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Inheritance of Ornithine Aminotransferase Gene, mRNA, and Enzyme Defect in a Family with Gyrate Atrophy of the Choroid and Retina

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Summary

We studied the human ornithine aminotransferase (OAT) gene, mRNA, and enzyme activity in fibroblasts from a family with gyrate atrophy (G.A.) of the choroid and retina, using a normal human OAT cDNA as a probe. The family consists of an affected patient, who is heterozygous for a partial deletion of the functional OAT gene and whose cells produce no mRNA, and of his father, mother, two sons, and a daughter. Southern blot analysis of the OAT gene showed the partial deletion in the patient and in his father and daughter and in one son. Northern blot analysis revealed no OAT mRNA in the patient and approximately 50% of the normal level of OAT mRNA in the father, mother, two sons, and daughter. Assay showed that the OAT activity in these individuals mirrored the OAT mRNA levels. The results indicate that an active allele of the OAT gene expresses 50% of the total normal OAT mRNA and activity and that both alleles of the gene are inactive in the patient in this pedigree, a situation resulting in a complete absence of the OAT mRNA, in accordance with the autosomal recessive mechanism of this disease; they also indicate a 50% decrease of OAT mRNA and enzyme activity in obligate heterozygous carriers carrying one defective allele and that these defects are stably inherited.

Introduction

Ornithine aminotransferase (OAT) is a mitochondrial enzyme deficient in patients with gyrate atrophy (G.A.), an autosomal recessive chorioretinal degenerative disease of the eye that leads to blindness (Simell and Takki 1973; Takki 1974). The virtual absence of OAT activity is observed in many tissues of patients with G.A., and intermediate OAT activity is found in heterozygous carriers (O'Donnell et al. 1977; Trijbels et al. 1977; Valle et al. 1977; Shih et al. 1978; Kennaway et al. 1980). We have previously constructed and characterized a cDNA for the human OAT, have used it to map the OAT gene sequences to specific chromosomes, and have analyzed the OAT gene and mRNA in patients with G.A. (Inana et al. 1986, 1988; Barrett et al. 1987). A patient with both a partial heterozygous deletion of the functional OAT gene and a complete absence of the OAT mRNA was identified (Inana et al. 1988). This case represents a complete loss of expression, by different mechanisms, of both alleles of the OAT gene, resulting in no OAT mRNA. If each allele of the OAT gene contributes half of the total OAT expression, as indicated by the OAT activity in heterozygous carriers, nonexpression of one allele should be reflected at the mRNA level in the obligate heterozygous carriers in this patient's family, and this defect should be inherited in a stable manner. To test this hypothesis, we examined the OAT gene, mRNA, and enzyme activity in fibroblasts from the family members of this patient.

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Material and Methods

The clinical characteristics of the patient have been described elsewhere (patient 4 of Weleber et al. [1982] and Weleber and Kennaway [1988]). The patient is of English ancestry. His visual acuity at age 40 years was

20/40 right eye and 20/200 left eye, and he had mild to moderate cataracts and maculopathy, both worse in the left eye. His visual fields were constricted to approximately 70° of remaining field, measured horizontally. Thus, his severity of disease was mild to moderate clinically, in comparison with most patients with G.A. He was classified as a B_6 nonresponder both by failure of serum ornithine levels to decrease after oral pyridoxine supplementation and by failure of pyridoxal phosphate to increase ornithine anminotransferase activity in vitro. With informal consent, skin biopsies were obtained from the patient and from his father, mother, two sons, and daughter, and fibroblasts were cultured under 7.5% CO_2 by using Dulbecco's modified Eagle medium with 10% FCS and antibiotics (GIBCO, Grand Island, NY).

Southern Blot Analysis

High-molecular-weight genomic DNA was extracted from cultured cells (5–8 × 10^6) by using proteinase K (Blin and Stafford 1976). Seven micrograms of genomic DNA isolated from the fibroblasts was digested with *Eco*RI, subjected to electrophoresis on a 0.8% agarose gel, transferred to nylon filter paper, hybridized with human OAT cDNA (Inana et al. 1986), labeled with ^{32}P by the method of random priming (Feinberg and Vogelstein 1983), washed, and autoradiographed as described elsewhere (Inana et al. 1983).

Northern Blot Analysis

Total RNAs were prepared from cultured cells (8–10 × 10^6) by the guanidine thiocyanate method (Chirgwin et al. 1979). Ten micrograms of total RNA was subjected to electrophoresis on a 0.8% denaturing agarose gel and was transferred to nylon filter paper, hybridized with human OAT cDNA labeled with ^{32}P by the method of random priming, washed, and autoradiographed as described elsewhere (Inana et al. 1982). Standardization of RNA quantity and quality with human actin cDNA was performed using the same blot washed after hybridization with the human OAT cDNA. Band intensities on the northern blot were quantified by densitometry, and the areas were calculated.

OAT Assay

Cells harvested 5–10 d after confluency were assayed for OAT activity by a modification of the method of Herzfeld and Knox (1968).

Results

To investigate the inheritance of OAT gene defects in G.A., cellular DNA, RNA, and proteins were isolated from skin fibroblasts of the family members of a patient with both a partial heterozygous deletion of the OAT gene and complete absence of the OAT mRNA (Inana et al. 1988). Southern blot analysis of the OAT gene in the family members indicated that the patient had inherited the partially deleted allele of the OAT gene, represented by the 5-kbp *Eco*RI fragment, from his father and had passed it on to his daughter and one of his sons (fig. 1).

Northern blot analysis of the fibroblast RNAs demonstrated the complete absence of OAT mRNA in the patient, as reported elsewhere (Inana et al. 1988), and decreased levels of OAT mRNA in the father, mother, two sons, and daughter (fig. 2). Densitometric standardization of the mRNA levels by using the amount of actin mRNA present in each lane indicated that the level of OAT mRNA present in the patient's parents and children, who are all obligate heterozygous carriers, is 41%–63% of normal (fig. 3).

The fibroblast cytosol preparations from the family members were used for assay of OAT activity. OAT activity in the patient's cells was 3 nmol/h/mg protein, and in the other family members it was 170–300 nmol/h/mg protein, which is 35%–61% of normal (490 ± 98 nmol/h/mg protein).

Discussion

Most of the G.A. cases so far examined at the molecular level have shown grossly intact OAT genes and mRNAs, suggesting that subtle point mutations are probably the most common defects in G.A., as is usually the case in genetic diseases (Ramesh et al. 1986; Inana et al. 1988). The patient discussed here is exceptional in that gross abnormalities, including a partial heterozygous deletion of the OAT gene and a complete absence of the OAT mRNA, are present (Inana et al. 1988). Both alleles of the OAT gene appear not to be expressed, so that no OAT mRNA is found. Inactivation of the OAT gene in this case may be due to mutations in the promoter regions of the gene, as well as to the partial deletion in one of the alleles. Because the molecular genetic defects of OAT are relatively easy to identify in this patient, the case offers an opportunity to confirm the autosomal recessive inheritance of OAT gene defects in G.A. and to examine the gene dose-

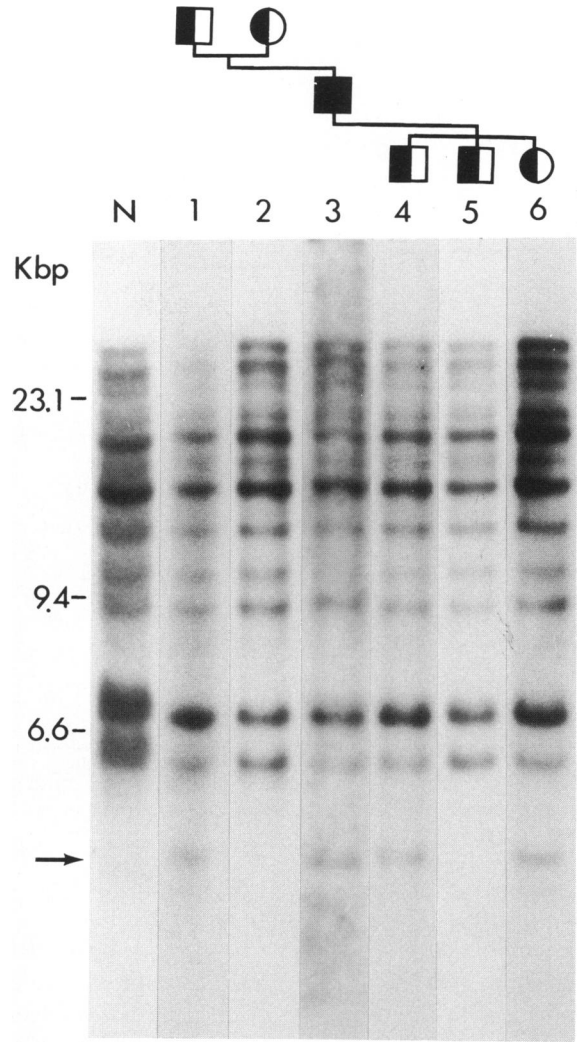


Figure 1 Southern blot analysis of the OAT gene in the G.A. family members. Shown are blots for DNA from normal human liver (lane N) and fibroblast DNA from the G.A. patient (lane 3) and from his father (lane 1), mother (lane 2), two sons (lanes 4 and 5), and daughter (lane 6). *Eco*RI OAT gene fragments are shown. The 6.7-, 6.6-, and 5.7-kbp bands represent the functional OAT gene (Barrett et al. 1987). The arrow at 5 kbp points to the band corresponding to the partially deleted allele of the 5.7-kbp functional OAT gene band, which shows a 50% decrease in hybridization intensity. Size markers are *Hin*dIII fragments of bacteriophage λ DNA.

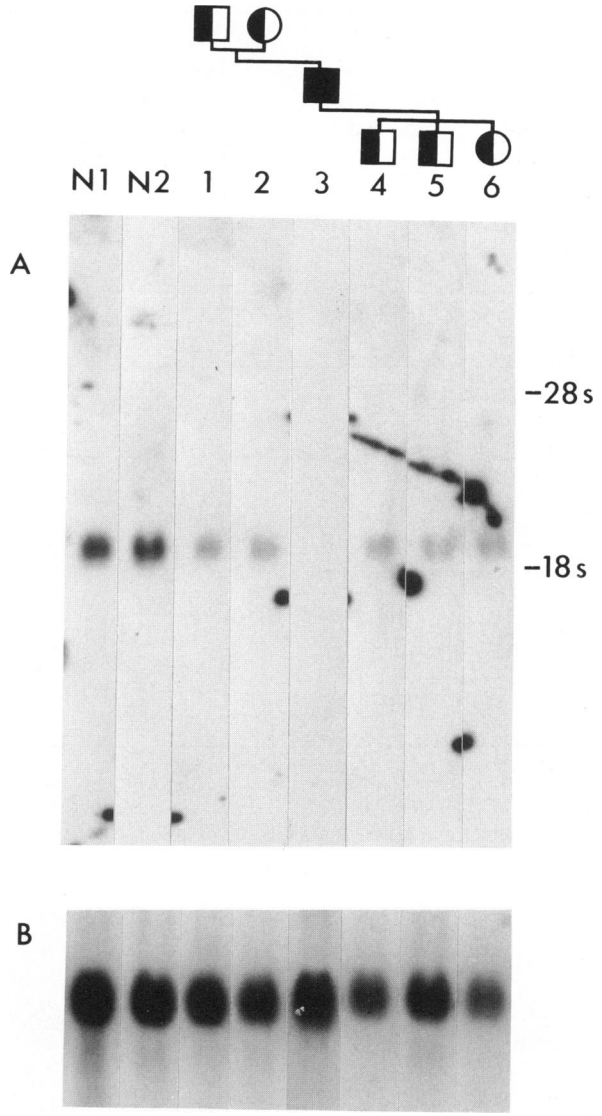
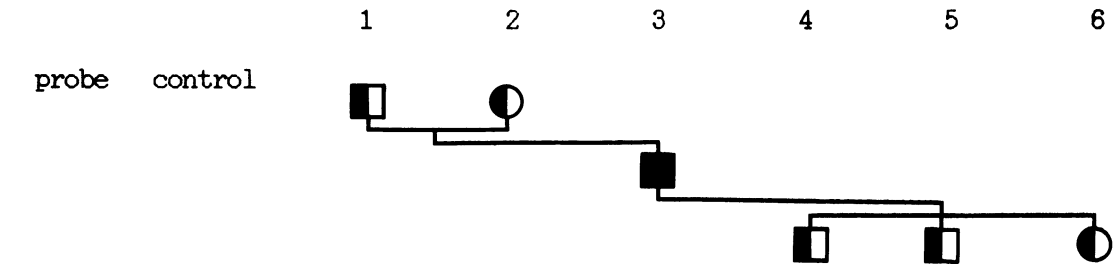


Figure 2 Northern blot analysis of the OAT mRNA in the G.A. family members. *A*, Probing for the OAT mRNA with OAT cDNA. Lanes N1 and N2, fibroblast RNAs from two normal individuals; lanes 1–6, fibroblast RNAs from the G.A. family members. Lanes 1–6 are the same as those described for fig. 1. *B*, Probing for the actin mRNA with actin cDNA for standardization of the quality and quantity of RNAs present. Positions of the 28s and 18s ribosomal RNA are indicated.

product relationship of the OAT gene, by examining the status of OAT gene expression in the patient's family members.

The result of the gene analysis identifies the patient's father as the source of the partially deleted allele of the OAT gene. The defective allele shows stable inheritance in the family, and the partial deletion could very likely result in inactivation of this allele of the OAT gene, as mentioned above. The other defective allele, coming from the patient's mother, is not readily identifiable, but its presence is clearly indicated by the half-



probe	control	1	2	3	4	5	6
OAT	1	0.447	0.353	0	0.393	0.440	0.353
Actin	1	0.994	0.869	0.493	0.670	0.768	0.560
OAT/Actin x100 (%)	100	45.0	40.6	0	58.7	57.3	63.0

Figure 3 Densitometric analysis of northern blot hybridization. The band intensities on the northern blot were measured by a densitometer, and the areas were calculated. Each value is presented as a proportion of the control value (normalized to 1), the latter being an average of two normal human mRNA levels shown in fig. 2.

normal expression of OAT at both the mRNA level and enzyme level. The defect in this allele may involve a point mutation, deletion, or insertion of the gene—especially in the regulatory region—that results in nonexpression.

The results of the mRNA and enzyme analyses clearly demonstrate the presence of two distinct heterozygous defects in OAT gene expression in the parents of the patient, their coming together to result in the loss of both functional alleles in the patient, and their transmission to the children to establish the separate heterozygous states. The data demonstrate the stable inheritance of the expression defects of the OAT gene in this family and confirm the autosomal recessive nature of this disease. The occurrence of two distinct molecular defects, both resulting in lack of expression of the OAT gene in this family, is so far unique among patients with G.A. The very small amount of OAT activity present in the patient (3 nmol/h/mg protein; normal = 490 ± 98 nmol/h/mg protein), if real, may be consistent with the barely detectable level of OAT protein demonstrated elsewhere immunologically in this patient (patient 35 of Inana et al. [1988]). As discussed in that other paper, there may be an extremely small amount of active OAT enzyme being made from an undetectably low level of OAT mRNA expressed in the patient. The data also point out both that a normal active allele of the OAT gene is responsible for 50% of the total OAT mRNA and enzyme activity in a cell, in agreement with the previous demonstration of half-normal level of OAT activity in heterozygous carriers (Trijbels et al. 1977; Valle et al. 1977). In this regard, the OAT gene allele is similar to many other mammalian gene alleles that show a codominant mode of action, such as in the hemoglobin gene (Honig and Adams 1986).

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References

Barrett, D. J., J. B. Bateman, R. S. Sparkes, T. Mohandas,

I. Klisak, and G. Inana. 1987. Chromosomal localization of human ornithine aminotransferase gene sequences to 10q26 and Xp11.2. Invest. Ophthalmol. Vis. Sci. **28**:1037–1042.

Blin, N., and D. W. Stafford. 1976. Isolation of high-molecular-weight DNA. Nucleic Acids Res. **3**:2303–2308.

Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry **18**:5294–5299.

Feinberg, A. P., and B. Vogelstein. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132**:6–13.

Herzfeld, A., and W. E. Knox. 1968. The properties, developmental formation, and estrogen induction of ornithine aminotransferase in rat tissues. J. Biol. Chem. **243**:3327–3332.

Honig, R. H., and J. G. Adams. 1986. Human hemoglobin genetics. Springer, Vienna and New York.

Inana, G., Y. Hotta, C. Zintz, K. Takki, R. G. Weleber, N. G. Kennaway, K. Nakayasu, A. Nakajima, and T. Shiono. 1988. Expression defect of ornithine aminotransferase gene in gyrate atrophy. Invest. Ophthalmol. Vis. Sci **29**:3–7.

Inana, G., J. Piatigorski, B. Norman, C. Slingsby, and T. Blundell. 1983. Gene and protein structure of a β-crystallin polypeptide in murine lens: relationship of exons and structural motifs. Nature **302**:310–315.

Inana, G., T. Shinohara, J. W. Maizel, Jr., and J. Piatigorsky. 1982. Evolution and diversity of the crystallins: nucleotide sequence of a β-crystallin mRNA from the mouse lens. J. Biol. Chem. **257**:9064–9071.

Inana, G., S. Totsuka, M. Redmond, T. Dougherty, J. Nagel, T. Shiono, T. Ohura, E. Kominami, and N. Katunuma. 1986. Molecular cloning of human ornithine aminotransferase mRNA. Proc. Natl. Acad. Sci. USA **83**:1203–1207.

Kennaway, N. G., R. G. Weleber, and N. R. M. Buist. 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.

O'Donnell, J. J., R. P. Sandman, S. R. Martin. 1977. Deficient L-ornithine: 2-oxoacid aminotransferase activity in cultured fibroblasts from a patient with gyrate atrophy of the retina. Biochem. Biophys. Res. Commun. **79**:396–399.

Ramesh, V., M. M. Shaffer, J. M. Allaire, V. E. Shih, and J. F. Gusella. 1986. Investigation of gyrate atrophy using a cDNA clone for human ornithine aminotransferase. DNA **5**:493–501.

Shih, V. E., E. L. Berson, R. Mandell, and S. Y. Schmidt. 1978. Ornithine ketoacid transaminase deficiency in gyrate atrophy of the choroid and retina. Am. J. Hum. Genet. **30**:174–179.

Simell, O., and K. Takki. 1973. Raised plasma-ornithine and gyrate atrophy of the choroid and retina. Lancet **1**:1031–1033.

Takki, K. 1974. Gyrate atrophy of the choroid and retina associated with hyperornithinaemia. Br. J. Ophthalmol. **58**:3–23.

Trijbels, J. M. F., R. C. A. Sengers, J. A. J. M. Bakkeren, A. F. M. DeKort, and A. F. Deutman. 1977. L-Ornithine-ketoacid-transaminase deficiency in cultured fibroblasts of a patient with hyperornithinemia and gyrate atrophy of the choroid and retina. Clin. Chim. Acta **79**:371–377.

Valle, D., M. I. Kaiser-Kupfer, and L. A. DelValle. 1977. Gyrate atrophy of the choroid and retina: deficiency of ornithine aminotransferase in transformed lymphocytes. Proc. Natl. Acad. Sci. USA **74**:5159–5161.

Weleber, R. G., and N. G. Kennaway. 1988. Gyrate atrophy of the choroid and retina. Pp. 198–220 *in* J. R. Heckenlively, ed. Retinitis pigmentosa. J. B. Lippincott, Philadelphia.

Weleber, R. G., M. K. Wirtz, and N. G. Kennaway. 1982. Gyrate atrophy of the choroid and retina: clinical and biochemical heterogeneity and response to vitamin B_6. Birth Defects **18**:219–230.