Complete suppression of haemoglobin A synthesis in haemoglobin D Los Angeles – beta thalassaemia

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SYNOPSIS A family study is reported in which all three siblings were shown to be doubly heterozygous for haemoglobin D Los Angeles and beta thalassaemia, which resulted in a complete suppression of haemoglobin A synthesis. This demonstrates the effects of genetic interaction which occur when the genes for haemoglobin D Los Angeles and beta thalassaemia are both transmitted to the offspring. The importance of family studies in the investigation of haemoglobin abnormalities is stressed.

Complete suppression of haemoglobin A synthesis seems to occur when the genes for haemoglobin D Los Angeles and beta thalassaemia are transmitted to one individual. In the three previous reports of haemoglobin D-thalassaemia, the laboratory results indicated that haemoglobin A was not found (Hynes and Lehmann, 1956; Sukumaran, Sanghvi, and Nazreth, 1960; Ghai, Varma, and Taneja, 1961). In each of these reports, descriptions of peptide mapping were not available, and Hynes and Lehmann could only obtain incomplete family data. Both investigations were carried out in this study of haemoglobin D Los Angeles-beta thalassaemia which occurred in the children of an Indian family from Bombay, who are now resident in Britain.

METHODS

Standard haematological methods were used (Dacie and Lewis, 1963). Total red and white cell counts were estimated in a Coulter blood cell counter. Serum iron was estimated by the method of Kok and Wild (1960). Haemoglobin F was estimated by the modification by West and McIver (1961) of the method of Singer, Chernoff, and Singer (1951). Solubility tests were carried out as described by Itano (1953). Qualitative and quantitative haemoglobin electrophoresis was performed on cellulose acetate strips (Oxoid) by the method of Marengo-Rowe (1965); the haemoglobin variant was also demonstrated by paper electrophoresis using Tris buffer at pH 8.9 (Cradock-Watson, Fenton, and Lehmann, 1959). Peptide

mapping studies were carried out by the M.R.C. Abnormal Haemoglobin Research Unit, Cambridge, as described by Ingram (1958), Baglioni (1961), and Beale (1966).

CLINICAL AND LABORATORY FINDINGS

The haemoglobin abnormality was first detected as an incidental finding in a girl aged 18, who was examined at Princess Mary's Royal Air Force Hospital, Halton, because of recurring episodes of respiratory infection. No clinical abnormalities were found in the girl, her parents, or her two brothers. There was no reason not to assume true paternity. The details of the haematological investigations are in Table I. The serum iron level in the patient was 100 μg per 100 ml and the iron-binding capacity 310 μg per 100 ml.

The father and the three siblings showed at least two of the haematological stigmata of thalassaemia (Malamos, Fessas, and Stamatoyannopoulos, 1962), namely, alterations in red cell morphology, low mean corpuscular haemoglobin, and increased osmotic resistance. Sickling tests were negative and solubility tests were normal in all members of the family. The results of haemoglobin electrophoresis are shown in Figures 1 and 2 and Table II. The father had a normal haemoglobin composition but a raised level of haemoglobin A₂ (5%). The mother had haemoglobin A and haemoglobin D. The patient and her two brothers had almost entirely haemoglobin D and no haemoglobin A. The slight increase in alkali-resistant haemoglobin (2.9%) which was present in the father was considered of doubtful significance because of inherent errors in the method

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TABLE I
HAEMATOLOGICAL FINDINGS IN THE FAMILY

Age					
	Patient 18	Brother 1 17	Brother 2 13	Mother 40	Father 41
Haemoglobin (g/100 ml)	12.8	13.8	10.5	13.1	14.9
RBC ($\times 10^6$ /cu mm)	5.6	6.2	5.1	4.5	6.4
PCV (%)	39	44	35	42	49
MCV (cu μ)	70	71	68	93	80
MCH (μ g)	23	22	21	29	24
Reticulocytes (%)	2	1	1	1	2
Platelets ($\times 10^3$ /cu mm)	2.5	3	3	2.1	3.2
Total WBC (per cu mm)	8500	10500	10000	6500	10000
Red cell fragility (g/100 ml NaCl)					
Begins at	0.45	0.45	0.45	0.50	0.50
Ends at	0.20	0.30	0.30	0.30	0.30
Peripheral blood morphology	Leptocytes, schistocytes, moderate aniso- and poikilo- cytosis	Leptocytes, schistocytes, marked aniso- cytosis	Leptocytes, schistocytes, ovalocytes, moderate aniso- cytosis	Normal appearances	Target cells
Serum bilirubin (mg/100 ml)	1.0	0.5	1.0	1.4	1.1

TABLE II
HAEMOGLOBIN COMPOSITION

	Patient	Brother 1	Brother 2	Mother	Father
Haemoglobin A (%)	nil	nil	nil	73	93
Haemoglobin A ₂ (%)	<3.0	<3.0	<3.0	<3.0	5.0
Haemoglobin F (%)	1.2	1.0	1.0	1.3	2.9
Haemoglobin D (%)	>95.0	>95.0	>95.0	23.0	nil

of estimation at low levels and because alkali-resistant haemoglobin and haemoglobin F are not necessarily synonymous in adults (Falbe-Hansen, 1961). The haemoglobin variant in the patient was identified as haemoglobin D Los Angeles by peptide mapping.

DISCUSSION

Beta thalassaemia is the most widespread abnormality of haemoglobin production and its distribution is worldwide (Lehmann and Huntsman, 1966). In this condition the rate of synthesis of the beta chains of the globin molecule is impaired and leads to deficient production of haemoglobin A. The 'excess' of alpha chains present becomes available for combination with delta and/or gamma chains and results in the increased formation of haemoglobin A₂ and/or haemoglobin F (Ingram and Stretton, 1959; Malamos *et al*, 1962). Haemoglobin D Los Angeles (formerly haemoglobin D Punjab) is found principally in Northwest India although there are instances in European populations; this haemoglobin is a variant in which glutamine is substituted for glutamic acid at position 121 in the beta chain (Lehmann and Huntsman, 1966).

In the father the haematological findings and raised haemoglobin A₂ level are consistent with beta

FIGS. 1 and 2. Cellulose acetate electrophoresis in Tris buffer at pH 8.9 of the haemoglobins from the father, mother, and patient.

thalassaemia. The mother's blood contained 23% haemoglobin D, the remainder being mainly haemoglobin A; this is consistent with haemoglobin D trait (Chernoff, 1958). In the three siblings haemoglobin A was completely lacking; the haemoglobin present was almost entirely haemoglobin D. These findings indicate that all three siblings inherited a D beta gene from the mother and a beta thalassaemia gene from the father. The substitution of D beta chains for normal beta chains in the haemoglobin composition of the three siblings represents genetic interaction between the two inherited abnormalities which in this instance led to a complete suppression of haemoglobin A synthesis.

Since the laboratory findings in haemoglobin D-thalassaemia are little different from those of homozygous haemoglobin D disease, it is essential to carry out a family study if the true nature of the abnormality is to be determined. It is interesting to note that all three siblings were doubly heterozygous for haemoglobin D and thalassaemia and that none of other possible genetic alternatives occurred.

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