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Articles

Chromosomal Localization of Human Ornithine Aminotransferase Gene Sequences to 10q26 and Xp11.2

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Gyrate atrophy is a hereditary chorioretinal degeneration associated with a deficiency of ornithine aminotransferase (OAT). By means of a complementary DNA clone encoding human OAT, the OAT gene sequences were mapped by somatic cell hybrids and in situ hybridization to human chromosome regions 10q26 and Xp11.2. A review of 80 biochemically confirmed cases of gyrate atrophy confirmed the autosomal recessive inheritance of this disease and supported the presence of a functional OAT gene on chromosome 10. Interestingly, the X chromosome OAT gene sequences (Xp11.2) map to the same region as L1.28 (Xp11.0-p11.3), a marker closely linked to X-linked recessive retinitis pigmentosa. Invest Ophthalmol Vis Sci 28:1037-1042, 1987

The major catabolic pathway of ornithine in human tissues is catalyzed by the mitochondrial matrix enzyme ornithine aminotransferase (OAT) (L-ornithine:2-oxo-acid aminotransferase, EC 2.6.1.13). Ornithine aminotransferase is a pyridoxal phosphate-requiring enzyme that catalyzes the interconversion of ornithine, glutamate, and proline. This enzyme is present in many mammalian tissues including liver, kidney, brain, and eye, with a high concentration in the retina, retinal pigment epithelium, and ciliary body.

A severe deficiency of OAT has been demonstrated in patients with gyrate atrophy, a rare hereditary ocular disease characterized by myopia, cataracts, and a severe progressive degeneration of the choroid and retina. The chorioretinal degeneration begins early in childhood and usually leads to blindness by the fourth decade. Biochemical abnormalities in this disease include pronounced serum hyperornithinemia and absence or severe deficiency of OAT, which is thought to be the primary defect. Gyrate atrophy is inherited in an autosomal recessive pattern; obligate heterozygotes show approximately 50% of normal OAT activity, and consanguinity in parents of affected individuals is common. The mechanism by which the OAT deficiency leads to the choroidal and retinal atrophy and cataract formation remains unclear.

Recently a complementary DNA (cDNA) clone for the mRNA encoding human OAT (pHOAT) has been isolated and characterized. We used this OAT cDNA and a panel of 17 human-mouse somatic cell hybrids to map the loci for human OAT on human chromosomes. In addition, we regionally mapped the OAT loci by in situ hybridization to human chromosomes.

Materials and Methods

The clone for human OAT contains a 2.1 kilobase (kb) cDNA insert with an open reading frame of 1371 nucleotides and represents a nearly full-length copy of the OAT messenger RNA (mRNA). This 2.1 kb insert was isolated from pHOAT, labeled with 32P, and used to probe Southern blots of Eco R1-digested somatic cell hybrid DNA. The preparation and char-
characterization of these somatic cell hybrids has been described previously.14

Genomic DNA was isolated from human and mouse parental cells and the hybrid cells, digested to completion with Eco R1, electrophoresed on either 0.8% or 1.2% agarose gels (approximately 10 μg/lane), and transferred and bound to a nylon membrane as previously described.14 The membrane was prehybridized in 7% sodium dodecyl sulfate (SDS), 0.25 M Na₂HPO₄, 0.25 M NaH₂PO₄, 1 mM ethylene di-
Table 1. Assignment of human ornithine aminotransferase (OAT) loci to human chromosomes 10 and X

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% Discordancy
OAT—10:  35 65 41 47 41 53 65 53 0 71 53 29 53 59 59 47 41 29 47 47 47 65 47
OAT—X:  41 24 53 71 35 82 71 71 12 65 41 71 35 59 53 18 88 59 47 65 41 41 0 18

* '+' indicates presence or absence of the OAT sequence in the hybrid clone.
† '++' indicates presence of the human chromosome in more than 30% of metaphases analyzed, (+) indicates presence of the human chromosome in 10-30% of metaphases analyzed, '-' indicates absence of the human chromosome.

amine tetraacetate (EDTA), 1% bovine serum albumin (BSA), and 250 μg/ml salmon sperm DNA for 8 hr at 65°C. The OAT cDNA insert was labeled with 32P-deoxycytidine 5'-α-32P triphosphate (32P-dCTP) by random priming,15 added to fresh prehybridization mixture, and hybridized with the membrane at 65°C for 20 hr. The blot was washed two times in 2X sodium chloride sodium citrate (SSC), 0.1% SDS at 57°C for 20 min each followed by two 20-min washes in 0.2X SSC, 0.1% SDS at 57°C. The filter was exposed to Kodak (Rochester, NY) XAR-5 X-ray film.

In situ hybridization of the labeled DNA probe to normal human chromosomes followed the method of Harper and Saunders16 as modified by Zabel and colleagues.17 The OAT probe was labeled with 3H-deoxynucleotides to a specific activity of approximately 5 × 107 cpm/μg. Slides were exposed for 7 days and silver grains on or touching chromosomes were scored.

Results

The human OAT probe hybridized to numerous Eco R1 fragments of human DNA including those of 23, 21, 19.2, 17.1, 16, 15, 13.4, 11.7, 8.8, 6.5, 6.35, and 5.8 kb (Fig. 1, lane 1). The OAT probe detected four Eco R1 fragments in the mouse of sizes 15.6,
Fig. 3a. Detailed distribution of the silver grains on chromosome 10. The major peak is at 10q26, indicating this as the site for the OAT locus.

13.5, 4.1, and 4.0 kb (Fig. 1, lane 2). All of the human fragments were easily distinguished from the mouse fragments. Analysis of the human chromosome composition of the hybrids revealed that human OAT sequence homology cosegregates with two different human chromosomes. The 6.5, 6.35, and 5.8 kb fragments cosegregate with human chromosome 10, and the 23, 21, 19.2, 17.1, 16, 15, 13.4, 11.7, and 8.8 kb fragments cosegregate with the human X chromosome. The chromosomal content of the somatic cell hybrid panel and the chromosomal assignment results are given in Table 1. We also confirmed the assignment of OAT loci to chromosomes 10 and X by Southern blot analysis of Hind III-digested somatic cell hybrid DNA (data not shown).

The in situ hybridization studies (Fig. 2) confirm the OAT gene sequence assignments made by the somatic cell hybrid panel and regionally map the OAT loci to the q26 region on chromosome 10 and the p11.2 region on the X chromosome (Fig. 3, a and b).

Discussion

The complexity of the hybridization pattern of human genomic DNA to the 32P-labeled OAT cDNA suggests the possibility that the OAT gene may be a gene family consisting of multiple copies. Our demonstration of the 10 and X chromosomal localization of the OAT gene sequence supports this hypothesis. Some of the gene copies may be pseudogenes or OAT-like gene sequences.

O'Donnell and colleagues18 have reported the localization of the human OAT gene to chromosome 10 by assaying for OAT activity in human-mouse somatic cell hybrids. Their data imply that a functional OAT gene is located on chromosome 10. However, the specific chromosomal composition of their somatic cell hybrids was not reported and therefore OAT gene activity on the X chromosome cannot be excluded on this basis. Our results are consistent with those of O'Donnell and colleagues18 in the localization of an OAT gene sequence to chromosome 10. The presence of a functional OAT gene on chromosome 10 is also consistent with the autosomal recessive inheritance of gyrate atrophy.

The significance of the OAT or OAT-like sequences present on the X chromosome and their relationship to the OAT deficiency found in gyrate atrophy patients is not known. To investigate the possibility that the OAT sequences present on the X chromosome may be functional, we reviewed reports of biochemically confirmed cases of gyrate atrophy that have appeared in the literature7,8,19-39; all pedigrees reported with these cases are consistent with autosomal recessive inheritance. Of the 80 patients reported, 44 (55%) were female and 36 (45%) were male. There was no significant quantitative differences in the OAT deficiency found in cultured fibroblasts and lymphocytes from male and female patients.10,11,19,33-40 The reported mean serum ornithine levels are greater in affected males (1026 nmol/ml) than in females (846 nmol/ml)7,11,24; we do not know whether this gender disparity is significant. The lack of involvement of an X chromosome gene in gyrate atrophy and the presence of a functional OAT gene on chromosome 10, which is presumably abnormal in gyrate atrophy patients, are supported by these observations. This does not exclude the possibility of functional OAT-like genes on the X chromosome.

We have demonstrated the localization of the OAT gene family to both an autosome and the X chromosome. Only nine other gene families have been mapped to both autosomes and the X chromosome.42 Two of the gene families, argininosuccinate synthetase-
tase\textsuperscript{43} and glutamate dehydrogenase,\textsuperscript{44} are involved in NH\textsubscript{4} metabolism and the urea cycle, as in OAT.

It is of interest that the X chromosome region containing the OAT sequence(s) (p11.2) is within the region of the DXS7 (L1.28) marker (Xp11.0-p11.3), which has been closely linked to X-linked recessive retinitis pigmentosa through the use of the DXS7 probe.\textsuperscript{45} The possibility that this OAT sequence may be linked to the X-linked recessive retinitis pigmentosa locus and even that it may be causally related requires further investigation. One may speculate that members of the OAT gene family are involved in multiple retinal degenerative diseases, and experiments are in progress to examine this possibility. The OAT gene sequences on chromosomes 10 and X require further characterization. We have isolated multiple genomic clones representing most of these OAT gene sequences and are in the process of characterizing them.

\textbf{Key words:} ornithine aminotransferase, gyrate atrophy, somatic cell hybrids, in situ hybridization, gene family

\textbf{Acknowledgments}

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\textbf{References}

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