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## Extremely High Frequencies of $\alpha$ -Globin Gene Deletion in Madang and on Kar Kar Island, Papua New Guinea

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### SUMMARY

Extremely high frequencies of the deletion form of  $\alpha^+$ -thalassemia ( $-\alpha/$ ), as studied by the DNA mapping technique, were found in the population of Madang, a coastal province in the north of Papua New Guinea (PNG) and in the population of Kar Kar, an island situated near Madang. Ninety-seven percent of the population tested from Madang and 89% of that from Kar Kar Island were either  $\alpha^+$ -thalassemia heterozygotes or homozygotes. By contrast, no examples of the deletion form were detected in the Eastern Highlands of PNG. The haplotype frequencies of  $\alpha^+$ -thalassemia ( $-\alpha/$ ) in Madang and Kar Kar Island were found to be 81.33% and 66.67%, respectively. A more detailed analysis of the gene deletion revealed that in both populations 96% were of the 4.2 kilobase (kb) type and 4% were of the 3.7-kb type. Thus, this group is the only example in which the 4.2-kb deletion is predominant over 3.7-kb defect. The presence in high frequencies of  $\alpha^+$ -thalassemia in the coastal area of Madang and on the neighboring island, where malaria has long been holoendemic or hyperendemic, and its virtual absence from the nonmalarious highlands of PNG suggest the role of malaria as the selective factor in maintaining  $\alpha^+$ -thalassemia. If this selective pressure is still operating, and since  $\alpha^+$ -thalassemia has no apparent homozygous disadvantage, the abnormal haplotype ( $-\alpha/$ ) will be in the process of fixation in this population.

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## INTRODUCTION

It has now been well established that normal individuals in human populations have duplicated  $\alpha$ -globin gene loci ( $\alpha\alpha/\alpha\alpha$ ) [1, 2]. However, before the arrangement of the  $\alpha$ -globin loci was completely elucidated, it was thought that population heterogeneity existed. Melanesians were often cited as an example of a group in which individuals normally had a single  $\alpha$ -globin gene locus. The evidence for this hypothesis was based on the fact that Hemoglobin (Hb) J Tongariki ( $\alpha$  115 Ala $\rightarrow$ Asp) made up 40%–50% of the hemoglobin in Hb J Tongariki heterozygotes and that Hb J Tongariki homozygotes lacked Hb A [3, 4]. This finding could later be explained by the linkage of an  $\alpha$ -globin deletion in *cis* to the  $\alpha^J$ -globin gene [5, 6]. The single  $\alpha$ -globin locus in Melanesians is, in fact, a known defective condition, namely,  $\alpha$ -thalassemia 2 or  $\alpha^+$ -thalassemia, caused by the deletion of an  $\alpha$ -globin gene ( $-\alpha$ ) [5–7]. However, little is known about  $\alpha$ -thalassemia in Melanesians, partly because it is difficult to identify  $\alpha^+$ -thalassemia in adults. Although examination of Hb Bart's in cord blood has been used for population screening, it has not been informative enough for accurate calculation of the gene frequency for  $\alpha^+$ -thalassemia [8, 9].

The availability of gene analysis by restriction endonuclease mapping makes it possible to detect the deletion form of  $\alpha$ -thalassemia and accurately define genotypes. A previously reported pilot study of Hb Bart's in cord blood samples and including  $\alpha$ -globin gene analysis of 30 samples from the Madang area of PNG suggested that there is a high frequency of  $\alpha$ -globin gene deletion in this population [7]. We have also used the restriction endonuclease mapping technique to study  $\alpha$ -globin genes in Melanesians from PNG. Our results have confirmed and extended the previous finding of extremely high frequency of  $\alpha$ -globin gene deletion in the Madang area and show that this is also true for the population on Kar Kar Island. In addition, when compared with the earlier findings [7], our data suggest the possibility of an age-related change in the frequency of  $\alpha$ -globin gene deletions.

## MATERIALS AND METHODS

Blood samples were collected from villagers living in Madang Province in the north of the PNG mainland, on Kar Kar Island, an island situated about 15 km from Madang, and in Goroka in the Eastern Highlands Province. Hb type was analyzed by starch gel electrophoresis, pH 8.6, and the relative amount of Hb A<sub>2</sub> was quantitated by DEAE-cellulose microchromatography [10]. DNA was prepared from lymphocytes or buffy coats by the method described by Grunebaum et al. [11].

Aliquots of 10  $\mu$ g of DNA were digested with 6 U/ $\mu$ g *Bam*HI or 2 U/ $\mu$ g *Bgl*II for 16 hrs in the buffer specified by the manufacturer (Boehringer-Mannheim, Mannheim, West Germany). After electrophoresis in 0.8% agarose in Tris-EDTA-acetate buffer, pH 8.5, the DNA fragments were transferred to Gene Screen *Plus*<sup>TM</sup> membranes (NEN, Boston, Mass.). The membranes were hybridized with a <sup>32</sup>P-labeled  $\alpha$ -globin specific probe, JW101 [12] or  $\zeta$ -globin specific probe, pBR $\zeta$  [2]. The method of hybridization as described by Nasmyth [13] was followed. The membranes were washed sequentially in 2  $\times$ , 1  $\times$ , 0.5  $\times$ , and 0.1  $\times$  SSC plus 0.1% SDS at 65°C for 30 min each and autoradiographed at –70°C with intensifying screens for 3–7 days.

DNA from normal individuals shows a single 14-kb *Bam*HI fragment containing both  $\alpha$ -globin genes when hybridized with an  $\alpha$ -globin specific probe. In the  $\alpha^+$ -thalassemia

heterozygote, which has one  $\alpha$ -globin gene deleted from one chromosome, both the normal 14-kb fragment and a smaller fragment of 10.5 kb are observed, and in the  $\alpha^+$ -thalassemia homozygote, only the 10.5-kb fragment is detected. The 3.7-kb (rightward) deletion was detected by the presence of a 16-kb fragment and the 4.2-kb (leftward) deletion by the presence of a 8.4-kb fragment when DNA was digested with *Bgl*II and hybridized with a  $\zeta$ -globin specific probe [14].

#### RESULTS

Table 1 shows the results of analysis of the  $\alpha$ -globin gene. No  $\alpha$ -globin gene deletions were detected in the 17 individuals from Goroka. Seventy-three out of 75 individuals (97%) from Madang and 24 out of 27 (89%) from Kar Kar Island had both the 14- and 10.5-kb *Bam*HI  $\alpha$ -globin gene fragments or the 10.5-kb fragment alone and therefore were deletion type  $\alpha^+$ -thalassemia heterozygotes ( $-\alpha/\alpha$ ) and homozygotes ( $-\alpha/-\alpha$ ), respectively. The haplotype frequencies of the  $\alpha^+$ -thalassemia ( $-\alpha/$ ) are 81.33% in Madang and 66.67% on Kar Kar Island. The observed frequencies of the genotypes in both groups fit well to those predicted for a population in Hardy-Weinberg equilibrium (table 1).

Two different patterns of gene organization responsible for  $\alpha^+$ -thalassemia genotype have been found [2, 15]. The 4.2-kb (leftward) deletion involves the loss of the whole of the 5'  $\alpha$ -globin gene, whereas the 3.7-kb (rightward) deletion is probably the result of a crossover involving 5' and 3'  $\alpha$ -globin genes on different chromosomes giving rise to a fusion product between the two  $\alpha$ -globin genes. To determine whether these populations carry the 4.2-kb or 3.7-kb deletion, 145 deleted chromosomes from the two groups were analyzed using *Bgl*II digestion and the  $\zeta$ -globin specific probe. The result shows that 96% of the deletions from both groups were the 4.2-kb type and 4% were the 3.7-kb type (table 2).

Among the individuals from Kar Kar Island, five were found to be Hb J Tongariki heterozygotes. Of four Hb J Tongariki heterozygotes studied by DNA analysis, three were heterozygous and one was homozygous for the single  $\alpha$ -globin gene deletion. This finding adds support to the theory that the mutation leading to Hb J Tongariki occurred on a chromosome which carried an  $\alpha$ -globin gene deletion ( $-\alpha^J/$ ) [5, 6]. One Hb J Tongariki heterozygote (the deletion homozygote) was further analyzed, and both the 4.2-kb and 3.7-kb

TABLE I  
INCIDENCES OF  $\alpha\alpha/\alpha\alpha$ ,  $-\alpha/\alpha\alpha$ , AND  $-\alpha/-\alpha$  GENOTYPES AND FREQUENCIES OF NORMAL ( $\alpha\alpha/$ ) AND DELETED ( $-\alpha/$ ) HAPLOTYPES AS STUDIED BY GENE-MAPPING TECHNIQUE

AREAS	No.	GENOTYPES			HAPLOTYPE FREQUENCIES (%)	
		$\alpha\alpha/\alpha\alpha$	$-\alpha/\alpha\alpha$	$-\alpha/-\alpha$	$\alpha\alpha/$	$-\alpha/$
Madang . . . . .	75	2(2.61)*	24(22.78)	49(49.61)	18.67	81.33
Kar Kar Island . . . . .	27	3(3)	12(12)	12(12)	33.33	66.67
Goroka . . . . .	17	17	0	0	100	0

\* The figures in parentheses are nos. predicted by Hardy-Weinberg equation.

TABLE 2  
PREVALENCES OF THE 4.2-kb AND 3.7-kb DELETIONS OF  $\alpha$ -GLOBIN GENES

Areas	No. chromosomes	4.2-kb deletion	3.7-kb deletion
Madang . . . . .	122	117 (95.9%)	5 (4.1%)
Kar Kar Island . . . . .	23	22 (95.6%)	1 (4.4%)
Total . . . . .	145	139 (95.8%)	6 (4.2%)

deletions were observed. No other structural variants of hemoglobin were detected in the Madang, Kar Kar Island, or Goroka populations.

$\beta$ -Thalassemia (as judged by increased levels of Hb A<sub>2</sub>) had a much lower frequency in these populations than  $\alpha$ -thalassemia. Only six individuals from Madang were found to be  $\beta$ -thalassemia heterozygotes, there were no homozygotes, nor was  $\beta$ -thalassemia found among samples from Kar Kar Island or Goroka.

#### DISCUSSION

The results of this study show that the frequencies of the deletion form of  $\alpha^+$ -thalassemia are extremely high in Madang and on Kar Kar Island. The incidence of the  $\alpha$ -globin gene deletion in homozygous and heterozygous individuals in Madang is 97%, the highest so far reported [7, 16]. The frequencies of the deletion haplotype ( $-\alpha/$ ) in both Madang and Kar Kar Island (81% and 67%, respectively) are higher than those of the normal haplotype ( $\alpha\alpha/$ ). In a previous report [7] of  $\alpha$ -globin gene analysis of 30 individuals, the incidence of  $\alpha^+$ -thalassemia heterozygotes plus homozygotes in Madang was found to be 90%, but many more of these were heterozygotes, the frequency of the  $-\alpha/$  haplotype being 65% compared with 81% in Madang in our study. This difference probably reflects a difference in the groups of subjects from whom blood samples were collected. In the present work, all the subjects from Madang were adult villagers selected as part of a study of genetic markers and the incidence of malaria in the area. In the previous report, the majority of the samples for DNA analysis were obtained from the cord blood of infants born at Madang Provincial Hospital. The gene frequencies could therefore have been affected by the inclusion in our study of subjects carrying other genetic markers (J. Cattani, manuscript in preparation) or by possible differences in regional origin of children born at the hospital compared with the adult villagers. An alternative explanation would be that there is differential mortality during childhood of individuals not carrying a deletion ( $\alpha\alpha/\alpha\alpha$ ) since this class has a much lower frequency in our results than in the previous report.

The selective factor resulting in high frequencies of the deletion in infants and even higher frequencies in adults could be malaria, which has had a holo- to hyperendemic pattern in the area [17]. This is also supported by the finding that  $\alpha^+$ -thalassemia was not detected in either study in Goroka, a nonmalarious highland area. If this selective pressure is still operating in this population, one

might speculate that the normal haplotype is being replaced by the  $\alpha^+$ -thalassemia haplotype, since homozygous  $\alpha^+$ -thalassemia is asymptomatic and is apparently not disadvantageous.

The incidence of the 4.2-kb  $\alpha$ -gene deletion subtype in the present study is higher than that reported in the previous in this area [7], which found 69% of the 4.2-kb deletion, 26% of the 3.7-kb deletion, and 5% of unclassifiable type. Comparison of the two groups shows a highly significant difference in haplotype frequencies ( $\chi^2_3 = 69$ ;  $P < .001$ ). Again, some disadvantage of the 3.7-kb deletion compared with the 4.2-kb deletion might be postulated to account for the much increased proportion of the latter deletion in the adults of the present study, although the possibility that the subjects represent different population groups must also be taken into account.

This Melanesian population is the only one in which the 4.2-kb deletion is more prevalent than the 3.7-kb defect. The predominance of the 3.7-kb type has been documented in Polynesians [14, 18], Malays [19], Thais [20], Chinese [15, 19, 21, 22], Saudi Arabs [23], Jews [24], South Africans [25], American blacks [15, 22, 26], and Mediterraneans [15, 27, 28]. It is not clear whether the situation in Melanesians has resulted from selection or from genetic drift through founder effect.

Only one DNA sample from an Hb J Tongariki heterozygote was available for analysis of the deletion subtypes. This individual was homozygous for the deleted  $\alpha$ -globin gene, but was found to carry both the 4.2- and 3.7-kb deletions. A previous report [6] has already demonstrated the association of the 3.7-kb deletion and Hb J Tongariki. Since the 4.2-kb deletion is predominant on Kar Kar Island (as shown in the present study), it seems likely that the Hb J Tongariki allele was introduced to Kar Kar Island, rather than originating there.

The doubly deleted  $\alpha$ -thalassemia I or  $\alpha^0$ -thalassemia haplotype ( $- -/$ ) was not detected in the present study. Hb H disease, found in heterozygotes for single and double gene deletions ( $- -/-\alpha$ ), is uncommon in PNG, and Hb Bart's hydrops fetalis, found in homozygotes for double gene deletion ( $- -/- -$ ), has never been reported. Thus, the double deletion must be very rare in this population, perhaps because the genetic load imposed by segregation of the  $- -/$  chromosome outweighs the possible selective advantage for the  $\alpha^0$ -thalassemia heterozygote ( $- -/\alpha\alpha$ ).

No hemoglobin structural variants other than Hb J Tongariki were found in this study, and the incidence of  $\beta$ -thalassemia was not raised over that found in other malarious areas [29]. Thus, if malaria is having an unusually strong selective effect on  $\alpha$ -gene deletion frequencies, this is not carried through to other hemoglobinopathies normally postulated to confer advantage in the presence of malaria. Two other red blood cell defects, G6PD deficiency and ovalocytosis, commonly found in Melanesians [30, 31] and also postulated to confer advantage in the presence of malaria, are observed in high frequencies in the populations studied (data not shown). Interactions of these three genetic determinants ( $\beta$ -thalassemia, G6PD deficiency, and ovalocytosis) with  $\alpha^+$ -thalassemia in playing a role to cope with malaria would be an interesting topic for a further study.

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