as the presence or absence of fine structure in these bands. The widths of the bands in Fig. 1, A–D, and the absence of extensive fine structure are consistent with the interpretation that each band arises from a large number of phosphorus atoms in slightly different chemical environments. Thus, in the spectra in Fig. 1, A–C, the widths of the phosphonate absorption bands are about 200 hz, whereas the width of the entire spectrum of 2-aminoethylphosphonic acid would be only about 60 hz (*4*, *8*). In the orthophosphate region two broad absorption bands are resolved for each sample, which reflects the presence of at least two kinds of orthophosphate. The total bandwidth in this region is approximately 180 hz, whereas that of a simple monoester of orthophosphoric acid would be about 15 hz. In contrast, both the phosphonate and phosphate regions of the spectrum of hydrolyzed lipid (Fig. 1E) have been altered as a result of hydrolysis. The spectrum in the phosphonate region appears to be essentially that of 2-aminoethylphosphonate; however, upon expansion of this region the presence of at least two other compounds was detected (*4*). The complexity apparent in Fig. 1A has been reduced, which enables the resolution of the comparatively few remaining resonance signals. Similarly, the orthophosphate region has been simplified to that of the signal of inorganic orthophosphate (−5 ppm) and a second upfield signal which may arise from an alkyl monoester of orthophosphate. Small amounts of other compounds were observed upon expansion of this region. The increased ratio of signal to noise in the spectrum in Fig. 1E as compared to that in Fig. 1A indicates that the large number of compounds in the unhydrolyzed lipid has been reduced, thereby significantly decreasing the number of unique chemical environments for the phosphorus atoms and concentrating the absorptive power of the sample into a few resonance bands.

In summary, ^{31}P NMR can be used for the detection of biological phosphate and phosphonate and for the determination of the relative amounts of each type. From the details of the respective resonance bands, the complexity of the phosphorus-containing mixtures can be established, and, with favorable conditions, specific structures can be identified. Further, this work has shown that problems exist in the classical colorimetric procedure for the determination of phosphonate-phosphorus (see Fig. 1E) (*4*). The technique is nondestructive, and therefore may be of value in the study of the mode of binding of phosphonic acid derivatives in intact proteins and lipids.

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References and Notes

1. L. D. Quin, in *Topics in Phosphorus Chemistry*, M. Grayson and E. J. Griffith, Eds. (Interscience, New York, 1967), vol. 4, p. 23; J. S. Kittredge and E. Roberts, *Science* **164**, 37 (1969); D. Hendlin, E. O. Stapley, M. Jackson, H. Wallick, A. K. Miller, F. J. Wolf, T. W. Miller, L. Chaiet, F. M. Kahan, E. L. Foltz, H. B. Woodruff, J. M. Mata, S. Hernandez, S. Mochales, *ibid.* **166**, 122 (1969).
2. V. Mark, C. H. Dungan, M. M. Crutchfield, J. R. Van Wazer, in *Topics in Phosphorus Chemistry*, M. Grayson and E. J. Griffith, Eds. (Interscience, New York, 1967), vol. 5, p. 227. α-Substituted-alkyl-, alkynyl- and arylphosphonates may show considerably higher chemical shifts. However, compounds of these types are not known to occur in biological systems.
3. L. D. Quin, *Biochemistry* **4**, 324 (1965).
4. T. Glonek, T. O. Henderson, R. L. Hilderbrand, T. C. Myers, in preparation. The spectrum of authentic 2-aminoethylphosphonate shows seven clearly resolved signals which result from overlap of the simple first-order couplings of the phosphorus atom with the α- and β-alkyl protons.
5. D. C. White and R. H. Cox, *J. Bacteriol.* **93**, 1079 (1967).
6. J. Folch, M. Lees, G. H. Sloane Stanley, *J. Biol. Chem.* **226**, 497 (1957).
7. Medium according to J. D. Smith: 2 percent proteose-peptone, 0.2 percent glucose, 0.1 percent yeast extract, and 0.003 percent sequestrin.
8. The intrinsic resolution obtainable from the sample as determined by observation of the proton lock-signal was about 1.0 hz.
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Homozygous Hb J Tongariki: Evidence for Only One Alpha Chain Structural Locus in Melanesians

Abstract. *A high frequency of Hb J Tongariki (α 115 Ala → Asp) was found in a Kilenge village in New Britain. Heterozygotes had 45 to 50 percent of the Hb J component (determined by cellulose acetate electrophoresis). Two homozygotes for Hb J had no Hb A, suggesting that in this family only one Hb_α structural locus is present.*

Many animal species possess more than one structural locus for the α chain of hemoglobin (*1*). Schroeder *et al.* (*2*) have demonstrated fractional ratios of glycine and alanine at residue 136 of the human fetal γ chain, which they interpret as evidence for the presence of two or more loci encoding for the γ chain.

While critical genetic data have been lacking for man, Lehmann and Carrell (*3*) have suggested that there may be two *Hbα* loci in this species on the basis of the observation that many heterozygotes for α chain variants have only 20 percent of the abnormal component, half the amount found in most heterozygotes for an abnormal Hb_β gene. Thus, only one of four Hb_α genes is presumed to have undergone mutation. We now, however, present genetic and biochemical evidence that in a Melanesian population there is a single Hb_α locus.

An Hb J migrating more rapidly than Hb A at *p*H 8.6 on starch-gel electrophoresis (*4*) has been discovered in the Kilenge, a group of natives who reside west of Cape Gloucester and north of Sacsac in New Britain (*5*). Samples from 67 natives in three villages were available for study as part of a malarial survey. Twenty-two individuals were heterozygous for Hb J (Fig. 1). Fourteen persons were direct descendants of I-2, of whom nine were heterozygotes along with I-2. In addition, two individuals, a father (II-3), now deceased, and his son (III-2), had only Hb J as the major component, and completely lacked Hb A. Although one parent of each apparent homozygote was not available for study, of the six spouses marrying into this family who were studied, four were heterozygotes for the abnormal hemoglobin. Thus, it is likely that the missing parents were also heterozygotes.

On a second visit to the area, we obtained hematologic data from the relatives (Table 1). Total hemoglobin concentration and hematocrits were measured in New Guinea within 5 hours of collection on seven samples. The hemoglobin concentration of all samples was measured upon receipt several days later in Ann Arbor. He-

matocrit and hemoglobin concentration were within normal range in each person studied, including the apparently homozygous son (III-2). Minor degrees of erythrocyte morphologic abnormalities—hypochromia, anisocytosis, or poikilocytosis—were observed in six persons (Table 1). Five of these were heterozygotes for the abnormal hemoglobin. Because the hematologic abnormalities of simple α-thalassemia heterozygotes are minimal (*6*) and because parasitism and nutritional deficiencies are ubiquitous in this area, these abnormalities do not by themselves furnish critical evidence either for or against the possibility of concomitant α-thalassemia. The normal hematologic values of III-2 support the interpretation that this is a homozygote for the Hb^J gene because complete suppression of Hb A synthesis by an α-thalassemia allele should also have produced hematologic abnormalities.

Evidence that this Hb J is an α chain abnormality is supported by the presence of a second minor component in the heterozygotes, designated Hb J_2, migrating anodally to Hb A_2 on electrophoresis at *p*H 8.6, with the same relationship to Hb A_2 as Hb J has to Hb A. No Hb A_2 was seen in the two individuals homozygous for Hb J, only Hb J_2 being present.

The hemoglobin components were measured by elution from cellulose acetate electrophoresis strips (*7*). Normally the Hb A_2 comprises 2 to 3.5 percent of the total hemoglobin. In the heterozygotes, Hb J comprised 45 to 50 percent of the total (Table 1). The Hb $A_2 + J_2$ levels varied between 2.7 and 4.0 percent, slightly higher than the normal range because of the proximity of the Hb J_2 to the contaminating Hb A band. The amount of Hb J_2 was also within the normal range in III-2, the only homozygote in which the concentration could be measured. If α-thalassemia were present, a low value might be expected for Hb $A_2 + J_2$ values of heterozygotes or for the Hb J_2 in the homozygote. Fetal hemoglobin concentration, as determined by the method of alkali denaturation, were all normal (*8*). Neither Hb H nor Hb Bart's was present on starch-gel or cellulose acetate electrophoresis.

These electrophoretic data, when considered together with the above hematologic data, argue against the possibility that II-3 and III-2 are heterozygotes for both $Hb_\alpha{}^J$ and α-thalassemia. This further supports the hypothesis that they are homozygotes for $Hb_\alpha{}^J$. The absence of Hb A in their red cells therefore indicates that only one Hb_α structural locus is present.

Whole hemoglobin was treated with chloromercuribenzoate at *p*H 6.0 (*9*), which causes cleavage of α and β chains; the chains were separated by starch-gel electrophoresis. The results indicated that the abnormality resides in the α chain. In the homozygotes all α chains are abnormal (*10*).

Globin was prepared (*11*) from the hemoglobin of II-3 and chromatographed on a carboxymethyl cellulose column with a linear gradient from 0.005 to 0.03*M* phosphate, *p*H 6.7, containing 0.43*M* mercaptoethanol and 8*M* urea (*12*). The abnormal α chains separated by this method were dialyzed against distilled water, lyophilized, and subsequently subjected to tryptic digestion in 0.4*M* ammonium bicarbonate with 0.25 percent trypsin for 90 minutes at 37°C. Descending chromatography was carried out in either of two chromatographic solvent systems: isoamyl alcohol, water, pyridine (35 : 30 : 35) (*13*) or butanol, acetic acid, pyridine, and water (90 : 18 : 60 : 72) (*14*). Two electrophoresis buffers at either *p*H 3.6 (*15*) or *p*H 6.4 (*16*)

Table 1. Hematologic values and electrophoretic distribution (%) of hemoglobins in a family with Hb J Tongariki. N, Normal morphology; H, slight hypochromia; A, slight anisocytosis; P, slight poikilocytosis.

Subject	Hb* (g/100 ml blood)	Hematocrit	Morphology	Hb electrophoresis (%)				
				Hb A	Hb J	Hb A_2	Hb J_2	Hb F†
II-4	13.7/11.7	39	A,P	55.6	40.6	1.7	1.4	0.7
II-5	13.7		N	53.5	42.6	1.6	1.5	0.8
II-6	11.8/11.2	38	H,A	52.3	44.4	1.6	1.1	0.7
II-8	15.4/14.1	43	N	96.1		2.8		1.2
II-9	13.0		N	47.9	47.1	1.6	1.8	1.6
III-1	18.3		A	49.9	46.5	1.4	1.6	0.6
III-2	14.8/12.2	40	N		96.2		2.3	1.5
III-3	13.5/11.7	43	H,A	41.1	55.0	1.2	1.9	0.8
III-4	15.0		A	96.7		2.2		1.1
III-5	12.5		N	52.9	43.3	1.6	1.5	0.7
III-6	12.5		A,P	96.5		2.6		0.9
III-7	12.6		N	96.3		3.2		0.6
III-8	12.9		A,P	53.2	42.3	1.6	1.4	1.6
III-9	12.3/12.2	39	N	52.2	42.1	1.7	2.3	1.6
III-10	13.1		H,A	50.6	44.9	1.7	2.3	0.6
IV-2	13.9/12.2	39	N	96.5		2.6		0.9

* Performed in Ann Arbor or New Guinea or both. † Alkali denaturation.

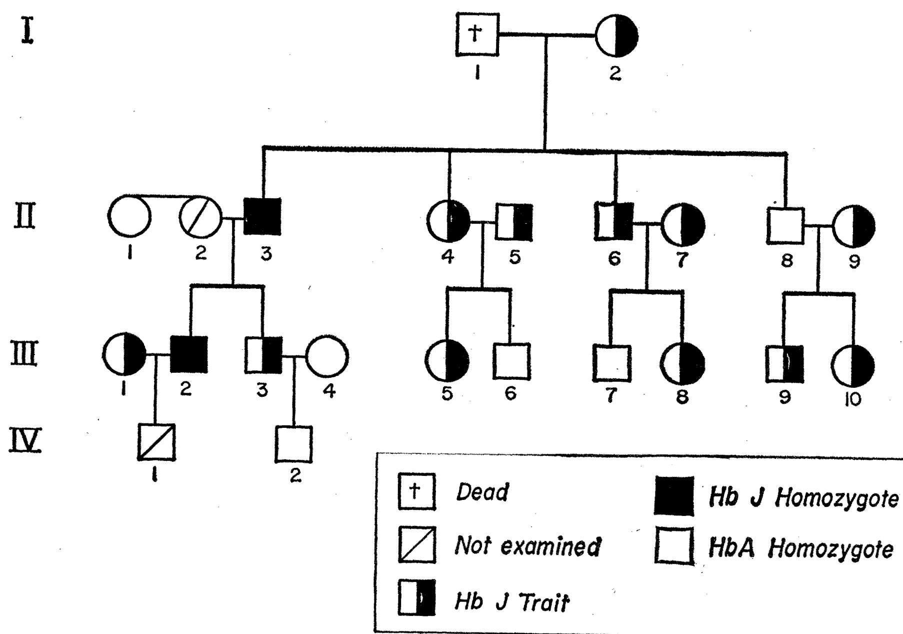

Fig. 1. Pedigree of Kilenge family with Hb J.

Table 2. Amino acid analysis of chymotryptic peptide 3 of α^J-Tongariki and α^A and Edman degradation of α^J-Tongariki.

Amino acid	Hb A		Hb J			
	α^A (rel. mole)	Integer No.	α^J (rel. mole)	Integer No.	Edman	
					Turn-1	Turn-2
Aspartic acid	0.10	0	1.22	1	1.22	1.13
Glutamic acid*	1.00	1	1.00	1	1.00	1.00
Proline	1.04	1	0.99	1	0.78	0.61
Alanine	2.41†	3	1.80	2	*1.16*	*0.58*
Leucine	1.05	1	1.09	1	1.19	0.83
Phenylalanine	1.23	1	0.79	1	0.84	0.73

* Glutamic acid is the standard for computation. † Low value due either to analytical error or degradation during acid hydrolysis.

were used for high-voltage electrophoresis. In no instance was any displaced peptide detected.

The remaining insoluble core was washed two times with an ammonium bicarbonate solution brought to *p*H 6.5 with 0.5*M* acetic acid (*17*). The washed core was suspended in 0.4*M* ammonium bicarbonate, *p*H 8.0, and digested with 0.25 percent chymotrypsin for 3 hours at 37°C. The chymotryptic peptide maps resulting from the second chromatographic and electrophoretic systems above contained one abnormal peptide which was displaced anodally. This peptide was isolated from the chromatograph, and by elution from Dowex-50 in a pyridine-acetate gradient, *p*H 4.8 to 5.6 (*18*). Acid hydrolysis was performed at 110°C for 24 hours in constant-boiling HCl, after which the amino acids were determined (Beckman Model 120 C automatic amino acid analyzer). The peptide contained two alanine, one aspartic acid, one proline, one glutamic acid, one phenylalanine, and one histidine residue (Table 2). The abnormal peptide was the third chymotryptic fragment of the 12th tryptic peptide, from residues 110 to 117. This fragment normally contains three alanine residues in the sequence Ala-Ala-His-Pro-Ala-Glu-Phe. To determine which of the three alanine residues had been replaced by aspartic acid, a subtractive Edman degradation was done (*19*). This showed that the first two alanine residues were present, indicating that the third alanine was replaced by aspartic acid (Table 2). This substitution, α 115 Ala → Asp is the same as in Hb J Tongariki, previously described by Gajdusek *et al.* (*20*).

Thus, the Kilenge in New Britain and the group studied in Tongariki, an island in the Shepherd group of the New Hebrides, have the same Hb J. Although these groups are 1920 km apart, they are both Melanesian. The proportion of individuals with Hb J Tongariki in both groups is large, 24 out of 67 in this study and 17 out of 228 in Tongariki (*20*). Since these are small endogamous communities it is difficult to be certain whether these are truly random samples and therefore whether the above-mentioned figures represent gene frequencies. Suffice it to say that the frequency of the gene in this area is high.

A number of alternative hypotheses must be considered in evaluating this family. First, the possibility that III-2 has α-thalassemia-Hb J disease is excluded, with reasonably high probability, by the absence of anemia or morphologic abnormality, the normal proportion of Hb J_2, and the absence of Hb H or Hb Bart's. Moreover, the consistency of the proportion of Hb J, 45 to 50 percent, in all heterozygotes examined excludes the possibility that some of them also have an α-thalassemia gene.

Second, it is possible that two α chain structural loci are, in fact, present, both of which have sustained mutational changes to Hb J and for which II-3 and III-2 are then homozygotes. If so, the hemoglobin peptide patterns and the structure studies indicate that only one type of α^J chain is present. This would require that an identical mutation had to occur spontaneously in two separate loci or that a $Hb_\alpha{}^J$ allele had to duplicate, both of which are improbable.

Third, the electrophoretic and hematologic findings and the prevalence of heterozygotes in the population support the notion that II-3 and III-2 are homozygotes for the structural mutant. The absence of Hb A suggests that only one Hb_α locus is present in this population. We believe that the evidence justifies accepting the third hypothesis. This does not preclude the possible of two Hb_α loci in other families or populations, especially in those where the abnormal component is only 20 percent of the total hemoglobin. In fact, Brimhall *et al.* (*21*) have described a Hungarian family in which individuals possess two Hb_α variants in addition to Hb A. We are also studying a family in which a Caucasian mother and child have two rapidly migrating hemoglobins with abnormal α chains in addition to Hb A. Thus, population heterogeneity for the number of structural loci for α chains is a possibility.

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References and Notes

1. T. H. J. Huisman, J. B. Wilson, H. R. Adams, *Arch. Biochem. Biophys.* **121**, 528 (1967); T. H. J. Huisman, A. M. Dozy, M. H. Blunt, F. A. Hayes, *ibid.* **127**, 711 (1968); G. Schapira, J. C. Dreyfus, N. Maleknia, *Biochem. Biophys. Res. Commun.* **32**, 558 (1968); J. V. Kilmartin and J. B. Clegg, *Nature* **213**, 269 (1967); K. Hilse and R. A. Popp, *Proc. Nat. Acad. Sci. U.S.* **61**, 930 (1968).
2. W. A. Schroeder, T. H. J. Huisman, J. R. Shelton, J. B. Shelton, E. F. Kleihauer, A. M. J. Dozy, B. Roberson, *Proc. Nat. Acad. Sci. U.S.* **60**, 537 (1968).
3. H. Lehmann and R. W. Carrell, *Brit. Med. J.* **4**, 748 (1968).
4. O. Smithies, *Biochem. J.* **71**, 585 (1959).
5. P. B. Booth, A. P. Vines, J. J. Saave, *Archaeol. Phys. Anthropol. Oceania* **4**, 115 (1969).
6. M. Pornpatkul, P. Wasi, S. Na-Nakorn, *J. Med. Ass. Thailand* **52**, 801 (1969).
7. D. L. Rucknagel, unpublished method.
8. K. Singer, A. I. Chernoff, L. Singer, *Blood* **6**, 413 (1951).
9. M. A. Rosemeyer and E. R. Huehns, *J. Mol. Biol.* **25**, 253 (1967).
10. D. L. Rucknagel, *Anal. Chem.* **40**, 36A (1968).
11. A. Rossi-Fanelli, E. Antonini, A. Caputo, *Biochim. Biophys. Acta* **28**, 221 (1958).
12. J. B. Clegg, M. A. Naughton, D. J. Weatherall, *Nature* **207**, 945 (1965).
13. R. L. Hill, R. T. Swenson, H. C. Schwartz, *J. Biol. Chem.* **235**, 3182 (1960).
14. C. Baglioni, *Biochim. Biophys. Acta* **48**, 945 (1961).
15. A. M. Katz, W. J. Dreyer, C. B. Anfinsen, *J. Biol. Chem.* **234**, 2897 (1959).
16. V. M. Ingram, *Biochim. Biophys. Acta* **28**, 539 (1958).
17. R. W. Carrell and D. Irvine, *Biochim. Biophys. Acta* **154**, 78 (1968).
18. W. Konigsberg and R. J. Hill, *J. Biol. Chem.* **237**, 2547 (1962).
19. W. T. Shearer, R. A. Bradshaw, F. R. N. Gurd, T. Peters, *ibid.* **242**, 5451 (1967).
20. D. C. Gajdusek, J. Guiart, R. L. Kirk, R. W. Carrell, D. Irvine, P. A. M. Kynoch, H. Lehmann, *J. Med. Genet.* **4**, 1 (1967).
21. R. Brimhall, S. Hollan, R. T. Jones, R. D. Koler, J. G. Szelenyi, *Clin. Res.* **18**, 184 (1970).

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