Am. J. Hum. Genet. 56:616–622, 1995

Pyridoxine-Responsive Gyrate Atrophy of the Choroid and Retina: Clinical and Biochemical Correlates of the Mutation A226V

Jacques Michaud,[1,2,*] Geoffrey N. Thompson,[3,†] Lawrence C. Brody,[4] Gary Steel,[4] Cassandra Obie,[4] Gisèle Fontaine,[1] Keith Schappert,[2,5,‡] C. Gregory Keith,[3,§] David Valle,[4] and Grant A. Mitchell[1,2]

[1]Service de génétique médicale, Hôpital Ste-Justine, Montréal; [2]Canadian Genetic Diseases Network; [3]Murdoch Institute, Royal Children's Hospital, Melbourne; [4]Departments of Pediatrics and Molecular Biology and Howard Hughes Medical Institute, Johns Hopkins University School of Medicine, Baltimore; and [5]Department of Medical Genetics, Hospital for Sick Children, Toronto

Summary

We discovered the missense mutation, A226V, in the ornithine-δ-aminotransferase (OAT) genes of two unrelated patients with gyrate atrophy of the choroid and retina (GA). One patient, who was a compound for A226V and for the premature termination allele R398ter, showed a significant ($P < .01$) decrease in mean plasma ornithine levels, following pyridoxine supplementation with a constant protein intake: 826 ± 128 μM ($n = 5$; no pyridoxine supplementation) versus 504 ± 112 μM ($n = 6$; 500 mg pyridoxine/d) and 546 ± 19 μM ($n = 6$; 1,000 mg pyridoxine/d). In extracts of fibroblasts from a second GA patient homozygous for A226V and from Chinese hamster ovary cells expressing an OAT-cDNA–containing A226V, we found that OAT activity increased from undetectable levels to ~10% of normal when the concentration of pyridoxal phosphate was increased from 50 to 600 μM. A226V is the fourth disease-causing pyridoxine-responsive human mutation to be reported.

Introduction

Gyrate atrophy of the choroid and the retina (GA) is a slowly progressive, blinding autosomal recessive disorder. The biochemical hallmark of GA is ornithine accumulation with plasma and urine levels ranging 5–15-fold above normal. The primary defect is deficiency of ornithine-δ-aminotransferase (OAT), a mitochondrial matrix enzyme that catalyzes the reversible interconversion of ornithine and Δ^1-pyrroline-5-carboxylate (Valle and Simell 1989). OAT monomers are synthesized as 49-kD precursors and are processed to 45 kD on entry into the mitochondrial matrix, where they assemble into the active homohexamers (Markovic-Housley et al. 1987). One molecule of cofactor pyridoxal-5′-phosphate (PLP) is bound covalently to each OAT monomer at lysine 292 (Simmaco et al. 1986). We and others have cloned and sequenced near-full-length human OAT cDNAs and have determined the organization of the OAT structural gene (Inana et al. 1986; Ramesh et al. 1986; Mitchell et al. 1988*b*). More than 50 mutations have now been identified at the OAT locus (Inana et al. 1988, 1989; Mitchell et al. 1988*a*, 1989, 1991; Ramesh et al. 1988, 1990; McClatchey et al. 1990; Akaki et al. 1992; Brody et al. 1992; Mashima et al. 1992; Michaud et al. 1992; Park et al. 1992).

GA is one of several inborn errors that affect enzymes with vitamin-derived cofactors. In some of these conditions, a small fraction of patients respond clinically and/or biochemically to treatment with pharmacological doses of the relevant vitamin. Some of the best-documented examples of vitamin-responsive inborn errors involve enzymes that, like OAT, utilize PLP as a cofactor. Five GA probands have been described in whom plasma ornithine levels decreased >33% in response to treatment with pyridoxine (Berson et al. 1978; Kennaway et al. 1980; Hayasaka et al. 1981); and a sixth in whom the GA phenotype was clinically mild and who had a marked in vitro response to PLP on OAT assay was described by Kennaway et al. (1989). OAT activity in fibroblast extracts from these patients increased when the concentration of PLP in the enzyme assay was increased 10–100-fold.

Despite the wealth of clinical and biochemical experience with vitamin-responsive inborn errors and the recent descriptions of mutations in pyridoxine-responsive cystathionine β-synthase–deficient patients (Hu et al. 1993; Kozich et al. 1993; Kraus et al. 1993), to date we are aware of only three mutations in humans that have been shown conclusively to be pyridoxine responsive. The first description of a vitamin-responsive mutation for any inborn error was the OAT missense mutation V332M identified in a pyridoxine-responsive GA patient by Ramesh et al. (1988). The two other proved pyridoxine-responsive mutations are

Received August 4, 1994; accepted for publication December 9, 1994.
Address for correspondence and reprints: Dr. Grant A. Mitchell, Service de génétique médicale, Hôpital Ste-Justine, 3175 Côte Ste-Catherine Road, Montréal, Québec, Canada H3T 1C5.
* Present address: Institut d'Embryologie Moléculaire et Cellulaire du Collège de France et du CNRS, Nogent-sur-Marne, France.
† Present address: 220 Melbourne Street, North Adelaide, S.A. 5006, Australia.
‡ Present address: Department of Medical Genetics, University of Uppsala, Uppsala.
§ Deceased.
© 1995 by The American Society of Human Genetics. All rights reserved.
0002-9297/95/5603-0009$02.00

in the erythroid-specific δ-aminolevulinate synthetase of patients with X-linked sideroblastic anemia (Cotter et al. 1992; Cox et al. 1994). In this report, we describe a new PLP-responsive OAT mutation, A226V, identified in two patients with GA.

Subject, Material, and Methods

Case Report and Clinical Evaluation of Pyridoxine Responsiveness in Patient GA084

GA084 is a 7-year-old Australian girl who presented with poor vision at 4 years of age. No other family members have severe eye problems. Her parents are unrelated healthy Caucasians with no known Italian ancestry. Ophthalmologic examination at 5 years of age revealed typical GA lesions in the periphery of the retina, moderate myopia, and mild astigmatism. At this time, corrected visual acuities were 20/20 (right eye) and 20/40 (left eye). Visual fields were clinically normal. Perimetry was not performed. Electroretinography showed no response under scotopic conditions and low potentials with lengthened b-wave implicit times under photopic conditions. Fasting plasma ornithine concentrations, measured at intervals of $\geqslant$1 wk, were 652, 596, and 620 μM (normal range 27–96 μM). Other amino acid concentrations were normal. Pyridoxine (500 mg/d) was administered, and, 1 mo later, fasting plasma ornithine concentrations, at weekly intervals, were 281, 213, and 201 μM (mean = 232 μM, or 37% of the prepyridoxine mean). Pyridoxine supplementation (500 mg/d) was continued, except during the trial described below. Dietary protein intake was unrestricted. Now, at 7 years of age, her corrected visual acuity has fallen to 20/60 in each eye. Fundoscopic appearances are essentially unchanged from those at the time of diagnosis.

We performed a second trial of pyridoxine responsiveness in GA084, divided into three phases, each separated by a 2-mo washout period. In phase 1, the dose of pyridoxine was 500 mg/d; in phase 2, 0 mg/d; and in phase 3, 1,000 mg/day. During each phase, a 2-mo stabilization period was followed by the collection of five or six blood samples taken at weekly intervals. The patient's protein intake was estimated with a 3-d diet record, the intake being kept as constant as possible, under dietetic supervision during each collection phase. Protein intake was 28, 30, and 27 gm/d during phases 1, 2, and, 3, respectively. The values from each phase were compared by the unpaired Student's t test.

Cell Lines, Cell Culture, and Enzyme Assays

Culture of patient and control fibroblasts and of the CHO-K1 Chinese hamster ovary clone NC5 (Mitchell et al. 1989) and OAT (Brody et al. 1992) and β-galactosidase (Miller 1972, pp. 352–355) assays were performed as described. Fibroblasts of GA026, a GA patient of Italian descent, were obtained from the National Institute of General Medical Sciences' Human Genetic Mutant Cell Repository (cell line GM6330).

OAT activity was measured radioisotopically, as we have described elsewhere (Brody et al. 1992), except that saturating concentrations of ornithine (15 mM) and α-ketoglutarate (3 mM) were used for all assays and the duration of the reaction was increased to 120 min. The concentration of PLP in this "high substrate" reaction routinely was 50 μM and was increased to 600 μM, so vitamin responsiveness could be assessed. The OAT activity of control fibroblast extracts is constant over this range of PLP concentrations. For assay of the OAT activity in transiently transfected NC5 cells, we utilized 40–60 μg of cellular protein/assay. For determination of the apparent Michaelis-Menten constant (K_m) of OAT for PLP in cell extracts, we used 125 μg protein/assay for control fibroblasts and 250 μg/assay for fibroblasts from the A226V homozygote GA026. We varied the concentration of PLP from 0 to 600 μM for the control fibroblast extracts and from 200 to 600 μM for the GA026 fibroblast extracts.

Plasmids

pGEM4 (Promega) was used for all cloning manipulations. phuOAT6 is a human-liver OAT cDNA containing the complete 1,317-bp open reading frame, 60 bp of 5′-nontranslated sequence, and 635 bp of 3′-nontranslated sequence (Mitchell et al. 1988*b*). mβ5 is a mouse β-tubulin cDNA probe used as a control for northern blot studies (Sullivan and Cleveland 1986). For β-galactosidase expression, we used the plasmid pCH110 (Hall et al. 1983), which contains the *Escherichia coli* lacZ gene. The eukaryotic expression vector p91023b (Oprian et al. 1987) was a gift from R. Kaufman (Genetics Institute).

RNA and DNA Isolation, Northern Blotting, Dideoxy Sequencing, and Probe Preparation

These techniques were performed by standard protocols. We have described this elsewhere (Brody et al. 1992).

Anti-OAT Peptide Antiserum and Immunoblotting

We used an antiserum directed against an OAT peptide spanning residues 32–50 to perform immunoblot analysis. We have described the technique elsewhere (Brody et al. 1992).

Identification of Mutations

The nine coding OAT exons of each patient were amplified from genomic DNA, with primers corresponding to flanking intronic regions, and SSCP analysis of these PCR products was performed under three different electrophoretic conditions, as described elsewhere (Michaud et al. 1992). For any exon showing abnormal migration with respect to control samples, genomic DNA from the patient was amplified, and the PCR products were sequenced directly in both directions. The presence of mutations discov-

ered in this fashion was confirmed by allele-specific oligonucleotide hybridization to the slot-blotted products of a different PCR amplification of the patient's genomic DNA.

Construction of Expression Vectors Containing OAT Mutations

We created an A226V-containing OAT cDNA as follows. We amplified OAT exon 7 from the genomic DNA of GA026 and subcloned the 60-bp exonic *Bam*HI fragment containing the A226V codon into a wild-type OAT cDNA from which this cassette had been excised. The A226V OAT cDNA was cloned into p91023b, sequenced in both directions over its entire length, including the regions flanking the cloning sites, and found to be identical to the wild-type sequence, except for the A226V mutation. We have elsewhere described the construction of expression plasmids containing wild-type and V332M- and R180T-OAT cDNAs in p91023b (Brody et al. 1992). R180T, which yields normal amounts of immunoreactive OAT peptide but has no detectable enzyme activity (Brody et al. 1992), serves as a negative control.

Transfections

NC5 cells were cotransfected with OAT-containing expression vectors and pCH110 as described elsewhere (Mitchell et al. 1989). The technique is a modification of the calcium phosphate precipitation method of Chen and Okayama (1987).

Results

Clinical Trial of Pyridoxine Supplementation in GA084

The effects of pyridoxine administration on plasma ornithine concentrations in GA084 are shown in figure 1. The mean ornithine concentration during the first period of pyridoxine treatment (500 mg/d) was 504 ± 112 μM (n = 6). During the second period, when pyridoxine was withdrawn, mean ornithine was 825 ± 128 μM (n = 6). In the third period, during pyridoxine supplementation at 1,000 mg/d, mean ornithine values were 546 ± 19.5 μM (n = 5). Plasma ornithine values during period 2 differed statistically from those in period 1 ($P < .01$) and in period 3 ($P < .001$). Plasma ornithine values did not differ significantly between periods 1 and 3.

Mutation Identification

SSCP analysis of amplified genomic DNA (not shown) revealed identical migration patterns for GA026, GA084, and a normal control in exons 3–6, 8, and 9. In exon 7, both GA084 and GA026 showed an identical abnormal pattern. GA026 appeared to be homozygous for the pattern, and GA084 appeared to be heterozygous. In exon 11, GA084 also showed an abnormal migration pattern, consistent with heterozygosity for a second mutant allele. In exon 10, each of these patients is homozygous for one polymorphic variant of the synonymous mutation N378N, GA026 for AAC and GA084 for AA*T* (Martin et al. 1991).

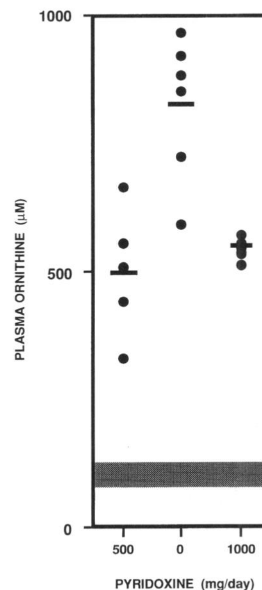

Figure 1 Biochemical response of GA084 to pyridoxine supplementation. Fasting plasma ornithine levels are indicated on the vertical axis, and the pyridoxine doses for each of the three phases are indicated on the horizontal axis. The dots (●) represent individual plasma levels obtained during each test period, and the horizontal bars indicate the mean ornithine level during each phase. The hatched zone indicates the normal upper and lower limits of plasma ornithine concentrations (21–77 μM).

Sequencing of cloned exon 7 amplified from genomic DNA showed GA026 to be homozygous and GA084 to be heterozygous for a C→T transition at position 677, which converts alanine 226 (GCG) to valine (G*T*G) (A226V) (fig. 2*A*). By allele-specific oligonucleotide hybridization, we did not find A226V in 83 other GA probands or in 32 controls. In GA084, we also identified a C→T transition at position 1192 in exon 11. This mutation creates a termination codon (CGA→*T*GA) and is designated "R398ter" (fig. 2*B*).

Analysis of Fibroblast OAT Activity, mRNA, and Antigen

OAT activity was undetectable (<1% of control) at both standard (50 μM) and high (600 μM) PLP concentrations in extracts of GA026 (A226V/R398ter) fibroblasts. By contrast, the OAT activity in extracts of GA084 (A226V/A226V) increased from 9 nmol product/mg/h (1.8% of control) to 44 nmol product/mg/h (8.9% of control) when the concentration of PLP in the assay was increased to 600 μM.

Northern blot analysis of fibroblast RNA from both GA026 and GA084 (not shown) showed normal amounts of a normal size OAT mRNA. By immunoblotting, fibroblasts from each patient contained reduced but detectable amounts of an OAT antigen with slightly slower migration than wild-type OAT (illustrated in fig. 3 for GA026).

Expression of A226V

To test directly whether A226V is a PLP-responsive mutation, we performed transient transfection studies compar-

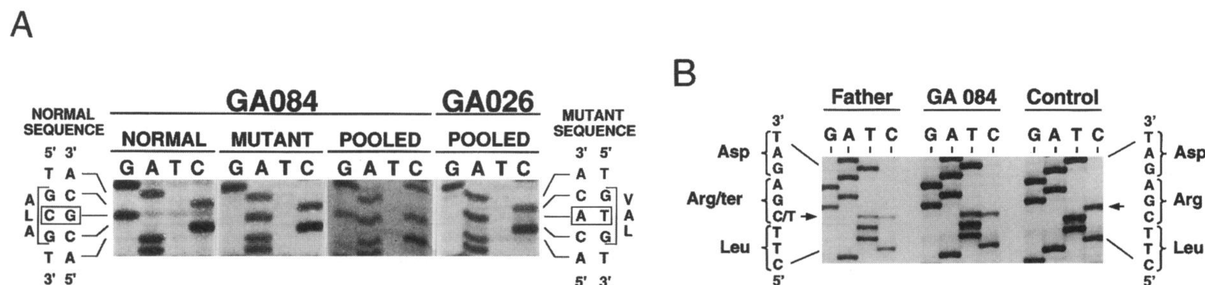

Figure 2 *a,* Sequence of cloned OAT exon 7 fragments of GA084 and GA026. The complementary strand sequence is shown. The template DNAs were individual exon 7 clones from GA084 ("normal" and "mutant") and pools of hybridizing exon 7 clones from GA084 (n = 16) and GA026 (n = 13). *b,* Results of direct sequencing of amplified exon II in the father of GA084, GA084, and a normal control, showing heterozygosity for R398ter in GA084 and her father.

ing its activity with that of the previously reported PLP-responsive allele (V332M) and a PLP-nonresponsive OAT allele that has detectable OAT antigen (R180T). NC5, a clonal line of CHO K1 cells that expresses no endogenous OAT mRNA, antigen, or enzymatic activity (Brody et al. 1992), was used as the recipient cell line in these studies. Cells transfected with A226V, V332M, R180T, and wild-type OAT expression vectors produced large amounts of OAT mRNA (not shown) and antigen (fig. 3). In cells transfected with the A226V construct, OAT antigen was detectable in amounts similar to those of cells transfected with the wild-type OAT construct. However, as in fibroblasts, the migration of the A226V peptide was slightly slower than that of wild-type OAT.

Despite normal amounts of OAT antigen, OAT activity normalized to β-galactosidase activity was barely detectable at 50 μM PLP for either A226V or V332M (table 1). At 600 μM PLP, the activity of wild-type OAT did not change, but that of V332M and A226V alleles increased to 15.8% and 12.9% of control, respectively. In extracts of R180T-transfected cells, OAT activity was undetectable at both PLP concentrations. The apparent K_m of OAT for PLP measured at saturating concentrations of ornithine and of 5-oxoglutarate was 122 μM in fibroblast extracts from GA026 and 6 μM in control fibroblast extracts.

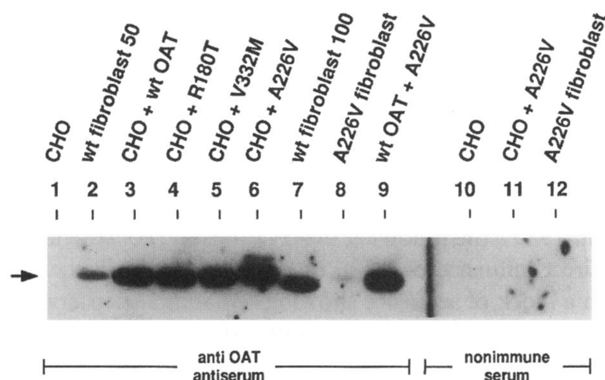

Figure 3 Immunoblot study of mutant and normal OAT expression in fibroblasts and transfected CHO cells. The lanes contain whole cell protein from the indicated sources. Fifty micrograms of protein were loaded in each lane, except lanes 7–9, which each contain 100 mg. Lane 8, Fibroblast protein from the A226V homozygote, GA026. Lane 9, Fifty micrograms each of protein from CHO cells transfected with wild-type OAT and with A226V-containing OAT constructs. On a shorter exposure both upper (A226V) and lower (wild-type) bands were visible. Negative controls include nontransfected cells (lane 1) and samples exposed to preimmune serum (lanes 10–12).

Discussion

A226V is the second PLP-responsive OAT mutation to be identified in GA and, to our knowledge, the fourth documented PLP-responsive mutation known for any human enzyme (Ramesh et al. 1988; Cotter et al. 1992; Cox et al. 1994). Three GA patients who have A226V mutant alleles have now been described: GA026, GA084, and the patient of Park et al. (1992), who is a genetic compound for A226V and a splice-site mutation. GA084, whom we showed to have a significant reduction in plasma ornithine levels following the administration of therapeutic doses of pyridoxine (fig. 1), is also a genetic compound for an A226V allele (fig. 2*A*) of maternal origin (data not shown) and a paternally derived premature-termination allele, R398ter (fig. 2*B*). The A226V homozygote GA026 was unfortunately not available for clinical trials of pyridoxine administration.

Pyridoxine responsiveness may be underdiagnosed in GA patients. Figure 1 demonstrates that, in GA084, multiple determinations of plasma ornithine were necessary in order to clearly demonstrate the pyridoxine response. In practice, we recommend that, in order to evaluate pyridoxine responsiveness, at least three fasting plasma ornithine levels be determined prior to and during pyridoxine supplementation in all patients with GA, under conditions of constant dietary arginine intake. Patients receiving pharmacological doses of pyridoxine should be tested for the development of peripheral neuropathy (Schaumburg and Berger 1988).

Detectable immunoreactive OAT peptide was present in the cells of only a small fraction of GA patients in our

Table 1

Enzymatic Activity of the Products of OAT Alleles Assayed at Standard and High PLP Concentrations

	OAT Activity			
	50-μM PLP		600-μM PLP	
Allele	Mean (Range)	% Detectable	Mean (Range)	% Detectable
Wild type	1.76 (1.80–2.01)	100	1.71 (1.20–1.98)	100
R180T	.01 (0–.02)	<1	.01 (0–.04)	<1
V332M	.01 (0–.02)	<1	.27 (.08–.42)	15.8
A226V	.03 (.01–.05)	1.7	.22 (.12–.32)	12.9

Note.—The values are the ratio of OAT activity (nmol product/h/mg protein) to β-galactosidase activity (μmol product/h/mg protein) in extracts of CHO NC5 cells cotransfected with an expression plasmid containing the indicated OAT allele (25 μg) and the β-galactosidase plasmid, pCH110 (5 μg). For each transient transfection experiment, OAT and β-galactosidase activity were measured in triplicate, and mean values were determined. These data are the means and ranges of the mean of three transient transfection experiments.

series (Brody et al. 1992). As expected, both A226V and V332M, the two PLP-responsive alleles reported to date, have detectable OAT antigen. GA patients whose cells contain stable OAT peptide should be evaluated carefully for pyridoxine responsiveness.

Expression studies of A226V confirmed PLP responsiveness to an extent similar to V332M, the previously reported PLP-responsive OAT mutation. The apparent K_m value of A226V for pyridoxal phosphate is similar to that reported in fibroblasts of other pyridoxine-responsive GA patients (Kennaway et al. 1989). Of >80 GA cell lines that we have studied, all have <6% residual OAT activity, suggesting that even the low levels of OAT activity observed in the expressed mutant OATs at high PLP concentration may prevent or slow the development of GA.

In fibroblast extracts, an increment of OAT activity in the presence of high PLP concentrations was demonstrated only for GA026. In the two other patients, both of whom are genetic compounds with a single A226V allele, Park et al. (1992) and we were unable to demonstrate an in vitro response to PLP. In GA084, we speculate that the single A226V allele results in a sufficient amount of OAT activity to reduce circulating ornithine levels during high-dose pyridoxine supplementation but that this level of OAT falls below the detection limit of our assay in fibroblast homogenates. GA084 cells also contain the R398ter mutation, which is predicted to have no detectable OAT activity, because we have elsewhere demonstrated that two premature termination alleles that flank R398 closely—R396ter and G401ter—have no detectable activity (Brody et al. 1992). It is possible that the mutant peptide produced by the R398ter allele may interfere with the function of the A226V peptide. Of note, the results suggest that clinical assessment of pyridoxine responsiveness is more sensitive than in vitro evaluation of the pyridoxine response of OAT activity in fibroblast extracts.

A226V results from a C→T transition at a CpG dinucleotide, a common occurrence in mammalian genomic DNA (Labuda and Striker 1989). GA026 and GA084, who are of different ethnic origins, are each homozygous for a different form of the intragenic polymorphism N378N. Together, these observations suggest that OAT alleles containing A226V may have arisen on at least two separate occasions. At present, we have no explanation for the somewhat slower migration of OAT peptides containing A226V, with respect to wild-type OAT in denaturing polyacrylamide gels (fig. 3).

It will be of interest to define the mutations and study the characteristics of OAT in the four other GA patients in whom a reduction of plasma ornithine levels, following pyridoxine supplementation, has been described elsewhere (Berson et al. 1978; Kennaway et al. 1980; Hayasaka et al. 1981). Since many residues may influence the position of the amino acids that react directly with PLP, further heterogeneity in the molecular basis of PLP responsiveness is expected.

Although we cannot eliminate an effect of PLP on the stability of OAT peptides containing A226V (Kennaway et al. 1989), the following observations are consistent with a direct influence of A226 on OAT catalysis. First, A226 is in a block of sequence showing remarkable conservation in all OATs and other ω-aminotransferases studied to date (fig. 4). In bacterial ω-amino acid pyruvate aminotransferase (ω-APT)—the only ω-aminotransferase for which the three-dimensional structure is known (Watanabe et al. 1989)—the residue corresponding to A226 is situated four residues from the conserved glutamate residue that interacts directly with the hydroxyl group of carbon 3 of PLP (fig. 4). In the analogous region of an α-aminotransferase, aspartate aminotransferase—the conformation of which closely resembles the PLP-binding region of ω-APT, although it bears no sequence similarity—mutations have also been shown

```
                            *       *
OAT (human)         P N V A A F M V E P
OAT (rat)           P N V A A F M V E P
OAT (yeast)         P N V A A I I V E P
OAT (plant)         P N V C A Y M V E P
OAT (P. malar.)     P N V C A F I V E P
GABAT (fungus)      P - V A A I I V E P
GABAT (pig)         K H G C A F L VDE V
APT (bacteria)      S N I A A V F V E P
DAPT (bacteria)     - E I A A V I I E P
```

Figure 4 Conservation of A226 and surrounding residues in OAT and other ω-aminotransferases. The sequences are aligned as by Watanabe et al. (1990). The sequences shown are OAT from human (Inana et al. 1988), rat (Mueckler and Pitot 1985), *Sacharomyces cerevisiae* (Degols et al. 1987), plant (*Vigna aconitifolia*) (Delauney et al. 1993), and *Plasmodium falciparum* (Schmid et al. 1993), as well as fungal gamma aminobutyric acid transaminase (GABAT) (Richardson et al. 1989), ω-APT (Yonaha et al. 1992), and bacterial 7, 8-diamino-pelargonic acid aminotransferase (DAPT) (Otsuka et al. 1988). The alanine residue corresponding to A226 in human OAT, and the glutamate residue known to interact directly with PLP in APT, are indicated by asterisks (*).

to alter PLP binding (Morino and Shimada 1990). On the basis of these observations, it seems likely that A226 influences the interaction of the OAT apoenzyme with PLP.

Acknowledgments

This research was funded in part by Medical Research Council of Canada grant MT-10541 (to G.M.) and National Institutes of Health grant EY02948 (to D.V.). D.V. is an investigator in the Howard Hughes Medical Institute. Dorothy Francis performed the dietary assessments, and Kaye Seller performed the plasma amino acid determinations, during the clinical trial of patient GA084. We thank David Danks for assistance in clinical follow-up. Micheline Patenaude and Sandy Muscelli provided excellent secretarial assistance.

References

Akaki Y, Hotta Y, Mashima Y, Murakami A, Kennaway NG, Weleber RG, Inana G (1992) A deletion in the ornithine aminotransferase gene in gyrate atrophy. J Biol Chem 267:12950–12954

Berson EL, Schmidt SY, Shih VE (1978) Ocular and biochemical abnormalities in gyrate atrophy of the choroid and retina. Ophthalmology 85:1018–1027

Brody LC, Mitchell GA, Obie C, Michaud J, Steel G, Fontaine G, Robert M-F, et al (1992) Ornithine-δ-aminotransferase mutations in gyrate atrophy: alleleic heterogeneity and functional consequences. J Biol Chem 267:3302–3307

Chen C, Okayama H (1987) High-efficiency transformation of mammalian cells by plasmid DNA. Mol Cell Biol 7:2745–2752

Cotter PD, Baumann M, Bishop DF (1992) Enzymatic defect in "X-linked" sideroblastic anemia: molecular evidence for erythroid δ-aminolevulinate synthase deficiency. Proc Natl Acad Sci USA 89:4028–4032

Cox TC, Botomley SS, Wiley JS, Bawden MJ, Matthews CS, May BK (1994) X-linked pyridoxine-responsive sideroblastic anemia due to a Thr^{388}-to-Ser substitution in erythroid 5-aminolevulinate synthase. N Engl J Med 330:675–679

Degols G, Jauniaux CC, Wiame JM (1987) Molecular characterization of transposable-element-associated mutations that led to constitutive L-ornithine aminotransferase expression in *Saccharomyces cerevisiae*. Eur J Biochem 165:289–296

Delauney AJ, Hu CAA, Kavi Kishor PB, Verma DPS (1993) Cloning of ornithine δ-aminotransferase cDNA from *Vigna aconitifolia* by trans-complementation in *Escherichia coli* and regulation of proline biosynthesis. J Biol Chem 268:18673–18678

Hall CV, Jacob PE, Ringold GM, Lee F (1983) Expression and regulation of *Escherichia coli* lack Z gene fusions in mammalian cells. J Mol Appl Genet 2:101–109

Hayasaka S, Saito T, Nakajima H, Takaku Y, Shiono T, Mizuno K, Ohmura K, et al (1981) Gyrate atrophy with hyperornithinaemia: different types of responsiveness to vitamin B_6. Br J Ophthalmol 65:478–483

Hu FL, Gu Z, Kozich V, Kraus JP, Ramesh V, Shih VE (1993) Molecular basis of cystathionine β-synthase deficiency in pyridoxine responsive and nonresponsive homocystinuria. Hum Mol Genet 2:1857–1860

Inana G, Chambers C, Hotta Y, Inouye L, Filpula D, Pulford S, Shiano T (1989) Point mutation affecting processing of the ornithine aminotransferase precursor protein in gyrate atrophy. J Biol Chem 264:17432–17436

Inana G, Hotta Y, Inouye L, Zintz C, Shiono T (1988) Single point mutation and amino acid change in ornithine aminotransferase from a gyrate atrophy patient. Invest Ophthalmol Vis Sci 29:14

Inana G, Totsuka S, Redmond M, Dougherty T, Nagle J, Shiono T, Ohura T, et al (1986) Molecular cloning of human ornithine aminotransferase mRNA. Proc Natl Acad Sci USA 83:1203–1207

Kennaway NG, Stankova L, Wirtz MK, Weleber RG (1989) Gyrate atrophy of the choroid and retina: characterization of mutant ornithine aminotransferase and mechanism of response to vitamin B6. Am J Hum Genet 44:344–352

Kennaway NG, Weleber RG, Buist NRM (1980) Gyrate atrophy of the choroid and retina with hyperornithinemia: biochemical and histologic studies and response to vitamin B_6. Am J Hum Genet 32:529–541

Kozich V, de Franchis R, Kraus JP (1993) Molecular defect in a patient with pyridoxine-responsive homocystinuria. Hum Mol Genet 2:815–816

Kraus JP, Le K, Swaroop M, Ohura T, Tahara T, Rosenberg LE, Roper MD, et al (1993) Human cystathionine β-synthase cDNA: sequence, alternative splicing and expression in cultured cells. Hum Mol Genet 2:1633–1638

Labuda D, Striker G (1989) Sequence conservation in Alu evolution. Nucleic Acids Res 17:2477–2491

Markovic-Housley Z, Zania M, Lustig A, Vincent MG, Jansonius JN, John RA (1987) Quaternary structure of ornithine aminotransferase in solution and preliminary crystallographic data. Eur J Biochem 162:345–350

Martin LS, Mitchell GA, Michaud J, Brody LC, Valle D (1991) A polymorphic synonymous mutation in human ornithine-δ-aminotransferase (N378N). Nucleic Acids Res 19:1962

Mashima Y, Murakami A, Weleber RG, Kennaway NG, Clarke L, Shiono T, Inana G (1992) Nonsense-codon mutations of the ornithine aminotransferase gene with decreased levels of

mutant mRNA in gyrate atrophy. Am J Hum Genet 51:81–91

McClatchey AI, Kaufman DL, Berson EL, Tobin AJ, Shih VE, Gusella JF, Ramesh V (1990) Splicing defect at the ornithine aminotransferase (OAT) locus in gyrate atrophy. Am J Hum Genet 47:790–794

Michaud J, Brody LC, Steel G, Fontaine G, Martin LS, Valle D, Mitchell GA (1992) Strand-separating conformational polymorphism (SSCP) analysis: efficacy of detection of point mutations in the human ornithine-δ-aminotransferase gene. Genomics 13:389–394

Miller JH (1972) Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Mitchell GA, Brody LC, Looney J, Steel G, Suchanek M, Dowling C, Der Kaloustian V, et al (1988*a*) An initiator codon mutation in ornithine-δ-aminotransferase causing gyrate atrophy. J Clin Invest 81:630–633

Mitchell GA, Brody LC, Sipila I, Looney J, Wong C, Engelhardt J, Patel A, et al (1989) At least two mutant alleles of ornithine-δ-aminotransferase cause gyrate atrophy of the choroid and retina in Finns. Proc Natl Acad Sci USA 86:197–201

Mitchell GA, Labuda D, Fontaine G, Saudubray J-M, Bonnefont J-P, Lyonnet S, Brody LC, et al (1991) Splice-mediated insertion of an Alu sequence inactivates ornithine-δ-aminotransferase: a role for Alu elements in human mutation. Proc Natl Acad Sci USA 88:815–819

Mitchell GA, Looney J, Brody LC, Steel G, Suchanek M, Engelhardt J, Willard H, et al (1988*b*) Human ornithine-δ-aminotransferase: cDNA cloning and analysis of the structural gene. J Biol Chem 263:14288–14295

Morino Y, Shimada K (1990) Mammalian aspartate aminotransferase isozymes from DNA to protein. Ann N Y Acad Sci 585:32–47

Mueckler MM, Pitot HC (1985) Sequence of the precursor to rat ornithine aminotransferase deduced from a cDNA clone. J Biol Chem 260:12993–12997

Oprian DD, Molday RS, Kaufman RJ, Khorana HG (1987) Expression of a synthetic bovine rhodopsin gene in monkey kidney cells. Proc Natl Acad Sci USA 84:8874–8878

Otsuka AJ, Bouncristiani MR, Howard PK, Flamm J, Johnson C, Yamamoto R, Uchida K, et al (1988) The *Escherichia coli* biotin biosynthetic enzyme sequences predicted from the nucleotide sequence of the bio operon. J Biol Chem 263:19577–19585

Park JK, Herron BJ, O'Donnel JJ, Shih VE, Ramesh V (1992) Three novel mutations of the ornithine aminotransferase (OAT) gene in gyrate atrophy. Genomics 14:553–554

Ramesh V, McClatchey A, Herron B, Weleber R, Kennaway NG, O'Donnell J, Berson EL, et al (1990) Analysis of ornithine aminotransferase mutations in B6-responsive and nonresponsive forms of gyrate atrophy. Invest Ophthalmol Vis Sci 31:310

Ramesh V, McClatchey AI, Ramesh N, Benoit LA, Berson EL, Shih VE, Gusella JF (1988) Molecular basis of ornithine aminotransferase deficiency in B-6-responsive and -nonresponsive forms of gyrate atrophy. Proc Natl Acad Sci USA 85:3777–3780

Ramesh V, Shaffer MM, Allaire JM, Shih VE, Gusella JF (1986) Investigation of gyrate atrophy using a cDNA clone for human ornithine aminotransferase. DNA 5:493–501

Richardson IB, Hurley SK, Hynes MJ (1989) Cloning and molecular characterisation of the amdR controlled gat A gene of *Aspergillus nidulans.* Mol Gen Genet 217:118–125

Schaumburg HH, Berger A (1988) Pyridoxine neurotoxicity. In: Leklen JE, Reynolds RD (eds) Clinical and physiological applications of vitamin B-6. Alan R Liss, New York, pp 403–413

Schmid SR, Linder P, Reese RT, Stanley HA (1993) Characterization of a putative ornithine aminotransferase gene of *Plasmodium falciparum.* Mol Biochem Parasitol 61:311–314

Simmaco M, John RA, Barra D, Bossa F (1986) The primary structure of ornithine aminotransferase: identification of active-site sequence and site of post-translational proteolysis. FEBS Lett 199:39

Sullivan KF, Cleveland DW (1986) Identification of conserved isotype-defining variable region sequences for four vertebrate β tubulin polypeptide classes. Proc Natl Acad Sci USA 83:4327–4331

Valle D, Simell O (1989) The hyperornithinemias. In: Scriver CR, Beaudet AL, Sly W, Valle D (eds) The metabolic basis of inherited disease, 6th ed. McGraw-Hill, New York, pp 599–627

Watanabe N, Yonaha K, Sakabe K, Sakabe N, Aibara S, Morita Y (1990) Crystal structure of ω-amino acid: pyruvate aminotransferase. In: Fukui T, Kagamiyama H, Soda K, Wada H (eds) International Union of Biochemistry Symposium 199: enzymes dependent on pyridoxal phosphate and other carbonyl compounds as cofactors. Pergamon, New York, pp 121–124

Watanabe W, Sakabe K, Sakabe N, Higashi T, Sasaki K, Aibara S, Morita Y, et al (1989) Crystal structure analysis of ω-amino acid: pyruvate aminotransferase with a newly developed Weissenberg camera and an imaging plate using synchroton radiation. J Biochem 105:1–3

Yonaha K, Nishie M, Aibara S (1992) The primary structure of ω-amino acid:pyruvate aminotransferase. J Biol Chem 267:12506–12510