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Asymptomatic type II hyperprolinaemia associated with hyperglycinaemia in three sibs

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Pavone, L., Mollica, F., and Levy, H. L. (1975). *Archives of Disease in Childhood*, **50**, 637. **Asymptomatic type II hyperprolinaemia associated with hyperglycinaemia in three sibs.** Three clinically normal sibs were discovered to have type II hyperprolinaemia in a routine serum amino acid screening programme in Sicily. In addition to the basic biochemical features of type II hyperprolinaemia, all 3 children had marked hyperglycinaemia, whereas their parents had both normal blood proline and glycine concentrations. Clinical normality in individuals with hyperprolinaemia may suggest that these two metabolic disorders (types I and II) are benign entities. Furthermore, the absence of clinical abnormality in these sibs, despite the presence of marked hyperprolinaemia and hyperglycinaemia, may suggest that neither of these findings alone causes brain damage. The hyperglycinaemia in these sibs is unexplained and is an unusual if not unique finding in association with hyperprolinaemia.

Hyperprolinaemia is a result of at least two inborn errors of amino acid metabolism (Fig. 1). Each of these disorders is characterized by raised plasma proline concentrations and by increased urinary excretion of proline, hydroxyproline, and glycine (Scriver and Rosenberg, 1973). In hyperprolinaemia type I liver proline oxidase activity is deficient and thus proline cannot be normally oxidized to Δ^1-pyrroline-5-carboxylic acid (PCA) (Efron, 1965). In type II hyperprolinaemia, Δ^1-pyrroline-5-carboxylic acid dehydrogenase activity is deficient, resulting in the accumulation of PCA and its excretion in urine (Valle, Phang, and Goodman, 1974). In both disorders the hyperprolinaemia seems to be a specific hyperaminoacidaemia.

Urinary measurement of PCA has served to distinguish these two disorders. In addition, type II hyperprolinaemics usually have had a greater degree of hyperprolinaemia than those with type I (Scriver and Rosenberg, 1973). Clinical differences have also been described. Among these are the nephropathy, deafness, photogenic epilepsy, mental retardation, and electroencephalographic (EEG) abnormalities in type I individuals, and mental retardation, seizures, and EEG aberrations in association with type II. However, several clinically normal individuals with type I disorder have been described (Scriver and Rosenberg, 1973; Fontaine, Farriaux, and Dautrevaux, 1970; Mollica, Pavone, and Antener, 1971; Potter and Waickman, 1973; Perry *et al.*, 1967/1968; Holton, 1973), and recently a girl with type II disorder was described as being normal (Goodman *et al.*, 1974).

Type II hyperprolinaemia seems to be a rare metabolic disorder. To our knowledge only 11 cases in nine different families have been described (Table I). An additional 2 cases, not including those in this report, are known to us (Shih, Levy, and Coulombe, unpublished data), and are included in Table I. Thus the full range of clinical and biochemical findings in this disorder has not yet been ascertained. Indeed, it is not at all certain that clinical abnormalities occur at all as a consequence of the disorder since those individuals discovered to have type II hyperprolinaemia have been studied precisely because of some known clinical abnormality.

In 1971, we reported a family in which several

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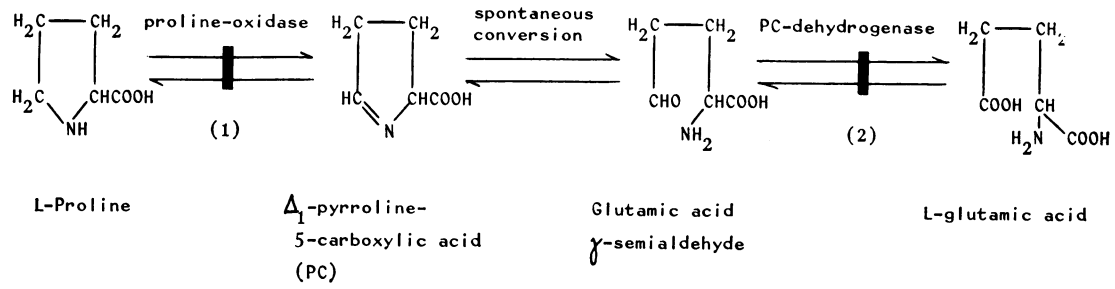

FIG. 1.—*Pathway of mammalian proline metabolism, with the two known human metabolic blocks indicated. (1) Metabolic block in type 1 hyperprolinaemia. (2) Metabolic block in type 2 hyperprolinaemia.*

members had type I hyperprolinaemia unassociated with clinical abnormalities (Mollica *et al.*, 1971). Now we report a family in which 3 sibs have type II hyperprolinaemia without clinical abnormalities. The hyperprolinaemia in this family is also associated with hyperglycinaemia, an unusual finding in any of the hyperprolinaemic disorders.

Case report

As in the case of type I hyperprolinaemia already reported (Mollica, *et al.*, 1971), this family with type II hyperprolinaemia was identified while screening for aminoacidopathies in the infant population of Eastern Sicily (Mollica *et al.*, 1970). The proband in whom hyperprolinaemia was noted is now an 8-year-old girl. She was born after a term, uneventful pregnancy, has not suffered from any particular illnesses, has been on a normal diet throughout life, and is presently clinically normal. Hearing, ophthalmological examinations, and electroencephalogram (EEG) were normal. Her intelligence quotient determined by the Wechsler Intelligence Scale for Children was 105. Laboratory examinations, including blood urea nitrogen and intravenous urography, were also normal. Chromosome count and karyotype were normal. Results of plasma amino acid screening showed a marked increase in proline.

The parents, who come from a small village in the province of Ragusa (Sicily), are first cousins (Fig. 2). Clinical examinations of the parents and 2 sibs of the proband were normal. Routine laboratory findings, hearing examination, and EEG were normal. All members of the immediate family were screened for amino acids and the 2 sibs were also found to have hyperprolinaemia.

Methods

Semiquantitative amino acid analysis of plasma was by the unidimensional paper chromatographic technique of Scriver, Davies, and Cullen (1964), and of urine by the unidimensional and two-way sequential paper chromatographic techniques of Efron *et al.* (1964) and Efron (1968). Plasma and urine amino acids were

TABLE

Pertinent clinical and biochemical characteristics of published

Case no.	Studies	Age	Sex	Origin	Consanguinity of the parents
1	Efron (1967)	10yr	M	—	—
2	Emery *et al.* (1968)	18yr	F	—	Yes
3	Selkoe (1969)	19m	M	Italy	No
4	Similä (1970)	2yr	M	—	Yes
5	Similä (1970)	—	M	—	Yes
6	Jeune *et al.* (1970)	18m	M	France	No
7	Jeune *et al.* (1970)	6m	F	France	No
8	Goodman *et al.* (1974)	9yr	F	Spanish-American	No
9	Applegarth *et al.* (1974)	5yr	M	—	No
10	Cooke and Raine (1973)	23m	M	—	—
11	V. E. Shih *et al.* (unpublished)	1yr	F	U.S.A (Irish)	No
12	H. L. Levy *et al.* (unpublished)	15yr	M	U.S.A (Irish)	No
13	Our proband	8yr	F	Italy	Yes
14	Her brother	10yr	M	Italy	Yes
15	Her sister	2yr	F	Italy	Yes

*Normal values: proline 0·11–0·44; glycine 0·12–0·51.

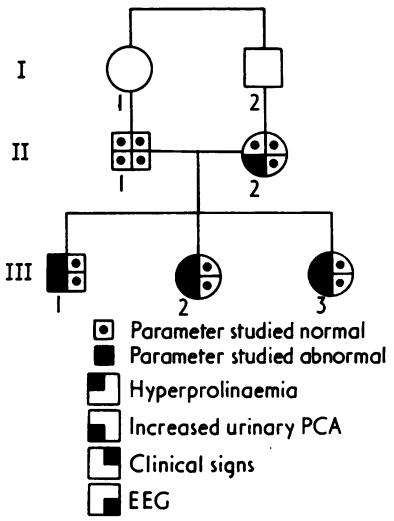

FIG. 2.—*Family tree showing those with biochemical abnormalities related to proline metabolism and lack of clinical signs.*

quantitated by ion-exchange column chromatography (Efron, 1966). Urinary excretion of PCA was measured by a colorimetric assay (Efron, 1965).

Results

Analyses were performed on plasma obtained in the fasting state and on aliquots of 24-hour urine collections of the proband, both sibs, and the parents while on a normal diet. The proband and both of her sibs showed a marked increase in plasma proline by paper chromatography, while the parents were normal. The result of quantitative plasma amino acid analyses (Table II) confirmed the marked increase in proline in all 3 sibs and also showed a marked increase in glycine. The magnitude of the hyperglycinaemia, however, was not proportional to the magnitude of the hyperprolinaemia. The proband, for instance, had the most marked hyperprolinaemia but not the most extreme hyperglycinaemia (Table II). All other amino acids, including hydroxyproline, were normal. All serum amino acids were normal in both parents.

All 3 hyperprolinaemic subjects showed a markedly increased urinary excretion of proline, hydroxyproline, and glycine (iminoglycinuria) while the urinary amino acid excretion was normal in the parents.

Urinary excretion of PCA was markedly increased in the 3 sibs, slightly increased in the mother, and normal in the father (Table II).

Discussion

Table I summarizes the major clinical and biochemical findings of individuals known to have type II hyperprolinaemia, including those reported here. These cases include 15 individuals in 11 different families. At least 4 have been of Italian origin.

Type II hyperprolinaemia has been associated with mental retardation, seizures, and EEG abnormalities, though not always (Table I). The convulsions have generally been grand mal in type, though in the patient described by Emery, Goldie, and Stern (1968) the seizures changed from grand

ases of type II hyperprolinaemia or cases known to the authors

Serum (mmol/l)*		Iminoglycinuria	Mental retardation	Convulsions	EEG abnormalities
Proline	Glycine				
>3·5	0·18	Yes	Yes	Yes	Yes
1·7–2·6	—	Yes	Yes	Yes	Yes
3·8	0·14	Yes	Yes	Yes	Yes
3·5	0·19	Yes	No	Yes	Yes
2·9	0·20	Yes	No	No	Yes
0·5–1·2	0·23	Yes (partial)	Yes	Yes	Yes
0·5–1·3	0·21	Yes (partial)	Yes	Yes	Yes
1·7	0·25	Yes	No	No	—
2·8	0·24	Yes	No	?Yes	Yes
3·5	—	Yes	Yes	Yes	Yes
3·2	0·35	Yes	No	No	No
2·2	0·29	Yes	Yes	Yes	Yes
1·3	1·25	Yes	No	No	No
1·7	1·86	Yes	No	No	No
2·7	1·26	Yes	No	No	No

TABLE II

Pertinent serum and urine amino acid concentrations and concentrations of PCA in urine of sibs with type II hyperprolinaemia and their parents

Subject*	Age (yr)	Serum amino acids (mmol/l)		Urine amino acids (mmol/g creatinine)			Urine PCA (mmol/g creatinine)
		Proline	Glycine	Proline	Hydroxyproline	Glycine	
II.1	41	0·25	0·50	0	0	0·96	0·01
II.2	38	0·12	0·13	0	0	0·87	0·04
III.2	10	1·69	1·86	22·63	2·54	14·65	0·24
III.2	8	2·68	1·26	29·59	4·23	21·31	0·10
III.3	2	1·34	1·25	26·11	3·39	23·98	0·20
Normal values		0·11–0·44	0·12–0·51	0·0·05	0–0·08	0·30–2·62	0–0·02

*See Fig. 2.

mal to petit mal at the age of 11 years. EEG has either been normal or had diffuse nonspecific aberrations, the single exception again being the case with petit mal seizures showing the characteristic 3 per second spike and wave pattern (Emery *et al.*, 1968).

In addition to the case described by Goodman *et al.* (1974), there are other cases of type II disorder in which biochemical findings are accompanied only by equivocally abnormal clinical signs. One of two type II hyperprolinaemic brothers (Similä, 1970) showed only EEG abnormalities and no clinical signs. The subject studied by Applegarth *et al.* (1974) was clinically healthy though he had had a febrile convulsion and abnormal EEG. In our family the affected members showed neither clinical signs nor EEG abnormalities. The mental level in each was quite high. These data suggest that high levels of plasma proline even when accompanied by accumulations of PCA may not cause clinically apparent alterations of the brain. As in the other published cases of type II hyperprolinaemia, the 3 sibs we report had a marked increase in urinary PCA. A slight increase of urinary PCA was also present in the mother, perhaps as a reflection of the heterozygotic state. However, the father, who is also an obligate heterozygote, had normal urinary PCA concentrations.

A striking characteristic of the family we describe is the markedly increased plasma glycine in those members with hyperprolinaemia. This is apparently a unique finding in type II hyperprolinaemia (Table I), though it has been noted in a much less severe degree in one family with type I hyperprolinaemia (Mollica *et al.*, 1971). The hyperglycinaemia is a peculiar finding and cannot readily be explained on the basis of the known metabolic pathway for proline, and distinct from the marked hyperglycinuria (but normal blood glycine concentration) noted in conjunction with the prolinuria and hydroxyprolinuria (iminoglycinuria) seen in individuals with hyperprolinaemia. The level of plasma glycine is even higher than has been observed in most patients with disorders that clearly result in a specific hyperglycinaemia, either ketotic or nonketotic type (H. L. Levy, V. E. Shih, and I. T. Lott, unpublished data). Since almost all of these latter cases have manifested mental retardation or other signs of brain damage not readily attributable to the hyperglycinaemia, it is of interest that our patients were clinically normal.

We believe that the association between hyperglycinaemia and hyperprolinaemia is not coincidental, since the hyperglycinaemia was present in each of our hyperprolinaemic cases and not present in the parents who had normal proline concentrations. However, since there is no known direct metabolic relation between glycine and proline, a plausible explanation is difficult. These different findings could conceivably be an expression of two different metabolic abnormalities due to a single gene with pleiotropic effects or to two linked genes. In this context may be cited the cases of Jeune *et al.* (1970) in which type II hyperprolinaemia was accompanied by hyperleucinisoleucinaemia and which were interpreted as sibs with double aminoacidopathy. However, precise interpretation of hyperglycinaemia in our cases awaits further definition of the mechanisms for hyperglycinaemia in general, a definition which could show an interrelation between proline and glycine degradation.

There is a further important point regarding hyperglycinaemia. Individuals with hyperglycinaemia have generally suffered brain damage when not treated. In ketotic hyperglycinaemia the basic defect is one of organic acid metabolism (Nyhan, Ando, and Rasmussen, 1972) and thus brain damage is usually ascribed to the resulting organic acid

derangement. In nonketotic hyperglycinaemia no such underlying disorder(s) has been described and the basic defect is believed by some to be due to an error in glycine metabolism (Ando *et al.*, 1968; de Groot, Troelstra, and Hommes, 1970). Thus, it has been presumed that the glycine accumulation alone could be responsible for the severe neurological manifestations. However, in the individuals whom we describe with relatively huge increases in the blood glycine concentration, no neurological damage is evident. This finding alone might challenge the aetiology of brain damage in the hyperglycinaemic states.

References

Ando, T., Nyhan, W. L., Gerritsen, T., Gong, L., Heiner, D. C., and Bray, P. F. (1968). Metabolism of glycine in the nonketotic form of hyperglycinemia. *Pediatric Research*, **2**, 254.

Applegarth, D. A., Ingram, P., Hingston, J., and Hardwick, D. F. (1974). Hyperprolinaemia type II. *Clinical Biochemistry*, **7**, 14.

Cooke, J. R., and Raine, D. N. (1973). *Treatment of Inborn Errors of Metabolism*, p. 97. Ed. by J. W. T. Seakins, R. A. Saunders, and C. Toothill, Churchill Livingstone, Edinburgh.

de Groot, C. J., Troelstra, J. A., and Hommes, F. A. (1970). Nonketotic hyperglycinemia: an in vitro study of the glycine-serine conversion in liver of three patients and the effect of dietary methionine. *Pediatric Research*, **4**, 238.

Efron, M. L. (1965). Familial hyperprolinemia. Report of a second case, associated with congenital renal malformations, hereditary hematuria and mild mental retardation, with demonstration of enzyme defect. *New England Journal of Medicine*, **272**, 1243.

Efron, M. L. (1966). Quantitative estimation of aminoacids in physiological fluids using a Technicon aminoacid analyzer. *Automation in Analytical Chemistry*, p. 637. Ed. by L. T. Skeggs, Jr. Mediad, New York.

Efron, M. L. (1967). Treatment of hydroxyprolinemia and hyperprolinemia. *American Journal of Diseases of Children*, **113**, 166.

Efron, M. L. (1968). High voltage paper electrophoresis. *Zone Electrophoresis, Chromatographic and Electrophoretic Techniques*, Vol. **2**, 2nd ed., p. 166. Ed. by I. Smith. Wiley Interscience, New York.

Efron, M. L., Young, D., Moser, H. W., and MacCready, R. A. (1964). A simple chromatographic screening test for the detection of disorders of amino acid metabolism. *New England Journal of Medicine*, **270**, 1378.

Emery, F. A., Goldie, L., and Stern, J. (1968). Hyperprolinaemia type 2. *Journal of Mental Deficiency Research*, **12**, 187.

Fontaine, G., Farriaux, J. P., and Dautrevaux, M. (1970). L'hyperprolinémie de type 1°. Etude d'une observation familiale. *Helvetica Paediatrica Acta*, **25**, 165.

Goodman, S. I., Mace, J. W., Miles, B. S., Teng, C. C., and Brown, S. B. (1974). Defective hydroxyproline metabolism in type II hyperprolinemia. *Biochemical Medicine*, **10**, 329.

Holton, J. B. (1973). *Treatment of Inborn Errors of Metabolism*, p. 103. Ed. by J. W. T. Seakins, R. A. Saunders, and C. Toothill. Churchill Livingstone, Edinburgh.

Jeune, M., Collombel, C., Michel, M., David, M., Guibaud, P., Guerrier, G., and Albert, J. (1970). Hyperleucinisoleucinémie par défaut partiel de transamination associée à une hyperprolinémie de type 2. Observation familiale d'une double aminoacidcpathie. *Annales Pédiatrie*, **17**, 85.

Mollica, F., Pavone, L., and Antener, I. (1971). Pure familial hyperprolinemia: isolated inborn error of aminoacid metabolism, without other anomalies in a Sicilian family. *Pediatrics*, **48**, 225.

Mollica, F., Pavone, L., Santonocito, B., and La Rosa, M. (1970). Richerche sulla diffusione delle aminoacidopatie mediante cromatografia su carta degli aminoacidi plasmatici secondo Scriver. *Reivista Pediatrica Siciliana*, **25**, 67.

Nyhan, W. L., Ando, T., and Rasmussen, K. (1972). Ketotic hyperglycinaemia. *Organic Acidurias*, p. 1. Ed. by J. Stern and C. Toothill. Churchill Livingstone, Edinburgh.

Perry, T. L., Hardwick, D. F., Lowry, R. B., and Hansen, S. (1967/1968). Hyperprolinaemia in two successive generations of a North American Indian family. *Annals of Human Genetics*, **31**, 401.

Potter, J. L., and Waickman, F. J. (1973). Hyperprolinemia I: study of a large family. *Journal of Pediatrics*, **83**, 635.

Scriver, C. R., and Rosenberg, L. E. (1973). *Amino Acid Metabolism and its Disorders*. Saunders, Philadelphia.

Scriver, C. R., Davies, E., and Cullen, A. M. (1964). Application of a simple micromethod to the screening of plasma for a variety of aminoacidopathies. *Lancet*, **2**, 230.

Selkoe, D. J. (1969). Familial hyperprolinemia and mental retardation. *Neurology*, **19**, 494.

Similä, S. (1970). Hyperprolinaemia type II. *Annals of Clinical Research*, **2**, 143.

Valle, D., Phang, J. M., and Goodman, S. I. (1974). The enzymatic defect in type II hyperprolinemia: absence of Δ^1pyrroline-5-carboxylic acid dehydrogenase activity. *Pediatric Research*, **8**, 396.

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