DEFICIENT L-ORNITHINE:2-OXOACID AMINOTRANSFERASE ACTIVITY IN CULTURED FIBROBLASTS FROM A PATIENT WITH GYRATE ATROPHY OF THE RETINA

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Summary. Gyrate atrophy of the retina is an autosomal recessive condition characterized by a specific progressive retinal degeneration and increased urine and plasma ornithine. This study demonstrates a deficiency of L-ornithine:2-oxoacid aminotransferase (EC 2.6.1.13) activity in cultured fibroblasts from a patient with this disease.

Gyrate atrophy of the retina is an autosomal recessive condition characterized by increased blood and urine ornithine and progressive retinal degeneration (1, 2, 3, 4). A recent in vivo metabolic study (5) suggests that this disease is an inborn error of L-ornithine:2-oxoacid aminotransferase (EC 2.6.1.13). This enzyme catalyzes the interaction of L-ornithine and α-ketoglutarate to produce glutamic-γ-semialdehyde and glutamate (6, 7). Spontaneous cyclization converts glutamate-γ-semialdehyde to Δ^1-pyrroline-5-carboxylate, a proline precursor.

The purpose of this investigation was to directly demonstrate in cultured fibroblasts the inborn error of L-ornithine:2-oxoacid aminotransferase activity in gyrate atrophy of the retina.

Case. The patient is the 14 year old daughter of clinically normal, non-consanguinous parents of Finnish ancestory. She has been well with the exception of visual problems noted at age 7. She has not had seizures. On examination, she has best corrected vision of 20/25 in each

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eye with myopic correction, impaired dark adaptation, extinguished scotopic electroretinogram, constricted visual fields, and peripheral chorioretinal atrophy with scalloped margins. Plasma ornithine was 1.16 μmoles/ml, a ten-fold increase over normal. Blood ammonia was 63 μg/100 ml (normal 40-100).

Methods. Skin fibroblast cultures from the patient, her obligate heterozygote father, and five normal lines were grown in Dulbecco's media with 10% fetal calf serum in a 5% CO_2 incubator at 37°. Cultures were free of mycoplasma contamination (8). Passage 11 was used for the patient, passage 10 for the heterozygote, and passages 10, 10, 11, 11, and 15 for the normals. For enzyme preparation, cells were harvested at mid-log growth observed by inverted phase microscopy and suspended in 0.1 M phosphate pH 8 buffer containing pyridoxal phosphate 4 μg/ml. This suspension was sonicated at 75 watts (Sonifier W185, Ultrasonics) for three 15 second intervals in an ice bath. L-ornithine:2-oxoacid aminotransferase was assayed with the sensitive radioisotopic method (9, 10) for the small amount of material available from cultured cells. Substrate concentrations were 0.7 mM L-ornithine and 0.7 mM α-ketoglutarate. L-[U-^{14}C] ornithine (200 μCi/μmole) was obtained from New England Nuclear. Radioactivity in the assay was 5-10 times the blank value without enzyme. The reaction was linear with time and with added enzyme. Protein was determined by the method of Lowry (11).

Results. As shown in the table, L-ornithine:2-oxoacid aminotransferase activity was markedly deficient in the patient's cells. Since the small activity measured in the patient is at the limit of sensitivity of the assay, no conclusion should be made as to whether the enzyme has very slight or zero activity. Enzyme activity in the heterozygote father's cells was significantly ($p < .01$) lower than normal. Although it is interesting that the heterozygote activity here is approximately one half the normal activity, more heterozygotes must be examined to conclusively prove the one-half level in heterozygotes.

Discussion. This mutation in L-ornithine:2-oxoacid aminotransferase may explain the hyperornithinemia seen in this

TABLE: L-ornithine:2-oxoacid aminotransferase activity in cultured fibroblasts.

	activity
normals (n=5)	126 ± 10 (S.D.)
heterozygote	67
patient	4.0

Activity is expressed as nmoles Δ^1-pyrroline-5-carboxylate reaction product x $hr.^{-1}$ x $mg.^{-1}$ protein. For the normals the mean and standard deviation (S.D.) are noted. The heterozygote activity is the mean of four determinations (range 57-72). The activity of the heterozygote is significantly ($p < .01$) lower than normal. The activity of the patient is ≤ 4.0 with the sensitivity limit of this assay.

disease. The deficient activity by itself does not explain the pathophysiology of the retinal degeneration. Product deficit of proline could impede synthesis of collagen in the basement membrane (Bruch's membrane) of the retinal pigment epithelium. Alternatively, precursor accumulation of ornithine could cause retinal toxicity, although the absense of gyrate atrophy in other hyperornithinemia conditions argues against this (4).

Prenatal diagnosis of this disease in the fetus of known heterozygous parents may be possible as L-ornithine:2-oxoacid aminotransferase has been measured in cultured amniotic fluid cells (12).

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