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Gyrate Atrophy of the Choroid and Retina: Assignment of the Ornithine Aminotransferase Structural Gene to Human Chromosome 10 and Mouse Chromosome 7

James J. O'Donnell,*,† Kaarina Vannas-Sulonen,*,‡ Thomas B. Shows,§ and David R. Cox†

*Department of Ophthalmology and †Departments of Pediatrics and of Biochemistry and Biophysics, University of California, San Francisco; ‡Department of Ophthalmology, University of Helsinki, Helsinki; and §Department of Human Genetics, Roswell Park Memorial Institute, New York State Department of Health, Buffalo

Summary

Gyrate atrophy of the choroid and retina is an autosomal recessive, blinding human disease caused by a deficiency of the mitochondrial matrix enzyme ornithine aminotransferase (OAT). Since human OAT cDNA hybridizes to DNA sequences on both human chromosomes 10 and X, a locus coding for OAT enzyme activity may be present on one or both of these human chromosomes. We have used a series of mouse-human somatic cell hybrids, in combination with starch gel electrophoresis and a histochemical stain for OAT enzyme activity, to assign the structural gene for OAT to human chromosome 10. Our results suggest that the human X chromosome does not contain a locus coding for OAT enzyme activity. In addition, we have used a panel of Chinese hamster-mouse hybrids to assign the murine *Oat* structural gene to mouse chromosome 7. Our findings, combined with recent molecular studies, indicate that human OAT probes specific for chromosome 10 will be useful for the diagnosis and genetic counseling of individuals at risk for gyrate atrophy.

Introduction

Gyrate atrophy of the choroid and retina is an autosomal recessive, blinding human disease which is distributed worldwide but which is most prevalent in Finland. This disorder is characterized by nightblindness, constriction of the field of vision, progressive peripheral retinal degeneration, an ultimately extinguished electroretinogram, and a marked elevation of plasma ornithine (Simell and Takki 1973; McCulloch et al. 1978; Takki and Milton 1981). A deficiency of the mitochondrial matrix enzyme ornithine aminotransferase (OAT; L-ornithine:2-oxo-acid aminotransferase; E.C.2.6.1.13) is the primary defect responsible for gyrate atrophy (Kennaway et al. 1977; O'Donnell et al. 1977, 1978; Trijbels et al. 1977; Valle et al. 1977; Shih et al. 1978).

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Address for correspondence and reprints: James J. O'Donnell, Department of Ophthalmology, Box 0730, K-301, University of California, San Francisco, CA 94143.

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This reduced OAT activity could be due to a mutation in the OAT structural gene or to a mutation in some other gene that results in OAT deficiency. Although the enzyme defect is expressed in many tissues in affected individuals, significant clinical abnormalities are restricted to the choroid and retina (Valle and Simell 1983). The molecular basis for the increased sensitivity of these cell types to OAT deficiency is unknown.

Recently, several groups have isolated human OAT cDNA clones that hybridize to human genomic DNA sequences clustered on chromosomes 10 and X (Mitchell et al. 1986; Barrett et al. 1987; Ramesh et al. 1987). However, it is not yet determined whether a locus coding for OAT enzyme activity is present on one or both of these human chromosomes. If the OAT structural gene is located on the human X chromosome, then a mutation in this gene could not be the genetic defect resulting in gyrate atrophy, since this disorder is inherited as an autosomal recessive rather than as an X-linked condition. To determine which human chromosome contains the human OAT structural locus, we have used a panel of mouse-human somatic cell hybrids in conjunction with starch gel electrophoresis and a histo-

chemical stain for OAT enzyme activity. In addition, we have used a panel of Chinese hamster-mouse somatic cell hybrids in combination with electrophoresis and the OAT activity stain to assign the murine OAT structural gene to a specific mouse chromosome. Elsewhere we have reported preliminary data concerning OAT mapping (O'Donnell et al. 1985).

Material and Methods

Somatic Cell Hybrids

The formation and characterization of the panel of mouse-human somatic cell hybrids used in the present study have been described elsewhere (Shows et al. 1984). The presence of human chromosomes in each hybrid was determined by karyotype analysis with trypsin/ Giemsa banding and/or by scoring for chromosome-specific enzyme markers (Shows et al. 1978). Chinese hamster-mouse somatic cell hybrids segregating mouse chromosomes were formed by polyethylene glycol fusion of mouse spleen or macrophage cells to an established Chinese hamster cell line (380-6) (Cox et al. 1980, 1982; Cox and Epstein 1985). These hybrids were characterized for the presence of specific mouse chromosomes by karyotype and/or enzyme analysis (Cox et al. 1982).

Electrophoresis and Histochemical Detection of OAT Enzyme Activity

OAT enzyme activity was analyzed in the supernatants of cell homogenates, prepared by suspending ~6 × 10^6 cells in 100 μl of lysis buffer (0.1 M potassium phosphate [pH 7.4], 1.5 × 10^{-5} M pyridoxal phosphate, 0.05% Triton X-100), freeze-thawing three times, and centrifuging at 30,000 *g* at 4 C for 15 min. Starch gel electrophoresis was performed as described elsewhere (Ruddle and Nichols 1971). Under these conditions, human, mouse, and Chinese hamster OAT migrated toward the anode. A 12.6% (w/v) solution of hydrolyzed starch was prepared in an aqueous buffer of 1.21 mM citric acid and 6.07 mM $K_2 + HPO_4$ adjusted to pH 7.0 with 1 N KOH. Thirty microliters of cell supernatant (containing extract from ~2 × 10^6 cells) was applied to filter paper and placed in each electrophoresis well. Electrophoresis was carried out at 155 V at 4 C for 20 h. Following electrophoresis, OAT enzyme activity was visualized using a modification of a histochemical stain described elsewhere (Pitot and Peraino 1963). This stain identifies glutamic semialdehyde, a product of the OAT reaction, through use of *o*-aminobenzaldehyde, which reacts with glutamic semialdehyde to form a yellow derivative. OAT activity was visualized by overlaying the gel with a 1% agarose solution containing 0.1 M L ornithine, 0.02 m α-ketoglutarate, 7 mM *o*-aminobenzaldehyde, and 0.25 M KPO_4, with the pH adjusted to 7.4 by the addition of 1 N KOH. The gel was incubated at 37 C for 90 min to develop the yellow color. Reaction mixtures containing buffer in place of ornithine or α-ketoglutarate failed to result in yellow color, establishing the specificity of the histochemical stain.

Results

When extracts from human, mouse, and Chinese hamster cells are analyzed by starch gel electrophoresis followed by histochemical staining for OAT activity, the enzyme activity from each species appears as a discrete band, with the mouse enzyme migrating more anodally than either the human or the Chinese hamster enzyme (fig. 1). Human and Chinese hamster OAT comigrate under these electrophoretic conditions. Occasionally, a second minor band of OAT activity is found in both mouse and Chinese hamster cell extracts, and this band migrates more anodally than the major band in each case. The molecular basis for this variable minor band of rodent OAT activity is not known.

When cell extracts of mouse-human somatic cell hybrids are analyzed for OAT activity following electrophoresis, all extracts contain activity corresponding to the mouse enzyme while some hybrids contain either one of two additional bands of enzyme activity. One of the additional bands comigrates with human OAT while the other migrates intermediately, between the mouse and human enzymes. When the band of OAT activity corresponding to the human enzyme is observed in a cell extract, it is always observed in association with the intermediate band of activity and is always much less intense than the intermediate band. In many hybrid extracts, only the intermediate band and the mouse band of OAT activity are observed (fig. 1, lane C). The intermediate band of activity is interpreted as a heterodimer of mouse and human OAT subunits (see Discussion). A mixture of mouse and human cell extracts does not result in an intermediate band of OAT activity (data not shown).

We assigned the structural OAT gene to a specific human chromosome by correlating expression of human OAT enzyme activity with the segregation of human chromosomes in a series of mouse-human somatic cell hybrids. Mouse-human hybrids were scored positive for human OAT if they contained both the inter-

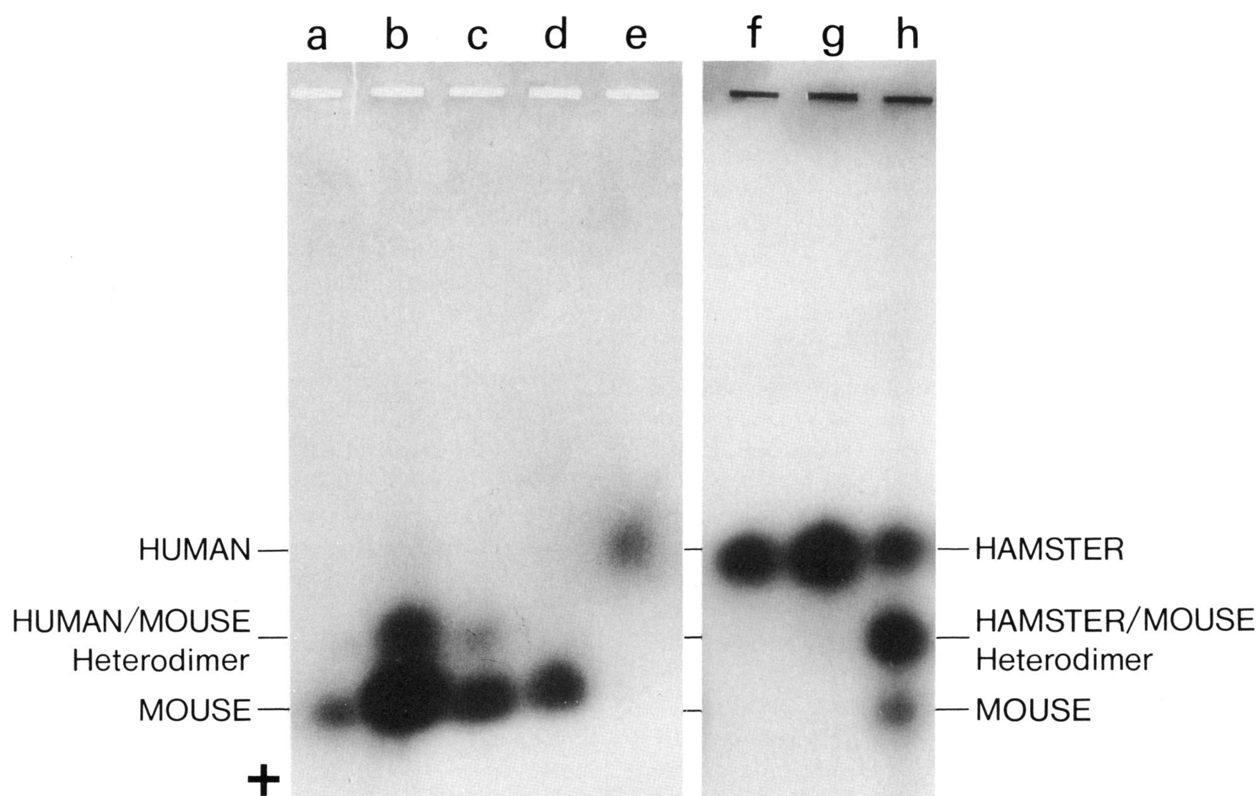

Figure 1 Starch gel electrophoresis of cell extracts followed by histochemical staining for ornithine aminotransferase (OAT) activity. Lane a, mouse L cells; lanes b and c, mouse-human hybrids with both mouse and human OAT activity; lane d, mouse-human hybrid with mouse but no human OAT activity; lane e, human fibroblasts; lane f, Chinese hamster cell line 380-6; lane g, Chinese hamster-mouse hybrid with hamster but no mouse OAT activity; lane h, Chinese hamster-mouse hybrid with both hamster and mouse OAT activity.

mediate band of OAT activity and a band of activity corresponding to mouse OAT, whether or not the hybrids contained a third band of activity comigrating with the human enzyme. Thirty-five mouse-human hybrids, which in aggregate contain all the human chromosomes except the Y, were scored for human OAT as described above. As shown in table 1, this enzyme segregated concordantly with human chromosome 10 with no exceptions, but it segregated discordantly with each of the other human chromosomes. These results demonstrate that human chromosome 10 is both necessary and sufficient for the expression of human OAT in mouse-human somatic cell hybrids and assign the structural locus for human OAT to chromosome 10. Furthermore, our results indicate that the human X chromosome does not contain a locus coding for a protein with OAT activity.

To assign the murine structural locus for OAT to a specific mouse chromosome, we analyzed a series of Chinese hamster-mouse somatic cell hybrids for the presence of mouse OAT enzyme activity following electrophoresis. Analogous to the results with mouse-human cell hybrids, we found that some Chinese hamster-mouse hybrids contain a band of OAT activity migrating intermediately between the Chinese hamster and mouse enzyme and that this intermediate band occurs either in the presence or in the absence of enzyme activity corresponding to mouse OAT (fig. 1, lane H). Mixing of Chinese hamster and mouse extracts does not produce an intermediate band of OAT activity following electrophoresis. Chinese hamster-mouse hybrids were scored as positive for mouse OAT if they contained the intermediate band of activity, whether or not they showed the presence of activity corresponding to mouse OAT. As shown in table 2, when a panel of Chinese hamster-mouse hybrid cell extracts was scored for mouse OAT, the enzyme segregated concordantly with mouse chromosome 7 but discordantly with each of

Table 1

Distribution of Human *Oat* in Mouse-Human Cell Hybrids

		Human Chromosome																							
Cell Hybrid	*OAT*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Translocation Chromosome(s)
REW-5[a]	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	−	+	+	+	
REW-7[a]	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+	
REW-81CSAz4	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	−	−	−	+	−	−	
REW-10[a]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	−	+	
REW-11[a]	−	−	−	−	+	−	−	−	−	−	−	+	+	+	−	−	+	−	−	−	+	+	−	+	
REW-13	+	−	+	+	−	−	+	−	−	+	+	+	−	−	+	−	−	+	+	−	−	+	−	+	
REW-14	+	−	+	+	−	+	−	−	−	−	+	+	+	−	+	+	−	+	+	+	−	+	−	+	
REW-15[a]	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+	
JWR-26C[a]	+	−	+	+	+	+	+	+	−	+	+	+	+	−	+	+	+	−	+	−	+	+	−	+	1p−
XER-1	+	−	−	+	+	+	+	+	−	+	+	−	+	+	+	+	−	+	−	+	−	+	−	+	
XER-9	+	−	+	+	+	−	−	−	+	−	+	−	+	−	+	−	−	+	+	−	−	+	−	+	
XER-11[a]	+	+	−	+	+	−	+	+	+	−	+	−	+	+	−	+	+	+	+	+	+	+	+	−	11/X,X/11
XER-11CSAzB	+	+	+	−	−	−	+	+	+	−	+	−	−	−	−	+	−	+	−	−	+	−	−	−	
XER-14	+	+	+	+	+	+	+	−	−	−	+	+	−	+	−	+	−	+	−	+	−	+	−	+	
XER-16	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	+	−	+	+	+	−	+	
SIR-1	−	−	−	−	+	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	
SIR-8[a]	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	
JVR-22[a]	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	2/1
JVR-32E	−	−	−	−	+	−	−	+	+	−	−	−	−	+	−	−	+	+	−	−	+	+	+	+	
DUM-6[a]	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−	+	+	−	+	+	+	+	+	X/15,15/X
DUM-13[a]	+	+	+	+	−	−	+	+	−	−	+	+	+	−	+	−	+	+	+	+	+	+	+	−	X/15,15/X
ATR-6CSAzE	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	
ATR-8BSAgMP	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	
ATR-11BSAgMP	+	−	−	+	−	+	−	−	+	−	+	−	−	+	+	−	−	−	−	−	−	−	+	−	
ATR-13[a]	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	−	−	−	−	5/X
JSR-2	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+	
JSR-9	+	+	+	−	−	+	+	−	+	−	+	+	+	+	+	−	+	−	+	+	+	+	−	+	
JSR-14	−	+	−	−	−	+	−	−	−	−	−	−	+	+	−	−	−	+	−	−	−	+	−	+	
JSR-17S[a]	+	+	+	+	−	+	−	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	7/9
JSR-26	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	−	−	−	+	−	+	−	+	
XTR-22[a]	+	−	+	−	+	+	+	−	+	−	+	+	−	−	−	+	−	−	+	+	+	+	+	−	X/3
ALR-3	+	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	+	+	−	+	+	+	−	+	
ALR-4BSAg	−	+	+	+	+	+	+	+	−	+	−	−	−	+	+	−	−	−	+	−	−	+	−	−	
EXR-1	+	+	−	−	+	−	−	+	+	+	+	+	−	+	+	−	−	+	−	+	+	−	−	+	
REX-57BSHB	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	+	+	−	+	−	−	−	−	
% Discordancy		23	14	17	34	23	23	37	29	49	0	17	26	40	17	14	49	26	29	23	34	26	43	23	

[a] Hybrids tested for human chromosomes and chromosome-specific enzyme markers. All other hybrids were tested only for chromosome-specific enzyme markers.

the other mouse chromosomes, including the X chromosome. The results assign the mouse structure locus of OAT to mouse chromosome 7.

Discussion

OAT is a mitochondrial matrix enzyme thought to be important in the intracellular production of glutamate and proline from ornithine in many mammalian tissues. In both humans and rodents, the enzyme is synthesized on cytoplasmic polysomes as a 49-kD precursor polypeptide that is processed to a mature polypeptide of 45 kD on transport to the mitochondria (Inana et al. 1986). Although all of the biochemical and molecular data indicate that the enzyme is composed of a single subunit, there is no published information concerning the subunit structure of the active enzyme in vivo. Our finding that mouse-human and Chinese hamster-mouse somatic cell hybrids contain a single discrete band of OAT activity that migrates intermediately

Table 2

Assignment of the *Oat* Structural Gene to Mouse Chromosome 7

Hybrid[a]	*OAT*[b]	Mouse Chromosome[c]																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	X
I-3A-2	+	−	+	−	+	−	−	+	+	−	−	−	+	+	−	+	+	−	+	+	−
I-8-5	+	−	+	−	+	−	+	+	−	−	+	−	+	−	−	−	−	−	−	+	−
III-14	+	+	−	−	+	−	(+)	+	−	−	−	−	−	−	−	+	(+)	−	−	+	+
VI-25	+	(+)	(+)	+	−	−	−	+	−	−	(+)	−	−	−	−	−	(+)	−	−	−	+
I-3A-3	−	+	+	−	+	−	−	−	−	−	−	−	−	+	−	+	−	−	+	+	−
I-7B-4	−	+	+	−	−	−	−	−	−	−	+	−	−	−	+	+	−	−	+	+	−
III-12	−	−	−	−	−	−	+	−	−	−	−	−	+	+	+	(+)	−	(+)	−	−	+
III-23	−	−	(+)	+	−	(+)	−	−	+	−	−	−	+	−	+	(+)	−	−	+	−	(+)

[a] Derivation of these cell lines has been described (Cox et al. 1980, 1982; Cox and Epstein 1985).

[b] + Indicates the presence of mouse OAT activity identified by histochemical staining following electrophoresis.

[c] The mouse chromosome complement of each hybrid was determined by isozyme and/or karotype analysis (Cox et al. 1982). + Indicates the presence of an intact chromosome; (+) indicates the presence of a partial or translocation chromosome.

between the two parental forms of the enzyme following electrophoresis suggests that active OAT enzyme consists of a homodimer in both humans and rodents. Both the electrophoretic migration of the intermediate band and its increased intensity compared with both human enzyme in mouse-human hybrids and mouse enzyme in Chinese hamster-mouse hybrids are consistent with this interpretation.

Our assignment of the OAT structural gene to human chromosome 10 by using starch gel electrophoresis followed by histochemical staining for OAT activity is consistent with the genomic mapping studies that have used human OAT cDNA probes (Mitchell et al. 1986; Barrett et al. 1987; Ramesh et al. 1987). Furthermore, our failure to identify a human X-linked locus coding for OAT enzyme activity is supported by recent molecular studies that indicate that some of the X chromosome DNA sequences that cross-hybridize with human OAT cDNA represent pseudogenes rather than related members of an expressed multigene family (Looney et al. 1987).

Our assignment of the OAT structural gene to human chromosome 10 rather than to the X chromosome suggests that a mutation in this gene may be the primary genetic defect resulting in gyrate atrophy. Several groups have identified a single mRNA species present in all human tissues analyzed that hybridizes with human OAT cDNA probes (Inana et al. 1986; Ramesh et al. 1986). However, when this mRNA was analyzed in cells from several patients with gyrate atrophy, it was found to be unaltered in size or amount, despite a 25–100-fold reduction in OAT enzyme activity (Ramesh et al. 1986). These results fail to confirm the hypothesis that a mutation in the OAT structural gene is the primary genetic defect in gyrate atrophy. Nevertheless, this hypothesis is supported by linkage analysis between gyrate atrophy and chromosome 10–specific OAT probes as well as by evidence of reduced heterozygosity of these probes in gyrate atrophy patients as compared with the general population (Ramesh et al. 1988). Furthermore, a point mutation in the initiation codon of human OAT mRNA has been found to be the genetic defect leading to gyrate atrophy in at least one family (Mitchell et al. 1988). Our assignment of *OAT* to chromosome 10, when combined with these additional studies, suggests that chromosome 10–specific OAT probes will provide useful tools for the diagnosis and genetic counseling of individuals at risk for gyrate atrophy.

Our mapping of the murine structural OAT gene to mouse chromosome 7 is consistent with the observation that mouse DNA sequences cross-hybridizing with a human OAT cDNA probe map approximately 17 centimorgans telomeric to the mouse hemoglobin beta-chain locus on chromosome 7 (Glaser et al., in press). This regional localization of *Oat* on mouse chromosome 7 places the gene in a segment of the mouse genome that has extensive homology with the distal arm of human chromosome 11 but no known homology with human chromosome 10 (Glaser et al., in press).

Although there has been significant progress in our understanding of the molecular defect underlying gyrate atrophy, little is known concerning the mechanisms by which OAT deficiency results in degeneration of the

choroid and retina. An animal model of OAT deficiency would be useful for studying the pathophysiology of gyrate atrophy, as well as for testing possible treatment regimens. While a number of mouse mutations are known that cause retinal degeneration, none of these mutations map to the region of chromosome 7 containing *Oat*. Similarly, while there exist a number of viable heterozygous deletions of mouse chromosome 7 that could be used as a starting point for the construction of an OAT-deficient mouse, none of these deletions include the region of chromosome 7 containing the mouse OAT structural gene (Glaser et al., in press). Nevertheless, by microinjecting the appropriate construct of a molecular clone of a mouse or human OAT gene into pluripotent mouse teratocarcinoma cells, it may be possible to use homologous recombination to produce cells with a mutation in one copy of the mouse cellular *Oat* gene (Thomas and Capecchi 1987). Such pluripotent cells could then be used to generate mice with a germ-line *OAT* mutation, and these mice could be interbred to produce a mouse model of gyrate atrophy (Hooper et al. 1987; Kuehn et al. 1987).

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