Genetic aspects in gyrate atrophy of the choroid and retina with hyperornithinaemia

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The relative importance of genetic degenerative eye lesions as causes of blindness or impaired vision has increased because of the lack of effective treatment for such conditions as compared with other diseases. Related to the large and not very well-defined group of degenerative eye lesions termed tapeto-retinal degenerations, is a choroidal form known as gyrate atrophy of the choroid and retina.

Night blindness is the most important subjective symptom in hereditary tapeto-retinal degeneration. Sharply-defined chorio-retinal atrophic areas of the fundus are typical of gyrate atrophy. In addition to constriction of the fields of vision, myopia, and complicated cataract, abnormalities in the electroretinogram, electro-oculogram, and fluorescein angiograms of the fundus form the basis for diagnosis.

A constant relationship between gyrate atrophy of the choroid and retina and massively increased plasma ornithine concentration was found in our earlier studies (Simell and Takki, 1973; Takki, 1974), in which it was suggested that the clinical symptoms and signs of the disease were due to large concentrations of ornithine in the plasma, aqueous humour, and cerebrospinal fluid or directly to the causative enzyme defect.

Though gyrate atrophy of the choroid and retina is a relatively rare ophthalmological disease and the published series of patients are small, inheritance has been suggested. Consanguinity of the parents, familial occurrence, and sporadic cases have been described (Cutler, 1895; McGuire, 1932; McGuire and McGuire, 1941; Waardenburg, 1939; Davis and Sheppard, 1940; Malbrán and Fonte, 1947; François, Barbier, and de Rouck, 1959; Kurstjens, 1965; Rieger, 1972; Botermans, 1972), but autosomal inheritance now seems to be a probable cause (Kurstjens, 1965; Botermans, 1972). The combination of clinical symptoms with inborn errors of metabolism was not understood until some relatives of the propositi were found to have the metabolic abnormality without the clinical disease (Ghadimi, Partington, and Hunter, 1961; Woody, Hutzler, and Dancis, 1966; Auerbach, DiGeorge, and Carpenter, 1967). The aim of this work was to confirm the regular combination of massively increased plasma ornithine concentration with the ophthalmological symptoms and signs by studying the eyes and plasma and/or urinary amino-acids of relatives of the patients, to test the autosomal recessive mode of inheritance in this group of families, and to find a clinical method for heterozygote detection in hyperornithinaemia by studying ornithine metabolism in probable heterozygotes of the disease and in controls.

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Material

CRITERIA
Typical chorio-retinal atrophic areas of the fundus and impaired retinal functions as described earlier (Takki, 1974) were used as ophthalmological criteria for the patients with gyrate atrophy of the choroid and retina. The quantitatively estimated plasma ornithine concentration of all these patients were ten to twenty times higher than normal values. All the patients with gyrate atrophy had come to an examination for ophthalmological complaints.

PATIENTS AND FAMILIES
A total of 71 family members from fourteen families with 22 patients with gyrate atrophy and hyperornithinaemia were studied; thirteen of them were parents, three children, 31 brothers and sisters, and the remaining 24 close relatives. The age and sex of the subjects studied are shown in Table I. All parents and brothers and sisters of the patients were included.

Table I Number, sex, age, and plasma ornithine concentration of patients and their relatives in families with gyrate atrophy of the choroid and retina with hyperornithinaemia

<table>
<thead>
<tr>
<th>Group of subjects</th>
<th>No. of cases</th>
<th>Age range (yrs)</th>
<th>Plasma ornithine concentration (µM)</th>
<th>Mean (range)</th>
<th>Daily urinary excretion of ornithine (µM)</th>
<th>Mean (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gyrate atrophy</td>
<td>13/9 = 22</td>
<td>9-53</td>
<td>1,015 (707-1,339)</td>
<td>1,856</td>
<td>(939-2,751)</td>
<td>(n = 5)</td>
</tr>
<tr>
<td>Parents</td>
<td>4/9 = 13</td>
<td>26-69</td>
<td>89.8 (43.8-142.6)</td>
<td>(n = 9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brothers and sisters</td>
<td>13/18 = 31</td>
<td>8-70</td>
<td>51.6 (31.1-72.0)</td>
<td>(n = 2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children</td>
<td>2/1 = 3</td>
<td>2-13</td>
<td>70.0 (61.8-78.1)</td>
<td>(n = 3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other relatives</td>
<td>17/7 = 24</td>
<td>16-75</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>49/44 = 93</td>
<td>2-75</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Methods

Ophthalmological examination
This included visual field studies, dark adaptation, colour vision, electroretinography, electro-oculography, and fluorescein angiography of the fundus as described earlier (Takki, 1974) in nine parents of patients with gyrate atrophy. Funduscopic examination was performed on 36 other relatives of the patients, including all relatives below the age at which clear impairment of vision appears.

Plasma amino-acids

Those of all patients with gyrate atrophy, of nine of their parents, of three of their children, and of two of their brothers and sisters were quantitatively estimated with an automatic amino-acid analyser (Beckman Unichrom Amino Acid Analyzer, with Beckman M 72 ion exchange resin for the basic amino-acids, by the method of Spackman, Stein, and Moore, 1958) after precipitation of protein with 0.1 ml. of 100 per cent (w/v) sulphosalicylic acid from a 3 ml. plasma sample. The amino-acids of plasma and/or urine of the remaining 57 subjects were measured semiquantitatively with a routine
high voltage electrophoretic technique (Visakorpi, 1960) or with a modification for better resolution of basic amino-acids (Holmgren, Jeppson, and Samuelson, 1970), if the lysine-ornithine spot on the normal analysis was more than 1 on a scale of 0 to 3.

**Peroral loading test**

This was performed by giving 100 mg./kg. body weight of L-ornithine after an overnight fast to seven patients with gyrate atrophy and hyperornithinaemia (age 9 to 51 yrs and weight 26 to 69 kg.; 5 male, 2 female), to four of their parents (age 29 to 52 yrs and weight 54 to 72 kg.; 2 male, 2 female), and to four voluntary controls* (3 male, 1 female). L-ornithine (anal. grade, Fluka AG, Buchs, Switzerland) was given in 200 ml. apple-juice and water. Blood was drawn for amino-acid analysis at the beginning of the test and at 30-min. intervals for 2 hrs, and at 3, 4, and 6 hrs; after protein precipitation, the plasma samples were kept at −23°C. until analysed.

**Family studies**

These utilized information from church registers in local parishes. Three pedigrees were studied in detail, since the beginning of 17th century, including five families. The first family was selected at random from those with one affected subject (Pedigree 1), the second was a family with four affected siblings (Pedigree 2), and the third had five affected cases in three families (Pedigree 3).

**Results**

**Ocular findings and plasma and/or urinary amino-acids in the relatives of the patients**

Nine of the parents of the patients with gyrate atrophy were examined ophthalmologically. None showed any of the signs exhibited in the patients. The visual acuity was 1.0 except in one eye, which was amblyopic on account of 6 D astigmatism. The ocular media, including the lenses, were clear. The fundus of the parents revealed normal-looking retina, pigment epithelium, and choroid. Only the oldest mother showed a little degenerative conus around the temporal disc. Retinal function tests gave normal results in all eyes.

The visual acuity and fundus appearance of fifteen brothers and sisters and of three children of the patients with gyrate atrophy were within normal limits, except that one brother (Family 4) suffered from congenital cataract because of trisomy 21.

Ocular examination of eighteen other relatives revealed only mild refractive errors, with normal fundus appearances.

The mean of the fasting plasma ornithine concentration of the nine parents studied slightly exceeded that commonly reported for normal subjects (Table I), but individual levels overlapped the normal range. The two sisters and three children of the patients had normal plasma amino-acid concentrations. The plasma or urinary high-voltage electropherogram, which was performed on 57 other relatives, revealed normal amounts of ornithine in every case.

**Family studies**

The fourteen families of the 22 patients included 59 children, six of whom had died before their second birthday from different causes. The three pedigrees studied in detail are shown in Figs 1 to 3. Two children in Family 4 (Pedigree 3, Fig. 3) were suffering from trisomy 21. Congenital primary amyloidosis (Meretoja, 1973) was present in relatives in Pedigree 3, but not in the families with gyrate atrophy.

The parents of the patients with gyrate atrophy in Pedigree 1 were third cousins. The same was also true in Pedigree 2, but in addition a more distant relationship was detectable.

* The controls were hospitalized for the following reasons:

Post-traumatic retinal detachment (age 19 yrs, weight 60 kg.).

Retinal detachment after an operation for congenital cataract (age 43 yrs; weight 70 kg.).

Traumatic contusion of one eye (age 17 and 23 yrs; weight 57 and 52 kg.). All controls were otherwise healthy.
between the parents (Fig. 2). In the third pedigree (Fig. 3) gyrate atrophy was present in three families. Two third cousins had married two siblings, all of whom were remote relatives. The mother of the patients in Family 3 of this pedigree was also descended from the common ancestors. This fact could not be proven for the father, although this family had the same surname and was living in the same village as the ancestors of the wife.

In twelve out of the fourteen families, the four grandparents of the patients in each family came from the same parish (Fig. 4). In the other two families, the patients were illegitimate and all grandparents could not be traced. The birthplaces of the ancestors were unevenly distributed on the map (Fig. 4). All ancestors were of Finnish origin, but one family belonged to Finland’s Swedish-speaking minority.
Autosomal recessive inheritance of gyrate atrophy with hyperornithinaemia

The error incurred when the series was collected and can be corrected by means of the *a priori* correction method. If we assume that gyrate atrophy has an autosomal mode of inheritance, the probable number of children in families produced by two heterozygous individuals who are not included in the series can be calculated by means of the following formula (Li, 1961):

\[ c_s = \frac{s n_s}{1 - (3/4)^s} \]

where \( s \) = size of sibship, \( n_s \) = number of families in a sibship of size \( s \), and \( c_s \) = theoretical number of children of such marriages.

Details of the analysis are presented in Table II. The number of patients with gyrate atrophy with hyperornithinaemia as a proportion of the corrected total number of children of all marriages between presumptive heterozygotes was 0.255, when all the families were included. Removal of illegitimate children and families with one child from the group gives the same size proportion, 0.256. The frequency of affected males to affected females was nearly equal, 13:9.
Table II  Proportion of affected individuals among all sibs by a priori correction method  
(Li, 1961)

<table>
<thead>
<tr>
<th>s</th>
<th>n_s</th>
<th>s/n_s = ts</th>
<th>r_s</th>
<th>c_s = ts / 1 - 2^s</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>16.00</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>6</td>
<td>3</td>
<td>10.48</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>8</td>
<td>4</td>
<td>11.70</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>15</td>
<td>4</td>
<td>19.67</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>7.30</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>20</td>
<td>6</td>
<td>21.19</td>
</tr>
<tr>
<td>Total 29</td>
<td>14</td>
<td>59</td>
<td>22 = R</td>
<td>86.34 = C</td>
</tr>
</tbody>
</table>

\[ B = \frac{R}{C} = 0.255 \]

**FIG. 4** Birth places of grandparents in fourteen families with gyrate atrophy. The birthplaces of thirteen grandparents could not be traced.

**Heterozygote detection with peroral L-ornithine load**

The somewhat increased fasting plasma ornithine concentration of the parents of the patients was suggestive of a disturbance of ornithine metabolism in the probable heterozygotes of the disease. The metabolism of ornithine was studied with peroral L-ornithine loads. The plasma ornithine concentration in all subjects studied clearly increased after a load of 100 mg./kg. body weight of L-ornithine (Fig. 5). The peak concentration was reached 1 hr after the intake of the load. The concentration then fell gradually and was close to the fasting levels 6 hrs after the load. The plasma ornithine concentrations of the parents at 30 to 120 min. were totally separated from those of the controls. The difference between these two groups was greatest at 60 and 90 min. (P for difference of groups <0.01). The heterozygotes and the controls differed even more if the surface areas covered by the 180-min. curves were compared (mean ± 1 SD for the controls and parents of the patients 79.85 ± 2.97 and 143.65 ± 22.63 respectively; P for difference <0.005).

From the beginning the patients had a much larger plasma ornithine concentration than the other subjects, and this difference was maintained throughout the study.

**Discussion**

Although gyrate atrophy of the choroid and retina has been known since the last century (Cutler, 1895; Fuchs, 1896), it is only rarely mentioned in the literature and confusion with
Gyrate atrophy of the choroid and retina

Other diseases still seem evident. We recently reported a series of fifteen patients with gyrate atrophy (Takki, 1974), in which the progressive nature of the chorio-retinal atrophy was clearly visible as in the present series of 22 patients. The atrophy begins in the first decade of life as sharply-defined, round atrophic areas in the midperiphery of the fundus. These atrophic areas fuse together to form garland-shaped areas extending towards the posterior pole of the fundus until the whole function of the retina disappears and only the whitish sclera remains visible. At a late stage of the disease there is fine pigmentation in the midperiphery of the fundus and macular area. Glittering crystals are situated on this pigmentation. The electroretinogram, electro-oculogram, and fluorescein angiogram are grossly pathological by the time the fundus changes have appeared. Function of the rods diminishes in dark adaptation and the concentric limitation of the fields of vision increases as the disease progresses. Macular function remains until a late stage. Myopia and a complicated type of cataract are typically also present.

The plasma ornithine concentration was 10 to 20-fold increased and a corresponding overflow ornithinuria was found in all 22 patients with gyrate atrophy of the choroid and retina but not in their relatives. Similarly, the normal ophthalmological findings in the parents confirmed the previous suggestion of healthy carriers of the disease (Franceschetti, François and Babel, 1963; Botermans, 1972). Thus the constant combination of the ocular disease with the abnormality of amino-acid metabolism seems evident and we have an accurate biochemical method for confirming the ophthalmological diagnosis.

Hyperornithinaemia seems to be unique among the known inborn errors of amino-acid metabolism, as it is obviously combined with constant ophthalmological findings with very
few systemic symptoms, although the few abnormal EEGs suggest some general involvement of the central nervous system (Takki, 1974). However, the higher levels of plasma ornithine did not correlate with mental subnormality or with advanced choroidal atrophy. A 3 to 4-fold increased plasma ornithine concentration has been described by Bickel, Feist, Müller, and Quadbeck (1968) in two patients (aged 3 and 7 yrs) with generalized aminoaciduria and mental retardation, but the funduscopic findings were normal.

Increased plasma ornithine concentrations similar to those in the present series were found by Shih, Efron, and Moser (1969) in a 3-year-old boy with an apparently different syndrome. This boy also had hyperammonaemia and homocitrullinuria combined with psychomotor retardation, infantile spasms, irritability, and intermittent ataxia. His ophthalmic findings were not reported.

Previously, many exogenous substances have been shown to produce tapeto-retinal-like degeneration in animals (François, 1964), and some metabolic disturbances are found together with tapeto-retinal degeneration in man (François, 1964). The only metabolic abnormality in gyrate atrophy was described by François and others (1959), who found lysinuria in their one patient. Since lysine and ornithine are not clearly separable in most urinary amino-acid screening methods, their patient may have suffered from ornithinuria.

Parental consanguinity is repeatedly mentioned even in early reports of gyrate atrophy. According to Waardenburg (1939), it is found in 40 per cent., and according to Franceschetti and others (1963) in about 35 per cent. of cases. A large number of familial cases was reported by at least fifteen authors from 1895 to 1971 (Cutler, 1895; Bednarski, 1900; Mori, 1914; Werkle, 1931; Lyle, 1932; Malbrán and Fonte, 1947; Saebö, 1948; Chams and Sadoughi, 1957; Sebestyén, 1959; Wu and Yang, 1960; Collier, 1962; Kurstjens, 1965; Hilsdorf, 1967; Schäfer and Tenner, 1970; Krill and Archer, 1971). The disease is present in only one generation and with an even distribution among females and males. Usher (1935) found eleven women and fifteen men in the literature suffering from gyrate atrophy, and eight of the thirteen cases of Kurstjens (1965) were females. Gyrate atrophy was regarded as an early stage of choroideremia by Böhm (1919), but this opinion was rejected by Waardenburg (1939), who first suggested a simple recessive mode of inheritance in gyrate atrophy.

In the present study, the parental consanguinity, the equal sex distribution (13 men and 9 women), the uneven regional distribution of the ancestors of the patients, the proportion of diseased individuals among all sibs, and the obviously detectable minor defect in the ornithine metabolism of probable heterozygotes confirm an autosomal recessive mode of inheritance. It seems obvious that the gene frequency of this disease is extremely high in Finland as is the frequency of many other inborn metabolic errors (Nevanlinna, 1972), compared with other countries.

None of the authors who reported cases of gyrate atrophy have found any signs of ocular change in the suspected heterozygotes of the disease, and similarly the fasting plasma ornithine concentrations of the heterozygotes overlapped the normal range in the present study. However, the metabolism of ornithine seems to be partially defective in the heterozygotes because they cannot normally eliminate extra loads of ornithine. This loading test makes it relatively easy to detect heterozygote carriers in families with known cases of the disease.

Two systems now seem to be available for the prenatal diagnosis of gyrate atrophy: (1) If the basic enzyme defect of hyperornithinaemia is present in fibroblasts and is detectable quickly from a small amount of cells and the difference between healthy subjects,
hetero- and homozygotes is large enough for separation of the groups, the disease can be shown in cultured fibroblasts from the amniotic fluid.

(2) The concentration of ornithine in the amniotic fluid of fetuses with hyperornithinaemia may be expected to be increased to the same degree as urinary ornithine concentration.

Summary

The plasma and/or urinary ornithine concentration in 71 relatives of 22 patients with gyrate atrophy of the choroid and retina and with massively increased plasma ornithine concentrations (range 707–1399 μM; mean 1015 μM) was within normal limits. The mean of the fasting plasma ornithine concentration of nine parents of the patients (898 μM) slightly exceeded that commonly reported for normal subjects, but individual levels (438–1426 μM) overlapped the normal range. The plasma ornithine concentration clearly increased after a peroral load of 100 mg./kg. body weight of L-ornithine in patients, heterozygotes, and controls. The plasma ornithine concentration of the parents at 30 to 120 min. was totally separated from that of the controls. The patients had a much larger plasma ornithine concentration than the other subjects throughout the study. This loading test enables heterozygous individuals in families with known cases of the disease to be detected, although the ocular findings in these heterozygotes are normal.

Parental consanguinity, equal sex distribution (13 men and 9 women), uneven regional distribution of the ancestors of the patients, calculations made on the ratio of affected sibs to the number of total sibs (0.255), and the obviously detectable minor defect in the ornithine metabolism of probable heterozygotes confirm an autosomal recessive mode of inheritance in gyrate atrophy of the choroid and retina with hyperornithinaemia.

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