Pyridoxine-responsive Anaemia Determined by an X-linked Gene

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Harris, Whittington, Weisman, and Horrigan (1956) described pyridoxine-responsive anaemia as a naturally occurring disease in man, and since then 28 further cases have been reported. They have been for the most part single case reports, and they suggest that pyridoxine-responsive anaemia is a syndrome with variable aetiology rather than a disease entity. There have been only two reported instances in which the disease could have been inherited.

The haematological picture is of a hypochromic anaemia with microcytic red cells which show anisocytosis and poikilocytosis and often target cells. In contrast there is evidence of excessive iron loading in plasma and tissues.

We report here details of a family in which a hereditary pyridoxine-responsive anaemia occurred. We have investigated 83 members of the family and studied in detail 6 affected members.

Methods

Through co-operation of local pathologists, 82 relatives of our patient were screened in our department or in the area of residence of the individuals. Haematological investigations were carried out to determine the haemoglobin levels and mean corpuscular haemoglobin concentrations, and stained blood films were examined for red cell morphology. Serum iron and total iron-binding capacity were determined by the method of Ramsay (1957a, b). (Normal range of serum iron 100–200 µg./100 ml., serum iron-binding capacity 200–400 µg./100 ml., percentage saturation 14–51.) In cases where borderline or abnormal results were obtained, the estimations were repeated a number of times. The individuals in whom an abnormal state was found were clinically examined by us, and further studies were undertaken, including osmotic fragility (Dacie, 1956), haemoglobin electrophoresis on paper (Goldberg, 1959),

and cellulose acetate using Goldberg's discontinuous barbiturate buffer system.

Results

The clinical details of the six patients have been described elsewhere (Bourne, Elves, and Israëls, 1965), but brief details of the clinical haematological states are given in Table I. From this Table it will be seen that the salient features of this disease are an anaemia that may vary from mild to severe, and evidence of iron loading. Two of the patients (Cases 4 and 5) had normal haemoglobin levels at the start of this study, but both showed iron loading which responded to pyridoxine therapy. Both of them have been closely followed and have had low haemoglobins (12 g./100 ml.) on a number of occasions. Osmotic fragilities and haemoglobin studies by electrophoresis revealed no departure from normal in any case.

Studies of other X-linked characters, i.e. colour vision, Xg blood groups, and glucose-6-phosphate dehydrogenase have been undertaken in three affected sibships, and the results are shown in Table II.

Discussion

The family studied showed a clear genetic basis for pyridoxine-responsive anaemia. From the pedigree (Fig.), it will be seen that no female relative of our patient has pyridoxine-responsive anaemia. Five male relatives of the propositus however have the anaemia, to varying degrees, and iron loading, both of which respond to pyridoxine. Three sibships contain affected males (IV.9, 10; V.4; V.7–9). There is no evidence of male-to-male transmission of the disease. These facts led us to postulate that the disease was an X-linked recessive condition. If this is so there are seven presumptive carriers in the family (I.2; II.8, 10; III.1, 4; and IV.3, 5). Thus in the kindred there are 18 sons of

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TABLE I
HAEMATOLOGICAL AND THERAPEUTIC DATA OF AFFECTED INDIVIDUALS—ALL MALE

Case	Pedigree Fig.	Age (yr.)	Before Treatment				Treatment								After Treatment			
			Hb (g./100 ml.)	MCHC (%)	Serum Iron (µg./100 ml.)	% Sat. Serum Iron-binding Capacity	Br ₁₂	Folic	Blood	Iron	Spleen	Liver	Pyridoxine (mg./day)	Marrow	Red cell morphology	Hb (g./100 ml.)	MCHC (%)	Serum Iron (µg./100 ml.)
1	IV.9	54	6.4	28.0	260	85	+	—	+	+	—	—	120	ZZ	A	13.7	31.5	80
2	IV.10	50	5.8	—	150	77	—	—	—	+	—	—	150	—	A	—	—	220
3	V.9	50	5.2	27.5	205	85	—	—	+	+	—	—	100	ZZZZ	A	12.8	—	79
4	V.7	52	14.2	31.5	200	64	—	—	—	—	—	—	100	—	ZZ	12.3	30.0	100
5	V.8	45	14.0	32.5	220	56	—	—	—	—	—	—	100	—	ZZ	—	—	100
6	V.4	41	11.7	29.0	290	94	—	—	—	—	—	—	100	—	A	14.0	32.0	270

N = Normal

A = Abnormal

TABLE II
LINKAGE STUDIES OF AFFECTED SIBSHIPS

Case No.	Generation No.	Xga	Colour Vision	Glucose-6-phosphate Dehydrogenase
1	IV. 9	+	ZZ	ZZ
2	IV. 10	+	ZZ	ZZ
3	V. 9	+	ZZ	ZZ
4	V. 7	+	ZZ	ZZ
5	V. 8	+	ZZ	ZZ
6	V. 4	—	ZZ	ZZ
7	V. 5	—	ZZ	ZZ
8	V. 6	+	ZZ	—

FIG. Pedigree of family showing incidence of pyridoxine-responsive anaemia.

these carriers who are potentially at risk to a significant degree (1/2). Unfortunately, we were able to study only 8 of them, but on questioning several members of the family independently, it was found that all the males in generation II lived to old age and did not suffer from anaemia. We were not able to examine III.3, who is alive and well and is at present in his 70's. His brother III.2 died at the age of 45 from septicaemia and cerebral haemorrhage and was not anaemic. In generation IV, No. 1 was dead and no details of him are available. IV.8 died at the age of 46 as a result of cerebral haemorrhage and was at that time haematologically normal. Of these 18 males, therefore, only 6 have been shown to have pyridoxine-responsive anaemia. If this disease were determined by a sex-linked recessive gene, then 9 members would have been expected to have been affected. The incidence of carrier females is more uncertain, as it is impossible to determine the number of possible carriers among those leaving no offspring, or those having female children who in turn have no evidence of being carriers. There are a total of 13 female offspring of presumed carrier females, and of these 6 are known to be carriers. On theoretical grounds 6.5 carrier females would have been expected.

These data would fit an X-linked recessive mode of inheritance. Well-documented reports of 28 cases of anaemia responding to pyridoxine, have been published, and we have studied a further isolated instance. These 29 cases, however, are heterogeneous, and only 19 resemble our group. All these 19 were males with marked anaemia and evidence of iron loading; the marrow in most showed normoblastic erythropoiesis, though there was some evidence of megaloblastic changes in 2 cases (Dawson, Leeming, Oelbaum, Pengelly, and Wilkinson, 1961; Heilmeyer, 1963).

The most constant feature was the failure of the anaemia to respond to haematinics other than pyridoxine, though haemoglobin levels were maintained temporarily by repeated blood transfusion.

In these 19 cases the very limited family studies revealed no affected relations in 11. In the case reported by Bernard, Bessis, Boiron, Malassenet, and Caroli (1960), a rather more extensive family investigation was made, that failed to reveal any affected relative of the *propositus*; though a number of the females showed slight anisocytosis and hypochromia, only two other male persons of the patient's generation were studied and found to be normal.

In four reports there is good evidence of a familial incidence. Sánchez Medal, Elizondo, Torres Gallardo, and Gittler (1961) reported a man

(Case 1), who had a pyridoxine-responsive anaemia, who relapsed on cessation of therapy. His brother (Case 2) had a similar anaemia and proven haemachromatosis, and the anaemia responded to pyridoxine. A haemoglobin level of 15-17 g./100 ml. has been maintained for 6 years without any therapy. Bottomley's (1962) first patient had a brother with anaemia and confirmed haemachromatosis, but as no therapeutic trial of pyridoxine was made (Byrd and Cooper, 1961) the diagnosis of pyridoxine-responsive anaemia was not certain.

A third possible occurrence of inherited cases is the two brothers reported by Redmond, Robertson, and Nelson (1963) in whom one had the anaemia, but the brother was mildly affected and may well have been shown to have responded to pyridoxine had it been given for longer than the 12 days. Earlier this year, a fourth report of a family affected by a pyridoxine-responsive anaemia was published (Losowsky and Hall, 1965). In this family eight males, of ages varying from 3 years to 52 years, had an anaemia of varying degrees accompanied by abnormalities in red cell morphology. However, only two were successfully treated with pyridoxine and showed some improvement in haemoglobin and iron loading. One other of the 'affected' males failed to respond to pyridoxine.

Losowsky and Hall (1965) found that some of their presumed carrier females showed abnormalities of the red blood cells, and ring sideroblasts were present among the normoblasts of the marrow. Only one presumed carrier in their family was anaemic, but the anaemia in this case was probably due to folic acid deficiency. Both in our family and in the published case reports, therefore, the affected persons have all been male. One possible exception is the female reported by Bottomley (1962: Case 2) who had a haematologically indistinguishable anaemia; unfortunately the family studies did not include the father of the patient, and he may well have been affected, and if so, this would not upset a sex-linked recessive mode of inheritance.

Linkage Studies. Glucose-6-phosphate dehydrogenase, colour vision, and Xg blood groups were determined for the living males in affected sibships (Table II). No individual had the G6PD deficiency or colour blindness. Only one sibship is of use for a study of linkage of pyridoxine-responsive anaemia (PRA) with these genes (V.4-6). In the two sibships containing affected individuals, it is not possible to determine whether the mothers are double heterozygote for PRA and Xg. In this useful sibship two subjects were Xg(a-), i.e. V.4 and 5, and one was Xg(a+), and there was one affected mem-

ber in the sibship (V.4). It may therefore be assumed that the mother IV.3 was a double heterozygote for the Xg/PRA gene combination, this being so it may be seen that if the mother was a double heterozygote, in repulsion phase for Xg/PRA genes, there would be 1 recombinant individual in 3 (V.5): if she was doubly heterozygous for these genes, but in the coupling phase, there would be 2 recombinant individuals (V.4 and 6). Recognizing the fact that we have very few useful sibs as far as these genes are concerned, it seems that the Xg and PRA genes are not closely linked because the minimum number of recombinants is 1 in 3.

Summary

A family is described of which 83 individuals have been investigated, a pyridoxine-responsive anaemia being found in 6 middle-aged men. It is suggested that the anaemia in this family is an X-linked recessive condition. Other published familial cases are discussed and all would be consistent with such a mode of inheritance. It was found that the gene responsible was not closely linked to that determining the Xg blood groups.

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