatment.

Because untreated OAT deficiency causes blindness and early vitamin B₆ administration retards the progress of visual impairment and blindness, early diagnosis is desirable. In order to accomplish this, it is necessary for ophthalmologists and pediatricians to have frequent contact and clear recognition of this disease.

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