A Hb J CAPE TOWN HOMOZYGOTE – ASSOCIATION OF Hb J CAPE TOWN AND ALPHA-THALASSAEMIA

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1. Introduction

When homologous mutations occurring, respectively, in the α- and β-chains of human adult Hb (A) are compared one finds that the product of the variant α-chain gene amounts to about one-half of that of the corresponding variant β-chain gene. The α-chain mutant contributes about one-quarter to the total and the homologous abnormal β-chain about one-half. This led to the suggestion that the α-chain gene is duplicated so that a mutation would affect only one-half of the available α-chain genes [1]. The duplication of the human α-chain gene is now accepted as a fact in view of the subsequent finding of several homozygotes for α-chain variants who also possess Hb A. These have been summarised recently [2]. However the question was raised whether the α-chain gene duplication is universal in man. Homozygotes for Hb Jα Tongariki have been described who possess the variant only and no Hb A [3], and a number of α-chain abnormal Hbs are found in the heterozygous state at a proportion of one-third or even one-half rather than one-quarter, i.e., more than one would expect from a mutation affecting merely one of four α-chain genes per individual. Lehmann and Lang [4] reviewed these observations and suggested that they did not rule out a universal duplication of the α-chain gene if one assumed that in these cases the second gene had been prevented from expression by an α-thalassaemia.

For the homozygote for Hb Jα Tongariki this has now been confirmed by demonstrating a reduced α-mRNA level [5].

Hb J Cape Town [6] is one of the α-chain variants which occur at a proportion of about 40% in heterozygotes. A hypochromic thalassaemia-like blood picture was noted on a number of occasions [7] but iron deficiency was also present and could have been the cause of the hypochromia of the red cells. The association of near-normal or normal Hb levels with hypochromia of the red cells [7] could well have been the outcome of a tendency to polycythaemia attempting to compensate for the high oxygen affinity of Hb J Cape Town [8].

We have now been able to study a family with this Hb variant in which two heterozygous parents had several heterozygous and one homozygous offspring.

2. Methods

Haematological investigations and serum iron estimations, followed standard methods [9]. The procedures for preparation of haemolysate, separation of haemoglobins by paper and cellulose acetate electrophoresis, at pH 8.9, quantitation of haemoglobin fractions including Hbs A_2 and F, preparation of globin, of tryptic peptides derived therefrom, their two-dimensional separation by high voltage electrophoresis and chromatography, elution of peptides and their analysis have been summarised [10]. DEAE–Sephadex column chromatography and globin chain separation on 8 M urea CMC 23–Sephadex columns followed established techniques

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[11,12]. In vitro biosynthesis of haemoglobin was carried out in reticulocyte-enriched cell suspensions incubated with [^{3}H]leucine (spec. act. 50 Ci/μmol) for 60 min as summarised [13,14].

3. Results

A 5 years old South African girl with a Dutch family name was found to be short of breath and to possess the high Packed Cell Volume (PCV) of 54% (normal range 37–42%). She was considered to suffer from polycythaemia. The father had a life long history of cardiac pulmonary problems which recently had become worse and he had a PCV of 55%. A haemoglobinopathy caused by a high oxygen affinity haemoglobin was suspected. On routine electrophoresis the father's haemoglobin pattern showed in addition to Hb A a fast moving band in the position of Hb J and also in addition to Hb A_2 a similar fraction moving faster towards the anode (Hb J_2). Whereas the father though not fully healthy had led a normal life working as a blacksmith on the South African Railways at Uitenhage, his daughter who was definitely ill showed on electrophoresis only Hbs J and J_2 and no normal haemoglobins. Further family study showed that her mother who was a first cousin of the father also had the haemoglobin pattern of a Hb Jα heterozygote, and so did her two younger siblings. A third had died of pneumonia at the age of 6 months.

The globin of the propositus was analysed and it will be seen from fig.1 that it had the characteristics of Hb Jα Cape Town.

Table 1 shows the haematological findings in this family and it will be seen that they show hypochromia of the red cells, but that the serum iron values (excepting the mother's) are normal. It was concluded that the family had an α^A thalassaemia gene for which, in view of the absence of Hbs A and A_2, the propositus was presumably a homozygote. The presence of α-thalassaemia was further confirmed by

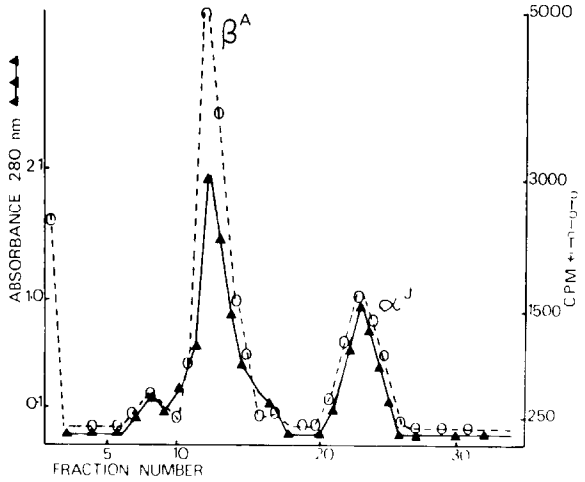

Fig.2. Elution profile [^{3}H]leucine and protein following CM cellulose chromatography of globin prepared from haemolysate of the propositus.

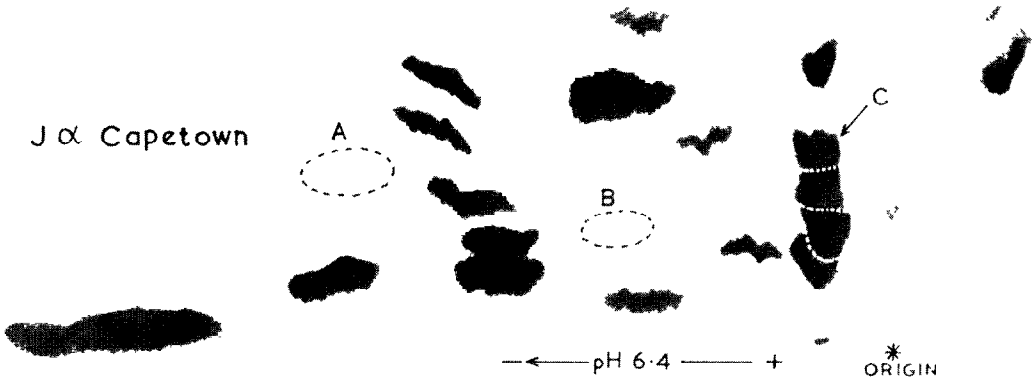

Fig.1. Fingerprint of the soluble tryptic peptides of haemoglobin J Cape Town. (A) Indicates where α^ATpX (α91–92) and (B) where α^ATpX–XI (α91–99) are missing. (C) Indicates α^JTpX–XI.

Table 1
Haematological data

	'RBC ($\times 10^{12}$/l)'	PCV (l/l)	Hb (g/dl)	MCHC (fl)	MCV (fl)	MCH (pg)	Serum F (μmol/l)	TIBC (μmol/l)	Hb phenotype	α^J/α^A (%)
Father	7.40	0.55	17.4	32	74	23.5	30.8	85	AJ	40
Mother	5.72	0.48	14.2	30	84	25	8.3	64	AJ	43
Propositus[a]	7.20	0.54	16.2	30	75	22.5	17.5	55	J	100
Sibling 1	5.59	0.43	13.2	31	77	24	28	–	AJ	40
Sibling 2	5.20	0.41	11.9	30	79	23	–	–	AJ	39
Normal range										
Male	4.5–6.3	0.41–0.51	14–18	31–35	77–93	26–32	13–32	45–70		
Female	4.2–5.5	0.37–0.47	12–16	31–35	77–93	26–32	13–32	45–70		

[a] Reticulocytes: 130×10^9/l (slightly elevated)

The Hb F level was within normal limits in all members of the family and the total Hb A_2 was in all within the lower range of normal (2.6–3.6% in Cambridge; 1.6–3.3% in Cape Town)

Table 2
Biosynthetic results in the homozygote in cpm

	Total	Spec. act/A_{280}
α^J	5904	1687
β	12 664	2097

Globin synthesis ratio 0.47

measuring the α/non-α globin ratio of incorporation of [^{3}H]leucine in her reticulocytes. Only radioactive α^J-chains and no α^A-chains were produced and there was an imbalance of the ratio of α^J/β globin synthesis seen with α-thalassaemia type 1 in which half of the four α-chain genes produce no globin (fig.2, table 2).

4. Discussion

There is a tendency towards polycythaemia in this family which certainly in the homozygote cannot be explained solely on the basis of a compensating thalassaemia. The mutation in Hb J Cape Town is at an $\alpha 1\beta 2$ contact – α92. In Hb Chesapeke where the mutation is α92 Leu→Arg [12] the oxygen affinity is considerably raised (8 times that of Hb A) [15]. In Hb J Cape Town the mutation α92 Leu→Gln 6 does not introduce a charged residue into the $\alpha 1\beta 2$ contact as it is the case for Hb Chesapeke. Some disturbance would nevertheless be expected from the introduction of a polar glutamine in place of a hydrophobic leucine. Correspondingly the oxygen affinity of pure Hb J Cape Town is only 2–3-times higher than that of Hb A and the cooperativity as expressed by the *n* value (2.23) also lies between those of Hb A (2.75) and Hb Chesapeke (1.37) [16]. Hb J Cape Town does therefore not cause serious clinical problems in the heterozygote as does Hb Chesapeke. However it is to be expected that it would cause a notable polycythaemia in the homozygote in whom the Hb J Cape Town is not diluted by Hb A.

Although it had been suspected that the high proportion of Hb J Cape Town in heterozygotes was the outcome of an associated α-thalassaemia which depressed the Hb A level and thereby raised the relative proportion of the α^J gene product above the expected 25%, convincing evidence for such α-thalassaemia had been lacking because of an additional iron deficiency which could have been a cause of the hypochromic blood picture. In the present family at least Hb J Cape Town is associated with α-thalassaemia, and this would explain that the homozygote unlike other individuals possessing two abnormal α-chain genes produced no Hb A and only the abnormal Hb. Thus the high proportion (40–45%) of Hb J Cape Town in heterozygotes and the absence of Hb A in the homozygote cannot be used to support the suggestion that some racial groups possess one rather than two α-chain genes per chromosome.

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