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Melanesians and Polynesians Share a Unique α-Thalassemia Mutation

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SUMMARY

Several genetic markers that provide information on population migrations and affinities have been detected by studies of proteins and cellular antigens in blood. Analysis of DNA polymorphisms promises to yield many further population markers, and we report here the distribution of a new α-globin gene deletion ($-\alpha^{3.7}$III) detected by a restriction enzyme mapping. This is found frequently in Melanesians and Polynesians but not in five other populations in which α-thalassemia is prevalent. We used restriction enzyme haplotype analysis to support a single origin for this mutation and propose that it is a useful population marker. Its geographical distribution supports a route through Island Melanesia for the colonizers of Polynesia.

INTRODUCTION

Extensive linguistic, archeological, and anthropological studies have contributed to our current understanding of population migrations in the Pacific [1]. Until recently, genetic data [2, 3] have provided less clear-cut information for several reasons [4]. In small island communities, the frequency of classical blood polymorphic markers is particularly susceptible to alteration by drift [5].

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Founder effects can lead to the total loss of such alleles or amplify uncommon genes in the parent population to high frequency on new islands. New mutations that produce population-specific markers are more useful in this regard and may be used to trace migration routes. Unfortunately, those discovered to date in the Pacific (reviewed in [2, 3, 6]) are relatively localized and are mostly present at low frequencies. Recently, investigation of the highly polymorphic HLA system has shown that linked polymorphisms in this complex can be used as population markers and provide results consistent with migration pathways based on linguistics and archeology [7]. Also, studies in north coastal Papua New Guinea [8] have now demonstrated greater genetic affinity between some neighboring groups who speak quite different languages than between other more widely separated groups speaking closely related languages. This indicates that linguistic studies do not provide an infallible guide to ancestral relationships and emphasizes the need for genetic data to complement such work.

With the advent of recombinant DNA methodologies, large numbers of restriction fragment length polymorphisms are being discovered [9]. Such DNA polymorphisms promise to increase enormously the number of useful population-specific markers. We report here the population distribution of a newly described [10] α-globin gene deletion, detected by restriction enzyme mapping. This is found frequently in both Melanesians and Polynesians and is the first genetic marker widely distributed within, but apparently confined to, Oceania.

The current view of prehistoric migrations in Oceania may be summarized as follows [1]: An Australoid population first moved south into New Guinea and down into Australia at least 50,000 years ago. With time and further waves of migration, a variety of Papuan languages evolved in New Guinea and speakers of these spread east into Island Melanesia at least as far as the Santa Cruz group (fig. 1). Four-to-five thousand years ago, Austronesian speakers, more Mongoloid in appearance and skilled in navigation, moved south to parts of coastal New Guinea and intermarried with resident Papuans. Their homeland remains unknown, but with them, the Austronesians brought pottery, pigs, and horticulture. Their characteristic Lapita pottery has been used to trace their spread through Island Melanesia, south to New Caledonia, and east to settle Fiji about 1500 B.C. The timing of their subsequent expansion into all parts of Polynesia has been clearly worked out from archeology and linguistics. Today, Austronesian languages are spoken in parts of Taiwan and Vietnam, in island Southeast Asia, and throughout Polynesia and Micronesia. In New Guinea, Papuan languages predominate, whereas Island Melanesians speak Austronesian languages. Howells [11] advocated an alternative Micronesian route for the colonization of Polynesia because of the marked physical differences between present-day Polynesians and Melanesians. However, linguistic analysis and the absence of Lapita pottery in Micronesia support the conventional view [12]. Data on the HLA complex [7] and other genetic markers tested [2] are also compatible with this general outline of colonization of Polynesia from the west.

The place of Vanuatu (formerly the New Hebrides) in this reconstruction is particularly uncertain. The inhabitants, the Ni-Vanuatu, are typical dark-skinned Melanesians in appearance, but they speak Austronesian languages

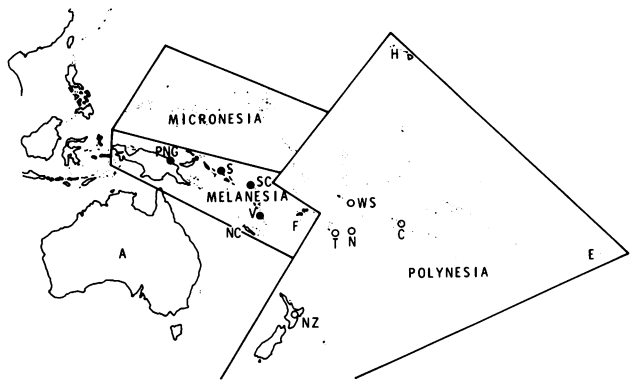

FIG. 1.—Map of the South Pacific showing Melanesia, Polynesia and Micronesia. Areas where both the $-\alpha^{3.7}$III deletion and Hb J Tongariki have been found are indicated by ●. Areas where $-\alpha^{3.7}$III deletions but not Hb J Tongariki have been found are indicated by ○. *A*—Australia; *C*—Cook Islands; *E*—Easter Island; *F*—Fiji; *H*—Hawaii; *N*—Niue; *NC*—New Caledonia; *NZ*—New Zealand; *PNG*—Papua New Guinea; *S*—Solomon Islands; *SC*—Santa Cruz Islands; *T*—Tonga; *V*—Vanuatu; *WS*—Western Samoa.

related to those in Polynesia. One possible explanation for this is that the islands were originally settled by migrants from Papua New Guinea who spoke Papuan languages. Subsequently, with the arrival of the more advanced Austronesian speakers, their languages and some of their cultural traits, such as pottery making, would have been adopted. Unfortunately, there is no archeological evidence from the archipelago earlier than 1300 B.C. to help determine who the first settlers were [1]. Linguistic analysis places the origin of the Eastern Oceanic subgroup of Austronesian in Northern Vanuatu [13]. All the Polynesian languages are members of this subgroup. However, if Polynesia was colonized from Vanuatu, as this would suggest, it is difficult to account for the marked physical differences between Polynesians and the dark-skinned Melanesian inhabitants of Vanuatu, unless there was a superficial assimilation of Austronesian language and cultural traits despite relatively limited genetic admixture.

Several genetic markers have been reported to occur, albeit at low frequency, in parts of Vanuatu [6, 15]. Some of these, such as phosphoglucomutase$_1^3$ and phosphoglycerate kinase[4] are considered Papuan, whereas others such as Albumin$^{\text{New Guinea}}$ and Hemoglobin J Tongariki, although apparently absent in Polynesia, have been associated with Austronesian speakers in Melanesia [3]. This suggests some genetic heterogeneity in Vanuatu, and recent HLA studies estimate an Austronesian admixture of 15%–18% into a Papuan stock in other parts of Island Melanesia [7].

We have accumulated genetic data relevant to this problem by analyzing the nature of various α-thalassemia deletions found in the Southwest Pacific [16]. Globally, the commonest type of α-thalassemia is that due to the single gene deletion ($-\alpha^{3.7}$) in which 3.7 kilobases (kb) of DNA are removed from between the two normal α-globin genes [17]. Recently, using detailed restriction enzyme mapping, we have been able to divide $-\alpha^{3.7}$ deletions into three types ($-\alpha^{3.7}$I,

II, and III) produced by different crossovers [10]. We report here the population distribution of these three types and of the related $-\alpha^{4.2}$ deletion [18]. The $-\alpha^{3.7}$I, $-\alpha^{3.7}$II, and $-\alpha^{4.2}$ deletions are found in many unrelated populations throughout the world. However, we find that the $-\alpha^{3.7}$III deletion is confined to Melanesia and Polynesia. We present evidence from genetic analysis that this mutation has probably had a single origin and, hence, is unlikely to have arisen independently in these two populations. We propose, therefore, that the $-\alpha^{3.7}$III deletion is a useful population marker and discuss the information its present distribution provides on population migrations and affinities in Oceania.

MATERIALS AND METHODS

Hemoglobin (Hb) electrophoresis was by standard methods [19]. DNA was extracted, digested with restriction enzymes, and run on 0.8% or 1.2% agarose gels. Southern blotting, prehybridization, hybridization, washing conditions, and autoradiography were as described [20]. The genomic probes used were: (1) a 2.85-kb *Eco*RI-*Bam*HI ζ fragment that includes the entire ζ gene, as well as 557 base pairs (bp) of 5′ and 100 bp of 3′ flanking sequence; (2) a 1,160-bp *Bgl*II-*Pvu*II fragment from intron-1 of the Ψζ gene; (3) an interzeta hypervariable region *Alu*I fragment containing 32 copies of a tandemly repeated 36-bp sequence [21]; and (4) 1.5-kb *Pst* fragment containing the entire α1 gene.

The human α-globin complex on chromosome 16 [22] consists of two adult α genes (α2 and α1), an embryonic ζ gene, and two pseudogenes (ψα and ψζ) arranged in the order 5′-ζ-ψζ-ψα-α2-α1-3′ (fig. 2 and [23]). The structure of this complex and deletions within it producing α-thalassemia have been analyzed extensively (reviewed in [17, 24]). The two α genes produce identical proteins and are contained in 4-kb homology blocks that have been subdivided into X, Y, and Z boxes (fig. 2 and [23]). The Z boxes containing the entire α genes are 98.5% homologous [25] and unequal crossing over in this region produces the common 3.7-kb deletion. Crossovers in the X box produce the 4.2-kb deletion [18]. Recently, we have been able to subdivide 3.7-kb deletions into three groups ($-\alpha^{3.7}$I, $-\alpha^{3.7}$II, and $-\alpha^{3.7}$III) with different points of crossing over in the Z box, on the basis of different restriction enzyme patterns generated using the enzyme *Apa*I [10].

All samples were initially digested with *Bgl*II and hybridized with the 2.85-kb ζ probe. The 3.7-kb deletion was detected by the presence of a 16-kb band and the 4.2-kb deletion by the presence of a 8.4-kb band. All samples with a 3.7-kb deletion were characterized further using *Apa*I and the *Pst* α probe [10, 25], allowing subdivision of the 3.7-kb deletion into three groups according to the point of crossover between the α2 and α1 genes (fig. 2). Normal (αα) chromosomes in all populations give bands of 2.7, 1.7, and 0.89 kb with this enzyme. Crossover I, which occurs 5′ to a 7-bp insert in intron-II of the α1 gene, produces a new 2.5-kb band [25]. Crossover II, 3′ to the 7-bp insert but 5′ to the nonhomology block in the 3′ untranslated region [25], produces a single 3.5-kb band [10]. Crossover III, in a 46-bp homology block surrounding the poly A addition site [10], produces a new 0.7-kb band.

Triplicated α genes were detected by rehybridizing the *Bgl*II filters with the *Pst* α probe; a new 3.7-kb band indicated the ααα haplotype, which is the counterpart of the 3.7-kb deletion [26, 27]. Further analysis of these chromosomes was performed using *Apa*I and the *Pst* α probe, a new 1.9-kb band indicating that a segment I crossover had produced the triplicated α gene arrangement [25].

The presence or absence of three restriction enzyme site polymorphisms and the size of four length polymorphisms in the α complex were determined. A group of such linked polymorphisms is termed a haplotype [28]. The sizes of the *Sac*I hypervariable region fragment lengths between the zeta genes [21], 3′ to the α genes [29] and in the ψζ first

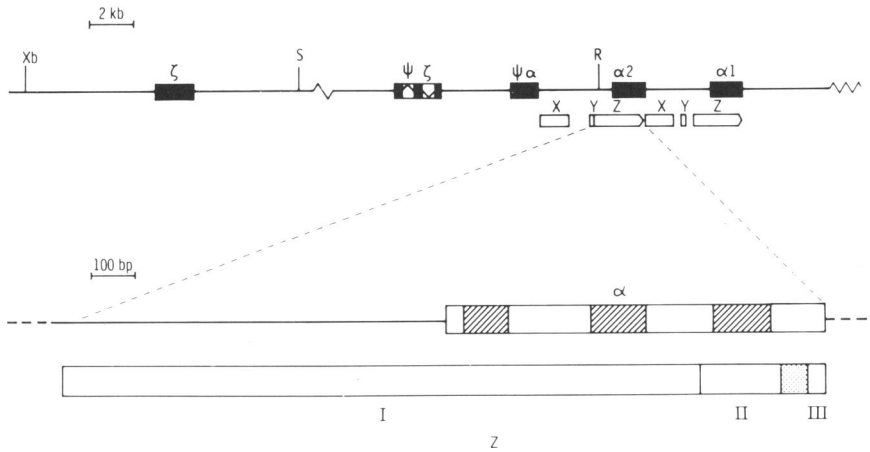

FIG. 2.—*Above:* Structure of the α-gene complex. Restriction enzyme site polymorphisms are marked: *Xb—Xba*I, *S—Sac*I, *R—Rsa*I. ∿ indicates length variation. The 4-kb homology blocks containing the α genes are divided into more homologous boxes labeled *X*, *Y*, and *Z*. *Below:* Detail of the Z homology box and α2 gene. Exons are indicated by ▭. 5′ noncoding region, introns, and 3′ noncoding region are indicated by □. The three regions in the *Z box* within which the $-\alpha^{3.7}$I, $-\alpha^{3.7}$II, and $-\alpha^{3.7}$III crossovers occur are shown. No crossover has been found to occur in the nonhomologous stippled area.

intron [21], were determined as described previously. A new ψζ intron-II length polymorphism was detected using a *Pvu*II digest and the 2.85-kb ζ probe. The band corresponding to the 3′ end of the ψζ gene varied from 2.0 to 2.2 kb in length. The ζ*Xba*I, interzeta *Sac*I, and α2 *Rsa*I site polymorphisms were detected as described [10, 30, 31].

RESULTS

Geographical Distribution of the $-\alpha^{3.7}$III Deletion

The types of single α gene deletions found in the different populations tested are shown in table 1. In Vanuatu, most (87/99) of the 3.7-kb deletions were of the $-\alpha^{3.7}$III type. We have found this type of deletion on 19 different islands in Vanuatu. It is also found in coastal Papua New Guinea, but there the 4.2-kb deletion, with a gene frequency of 50% ([32] and our unpublished data, 1984), is far more common. In Polynesia, however, the $-\alpha^{3.7}$III deletion is by far the most common type and no 4.2-kb deletion has been found. Given gene frequencies of all types of $-\alpha$ deletions in Vanuatu of approximately 20% ([16] and D. K. Bowden et al., in preparation) and in Polynesia of 8% [33], the gene frequencies of the $-\alpha^{3.7}$III deletion in these populations are 11.5% and 7.5%, respectively.

A previous study [25] found no example of this deletion in 16 $-\alpha^{3.7}$ samples from various black, European, and Oriental individuals. We have extended and confirmed this by examining 177 samples from five non-Oceanic populations in which α-thalassemia is prevalent (table 1). The predominant deletion in all these is the $-\alpha^{3.7}$I type. However, eight examples of the rare $-\alpha^{3.7}$II type [10] were found. No case of the $-\alpha^{3.7}$III deletion was found outside Melanesia and

TABLE 1

NO. OF CHROMOSOMES WITH EACH TYPE OF DELETION IN DIFFERENT POPULATIONS

	$-\alpha^{3.7}$			$-\alpha^{4.2}$
	I	II	III	
Vanuatu	12	0	87	49
Papua New Guinea	7	0	4	55
Polynesia	2	0	30	0
Southeast Asia	39	5	0	5
India	8	0	0	1
Saudi Arabia	27	0	0	1
Mediterranean	34	1	0	0
Jamaica	53	2	0	1

NOTE: Although the sample sizes were different and the frequency of α-thalassemia differs among the populations [17], the relative proportions of each type of deletion in each population are correct. Samples from Vanuatu were collected on numerous islands throughout the archipelago. Papua New Guinean samples were from north coastal populations including some from Karkar Island, New Britain, and New Ireland. The Polynesians comprise 16 Maori deletions, 10 from the Cook Islands, three from Western Samoa, two from Tonga, and one from Niue. The Southeast Asians were 39 Thais, six Southern Chinese, three Indonesians, and one Malay. Indian samples were from immigrants to the United Kingdom from the Punjab, Gujarat, and Kashmir. Saudi Arabs were from Eastern Oases populations. The Mediterraneans comprise Greek Cypriots and Italians. The Jamaican samples were from families attending the Sickle Cell Clinic at the University of the West Indies, Kingston, Jamaica.

Polynesia. In particular, no case was found in the Southeast Asian group including three deletions from island Southeast Asia.

Hemoglobin electrophoresis was performed on a group of samples from Vanuatu that included 44 chromosomes with the $-\alpha^{3.7}$III deletion. Six of these had the superimposed Hb J Tongariki mutation [16]. Hb J Tongariki was not found in association with $-\alpha^{3.7}$I, II, or normal αα chromosomes.

If single α gene deletions are produced by unequal crossing over between chromosomes, rather than by intra-chromosomal recombination, triple α chromosomes should be produced simultaneously with single α gene chromosomes. We have therefore looked for the triplicated α gene counterpart to the $-\alpha^{3.7}$III deletion. In eight examples from Vanuatu and 10 from Polynesia, every ααα chromosome was the counterpart of the $-\alpha^{3.7}$I deletion. So the $\alpha\alpha\alpha^{3.7}$III chromosome, if it exists, must be rare.

A Single Origin for the $-\alpha^{3.7}$III Deletion

The $-\alpha^{3.7}$III deletion results from a crossover within a 46-bp region of homology at the 3′ end of the α genes [10] (fig. 2). Given the small size of this homology block, one might expect this crossover to be a relatively rare genetic event. This, together with the limited geographical distribution of the deletion, suggests that it probably had a single origin. To test this hypothesis, we analyzed seven restriction fragment length polymorphisms in the α complex of ho-

mozygotes for the $-\alpha^{3.7}$III deletion. In all 18 homozygotes examined—13 from Vanuatu, one from Papua New Guinea [34], and two from Polynesia—the following haplotype was present on both chromosomes: the *Xba*I, *Sac*I, and *Rsa*I polymorphic sites were absent; the *Sac*I interzeta fragment length was 6.9 kb, the *Sac*I ψζ intron-1 fragment length was 1.6 kb, the *Pvu*II ψζ intron-II fragment length was 2.2 kb, and the *Sac*I 3′ α fragment length was approximately 14 kb. We have haplotyped 40 αα chromosomes from Vanuatu, and this haplotype was found on 12 of these (30%). In contrast, in Jamaica, $-\alpha^{3.7}$I deletions are found on at least eight different haplotypes (D. R. Higgs et al., manuscript in preparation). Thus, the observation that the $-\alpha^{3.7}$III deletion is associated exclusively with a particular haplotype that is found on less than a third of normal chromosomes supports the idea that it had a single origin.

DISCUSSION

This analysis of 180 single α-gene deletions from Vanuatu and Polynesia shows that the most common form of α-thalassemia in both these populations is the newly described $-\alpha^{3.7}$III deletion [10]. This gene is also found in coastal Papua New Guinea, but there the 4.2-kb deletion is more common. In the five other world populations that we have screened, no example of the $-\alpha^{3.7}$III defect was found among 177 $-\alpha$ chromosomes. With gene frequencies of 11.5% in Vanuatu and 7.5% in Polynesia, this deletion is a relatively high-frequency population marker.

Hb J Tongariki is an α-chain mutant found in Vanuatu and coastal Papua New Guinea but not, so far, in Polynesia, Micronesia, the Philippines, or Indonesia [2, 14, 35–37]. It results from a single base mutation on the $-\alpha^{3.7}$III chromosome [10] and was found on 14% of the Ni-Vanuatu $-\alpha^{3.7}$III chromosomes. Hb J Tongariki is, thus, a lower frequency Melanesian marker.

The limited geographical distribution of the $-\alpha^{3.7}$III defect suggests that it may have had a single origin. Consistent with this idea is the observation that a crossover producing the $-\alpha^{3.7}$III deletion must occur in a much smaller (46 bp) region than that producing the $-\alpha^{3.7}$I (1,436 bp) or $-\alpha^{3.7}$II (171 bp) deletions (fig. 1). To test this hypothesis further, we have mapped seven restriction fragment length polymorphisms around the $-\alpha^{3.7}$III deletion. The finding of a β-globin gene deletion associated with a single β complex haplotype has been used to support a single origin for that mutation [38]. Similarly, our observation of a particular α haplotype on all 36 $-\alpha^{3.7}$III chromosomes tested is consistent with a single origin for this defect. Since this haplotype is present on less than 35% of normal (αα) chromosomes in Melanesia, the $-\alpha^{3.7}$III deletion would be unlikely to have occurred twice on this haplotype. In contrast, $-\alpha^{3.7}$I deletions in Jamaica are found on at least eight different haplotypes and $-\alpha^{4.2}$ deletions in Melanesia on at least two haplotypes (our unpublished data, 1984).

The triplicated α-gene counterpart of the $-\alpha^{3.7}$III deletion was not found in this survey and, if present in the Pacific, must be rare. Its absence could be explained by loss of the $\alpha\alpha\alpha^{3.7}$III chromosome in the germline cells of the individual in whom the original deletion occurred.

A single origin for a gene shared by Ni-Vanuatu and Polynesians is consistent

with evidence from other fields [1] that Polynesians migrated eastwards into the Pacific through Island Melanesia. The amplification of this thalassemia gene to its present frequency of more than 10% in Vanuatu may be due to positive selection in a malarious region. Its relatively high frequency in Polynesia of 7.5%, where malaria is absent, is more likely due to founder effects and genetic drift. It would appear that the Austronesian speakers who colonized Fiji, Tonga, and then Samoa 3,000 years ago took this gene with them. The alternative explanation of more recent backflow of this gene following a Polynesian origin is difficult to reconcile with its widespread distribution in both Polynesia and Melanesia. The occurrence of the Hb J Tongariki mutation on this chromosome only in Melanesia also argues against a "recent" introduction of the $-\alpha^{3.7}$III gene into the region.

This genetic evidence for substantial contact between the Ni-Vanuatu and the colonizers of Polynesia complements linguistic research, which places Vanuatu as the focus of the spread of Eastern Oceanic, the Austronesian language subgroup to which Polynesian languages belong [11]. Further evidence for genetic affinity between these populations is the similarity of their β^A-globin haplotypes (A. V. S. Hill et al., in preparation). In particular, we have found a new *Hin*dIII polymorphism, 11 kb 3′ to the β-globin gene, not present in other populations [39, 40], in both Vanuatu and Polynesia. It is clear from these studies that Island Melanesians share some genetic affinity with Polynesians through unique genetic markers. Hence, they are not simply Papuan stock with an adopted language. Our findings support the linguistic hypothesis of Polynesian evolution within eastern Melanesia [13].

Although the $-\alpha^{3.7}$III mutation probably arose in Island Melanesia, it may have been brought there by Austronesian speakers as they spread southeast 4,000 years ago. Hence, it will now be of particular interest to look for this deletion in island Southeast Asia where α-thalassemia is known to be present [37, 41]. In particular, the presence of the $-\alpha^{3.7}$III deletion in parts of the Philippines or Indonesia might help to define the northern part of the Austronesians' migration route.

Differentiation of the three types of $-\alpha^{3.7}$ deletion is only possible by DNA analysis. It appears likely that we have found just one of very many useful population specific markers that will be discovered with the increased use of restriction enzyme analysis. For example, we have found a frequency of γ-globin gene deletions [42], which are rare in most populations, of 20%–25% in the two larger islands in Vanuatu considered to be Polynesian "Outliers"—Futuna and Emae (D. K. Bowden et al., in preparation). These islands are believed to have been colonized by recent back migration from Polynesia [1], and analysis of single γ-gene frequencies on other Polynesian islands may indicate a precise origin. Similarly, the $-\alpha^{4.2}$ deletion with a gene frequency of 50% in north coastal Papua New Guinea represents a suitable marker for this population.

In conclusion, we have shown that most α-thalassemia in Island Melanesia and Polynesia is due to an α-globin gene mutation unique to this region. This indicates genetic affinity between these populations and supports a migration route through Melanesia for the colonizers of Polynesia.

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