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Point substitutions in albumin genetic variants from Asia

(bisalbuminemia/alloalbumins/genetic polymorphism/population markers/point mutations)

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ABSTRACT **Despite their rarity and physiologically neutral character, more inherited structural variants of serum albumin (alloalbumins) are known than for any other human protein except hemoglobin. Including three previously unreported examples described here, we have identified 13 different point substitutions in alloalbumins of Japanese origin. Of these only albumin B and two proalbumins have been reported in other ethnic groups, and these are the most common variants of European origin. Some alloalbumins of Asiatic origin, but not yet identified in Japanese, are present in diverse ethnic groups. An alloalbumin found in indigenes of New Guinea (lysine → asparagine at position 313) is also present in Caucasians of various European descents. Albumin Lambadi, occurring in a tribal group in south India, has a mutation (glutamic acid → lysine at position 501) also found as a rare variant in individuals of diverse ethnic origin resident on four continents. These results suggest that some alloalbumins with the same substitution may have originated by independent mutations in various populations. This, together with the apparent clustering of point substitutions in the protein structure, may reflect hypermutability of the albumin gene.**

Because of recent studies (1–17) more heritable structural variants of serum albumin (alloalbumins) are known than for any other human protein except hemoglobin. However, unlike the situation with hemoglobin variants, no adverse effect on molecular function has been attributed to alloalbumins. Alloalbuminemia is rare and has a cumulative frequency of only 1:3000 to 1:10,000 in most populations (18–25); thus, it is usually expressed in a heterozygous fashion. More than 100 alloalbumins that have been given geographical or tribal names have been identified by genetic screening (18–20), by blood donor surveys (21), or by clinical electrophoresis (22, 23), and at least 20 different sites of structural change have been determined (1–17). Four types of alloalbumins have been identified: (*i*) a series of single-point mutants (1–7, 12–14, 16), (*ii*) several chain-termination mutants (10, 11), (*iii*) proalbumins (variants that retain a basic amino-terminal hexapeptide because of a mutation in the Arg-Arg propeptide sequence required for post-transcriptional processing) (8, 15), and (*iv*) arginyl-albumin, a variant that begins with arginine followed by a polypeptide sequence of the usual length (585 amino acid residues) (ref. 17; C. B. Laurell and F.W.P., unpublished results).

Recently we have undertaken a series of collaborative studies on alloalbumins from populations throughout the world (1–3, 5, 6). Our objectives have been to determine the structural changes and to correlate these with the molecular properties of albumin and with such genetic characteristics as the site and type of mutation, heritability, and frequency. In this paper we report structural studies on alloalbumins from eight unrelated Japanese residents of Japan or Hawaii and also on one specimen representative of certain New Guinea indigenes (19) and on an alloalbumin identified in the Lambadi tribal population in India (24). We also report the substitution in albumin Manaus I from Brazil (20) because it is the same as that in albumin Lambadi and several other alloalbumins of Asiatic origin (3).

MATERIALS AND METHODS

Sources of Sera. In this study all the sera from Japanese resident in Japan were identified as bisalbuminemic during routine clinical electrophoresis. The sera (generally only 1–2 ml) were from heterozygous individuals and contained approximately equal proportions of the alloalbumin and of normal (common) albumin (called albumin A). The Japanese sera were received from several sources listed in the text, and the alloalbumins are designated by place of origin (city or region). All sera from outside Japan were collected in the course of genetic or epidemiological surveys described in the text.

Methods and Strategy for Structural Study. Because of the number and variety of the specimens reported here and the fact that most of the methods have been published in detail (1–9), only a summary of the methods is given for each individual albumin. The general procedure consists of seven steps and was modified as required in each case. The steps are as follows: (*i*) cellulose acetate (Microzone) electrophoresis at pH 8.6 of the serum or the purified albumin; (*ii*) purification by HPLC of the total albumin (normal albumin A plus variant) by a method later referred to as the two-step procedure (4), reduction and carboxymethylation of the purified albumin and cleavage with CNBr (3, 4, 9); (*iii*) analytical isoelectric focusing of the CNBr digest to identify the CNBr fragment in which the substitution was localized (3–8); (*iv*) HPLC peptide mapping on a preparative scale to purify the variant CNBr fragment (1–4); (*v*) HPLC peptide mapping of a tryptic or *Staphylococcus aureus* V8 protease digest of the purified CNBr fragment (1–5); (*vi*) amino acid analysis of the variant peptide(s) with the Beckman model 121M amino acid analyzer; and (*vii*) automated sequence determination with the Beckman model 890C sequencer (9) or with the Applied Biosystems model 477B sequencer. After K.A. returned to Japan he used the Jeol model JLC-200A amino acid analyzer, and he determined the sequence of some peptides by manual Edman degradation (26). Tryptic peptides are designated T and V8 protease peptides are designated S, and both types are numbered consecutively in their predicted order in the amino acid sequence (9).

The two-step procedure for purification of albumin consists of a combination of anion-exchange HPLC on a TSK-DEAE 5PW column (7.5 × 75 mm; Anspec, Ann Arbor, MI) and gel filtration with a TSK 4000SW column (7.5 × 600 mm; Anspec) under conditions described elsewhere (3, 4). The

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HPLC purification of CNBr fragments and of the tryptic and V8 peptides was done with a Vydac C_{18} column (4.6 × 250 mm; Anspec) as described (1–5). As indicated in the text, some alloalbumins were compared with reference standards by several collaborators, who used starch-gel electrophoresis in a number of buffer systems (2, 6, 19, 21). The amino acid sequence of albumin A and the codons at each position are based on the genomic sequence of Minghetti *et al.* (27).

RESULTS AND DISCUSSION

Structural Studies of Japanese Alloalbumins with Point Substitutions. Three previously unreported point substitutions were identified in Japanese individuals living in various cities in Japan. As described below, these are 119 Glu → Lys in albumin Nagoya, 563 Asp → Asn in albumins Fukuoka-1 and Ube-1, and 565 Glu → Lys in albumin Osaka-1. Several other alloalbumins were found to have previously reported substitutions. Table 1 gives a summary of single amino acid exchanges reported here and elsewhere for alloalbumins of various ethnic and geographic origins. Fig. 1 gives the amino acid sequence of human serum albumin and identifies the location of the substitutions.

Albumin Nagoya. (Serum H160007.) This alloalbumin was discovered in a Japanese family and was present in five members, including the index case (29). Serum was provided by Makoto Sasaki (Department of Biochemistry, School of Medicine, Nagoya City University, Nagoya, Japan). In clinical electrophoresis at pH 8.6 the variant had a slow mobility (+2 net charge relative to normal albumin; denoted slow, +2). Chiyoko Satoh (Department of Genetics, Radiation Effects Research Foundation, Hiroshima; personal communication) used starch gel electrophoresis at pH 4.05 and pH 8.6 (5, 6) to compare albumin Nagoya with several reference albumins we supplied and with the five alloalbumins discovered in Nagasaki and Hiroshima by the Biochemical Genetics Study of the Radiation Effects Research Foundation. These are designated Nagasaki-1, -2, and -3, and Hiroshima-1 and -2 (5, 6). She reported that the six Japanese variants showed different combinations of mobilities in the two buffer systems and also were different from albumin B and proalbumin Pollibauer (1, 5).

The two-step HPLC procedure was used to purify the total albumin (albumins A and Nagoya denoted A/Nagoya) from serum H160007. Analytical isoelectric focusing of the CNBr digest did not reveal an abnormal pattern, but the HPLC peptide profile of the CNBr digest suggested the substitution might be in the small fragment CB2 (residues 88–123; see Fig. 1). The tryptic peptide profile of purified CB2 was obtained by HPLC on a Vydac C_{18} column and the amino acid compositions of the separated peptides were determined. This revealed the presence of normal peptide T14 (residues 115–123 of albumin A) and of two new peptides, T14A* and T14B, arising from the point substitution in albumin Nagoya. Peptide T14 from CB2 has the sequence Leu-Val-Arg-Pro-Glu-Val-Asp-Val-Met (see Fig. 1). Amino acid analysis of peptides T14A* and T14B followed by manual sequence analysis gave the sequences Leu-Val-Arg-Pro-Lys and Val-Asp-Val-Met, respectively, thus indicating a previously unreported substitution 119 Glu → Lys. This was confirmed by automated sequence analysis. This result fits with the slow (+2) mobility of albumin Nagoya and can be explained by a point mutation in the codon for residue 119, GAG → AAG.

Albumins Fukuoka-1 and Ube-1. Although found in unrelated individuals from different cities in Japan, these alloalbumins are discussed together because structural study was done concurrently and showed that they had the same substitution (563 Asp → Asn). Both alloalbumins had a slow (+1) mobility at pH 8.6. Serum containing Fukuoka-1 was obtained from Shunsuke Migita (Cancer Research Institute,

Table 1. Examples of proven point substitutions in alloalbumins of diverse ethnic origins

Substitution	Japanese	Other Asian	European descent	Amerindian
−2 Arg → His	Fukuoka-2 (1)	Taipei (8)	Lille (12)	
−1 Arg → Gln	Honolulu-2* (1, 5)		Christchurch (1, 8, 15)	
−1 Arg → Pro	Honolulu-1* (8)			
1 Asp → Val			Bremen†	
3 His → Gln	Nagasaki-3 (6)			
114 Arg → Gly				Yanomama-2 (6)
119 Glu → Lys	Nagoya*			
269 Asp → Gly	Nagasaki-1 (5)			
313 Lys → Asn		New Guinea*	Tagliacozzo (1, 4, 12, 16)	
320 Ala → Thr			Redhill (17)	
321 Glu → Lys			Roma (12)	
354 Glu → Lys	Hiroshima-1 (5)			
358 Glu → Lys			Porto Alegre I (2)	
365 Asp → His			Parklands (14)	
372 Lys → Glu		Mersin (7)		Naskapi (7)
375 Asp → Asn	Nagasaki-2 (6)			
376 Glu → Lys	Tochigi (5)			
382 Glu → Lys	Hiroshima-2 (5)			
501 Glu → Lys		Lambadi* (3)	Manaus I* (2)	
541 Lys → Glu				Maku (2, 6)
550 Asp → Gly				Mexico (7)
563 Asp → Asn	Fukuoka-1*			
565 Glu → Lys	Osaka-1*			
570 Glu → Lys	Osaka-2* (1, 5)	Phnom Phen (1)	B (1, 13)	
573 Lys → Glu			Gent (12)	
574 Lys → Asn			Vanves (12)	

Alloalbumins are denoted by geographical or tribal name, and references are given in parentheses. Listing of two or more references indicates that variants with different names but the same substitution have been reported. In all cases the single amino acid substitution listed is attributable to a single base change in the corresponding codon.

*Described in this article. Note Manaus I is from a biracial person.

†Unpublished work of K.A., J.M., and F.W.P.

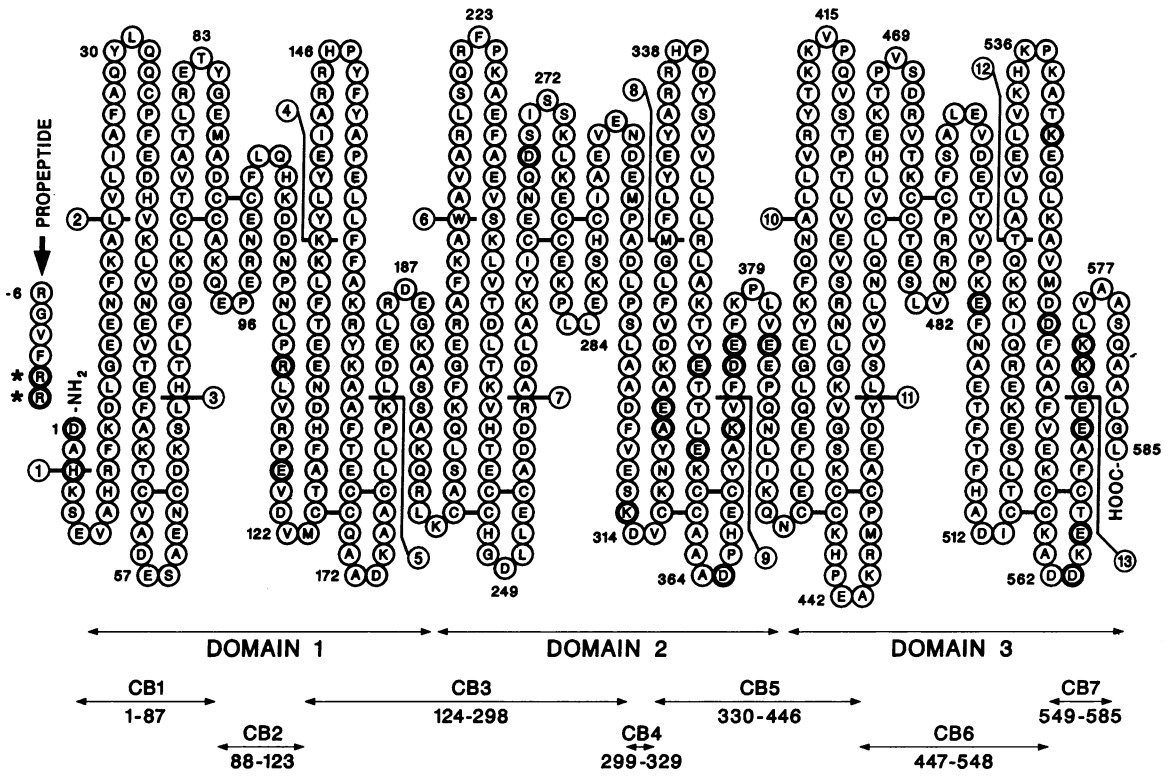

FIG. 1. Amino acid sequence of human serum albumin. The locations of substitutions in genetic variants are identified by bold circles. The protein sequence is given in the one-letter code and is based on the genomic sequence analysis of Minghetti *et al.* (27). The layout of the polypeptide chain, including the domain structure and disulfide bonds, is according to Brown and Shockley (28). CB1–CB7 refer to CNBr fragments, for which the positions of the amino acid residues are given. The location of the introns (designated 1–13, circled) is from Minghetti *et al.* (27). The normal hexapeptide propeptide (Arg-Gly-Val-Phe-Arg-Arg, residues −6 to −1) is illustrated. Substitutions in the propeptide and in the mature albumin are summarized in Table 1 and references are given. Asterisks denote positions where more than one substitution has been established.

Kanazawa University, Kanazawa), who had received it from Kazuo Ookouchi (Department of Clinical Chemistry, Kyushu University, Fukuoka). S. Migita also provided the Ube-1 serum, which he had received from Kyuukoh Miyaji (Department of Clinical Pathology, Yamaguchi University, Yamaguchi). In both cases the albumin was purified by HPLC by the two-step procedure. Analysis by isoelectric focusing indicated that in both specimens the substitution was in CNBr fragment CB7 (residues 549–585; see Fig. 1). The variant CB7 fragment was purified by HPLC and was analyzed directly in the Applied Biosystems sequencer. In the case of Ube-1 the entire variant CB7 fragment was sequenced, but only the first 19 residues were analyzed for the variant CB7 of Fukuoka-1. In both specimens the previously unreported substitution 563 Asp → Asn was clearly demonstrated. This correlates with the slow (+1) mobility at pH 8.6 and is attributable to a point mutation of the codon GAT to AAT. Although the two subjects were apparently unrelated, it should be noted that Fukuoka and Ube are less than 200 km apart. Hence, the mutation may have arisen in a common ancestor.

Albumins Osaka-1 and Osaka-2. Two bisalbuminemic sera each having a slow-mobility (+2) component in clinical electrophoresis at pH 8.6 were obtained by A.S. from unrelated patients in hospitals in Osaka Prefecture. The variants were designated Osaka-1 and Osaka-2. Purification of Osaka-1 and Osaka-2 was done by the two-step method and was followed by reduction and cleavage with CNBr. In both cases CB7 was identified as the variant fragment by use of isoelectric focusing, and the variant CB7 was isolated by HPLC and digested with V8 protease. In the case of Osaka-1 the HPLC peptide map of the V8 digest revealed a new peak. Amino acid analysis confirmed by automated sequence analysis proved that the variant peptide (S57-58-59A*) had a previously unreported substitution, 565 Glu → Lys. The peptide sequence, which corresponded to residues 557–571, was Lys-Cys-Cys-Lys-Ala-Asp-Asp-Lys-Lys-Thr-Cys-Phe-Ala-Glu-(Glu). The first nine residues correspond to V8 peptide S57 except for the 565 Glu → Lys substitution that prevents action of the V8 protease. Partial cleavage appeared to occur after each of the last two glutamic residues, resulting in the composite peptide S57-58-59A*. The 565 Glu → Lys substitution accounts for the slow (+2) mobility and is attributable to a point mutation in the codon GAG to AAG.

On the other hand, the V8 digest of Osaka-2 yielded a variant peptide that automated sequence analysis showed to be S58-59*. This has the sequence Thr-Cys-Phe-Ala-Lys-Glu, corresponding to residues 566–571, and results from the substitution 570 Glu → Lys, which is characteristic of albumin B (1).

Albumin Nagano. This serum was from an individual born in Nagano Prefecture northwest of Tokyo and was provided by I. Sakurabayashi (Department of Clinical Pathology, Jichi Medical School, Tochigi-ken). As shown by the procedure described above for Osaka-2 and detailed elsewhere (1), this slow (+2) variant also was an albumin of the B type with the substitution 570 Glu → Lys. In this case also, the V8 peptide S58-59* was isolated, and analysis with the Applied Biosystems sequencer showed that it had the sequence Thr-Cys-Phe-Ala-Lys-Glu. Including Osaka-2 described above, this is the fifth instance of an albumin B in Japanese for which we have established the change in structure. Four of these came from an area not far from Tokyo, in the central part of the island of Honshu. Although rare, this is also one of the most common albumin variants in Caucasians, and the struc-

tural change has now been proven in at least 15 unrelated individuals of varied ethnic and geographical origins (1).

Bisalbuminemia in Hawaii-Japanese. In the course of the Japan–Hawaii Cancer Study, Fukunaga and Glober (22) detected two instances of bisalbuminemia during routine Microzone electrophoresis on cellulose acetate at pH 8.6 of sera from Japanese living in Hawaii. We received the sera from Abraham N. Y. Nomura (Kuakini Medical Center, Honolulu). We designated them Honolulu-1 and Honolulu-2. These two were the only instances of alloalbumins out of 12,500 serum specimens electrophoresed from patients at Kuakini Hospital in the period from January 1969 to December 1972 (F. H. Fukunaga, personal communication). In both cases family studies showed the albumin variant was transmitted as an autosomal codominant trait. As reported below, both variants were proalbumins and migrated as slow (+2).

Proalbumin Honolulu-1. (Serum HHP#A06940I/03552040309, case 2 of ref. 22.) This slow (+2) variant did not behave as a proalbumin after limited tryptic cleavage followed by Microzone electrophoresis (8). The variant albumin was purified by the two-step procedure. Isoelectric focusing of a CNBr digest of it implicated CB1 as the site of substitution. Analysis of the intact variant albumin in the Applied Biosystems sequencer gave the sequence (Arg)-Gly-Val-Phe-Arg-Pro-Asp-Ala-His-Lys. This established the substitution −1 Arg → Pro, the same as in proalbumin Takefu (8). The −1 Arg → Pro exchange prevents the action of trypsin and of the hepatic proalbumin convertase. This substitution accords with the mobility change and is attributable to mutation of the codon CGA to CCA. This is only the second instance of the finding of a proalbumin of the Takefu type, and both cases were in Japanese. The parents of the propositus migrated to Hawaii from Fukushima Prefecture in Japan, which is north of Tokyo and about 300 km from Takefu.

Proalbumin Honolulu-2. (Serum HHP#A09027F/11190021501, case 1 of ref. 22.) This alloalbumin was readily shown to be a proalbumin of the Christchurch type (−1 Arg → Gln) (8, 15). The slow (+2) mobility and the susceptibility of the variant to limited tryptic digestion indicated it was a proalbumin. The variant protein was purified by the two-step procedure and was analyzed (without prior carboxymethylation) for 10 cycles in the Beckman sequenator. This gave an unambiguous sequence for a proalbumin with the substitution −1 Arg → Gln: Arg-Gly-Val-Phe-Arg-Gln-Asp-Ala-His-Lys. This substitution explains the slow (+2) mobility and is attributable to the point mutation CGA → CAA. The parents of the propositus for Honolulu-2 migrated from Kumamoto Prefecture, which is on the same island as Fukuoka. We conclude that these Asian examples of proalbumin Christchurch may have arisen from a common ancestor in Japan, but they represent a mutation independent of that which led to the Caucasian proalbumins of the Christchurch type. On the other hand, proalbumin Takefu has thus far been reported only for Japanese.

Bisalbuminemia in India and New Guinea. Although bisalbuminemia has been reported in populations in India and nearby countries (18, 19, 24) and also in New Guinea indigenes (19, 25), structural study of alloalbumins from defined population groups in these regions has not been published. Two examples from anthropologically distinct tribal groups in India and New Guinea are described below.

Albumin Lambadi and Albumin Manaus I (501 Glu → Lys). In 1980 Walter *et al.* (24) reported an apparently new, slow-moving albumin variant occurring in the Lambadi tribal group that lived in the Khamman district of Andra Pradesh in southern India but originated in northern India. Recently we received serum specimens from 12 individuals of the Lambadi tribal group that had been freshly collected by P. Veerraju (Department of Genetics, Andhra University, Waltair, India). One of the sera exhibited bisalbuminemia with a variant of the slow (+2) type.

The total albumin (A/Lambadi) in the serum containing the variant was purified by the two-step procedure. Isoelectric focusing of a CNBr digest indicated the substitution was in CNBr fragment CB6 (residues 447–548). The variant fragment was purified by HPLC on a Vydac C_{18} column and digested with *S. aureus* V8 protease. The HPLC profile on Vydac C_{18} of the V8 protease digest revealed the presence of the variant peptide denoted S50-51* that we had found for albumin Vancouver (see figure 5 of ref. 3 for the HPLC peptide profile and figure 6 for the amino acid sequence). The S50-51* peptide was rechromatographed on Vydac C_{18} and submitted to amino acid analysis and to manual sequence analysis. The results, which were confirmed by automated sequence analysis, established the amino acid sequence of S50-S51* as Thr-Tyr-Val-Pro-Lys-Lys-Phe-Asn-Ala-Glu-. Except for the substitution 501 Glu → Lys, this is the same as the sequence of peptide S50-51 for residues 496–505 in albumin A (see Fig. 1). We had earlier identified the same substitution in three Asiatic variants—i.e., albumins Vancouver, Birmingham, and Adana (3) and also in albumin Porto Alegre II from Brazil (2). The substitution 501 Glu → Lys explains the slow mobility of albumin Lambadi and accords with a point mutation in the codon GAG to AAG.

Serum with albumin Manaus I (20) was received from F. M. Salzano (Department of Genetics, Federal University of Rio Grande do Sul, Porto Alegre, Brazil). This variant had been discovered in an individual of mixed race from the city of Manaus in the state of Amazonas in northwestern Brazil and was present in other members of the family (20). It was difficult to differentiate Manaus I electrophoretically from the Brazilian variant Porto Alegre II and from albumin Vancouver, which was of Asiatic origin (see figure 1 of ref. 2). Structural study of Manaus I was done by the procedures summarized above and described elsewhere in detail (2, 3). The results were identical to those found for albumin Lambadi. That is, the substitution was localized to CB6, and the variant peptide S50-51* showed the substitution 501 Glu → Lys, which was confirmed by automated sequence analysis.

Identity of Albumins Lambadi, Manaus I, Porto Alegre II, Vancouver, Birmingham, and Adana. This study, together with earlier work (2, 3), has shown that the same amino acid substitution (501 Glu → Lys) is present in six named alloalbumins identified in unrelated individuals of diverse ethnic origin and living on four different continents. Four of these clearly have an Asiatic origin: Adana in Turkey (3), Lambadi in India, and Birmingham and Vancouver present in unrelated families that emigrated from the Punjab and northern India, respectively (3, 9). Two are from individuals of mixed race in Brazil. Porto Alegre II is from a Brazilian of African/Caucasian (Portuguese) ancestry (2). The racial background of the carriers of Manaus I is unclear. The other genetic markers cannot exclude an Asiatic (through Amerindian) ancestry (F. M. Salzano, personal communication). The four variants that clearly have an Asiatic origin may have arisen from an early common ancestor in the vicinity of northern India. However, the Brazilian variants appear to have had an independent origin.

Albumin New Guinea. This slow (+1) variant is present in sera that had been collected in a genetic and anthropological study of New Guinea indigenes (19, 25). The variant was designated New Guinea although it could not be distinguished in a series of electrophoretic systems (25) from albumin Reading present in an English family (23) or from a variant (Westcott) in a U.S. English/Amerindian family that we later studied and call albumin Cooperstown (4). Fifteen examples of the New Guinea variant were found among 2809 indigenes in various locations (occurrence of 0.5%) (25). Two serum specimens from Kar Kar Island (northeast of Papua, 4°38′ S,

145°58′ E) were provided by Philip G. Board (Human Genetics Group, the John Curtin School of Medical Research, The Australian National University, Canberra City). These came from the collection of the Human Biology Department in conjunction with the International Biological Program Study on Kar Kar Island. Our work was done on a single serum specimen (KKC5380).

Our structural study was done much as described by Huss *et al.* (4) for albumin Cooperstown. In summary, isoelectric focusing of the CNBr digest showed that the substitution was present in CB4 (residues 299–329, see Fig. 1). A variant of peptide S34 was isolated by HPLC from a V8 protease digest of a mixture of the variant CB4 fragment and the normal CB7. Amino acid analysis of this peptide followed by analysis in the Applied Biosystems sequencer showed that it had the sequence for positions 312–321 as follows: Ser-Asn-Asp-Val-Cys-Lys-Asn-Tyr-Ala-Glu. In this case the lysine at position 313 is replaced by asparagine (313 Lys → Asn).

The substitution found for albumin New Guinea has also been reported for a series of albumin variants from diverse sources (4, 12, 16, 21). This variant is called the Reading type (4, 23), and is rare, but it is one of the more frequent European variants (21, 23). The spread of this mutation of the albumin gene among carriers of European descent might possibly be attributed to the Roman migrations, but in such case the mutation would have occurred millennia ago. However, the appearance in New Guinea indigenes must signal an independent genetic event.

Alloalbumins—Markers of Migration or Independent Mutation? We have previously documented our findings that identical structural changes may be present in some alloalbumins from diverse and widely separated ethnic groups (1–8). Here we report additional examples where the same point mutation in the albumin gene has apparently occurred independently in populations that are genetically distinct and geographically distant. Table 1 summarizes the present and previous results from our laboratory and other sources. Certain examples are discussed in the text. The four most common point substitutions in albumins from individuals of different European origins are −2 Arg → His and −1 Arg → Gln (present in two proalbumin types) and also 313 Lys → Asn and 570 Glu → Lys. Their distribution in European nationalities might plausibly be attributed to early migrations. However, the same four variants are also present in Asian populations that are unusually homogeneous ethnically. Thus, the most common alloalbumins in Europeans (combined frequency only ≈1:3000) (21, 22) appear to have originated by independent mutations in certain Asian populations. However, other variants appear to be associated with, if not confined to, particular ethnic groups; for example, none of the Amerindian variants have yet been identified in other races. Altogether we have established 13 different point substitutions in alloalbumins from Japanese (Table 1), and 10 of these have not yet been identified in other races.

Distribution of Point Substitutions in the Albumin Structure. We have previously called attention to the apparent clustering of point substitutions in three short segments of the albumin molecule: (*i*) the propeptide and the amino terminus, (*ii*) the connecting segment at the end of the second domain, and (*iii*) the carboxyl-terminal tailpiece, for which two previously unreported replacements have been added in this work (563 Asp → Asn and 565 Glu → Lys). Fig. 1 shows that the latter two segments appear to be homologous in position in the second and third domains. Progress is being made in elucidation of the crystallographic structure of human serum albumin (30). When a higher-resolution structure is determined, it will be of much interest to locate in it the point substitutions listed in Table 1. Of even greater interest is the question whether the clustering of point substitutions in the protein structure reflects hypermutability of segments of the albumin gene.

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