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HAEMOGLOBIN Q AND THALASSAEMIA

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Summary

A description is given of the association of haemoglobin Q with β thalassaemia in a woman of Chinese extraction. The haematological and biosynthetic data are discussed in relation with the number of α chain genes per chromosome.

It is a pleasure to contribute to a new journal in Thailand and this article comes with our special regards and good wishes. One of us (H.L.) is a friend who has visited Thailand twice and who has long standing ties with many Thai workers, medical and biochemical. We have chosen as our tribute a subject which is of particular interest in Thailand and the development of which owes much to investigators from that wonderful country.

Introduction

Haemoglobin Q (HbQ), first described in a Chinese person by Vella *et al.*[1] has now been identified as $\alpha_2^{74(EF3)\ Asp-His}\beta_2$ in individuals of both Chinese and Thai origins[2–4]. In association with α thalassaemia trait, HbQ gives rise to a moderately severe thalassaemic disorder, HbQ–H disease[1,5–8]. In this communication we describe the association of HbQ with β thalassaemia in a woman of Chinese extraction. In contrast to HbQ α thalassaemia, HbQ β thalassaemia is associated with only mild thalassaemic features.

Methods

Haematological Investigations

Haematological studies were performed by standard techniques[9].

Haemoglobin Analysis

The abnormal haemoglobin was purified from haemolysates by DEAE Sephadex chromatography[10] followed by paper electrophoresis at pH 8.9[11]. It was identified as HbQ Thailand (α_2 $^{74(EF3)\ Asp-His}$ β_2) as previously described[3]. HbQ, HbA_2 and HbQ_2 were quantitated both by elution from cellulose acetate strips[12] and by DEAE Sephadex charomatography. HbF levels were measured by the method of Betke *et al.*[13].

Haemoglobin Biosynthesis

The incorporation of ^{3}H–leucine into the α and non α chains was measured in reticulocyte preparations from the peripheral blood of the propositus and her mother[14]. After incubation the washed cells were lysed and globin was prepared directly from the lysates by precipitation from acidified acetone at–20°C. Individual globin chains were separated by CM Cellulose chromatography[15] using a linear gradient 0.005–0.05M Na+ 8M urea/mercaptoethanol-phosphate buffer pH 6.7. The fractions corresponding to each chain were pooled and the radioactivity incorporated measured as previously described[16].

Results

Haematological Studies

The propositus (IIi) is 26 years old–the only child of Chinese parents. Her haematological values and those of her mother (Ii) are given in Table I. The findings in her mother were normal but in the propositus they indicated the presence of thalassaemia. These were a slightly elevated red cell count, a reduced mean cell haemoglobin and a mean cell volume. The peripheral blood smear, however, did not show obvious morphological abnormalities. In both mother and daughter the serum iron values were normal.

The haemoglobin pattern of the mother was normal, HbA being the major haemoglobin and HbA_2 levels being within the normal range. The propositus had, in addition to HbA, a slow moving haemoglobin which was characterised as HbQ Thailand. HbQ amounted to 22% of the total haemoglobin when quantitated by both cellulose acetate electrophoresis and DEAE Sephadex chromatography. The δ chain haemoglobins HbA_2+HbQ_2 amounted to 5.42, well above the range of normal HbA_2 values and HbF level was slightly elevated (Table I).

Biosynthetic Studies

Fig. 1 shows the synthesis of α^A, α^Q and β^A globin chains in reticulocytes from the propositus over a period of 60 minutes incubation of the cells with ^{3}H-leucine. On comparing α chain synthesis ($\alpha^A + \alpha^Q$) with β^A chain synthesis (Table II) the mean value of the ratio $(\alpha^A + \alpha^Q)/\beta^A$, was 1.19, just above the normal range indicating a slight deficit of α chain production. α^Q chain synthesis expressed as a per cent of total α chain systhesis, ie. ratio $\alpha^Q/(\alpha^A + \alpha^Q)$ was 23% which agrees with the proportion of HbQ found in the peripheral blood.

Table I

Haematological and haemogiobin data on the propositus (II) and her mother (I)

	Hb (g/100 ml)	PCV (%)	RBC (x10^6/µl)	MCH (pg)	MCHC (%)	MCV (fl)	Rehcs (%)	Serum Fe (µg/100 ml)	HbF (%)	HbA_2 (%)	$(HbA_2 + HbQ_2)$ (%)	HbQ (%)
I (i)	12.3	40.0	4.4	27.8	30.8	90.5	0.5	90	0.6	3.0	—	—
II (i)	11.2	39.5	5.9	19.0	28.5	67.0	1.3	67	1.3	—	5.3	22

Normal ranges for HbF $< 0.8\%$
HbA_2 $2.5\% - 3.5\%$

Table II

Globin chain synthesis studies on blood from the propositus (IIi) and her mother (Ii).

At all time intervals the total radioactivity incorpated into the globin chains was measured.

	Incubation time (min)	5	10	20	40	60	MEAN
IIi	1. $(\alpha^Q + \alpha^A)/\alpha^A$	1.13	1.22	1.18	1.17	1.23	1.19
	2. $\alpha^Q/(\alpha^Q + \alpha^A)$	0.23	0.27	0.24	0.22	0.21	0.23
Ii	1. α^A/β^A			0.81	0.79	0.87	0.82
Normals	1. α^A/β^A					Range 1.00 — 1.15	1.08
β Thalassaemia heterozygotes	1. α^A/β^A					Range 1.44 — 2.16	1.84

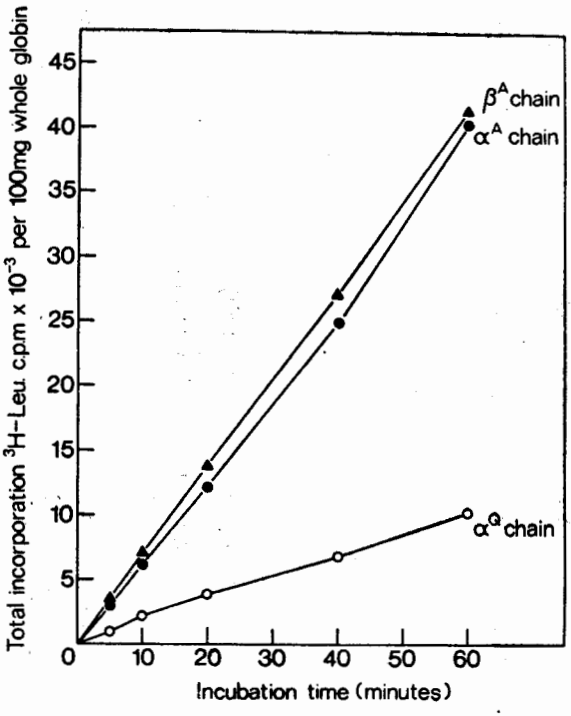

Fig. 1 Incorporation of ^{3}H-Leucine into α^A, α^Q and β^A chains in peripheral blood from the propositus over 60 min. incubation at 37°C.

The α chain/β chain synthesis ratio in the mother's reticulocytes was 0.82 over the period of incubation. This value indicates a small deficit in α chain synthesis and falls within the range of values reported for silent carriers of α thalassaemia[17].

Discussion

The haematological data and the elevated HbA_2+HbQ_2 levels suggest that the propositus is heterozygous for β thalassaemia. However, the biosynthetic data give an $(\alpha^A + \alpha^Q)/\beta^A$ chain production ratio of 1.2 which is significantly lower than the values we have recorded for other β thalassaemia heterozygotes (α/β ratios 1.5–2.0). The propositus is likely, therefore, to be a double heterozygote for α thalassaemia and β thalassaemia. Such α/β thalassaemia has been described several times before[18–20] and the propositus presents with haematological and biosynthetic values similar to those of one reported by Knox-Macauley *et al.*[20] with the exception that the morphology of our propositus' red blood cells appeared normal. However, unlike the other cases reported, the propositus has also inherited the abnormal α chain gene responsible for HbQ and the biosynthetic data show that HbQ production is only 30% that of HbA production during erythroid cell maturation. The problem then, is to establish the inheritance pattern of the abnormal genes and explain the apparent suppression of α^Q chain synthesis from the abnormal α chain locus.

The propositus' mother has a "silent" α thalassaemia—normal haematological parameters and an α/β chain production ratio of 0.82. Her father, although he was not available for investigation, presumably has HbQ β thalassaemia. This being the case, the pattern of inheritance of the abnormal genes would appear to be staightforward. How ever, the transmission of an α^Q chain gene and α thalassaemia to one person has, in recent years, been at the centre of the controversy over the number of α chain genes per chromosome and the relationship between these loci and the α thalassaemia genes. It is now generally accepted that there are two phenotypes of α thalassaemia trait[21], a severe form or α thalassaemia 1 and a mild or "silent" form, α thalassaemia 2. The simultaneous inheritance of both forms of α thalassameia produces HbH disease in the affected individual. Likewise children with HbQ–H disease have one parent with α thalassaemia 1 but the other parent has HbQ trait with no signs of α thalassaemia[5,22,23]. The exacerbation of α the thalassaemia 1 by the simultaneous inheritance of the α^Q chain gene suggests an association between the α^Q gene locus and a "silent" thalassaemia. The report of 1–2% Hb Barts' in a newborn child heterozygous for HbQ[23] provides some evidence of this. This being the case, the propositus could not have inherited α thalassaemia from her mother since the experimental data are not consistent with the presence of the classical α thalassaemia 1 which would be expected if she had acquired two α thalassaemia genes—one from her father with the α^Q chain gene and one from her mother.

There are two genetic models to explain any association between the α^Q chain gene and α thalassaemia.

1. *The single α chain locus*[21–24].

According to this model there is one α chain gene per chromosome and the two phenotypes of α thalassaemia trait are related to the degree of suppression of α chain production directed by the single locus. Thus α thalassaemia 1 results from complete suppression of α chain synthesis whereas α thalassaemia 2 is due to a reduction in α chain synthesis from the affected locus. This model predicts α chain variations should be 50% of the total haemoglobin in heterozygotes. However, of the known variants only three have been reported in proportions close to 50%: HbG Philadelphia, HbG Chinese and HbJ Tongariki. The other 62 α chain variants have been found at levels usually ranging from 20–35% (including some cases of HbG Philadelphia at 33%) with the majority clustering around 25%. According to the single locus model 95% of the known α chain variants must have defective synthesis compared to the normal α^A chain and, therefore, should be associated with mild or "silent" thalassaemia. The level of HbQ in the propositus, 13%, would be the result of reduced synthesis by the abnormal locus and directly responsible for the α thalassaemia. While this model certainly explains the experimental results, it is felt that it does not adequately explain the proportions at which α chain variants are usually found.

2. *The two α chain loci model*[25–27].

By this model, α chain variants are not present at 25% of the total haemoglobin in heterozygotes as a result of suppressed synthesis. The 25% level represents the normal production rate of a single α chain locus during erythroid cell maturation. Balanced globin

Phenotype	α chain gene camplement	Expected % Hb Variant	Possible Examples
Normal	$\alpha^V \alpha^A; \alpha^A \alpha^A$	25 %	Majoriıy of α chain Hb variants
α Thalassaemia 2	(1) $\alpha^V \alpha^T; \alpha^A \alpha^A$ (2) $\alpha^V \alpha^A; \alpha^A \alpha^T$	30 – 35 %	(1) HbQ, HbG Philadelphia, HbJ Tongariki, Hb Stanleyville II Hb Memphis (see ref. 27)
α Thalassaemia 1	(1) $\alpha^V \alpha^T; \alpha^A \alpha^T$ (2) $\alpha^V \alpha^A; \alpha^T \alpha^T$	40 – 50 %	(1) Hb G Philadelphia, Hb J Tongar iki Hb G Chinese (see ref. 27)
Hb H disease	$\alpha^V \alpha^T; \alpha^T \alpha^T$	100 %	Hb Q – H disease

Fig. 2 The possible inheritance patterns of an α chain haemoglobin variant (α^V) and α thalassaemia (α^T) based on the two α chain loci model.

synthesis can then only be explained by two closely linked α chain genes per chromosome. Given a population with this genotype the phenotypes of α thalassaemia and the proportions of abnormal haemoglobins are explained (Fig. 2). In general, therefore, according to this model, heterozygotes for α chain variants at levels greater than 25% have an α thalassaemia gene linked to the abnormal α chain locus; of course there is also always a possibility that it is present on the allelic chromosome. HbQ would be linked to an α thalassaemia gene and in straightforward heterozygotes (HbQ+HbA) it should amount to 30–35% of the total haemoglobin. This level of HbQ has, in fact, been reported in several heterozygotes[2,3] and thus supports the model. The propositus, however, with a HbQ level of 23% apparently does not fit the model. However, interaction between a β thalassaemia gene and the amount of α chain variant produced, has been reported. HbQ India ($\alpha_2{}^{64\ Asp-His}\ \beta_2$) is normally found at 20–25% in heterozygotes but falls to 10–15% when inherited together with a gene for β thalassaemia– both proportions being found within a single family[28]. Therefore, the the low proportion of HbQ in the propositus is the result of the β thalassaemia gene. Although HbQ is virtually always reported as being present at 30–35% in the HbA and HbQ heterozygotes, Pootrakul *et al.*[29] report cases with 23%. It has to be further investigated whether these were cases of HbQ/β thalassaemia. Unlike in our case, there was no obviously high HbA_2 and a final answer would require analysis by biosynthesis.

In conclusion, while the genetics of the propositus can be adequately explained by both models, we feel that the two loci model explains best the proportions of the abnormal α chain variants. Furthermore, the discovery of Hb Constrant Spring [30,31] in South East Asia and its presence in HbH disease[32] suggest the existence of two α chain loci, at least in South East Asia, and the linkage of an α thalassaemia gene to a normal α chain locus. The recent description of a homozygote for Hb Constant Spring with an associated α thalassaemia[33] furnish the final proof since the single locus model would postulate hydrops fetalis for a homozygous Hb Constant Spring infant.

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บทคัดย่อ

บทความนี้บรรยายการมี haemoglobin Q และ β thalassaemia อยู่ร่วมกันในหญิงเชื้อจีนคนหนึ่ง ได้นำข้อมูลด้านโลหิตวิทยาและการสังเคราะห์ มาพิจารณาในแง่ที่เกี่ยวกับจำนวนจีนส์ของเส้น α ต่อโครโมโซม