Non-deletion haemoglobin H disease in Papua New Guinea

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SUMMARY Analysis of DNA from members of a Melanesian family from Papua New Guinea with haemoglobin (Hb) H disease revealed that all four α globin genes are intact in affected subjects. Study of restriction enzyme site and length polymorphisms and the use of oligonucleotide probes indicated that the molecular basis of this Papuan form of non-deletion Hb H disease differs from the previously described Middle Eastern and Mediterranean types.

Alpha thalassaemia is a genetic disorder which is common in most tropical and subtropical populations.^{1,2} It results from diminished (α^+ thalassaemia) or absent (α^0 thalassaemia) α globin chain production from the duplicated α globin genes ($\alpha 2$ and $\alpha 1$) on chromosome 16 (fig 1). Whereas heterozygotes for α^+ and α^0 thalassaemia show only minor haematological abnormalities and are clinically asymptomatic, compound heterozygotes for α^+ and α^0 thalassaemia have haemoglobin (Hb) H disease, a moderately severe haemolytic anaemia. Homozygotes for α^0 thalassaemia, in which α chain synthesis is completely abolished, have the Hb Bart's hydrops syndrome and are usually stillborn or die a few hours after birth. Most α^+ thalassaemia homozygotes, on the other hand, have only minor haematological abnormalities, a reduced MCV and MCH, and are asymptomatic.

Unlike α^+ thalassaemia, which is found at high frequencies throughout the malaria belt,¹ Hb H disease is rare except in South-east Asia,³ the Mediterranean, and certain Middle Eastern popula-

tions. Only three cases of Hb H disease have been reported in Pacific Islanders, all from Papua New Guinea.⁴⁻⁶ The molecular basis of Hb H disease has been extensively investigated in Euroasian populations (reviewed in reference 2). In most cases it results from the presence of a single α gene deletion ($-\alpha/$) on one chromosome 16 and also a larger deletion which removes both the duplicated α globin genes ($--$) on the other chromosome 16. The larger deletion which is the α^0 thalassaemia determinant is remarkably population specific. In South-east Asians a 20 kilobase (kb) deletion is commonly found⁷ and in Mediterraneans either a 17.5 kb, a 20.5 kb, or a 5.2 kb deletion is found. Less commonly, much larger deletions removing the whole α globin gene complex or non-deletion defects, point mutations, have been shown to cause Hb H disease.

As part of a larger study of the distribution and molecular basis of α thalassaemia in Melanesian populations,⁸ we have studied the molecular defect in one⁵ of the three previously identified families with Hb H disease in Papua New Guinea. DNA analysis of other globin gene variants in Melanesians has revealed new genetic markers useful in retracing

FIG 1 Structure of the α globin gene complex showing polymorphic restriction enzyme sites and regions of length variation.¹³ Zigzag lines indicate regions of length variation. X=XbaI, S=SacI, B=BglII, A=AccI, R=RsaI, P=PstI. H indicates the interzeta hypervariable region. Z indicates the region of length variation in intron 1 of the $\psi \zeta 1$ gene. The region of length variation at the 3' end of the complex, 3'HVR, is also indicated.

prehistoric population movements in this region.⁹ In view of the known high frequencies of single α gene deletions in coastal Papua New Guinea¹⁰ and the proximity of these populations to Island South-east Asia, it was therefore of interest to determine whether such cases of Hb H disease were caused by the presence, at low frequency, of the characteristic South-east Asian α^0 thalassaemia deletion, since other South-east Asian genetic markers are strikingly absent in New Guinea.¹¹ DNA analysis indicated that all four α globin genes are intact in this Melanesian form of Hb H disease and that the non-deletion defect is different from those described previously.

Methods

With the assistance of P and K Booth it was possible to trace the Papuan family from Kairuku with Hb H disease studied in 1966.⁵ The names of subjects are given here to allow comparison with the original report.⁵ The proband, Kahiri, is still alive and in her early forties. Of the four other subjects with Hb H disease described in the 1966 report, the two brothers of Kahiri, Apa and Aihi, are well but their half brother and aunt have died. Kahiri and her two brothers with Hb H disease have had a total of 19 children of which two died in infancy but the others remain well. With informed consent it was possible to collect blood samples from 12 members of the extended family. Samples were stored at 4°C for a maximum of seven days and then blood counts were performed on a Coulter counter model S at the Royal Prince Alfred Hospital in Sydney. DNA extraction and Southern blot analysis were performed as previously described.¹² The genomic probes¹³ used were (1) a 1.5 kb *Pst*I fragment containing the entire $\alpha 1$ globin gene sequence; (2) a 2.85 kb *Eco*RI/*Bam*HI fragment containing the entire $\zeta 2$ gene; (3) an *Alu*I fragment spanning the interzeta hypervariable region (HVR); (4) a 4 kb *Hinf*I fragment which includes the hypervariable region 3' to the α gene complex (the 3'HVR probe); (5) a 0.6 kb *Bam*HI/*Eco*RI fragment 9 kb 5' to the $\zeta 2$ gene; and (6) a 0.8 kb *Bam*HI fragment which contains single copy sequences 3 kb 3' to the $\alpha 1$ globin gene. Restriction enzyme site and length polymorphisms in the α gene complex were determined using these probes as previously described.¹³ Oligonucleotide analysis using two 21 base oligomers (5'-TTTGAATAAAGTCTGAGTGGG-3' and 5'-CCCACCTCAGACCTTATTCAA-3'), which differ only at the single base change characteristic of the polyadenylation signal mutation responsible for the Saudi Arabian form of Hb H disease, was as described previously.¹⁴

Results

HAEMATOLOGICAL DATA

Haematological data on the 12 members of the family studied are shown in table 1. It is clear from the results of haemoglobin electrophoresis and inspection of their red cell indices that none of the children studied of either the proband or her two affected brothers has Hb H disease.

α GLOBIN GENOTYPES

Southern blot analysis of DNA from all subjects was performed using the restriction enzymes *Bam*HI and *Bgl*III and the α specific and ζ specific probes.⁹ All except two subjects showed normal band patterns (table 2). The two exceptions, I.5, the wife of one of the brothers of the proband, and her son, II.7, were both heterozygous for the 'leftward' single α gene deletion ($-\alpha^{4.5}/\alpha\alpha$).¹⁵ Hence, remarkably, all three sibs with Hb H disease appeared to have four intact α globin genes ($\alpha\alpha/\alpha\alpha$). This contrasts with Hb H disease in most other populations where, usually, only one α globin gene is found ($-\alpha/-$).

However, it remained possible that the subjects with Hb H disease in this family might be heterozygous for the normal $\alpha\alpha$ arrangement with a non-deletion determinant and a total deletion of the α globin complex ($\alpha\alpha/-$). Such total deletions produce no abnormal bands on Southern blot analysis and have been identified on rare occasions in other

TABLE 1 Haematological data on the Papuan Hb H disease family.

Subject	Age	Sex	Hb	MCV	MCH	% Hb H
I.1 Kahiri	41	F	ND	ND	ND	17.9
I.2 Apa	37	M	11.4	67.1	20.2	13.6
I.3 Gahusi	Adult	F	11.9	85.9	28.6	0
I.4 Aihi	35	M	10.9	60.0	18.2	ND
I.5 Kila	Adult	F	10.5	70.0	22.7	0
II.1 Naime Jim	20	F	11.5	75.6	26.9	0
II.2 Aiwa	16	F	12.3	73.9	25.0	0
II.3 Eru	13	F	12.8	74.1	24.1	ND
II.4 Avia	14	F	12.9	69.6	23.0	0
II.5 Ginate	12	M	12.6	72.8	24.0	ND
II.6 Lilee	11	F	13.0	70.4	25.7	0
II.7 Peter	7	M	11.4	60.2	19.7	0

Data from two generations of the Hb H disease family previously studied in 1966 by Booth.⁵ The proband (I.1) and her two brothers (I.2 and I.4) were shown in that report to have Hb H disease. II.1, II.2, and II.3 are three of the children of the proband and her husband (not studied). Similarly II.4, II.5, and II.6 are the children of I.2 and I.3; II.7 is the son of I.4 and I.5. Hb A₂ levels were normal in all those studied, excluding the presence of β thalassaemia.

ND= not determined.

MCV= mean cellular volume in femtolitres.

MCH=mean cellular haemoglobin in picograms.

TABLE 2 Molecular data on the Papuan family.

Subject	α genotype	IZ HVR	3'HVR
I.1	$\alpha\alpha/\alpha\alpha$	MM	<u>2.3</u> , <u>2.1</u>
I.2	$\alpha\alpha/\alpha\alpha$	MM	<u>2.3</u> , <u>2.1</u>
I.3	$\alpha\alpha/\alpha\alpha$	MS	6.5, <u>2.3</u>
I.4	$\alpha\alpha/\alpha\alpha$	MM	<u>2.3</u> , <u>2.1</u>
I.5	$-\alpha^{+2}/\alpha\alpha$	MM	2.4, <u>2.0</u>
II.1	$\alpha\alpha/\alpha\alpha$	MM	<u>2.3</u> , 3.8
II.2	$\alpha\alpha/\alpha\alpha$	MM	3.8, <u>2.1</u>
II.3	$\alpha\alpha/\alpha\alpha$	MS	3.1, <u>2.1</u>
II.4	$\alpha\alpha/\alpha\alpha$	MM	<u>2.3</u> , <u>2.1</u>
II.5	$\alpha\alpha/\alpha\alpha$	MM	<u>2.3</u> , <u>2.3</u>
II.6	$\alpha\alpha/\alpha\alpha$	MM	<u>2.3</u> , <u>2.1</u>
II.7	$-\alpha^{+2}/\alpha\alpha$	MM	2.4, <u>2.3</u>

Results of DNA analysis showing α globin genotype, the size of the interzeta hypervariable region alleles, and the size of the 3'HVR alleles after *PvuII* digestion (fig 2). M and S refer to medium and small length alleles of the interzeta HVR.¹³ *PvuII* length alleles are shown in kilobases; alleles which must be present on α thalassaemia chromosomes are underlined.

populations.^{7 16} In an attempt to show that both α gene complexes were present in the proband we first analysed known restriction enzyme site polymorphisms in the α globin gene complex. Heterozygosity for the presence of any of these would have confirmed the presence of both complexes. However, the proband failed to show heterozygosity at any of the seven previously reported sites.¹³ Similarly, analysis of the length polymorphism between the ζ globin genes and of the length polymorphism in the first intron of the $\psi\zeta 1$ gene (fig 1)¹³ failed to resolve the question. The restriction enzyme haplotype determined thus was IIIa in the nomenclature of Higgs *et al.*¹³ This is the commonest α globin haplotype in Melanesia¹³ and it was still unclear whether the proband was homozygous or effectively hemizygous for this haplotype. However, analysis of the region of length variation 3' to the α genes, the 3'HVR, showed that the proband was in fact heterozygous for this length polymorphism (fig 2), displaying band sizes of 2.3 and 2.1 kb after digestion with the restriction enzyme *PvuII* (table 2). Analysis of the 3'HVR of the proband with several other restriction enzymes also showed two bands differing in length by 0.2 kb. Hence, both α globin gene complexes are intact and her genotype is $\alpha\alpha/\alpha\alpha$.

MOLECULAR ANALYSIS

Two types of non-deletion forms of Hb H disease have been described previously.^{17 18} The Arabian type is not detectable by Southern blot analysis.^{14 17} However, the Sardinian type has been shown to be produced by homozygosity for an initiation codon

FIG 2 Southern blot analysis of DNA from 12 members of the Papuan family using the 3'HVR probe after *PvuII* digestion. The samples are in the following order: lane 1, I.1; lane 2, II.2; lane 3, II.1; lane 4, I.3; lane 5, II.4; lane 6, II.5; lane 7, II.6; lane 8, II.3; lane 9, I.5; lane 10, II.7; lane 11, I.4; lane 12, I.2. Hence, the samples in lanes 1, 11, and 12 are from the subjects with Hb H disease. Band sizes are indicated in kilobases.

mutation of the $\alpha 2$ globin gene (ATG→ACG) which abolishes a site for the restriction enzyme *NcoI*. DNA from the proband in this Papuan family was digested with *NcoI* and hybridised with the α globin probe. Only the normal 4.8, 3.7, and 2.1 kb bands were observed, indicating the absence of the Sardinian mutation.

The relatively common type of non-deletion Hb H disease first reported in Saudi Arabians,¹⁷ and now known to be more widely distributed in the Middle East and parts of the Mediterranean,¹⁴ is due to homozygosity for a point mutation in the polyadenylation signal sequence of the $\alpha 2$ gene (AATAAA→AATAAG); the sequence of the $\alpha 1$ gene is normal on these chromosomes.¹⁴ Because there is no restriction enzyme which recognises this sequence change, two oligonucleotides were used, one ($\alpha 2$ normal) is completely homologous to the 3' end of the normal $\alpha 2$ gene sequence, the other ($\alpha 2$ mutant) to the corresponding sequence on the chromosome with the polyadenylation signal mutation. After digestion with *PvuII* the $\alpha 2$ normal oligonucleotide but not the $\alpha 2$ mutant oligonucleotide hybridised to the 2.0 kb $\alpha 2$ specific fragment.

Hence, a polyadenylation signal mutation is not responsible for this Papuan form of Hb H disease.

Discussion

This study of a Melanesian family has revealed that, unusually, none of the α globin genes is deleted in subjects with Hb H disease. Further analysis showed that the molecular defect responsible for Hb H disease in this Papuan family is different from those found in the Middle East and Sardinia. A common problem in this sort of molecular analysis is to distinguish between the presence of two intact but dysfunctional gene complexes (denoted $\alpha\alpha/\alpha\alpha$ or sometimes $\alpha\alpha^T/\alpha\alpha^T$) and heterozygosity for a total deletion of one gene complex ($-/-\alpha\alpha$ or $-/-\alpha\alpha^T$). Judgements based solely on band intensities are often unreliable. Here, although analysis of numerous restriction enzyme site polymorphisms failed to distinguish between these interpretations, it was possible to show the presence of both complexes by analysis of the very informative length polymorphism 3' to the α genes.

The heterozygosity at this 3'HVR of these Papuans with Hb H disease raises the question of whether the non-deletion defect on each of their chromosomes is the same. This has been shown to be so in both the Saudi Arabian and Sardinian cases of non-deletion Hb H disease. However, such persons from Saudi Arabia have been shown to be homozygous for a particular 3'HVR as well as a particular restriction enzyme haplotype (haplotype Ia).¹⁴ This is as might be expected assuming a single, relatively recent origin for a particular point mutation. In this Papuan form of Hb H disease it is possible that the mutations on the two chromosomes are identical and that a length mutation of the 3'HVR occurred after the mutation affecting α globin production. Alternatively, the red cell indices of the children who have inherited one or other non-deletion allele in this family (tables 1 and 2) might be seen to support the possibility that the proband is a compound heterozygote, in that it appears that the chromosome with the 2.1 kb 3'HVR allele may be associated with a more severe form of α thalassaemia than the chromosome with the 2.3 kb allele. Clearly, the latter chromosome does not carry an α^0 thalassaemia determinant because when found in association with a $\alpha^{4.2}$ deletion (subject II.7) it does not produce the phenotype of Hb H disease. However, further data are required and it should be possible to resolve this question by sequence analysis of the α genes from both chromosomes of the proband.

A previous survey of α globin genotypes in Island Melanesia found numerous persons with normal α

globin gene maps on restriction enzyme analysis, but detectable amounts of Hb Bart's in umbilical cord blood.¹⁹ The presence of Hb Bart's indicates that α globin chain production is deficient. It was thus possible to estimate that the gene frequency of non-deletion α thalassaemia determinants in that population is about 0.06, and marked differences in the percentage of Hb Bart's between persons suggested the presence of different types of mutation. This study provides further evidence for the presence of a diversity of non-deletion α thalassaemia determinants in Melanesia. High frequencies of a variety of different single gene deletion forms of α thalassaemia have been extensively documented in these islands,^{8-10 19 20} and the remarkable distribution of these various alleles appears to be related to both the presence of endemic malaria²⁰ and the historical isolation of Melanesian populations.

It is clear that Melanesian Hb H disease, at least in this Papuan family, does not result from admixture of the common South-east Asian α^0 thalassaemia determinant into Melanesia. This provides another example of the marked change in the spectrum of haemoglobinopathies that occurs between South-east Asia and Melanesia. In the former, Hb E, Hb Constant Spring, and the South-east Asian α^0 thalassaemia deletion are all common,³ whereas in Melanesians and Polynesians they have never been found.²¹ Similarly, although single α gene deletions found at very high frequency in parts of Oceania^{20 22 23} are also present at lower frequency in South-east Asians,³ molecular analysis has suggested independent origins for the deletions in the two regions.²⁰

We are grateful to P and K Booth for assistance in finding this family, to S Naraqui, P R Sill, and J M White for assistance in Port Moresby, to R J Trent for the Coulter counter analyses, to J Sharpe for haemoglobin analysis, and to D R Higgs for providing cloned probes. This work was supported in part by a grant from the Rockefeller Foundation. AVSH is an MRC Training Fellow and SLT is supported by the Wellcome Trust.

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