High frequency of β thalassaemia in a small island population in Melanesia

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SUMMARY A study of the causes of anaemia in the south west Pacific archipelago of Vanuatu has identified one island, Maewo, where the carrier rate for β thalassaemia exceeds 20%, one of the highest recorded incidences in the world. Homozygous β thalassaemia is a major cause of infant mortality and a serious drain on health resources on this island. Interactions of β thalassaemia with various forms of α^+ thalassaemia were common in this population. Coexistent α^+ thalassaemia leads to better haemoglobinised and larger red cells than are seen in simple β thalassaemia heterozygotes and screening for the latter can only be reliably carried out by Hb A₂ estimation.

In a recent study of the causes of hypochromic anaemia in the south west Pacific archipelago of Vanuatu we identified a variety of haemoglobinopathies on many of the islands.¹ Most common were deletion forms of α^+ thalassaemia, which occur throughout the country, but reach their highest gene frequency of 40% on the northern island of Espiritu Santo. Apart from being misdiagnosed as iron deficiency they have had few direct clinical consequences. In contrast, β thalassaemia was found to an appreciable extent only on two islands, Malekula, and, in particular, Maewo, where it has reached a carrier rate of over 20%, one of the highest recorded incidences in the world. This probably results from a combination of malarial selection and genetic drift in a small isolated community, and homozygous β thalassaemia is now a serious health problem and a major cause of infant mortality on this island.

Although there is a considerable amount of circumstantial evidence that high frequencies of β thalassaemia result from malarial selection,² conclusive microepidemiological and in vitro studies such as those that established the interaction between the sickle cell gene and malaria^{3,4} are lacking. Furthermore, in many countries vector control programmes, prophylactic treatment, and the availability of sophisticated medical services have ended by now any possible interaction between β thalassaemia and the malarial parasite. These considerations unfortunately do not yet apply in many of the remote

malarious islands of the south west Pacific. The extremely high frequency of β thalassaemia in the isolated community of Maewo thus affords a rare opportunity to study the natural history of β thalassaemia in its 'natural' environment. The clinical and haematological characterisation of the variant are presented in this paper.

Materials and methods

A total of 2289 subjects from the 17 larger islands of Vanuatu, formerly the New Hebrides (fig 1), was investigated for the presence of β thalassaemia. Blood was collected by venepuncture from volunteers of all ages and both sexes as previously described.¹ Detailed clinical histories were collected and physical examinations carried out whenever possible. Populations were studied without any deliberate selection procedures and on the largest islands collections from different geographical areas were made to prevent unrepresentative sampling. When the high incidence of β thalassaemia in the central coastal region of Maewo became known, further blood collections were carried out in the northern and southern regions of the island to determine its overall distribution and frequency. Blood samples were routinely collected into heparin, EDTA, and plain tubes and blood films were made at the time of the initial collection. Samples were immediately placed on melting ice for transportation to the laboratory, where buffy coat samples were prepared from heparinised blood and deep frozen

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FIG 1 Map of Vanuatu, south west Pacific. Left: south west Pacific. V=Vanuatu, A=Australia, PNG=Papua New Guinea, SI=Solomon Islands, NC=New Caledonia, F=Fiji. Right: Vanuatu. ES=Espiritu Santo, M=Maewo, P=Pentecost, Pa=Paama, Mk=Malekula, Em=Emae, MM=Makura-Matato, E=Efate, T=Tanna, F=Futuna, A=Aneityum.

for later DNA extraction.⁵ A Coulter counter model S or S plus was used to determine the haemoglobin level and red cell indices. Haemoglobin electrophoresis and quantification of haemoglobin A₂ was carried out by standard methods.⁶ Hb F levels were determined by the modified Betke method⁷ and serum ferritin levels were measured by a standard radioimmunoassay method.⁷ Two untransfused infants with homozygous β thalassaemia were studied by standard methods (biosynthesis studies⁸ and isoelectric focusing⁹) to determine whether or not β globin chain synthesis and the formation of Hb A was occurring. Alpha thalassaemia is commonly found in this population so Southern blot hybridisation studies for the presence of deletion forms of the disorder were routinely carried out on all samples as previously described.¹

Results

The island by island distribution and frequency of β

thalassaemia heterozygotes in Vanuatu are given in table 1. While sporadic cases are seen on many islands, the disease reaches very high frequencies only on Malekula and Maewo. A detailed survey of Maewo revealed that it was largely confined to the coastal populations, with a uniform incidence in all areas sampled (table 1).

Haematological data on 249 Ni-Vanuatu, including 63 β thalassaemia heterozygotes and compound heterozygotes with α thalassaemia, are given in tables 2, 3, and 4. These values fall within the range seen for β thalassaemia in other populations.^{6, 10} The effects of interacting α thalassaemia genes in minimising the reduction in MCH and MCV values in heterozygous β thalassaemia have been noted previously and are in agreement with the data presented here.¹⁰ Both the $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletion forms of α^+ thalassaemia are found in this population.¹ It is evident from table 2 that they result in very similar haematological phenotypes upon interaction with heterozygous β thalassaemia.

There were no significant differences in serum ferritin levels between β thalassaemic and normal subjects (table 5).

Six subjects, five from Maewo and one from Malekula, who were homozygous for β thalassaemia were identified during this study. All presented

TABLE 1 β thalassaemia heterozygote frequencies in Vanuatu.

Island	Population (1979 census)	β thalassaemia*	
		%	No studied
Espirito Santo	16 242	0	160
Maewo (North)	{ 1771 }	20.1	154
(Central)		20.7	179
(South)		21.9	73
Ambae	7820	0	303
Pentecost	9544	0	57
Malekula	15 935	6.2	113
Ambrym	6324	4.5	22
Paama	2354	0	66
Epi	2672	0.7	288
Tongariki	362	—	—
Emae/Tongoa	754/2892	—	—
Makura/Matato	162/102	—	—
Efate	20 110	3.3	61
Tanna	15 715	0.9	306
Futuna	357	2.0	102
Ancityum	465	0	260
Average (total)		5.7	130/2289

*Defined by Hb A₂ levels >3.5%.

before the age of one year with severe anaemia requiring regular blood transfusion. However, four of the six children were eventually withdrawn from the transfusion programme by their parents and later died before the age of six. The fifth child died suddenly at the age of six years, five months after undergoing splenectomy, despite continuous anti-malarial and antibiotic prophylaxis postoperatively, and one child remains alive but requires regular blood transfusion.

In addition to these patients there is evidence of considerable childhood mortality in families in which children known to have β thalassaemia have been born. Thus, in family A (fig 2), in which two children were proven β thalassaemia homozygotes, two other infants died of unknown causes, although they were known to be anaemic. Similarly, in family B, in which two homozygotes died in infancy, a further eight infants (all with a clinical history of anaemia) had died before this study was undertaken. In contrast, we have no evidence of any significant childhood mortality in families in this population in which only one or neither parent carries a β thalassaemia gene.

Haemoglobin analysis and biosynthesis was carried out on peripheral blood samples from two untransfused infants. In both cases there was un-

TABLE 2 Haematological data in 249 Ni-Vanuatu according to α and β thalassaemia genotypes.

Adult	No	Genotype	Hb(g/dl)	MCV (fl)	MCH(pg/cell)
M	45	No α or β thalasaemia detected	14.7 $\pm$ 1.2	89.4 $\pm$ 4.7	29.1 $\pm$ 1.5
F	47		13.5 $\pm$ 0.9		
M	6	β thalasaemia heterozygote only	12.4 $\pm$ 1.2	65.8 $\pm$ 5.3	20.7 $\pm$ 1.7
F	13		11.3 $\pm$ 1.0		
M	7	$-\alpha^{3.7}/\alpha\alpha$ plus heterozygous β thalassaemia	12.7 $\pm$ 0.9	69.0 $\pm$ 4.0	21.7 $\pm$ 1.4
F	10		11.8 $\pm$ 1.1		
M	3	$-\alpha^{3.2}/\alpha\alpha$ plus heterozygous β thalassaemia	13.3 $\pm$ 1.2	68.8 $\pm$ 2.2	21.8 $\pm$ 1.3
F	2		11.9 $\pm$ 1.2		
M	4	$-\alpha/\alpha$ plus heterozygous β thalassaemia	12.9 $\pm$ 1.0	74.3 $\pm$ 3.5	23.6 $\pm$ 1.5
F	3		13.0 $\pm$ 0.7		
10-15 years					
M	40	No α or β thalassaemia detected	12.7 $\pm$ 1.0	86.3 $\pm$ 5.4	27.1 $\pm$ 1.3
F	39		12.8 $\pm$ 0.9		
M	3	Heterozygous β thalassaemia	12.0 $\pm$ 0.4	63.3 $\pm$ 3.0	19.1 $\pm$ 0.6
F	1		—		
M	3	$-\alpha^{3.7}/\alpha\alpha$ plus heterozygous β thalassaemia	11.6 $\pm$ 0.1	68.6 $\pm$ 3.0	21.0 $\pm$ 1.0
F	2		—		
5-9 years					
M	10	No α or β thalassaemia detected	12.8 $\pm$ 1.1	80.1 $\pm$ 4.5	26.1 $\pm$ 1.8
F	7		12.5 $\pm$ 0.9		
M(2)+F(2)		Heterozygous β thalassaemia	—	63.0 $\pm$ 2.0	19.3 $\pm$ 0.8

TABLE 3 Hb F levels in 361 Ni-Vanuatu of all ages (above five years) according to α + β thalassaemia genotypes.

No	Genotype	Hb F (%)
277	No β thalassaemia detected	0.48 $\pm$ 0.18
84	Heterozygous β thalassaemia	0.79 $\pm$ 0.38
42	Heterozygous β thalassaemia and no α thalassaemia detected	0.81 $\pm$ 0.44
8	Heterozygous β thalassaemia plus — α/α of all types	0.39 $\pm$ 0.10

(includes all — α/α genotypes)
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equivocal evidence for small amounts of β chain synthesis, or the presence of Hb A, indicating that these subjects have a form of homozygous β^+ thalassaemia. Subsequent characterisation of the defective β gene has shown that it carries a G→C change at position 5 in IVS-1 (Hill *et al*, in

preparation), a mutation previously described¹¹ only in Asians and known to result in inefficient splicing of β mRNA transcripts with consequent reduced output of β globin.¹² However, the variant present in the Maewo population has a different β gene complex haplotype than that previously described^{11 13} and is presumably a new mutation.

Discussion

Sporadic cases of β thalassaemia in Vanuatu had originally come to our attention during investigations of hospital patients with anaemia of pregnancy. However, it was not until a widespread survey of the entire archipelago had been completed¹⁴

TABLE 4 Hb A₂ levels in 510 Ni-Vanuatu of all ages (above five years) from the islands of Maewo and Aneityum.

No	Genotype	Hb A ₂ (%)	Island
129	No β thalassaemia detected	2.4±0.4	Aneityum
298	No β thalassaemia detected	2.4±0.3	Maewo
83	Heterozygous β thalassaemia	4.8±0.7	Maewo

TABLE 5 Serum ferritin levels in 217 Ni-Vanuatu from the island of Maewo.

Age	Sex	No	Genotype	Serum ferritin (μ g/l)
Adult	M	42	No β thalassaemia detected	{ 72.1±37.0
	F	67		{ 56.7±39.0
	M	14	Heterozygous β thalassaemia	{ 63.0±41.8
	F	25		{ 57.5±36.8
10–15 years	M	13	No β thalassaemia detected	{ 41.2±14.6
	F	14		{ 36.1±9.5
	M	8	Heterozygous β thalassaemia	42.4±13.9
	F	2		
5–9 years	M	15	No β thalassaemia detected	{ 39.0±22.0
	F	13		{ 43.3±27.7
	M	4	Heterozygous β thalassaemia	39.8±23.0

FIG 2 Pedigrees of two unrelated families in which children with proven homozygous β thalassaemia have been born.

that the grossly uneven distribution of the disease became apparent. On Maewo, over 20% of the population are carriers whereas, apart from Malekula, most other islands are largely free of the disease.

The clinical and laboratory findings are in keeping with β^+ thalassaemia as seen in other populations,⁶ in which heterozygotes have a mild hypochromic microcytic anaemia and are usually asymptomatic, except for females in pregnancy when there is an exaggerated fall in haemoglobin level in the mid-trimester. Homozygotes are affected by severe anaemia within the first six months requiring four- to six-weekly blood transfusions to maintain adequate haemoglobin levels.

Because there is a high incidence of deletion forms of α^+ thalassaemia in Vanuatu, their interactions with β thalassaemia are frequently encountered on Maewo. As seen in table 2, MCV and MCH indices in these cases are usually higher than for the simple heterozygous β thalassaemia, especially when the interacting α^+ thalassaemia is present in the homozygous state (β thal $-\alpha/-\alpha$). Screening for β thalassaemia can not be carried out satisfactorily by electronic cell counting in these circumstances; raised Hb A₂ levels are, however, a reliable indication of β thalassaemia in the simple or compound heterozygous state.¹⁴

Malaria is endemic and widespread in Vanuatu. Hence, it might have been expected that, as in malarial areas elsewhere, haemoglobinopathies would not be uncommon. The restricted distribution of β thalassaemia probably points to a recent introduction on Maewo which, because of relatively limited inter-island population movement, has not yet had time to spread throughout the archipelago. Thus, homozygous β thalassaemia, while of minor importance nationally in Vanuatu, is a serious public health problem for the 2000 inhabitants of Maewo; it accounts for a high infant mortality in affected families while seriously stretching the limited resources of local health services. Although transfusion has proved practicable and well tolerated, chelation therapy is not yet considered feasible for both social and practical reasons. On the other hand, if in the future the island population requested genetic counselling and prenatal diagnosis for homozygous β thalassaemia, it should now be possible to use chorion biopsy and DNA analysis with oligonucleotide probes for this purpose.^{15 16}

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