CLINICAL RESEARCH

 α thalassaemia in British people

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Abstract

Although α thalassaemia is rare in north Europeans, it has been identified in British people with no known foreign ancestry. Twelve such patients were studied, of whom eight shared a distinctive molecular defect, which was clearly different from defects seen in subjects of Mediterranean or South East Asian origin. A rare but specific form of α thalassaemia is therefore present in the British population. In addition, two patients from families of mixed racial origin were encountered who had a moderately severe form of thalassaemia (HbH disease) due to the inheritance of one form of α thalassaemia

from the British parent and another type from the foreign parent. This shows the importance of careful genetic counselling of British patients with haematological findings of thalassaemia.

Introduction

The α thalassaemias are common genetic disorders of synthesis of the α globin chains, which are common to both fetal and adult haemoglobins (HbF ($\alpha_2\gamma_2$) and HbA ($\alpha_2\beta_2$), respectively). Reduced synthesis of α globin chains in fetal or adult life gives rise to an excess of γ or β chains, which may form Hb Barts (γ_4) and HbH (β_4) tetramers.¹

The structural genes for the α globin chains are located on chromosome 16, where they are part of a multigene family consisting of two α genes separated from an embryonic α -like gene (ζ) by two non-functional pseudogenes ($\psi\alpha$ and $\psi\zeta$) in the order 5' ζ - $\psi\zeta$ - $\psi\alpha$ - $\alpha 2$ - $\alpha 1$ 3'.^{2,3} thus the normal α globin genotype may be written $\alpha\alpha/\alpha\alpha$.¹ Molecular analysis has shown that normal people have two functional α genes on each chromosome ($\alpha\alpha$) but that most α thalassaemia defects are due to deletions of one ($-\alpha$) or both ($--$) of these genes.⁴ Heterozygotes for these defects ($-\alpha/\alpha\alpha$ and $--/\alpha\alpha$), although clinically unaffected, have a reduced mean cell volume and mean cell haemoglobin and may produce small amounts of Hb Barts in fetal life. Compound heterozygotes for α thalassaemia ($--/-\alpha$) have HbH disease, which is a moderately severe form of haemolytic anaemia with readily detectable amounts of HbH in the peripheral blood. Homozygotes ($--/--$) have a lethal condition known as the Hb Barts hydrops syndrome.¹

The prevalence of α thalassaemia in different populations varies greatly,⁴ the prevalence of carriers ranging from less than 1%⁵ to over 90%.^{6,7} Furthermore, though deletions of one α gene ($-\alpha$) are commonly found in most populations within the "malaria belt," deletions of both α genes ($--$) usually occur only in people from South East Asia and the Mediterranean.^{1,4}

As judged by surveys of red cell variables^{8,9} and cord blood concentrations of Hb Barts (H Lehmann, personal communication) α thalassaemia is probably rare in north Europeans; only two British families with the common type of HbH disease ($--/-\alpha$) have been described.^{10,11} Four other cases of HbH

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disease in north Europeans have been seen in patients with mental retardation, although the genetic and molecular bases of α thalassaemia in these cases were quite different from those of inherited forms of α thalassaemia.¹² Hitherto, it has been assumed that the occasional examples of thalassaemia in north European subjects resulted from racial admixture. We studied 12 British subjects with α thalassaemia in whom there was no evidence of such admixture in the recent past. Characterisation of the molecular defect in eight of them showed that it resulted from a deletion of both α genes on one chromosome (--) but was quite different from that found in either the South East Asian or the Mediterranean forms of α thalassaemia.

Patients and methods

Twelve British patients were referred independently. They were identified by a persistently reduced mean cell volume and mean cell haemoglobin concentration, although they did not have iron deficiency or raised concentrations of HbA₂ ($\alpha_2\delta_2$) in the peripheral blood (which is indicative of β thalassaemia). In six cases (cases 2, 4, 7, 9, 10, and 12; see table) the diagnosis of α thalassaemia was confirmed by identification of typical HbH inclusions in red cells that had been incubated

whereas chromosomes from which both α genes were deleted (--) produced no α specific fragments.¹⁷ Consequently, using a combination of the phenotype and the pattern of Bam HI α specific fragments, we could assign provisional genotypes to the patients studied (table). DNA samples from 11 patients were analysed: in three cases we could not positively determine the genotype, which may have involved a different deletion of the α gene complex or a non-deletion defect⁴; in five of the carriers of α thalassaemia (cases 1, 2, 3, 4, and 12) the genotype was --/ $\alpha\alpha$; and in the three cases of HbH disease (cases 5, 6, and 11) the genotype was --/- α . Studies of the families of two of these patients with HbH disease showed that in each case the chromosome with no α genes (--) came from the British parent, whereas the more common - α defect was inherited from a parent of known foreign ancestry (fig 1).

DETAILED ANALYSIS OF THE BRITISH FORM OF α THALASSAEMIA (-- BRIT)

Figure 2 shows a comprehensive restriction enzyme map of the human α globin gene complex. In the five heterozygotes for α thalassaemia described here (--/ $\alpha\alpha$) new fragments from the abnormal chromosome (--) were identified by a ζ specific probe, using the enzymes Bgl II, Hind III, and EcoRI in addition to the usual restriction fragments produced from the normal chromosome. As neither these nor any other abnormal fragments were identified by the α specific probe or the

Results of standard haematological investigations

Case No	Haemoglobin (g/dl)	Mean cell volume (fl)	Mean cell haemoglobin (pg)	α : β globin chain synthesis ratio	HbH	Bam HI α specific fragments (kb) (putative haplotypes)			Referral centre
						($\alpha\alpha$)	(- α)	(--)	
1*	12.7	71	21.7	Not done	Not done	14.0		+	Mater Misericordiae Hospital, Dublin
2	11.6	66	20.6	Not done	Occasional cell	14.0		+	Royal Lancaster Infirmary, Lancaster
3	15.3	73	25.0	0.61	Not done	14.0		+	Princess Alexandra Hospital, Swindon
4	13.8	68	21.5	Not done	1:3000 cells	14.0		+	Royal Albert Edward Infirmary, Wigan
5	9.0	54	16.7	0.64	12%		10.5	+	Royal Victoria Infirmary, Newcastle
6	8.9	59	20.4	Not done	Present on electrophoresis		10.5	+	Manchester Royal Infirmary, Manchester
7	13.2	64	21.8	Not done	Occasional cell	14.0		++	Princess Margaret Hospital, Swindon
8	8.4	62	19.5	Not done	Not done	14.0		+++	Queen Charlotte's Hospital, London
9	15.0	76	26.0	Not done	1:3000 cells	14.0		+++	Royal Albert Edward Hospital, Wigan
10	11.7	63	20.6	0.85	Occasional cell	DNA not studied			Manchester Royal Infirmary, Manchester
11	9.9	67	18.8	0.46	17.5%			§	University Hospital of Wales, Cardiff
12	11.5	64	20.6	Not done	Occasional cell			§	Royal Lancaster Infirmary, Lancaster

*Patient originally thought to be of Irish extraction, but family subsequently found to have originated from England.

†No α specific band detected.

‡Precise molecular defect of α thalassaemia chromosome not determined; could be deletion or non-deletion defect of α cluster.

§Molecular defect determined using restriction enzymes other than Bam HI.

with a redox dye (1% brilliant cresyl blue or 1% methylene blue in isotonic saline).¹³ In three other cases (cases 5, 6, and 11) HbH was readily shown by haemoglobin electrophoresis, proving that these subjects had HbH disease.

Deoxyribonucleic acid (DNA) was prepared from the white cells of the peripheral blood of each patient by phenol chloroform extraction as described previously.¹⁴ The DNA was digested with various restriction enzymes (Bam HI, Bgl II, EcoRI, Hind III, Pst I, Pvu II, Rsa I, or Sac I), fractionated on 0.8% agarose gel, and transferred to nitrocellulose filters. DNA fragments on the filter were hybridised to probes labelled with phosphorus-32, which represented various regions of the cluster of α globin genes, including a 1.5 kb Pst I α specific fragment,³ a 1.16 kb Bgl II/Pvu II ζ specific fragment,¹⁵ and a 0.6 kb Alu I fragment which corresponded to the interzeta hypervariable region (see fig 2). Subsequently when the nitrocellulose filters had been washed under appropriately stringent conditions specific DNA fragments were identified by autoradiography.¹⁴

Results

The table shows the results of standard haematological investigations and α globin gene analysis.

GENOTYPE ANALYSIS

Chromosomes with one (- α) or two ($\alpha\alpha$) α genes were represented by Bam HI α specific fragments of 10.5 kb or 14.0 kb, respectively,

FIG 1—Family pedigrees of two families in which proband had HbH disease (cases 5 and 6). Mild and severe changes in haematological variables do not indicate clinical severity.

interzeta hypervariable region probe, however, it is likely that the abnormal chromosome resulted from a deletion that included the α genes, the $\psi\alpha$ gene, the $\psi\zeta$ gene, and the interzeta hypervariable region but spared the ζ gene (fig 2). In support of this we noted that there was no variation between the two chromosomes of each patient

at known polymorphic markers within the segment comprising the putative deletion (fig 2), which suggested either that the subjects were homozygous at each of these loci, which is unlikely,¹⁸ or that there was a deletion of this region of the α gene complex on one chromosome.

As the abnormal chromosome could be identified with a ζ specific probe we used this to locate the break point of the deletion more precisely. Although the Pvu II and Bam HI sites, which lie at the 3' end of the ζ gene, appeared to be intact, the EcoRI site between the ζ and $\psi\zeta$ gene was not present, as shown by the finding of an entirely

new EcoRI fragment from this chromosome (figs 2 and 3). Thus the break point of the deletion in the abnormal chromosome lay between the Pvu II site and EcoRI site (fig 2). Comparison of the restriction maps of the abnormal chromosomes (--) from the five α thalassaemia heterozygotes (--) and the three subjects with HbH disease (--) showed that all eight chromosomes (--) were identical. Figure 3 shows a direct comparison of DNA from six subjects. The restriction map of this defect was compared with previously established maps of the Mediterranean and South East Asian deletions (fig 4).¹⁹ Clearly,

FIG 2—Detailed restriction map of α globin gene cluster in normal subject and British α thalassaemia. $\sim\sim\sim$ indicates hypervariable regions of DNA.^{16,18} Symbols representing restriction enzymes are: B, Bam HI; Bg, Bgl II; E, EcoRI; H, Hind, III; and P, Pvu II. $\blacksquare$ indicates various probes described in Patients and methods. Polymorphic enzyme sites Pst I, Rsa I, and Sac I (not shown) were monomorphic in subjects with the British defect. Abnormal Bgl II, EcoRI, and Hind III fragments are shown below. --- indicates region of break point in British defect.

FIG 3—Autoradiograph of EcoRI digests from five patients (cases 1, 2, 3, 4, and 6), one normal subject (N), and one relative of patient in case 1 (R). ζ specific probe was used to hybridise to filter, identifying both $\psi\zeta$ (23 kb) and ζ (4.3 kb) specific fragments in addition to abnormal (6.7 kb) fragment. Smaller $\psi\zeta$ specific band in case 6 (~19 kb) was due to 3.7 kb deletion that removed one α gene from -- α chromosome.

this deletion was quite different and did not correspond to any of the previously described deletions of the α gene complex.²⁰ We therefore called this new mutant British (--) in accordance with previous nomenclature.⁴

FIG 4—Comparison of molecular defects in common severe types of α thalassaemia. --- indicates break points of deletions.

Discussion

The remarkable geographical variation in the prevalence of genes for both α and β thalassaemia is now well documented. In particular, both types of thalassaemia seem to be rare in north European populations.¹ The form of α thalassaemia in which one α gene is deleted (--) is common in many different regions, including the Mediterranean, Africa, the Middle East, India, South East Asia, and Melanesia, possibly due to a selective advantage of this allele in the presence of endemic falciparum malaria. This mutation (--) probably arises by a simple homologous recombination event, and a high prevalence of mutation,

leading to multiple independent origins of this defect, could also partly account for its wide geographical distribution. On the other hand, the more severe α thalassaemia defect (--) is found only in South East Asia and the Mediterranean.⁴ Although deletions of both α genes (--) may offer a similar selective advantage to the $-\alpha$ defect, they probably arise from a less common type of illegitimate recombination.²¹

The British defect is of this less common type and, like the Mediterranean and South East Asian defects, is of limited geographical distribution. By contrast, however, the overall prevalence of carriers for all types of α thalassaemia in the British population is almost certainly much less than 1%, compared with 20% in South East Asia and up to 15% in the Mediterranean. This is consistent with the lack of an obvious selective advantage for α thalassaemia in the British population. The most likely interpretation is therefore that the Mediterranean, South East Asian, and British defects each arose just once during human evolution, and though the Mediterranean and South East Asian defects were selected in their respective populations, the British was not. If this interpretation is correct we must conclude that all of the patients with the British defect are at least distantly related.

Accurate estimates of the prevalence of α thalassaemia in the British population are not available. We found that among the patients referred to us with a putative diagnosis of α thalassaemia, when foreign ancestry was known the molecular defect was usually consistent with the racial background of the subject. The patients described here did not know of any foreign ancestry and all had a previously unrecognised and new α thalassaemia defect, which appears to be limited to people of British origin. This therefore raises two practical points for genetic counselling: firstly, α thalassaemia may occur, albeit rarely, in patients of British origin with no known foreign ancestry; and, secondly, in British families in which there is racial admixture it is possible, as we have observed here, to encounter a clinically severe form of α thalassaemia (HbH disease). In families of British and Mediterranean or British and South East Asian mixture the

Hb Barts hydrops syndrome could occur despite the British parentage.

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100 YEARS AGO

The following quotation from a letter, recently received by a correspondent, from a gentleman who sailed early last December to avoid an English winter, conveys a needed warning. "The weather was very pleasant for some days after we left Teneriffe; but, as we had a following wind, the north-east trade, it got gradually warmer until the thermometer touched 83° in the cabins at 10 degrees north, which at that season means 33 degrees north of the sun. Then began a process which astonished us very much. So far, we had not been any great distance from land; but, as soon as we passed into the Gulf of Guinea, and while still north of the equator, the temperature began to fall, 82, 80, 79, 77, 75, 71, 71, 69, 69, 68. This, with a strong south-east trade-wind in our teeth, made the ship very cool, if not cold; and, when under the vertical sun, greatcoats and rugs were required on deck after dinner. As we approached land again, towards the Cape, the thermometer rose a few degrees, touching 74°; but, as soon as the land was left behind, it went to 71, 63, 57, 55, 52, 50, 45. These are cabin readings, and, on deck, 37° and 36° were the midday temperatures. The regular steamer-route goes to 51 degrees south, and travels on that parallel for 65 degrees; and one soon begins to wonder whether doctors know what they are about when they recommend a voyage round the Cape. The weather, we are told, is quite normal, and a shower of sleet is just what may be looked for at midsummer here. The ships' officers consider it a very good voyage, and the captain, when he turns out of his cabin, after a week of congestion of the lungs, says the weather is beautiful. So it is, according to the log; for there is generally plenty of fair wind, and the runs are long, but it is not invalids' weather. I wear both summer and winter flannels, a chamois waistcoat, and, with a greatcoat turned up at the neck, and lined gloves on, I manage to walk for an hour once, or it may be twice, a day; but one day this week I did not get out at all, and I had to pay for one of my walks with a mustard-blister. I am not prepared to say that the cold weather has been bad for me; for I had a troublesome cough when the weather was warmer, which I have not got now; but it is risky, and, for a

weaker man, would be dangerous. One poor fellow, sent off by eminent London doctors, will, I fear, never see New Zealand. He came almost from his bed to the ship, and, being over middle age, he had little rallying power, and a very poor idea of what to eat and drink. He was left to order just what he wanted, and, having no appetite, I do not think he has had enough of food. The ship has been healthy, on the whole; a sailor who came on board with a cold died, and there have been some cases of bronchitis and sore-throat; but, amongst five hundred, the patients have not been numerous." (*British Medical Journal* 1885;i:953.)

There will be no "-isms" in the future of scientific enlightenment—no teetotalism, no vegetarianism, no antitobaccoism—in short, nothing of specialism in the ordering of a moderate life on natural principles. Obviously, man was made to eat a mixed diet, including both animal and vegetable food. Food is more a matter of climate than anything else. Climate determines development, both as regards demand and supply. Ruskin is right when he makes even the moral character of man depend directly upon his surroundings. We have no sort of sympathy with the "fads" of the day. Our standpoint is the simply and severely rational. Nothing would be gained by rehearsing the stock and familiar arguments of the vegetarians. They one and all contain much of truth, but they one and all err in mistaking the particular for the general. There are, probably, some persons who do nourish their organisms as well with vegetable materials as with animal food; but this we take to be a peculiarity in the individual apparatus and function, not an excellence of quality to glory in and be proud of. It would be better if we could learn to look on the eccentricities of appetite as abnormal, instead of emulating the fox who, having lost his own tail, went about trying to persuade other creatures of his species to get rid of theirs also. (*British Medical Journal* 1885;i:188.)