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Point substitutions in Japanese alloalbumins

(bisalbuminemia/albumin genetic variants/genetic polymorphism/population markers/point mutation)

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ABSTRACT **We have completed the structural study of five rare types of inherited albumin variants (alloalbumins) discovered in the Biochemical Genetics Study of 15,581 unrelated children in Hiroshima and Nagasaki. We have also identified the structural change in five other alloalbumin specimens detected during clinical electrophoresis of sera from Japanese living near Tokyo. Each of the five albumin variants from Nagasaki and Hiroshima has a single amino acid substitution. All of these substitutions differ, and none has been reported in non-Japanese populations. No instances of proalbumin variants or of albumin B (the most frequent alloalbumins in Caucasians) were detected in the children in Hiroshima and Nagasaki. However, one instance of a variant proalbumin and two examples of albumin B occurred in Japanese from the vicinity of Tokyo. In addition a previously unreported point substitution was found in albumin Tochigi, which is present in two unrelated persons from Tochigi prefecture. Four of the point mutations in the Japanese alloalbumins are in close proximity in a short segment of the polypeptide chain (residues 354–382) in which three additional point substitutions have been reported in diverse populations. These results, combined with earlier data, suggest that point substitutions are grouped in certain segments of the albumin molecule.**

Many rare genetic variants of human serum albumin (alloalbumins) that differ in electrophoretic mobility from normal (common) albumin (albumin A) have been identified through population genetics surveys (1–5), in the course of clinical electrophoresis (5–7), or in blood donor surveys (8). As markers of mutation and migration, alloalbumins are of interest to geneticists, biochemists, and anthropologists, but they are not associated with disease. For want of structural information such variants generally have been assigned ethnic or geographical names. Recently we (4, 9–15) and others (16–21) have determined the structural change in a number of variant albumins. We have now completed the structural study of the five rare types of inherited albumin variants discovered in the Biochemical Genetics Study of the Radiation Effects Research Foundation (RERF); this surveyed some 30 blood and plasma polypeptides in a cohort of 15,581 unrelated children in Nagasaki and Hiroshima (2). Family studies on the Japanese albumin variants revealed that they were in each case inherited from one parent or the other and hence were unrelated to parental exposure at the time of the bombing. We have also identified the structural change in five other alloalbumin specimens detected by one of us (I.S.) during clinical electrophoresis of sera from Japanese living in prefectures near Tokyo.

Each of the five types of albumin variants from Nagasaki (Nag-1, Nag-2, and Nag-3) and Hiroshima (Hir-1 and Hir-2) has a single amino acid substitution. All of these substitutions differ, and none has yet been reported in non-Japanese populations. Earlier we described two of these changes (12). In albumin Nag-2 the aspartic acid at position 375 is replaced by asparagine (denoted 375 Asp → Asn); in Nag-3 the exchange is 3 His → Gln (12). Here we report the following substitutions: 269 Asp → Gly in Nag-1, 354 Glu → Lys in Hir-1, and 382 Glu → Lys in Hir-2. No instances of proalbumin variants or of albumin B (Glu 570 → Lys), which are the most common Caucasian alloalbumins (8, 15), were detected in the nearly 16,000 children in the RERF study. However, two instances of albumin B and one example of a variant proalbumin occurred in the Japanese alloalbumins we identified by clinical electrophoresis. In addition, a previously unreported substitution (376 Glu → Lys) was found in the alloalbumin (albumin Tochigi) carried by two unrelated persons living in Tochigi prefecture.

The results described here support our suggestion that point substitutions are grouped in certain segments of the albumin polypeptide chain (4, 14, 15). Four of the mutations in the Japanese described here are in close proximity in the albumin polypeptide chain and gene (354 Glu → Lys, 375 Asp → Asn, 376 Glu → Lys, and 382 Glu → Lys). Other alloalbumin mutations reported for the same segment are as follows: 358 Glu → Lys in albumins Coari I and Porto Alegre I, from Brazil (4); 365 Asp → His, in albumin Parklands from New Zealand (17); and 372 Lys → Glu in albumins Naskapi (North American Indians) (10) and Mersin (Eti Turks) (10). Thus, seven independent substitution sites have so far been identified in the alloalbumins of diverse populations in a sequence of only 29 amino acids; this frequency may be compared to a total of five sites (excluding proalbumin variants) reported thus far for the first 353 amino acids. Such a cluster of substitutions may reflect vulnerability of the albumin gene to mutation in this region or the ease of accommodation to structural changes in the affected area of the protein.

MATERIALS AND METHODS

Plasmas and Sera. The Japanese plasmas are from the collection of the RERF Biochemical Genetics Study (2) and had been preserved in liquid nitrogen for up to 11 years. Five different alloalbumins were identified by vertical starch gel electrophoresis (2, 3) using 0.125 M sodium acetate/0.018 M EDTA buffer, pH 5.6 (5) and 0.125 M sodium acetate buffer,

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Abbreviations: RERF, Radiation Effects Research Foundation; Nag, Nagasaki; Hir, Hiroshima.

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pH 4.0. The variants were designated Nag-1, Nag-2, Nag-3, Hir-1, and Hir-2 on the basis of the city and the order in which they were identified. All specimens studied were from heterozygous individuals, and inheritance was established (2, 12). Some properties of the five albumin variants, and also the frequencies in the children, have been reported (12). The five bisalbuminemic sera provided by I.S. were detected during routine clinical electrophoresis. They are all recent specimens, and the cases have not previously been reported. The frequencies of these variants are not known; however, the variants must be rare because none of them were encountered in the RERF survey.

Methods. The following methods previously described (9–15) were used for characterization and structural study and are referred to hereafter as the standard procedure: (*i*) cellulose acetate (Microzone) electrophoresis at pH 8.6 in a Beckman model R101 apparatus (13); (*ii*) purification by HPLC of the total albumin (A and the variant) by use of the two-step method (13) followed by reduction and carboxymethylation and cleavage with CNBr (9, 13, 14); (*iii*) analytical isoelectric focusing of the CNBr digest to identify the variant fragment (10–14); (*iv*) HPLC peptide mapping of the CNBr digests of the carboxymethylated albumins (13–15); (*v*) HPLC peptide mapping of tryptic or *Staphylococcus aureus* V8 protease digests of purified CNBr fragments (4, 13–15); and (*vi*) amino acid analysis of the variant peptide(s) with the Beckman model 121M amino acid analyzer and automated sequence determination with the Beckman model 890C sequencer (9) or with the Applied Biosystems model 477B sequencer. The amino acid sequence of normal albumin A and the codons at each position are based on the genomic sequence of Minghetti *et al.* (22).

RESULTS AND DISCUSSION

Electrophoretic Classification of Albumins. Previous reports have described the differentiation of the five alloalbumins from Nagasaki and Hiroshima by means of vertical starch gel electrophoresis at pH 4.0 and 5.6 (2, 12). This was illustrated for pH 5.6 in figure 2 of Takahashi *et al.* (12). In the present work the five variants were compared at pH 4.0 and 5.6 with reference alloalbumins for which the substitution was known. The reference variants with known substitutions are albumin Mexico (slow +1, 550 Asp → Gly) (10), albumin B Lübeck (slow +2, 570 Glu → Lys) (15), and proalbumin Pollibauer (Lille type, slow +2, −2 Arg → His) (11). The five Japanese variants showed different combinations of mobilities in the two buffer systems; all were also different from albumin B and proalbumin Pollibauer. Albumin Mexico was not differentiated from Nag-1 in either system but was distinguished from all the others by use of both systems. An example is given in Fig. 1. An important point is that this illustrates that Hir-2, the slowest moving of the five RERF variants, can be differentiated from albumin B and proalbumin Pollibauer, which move even more slowly than Hir-2 at pH 5.6. Thus, if albumin B or proalbumins were present in the RERF study, they would easily have been differentiated and detected. Note also that variants that have the same nominal charge difference (e.g., B, Hir-1, and Hir-2) may exhibit different mobilities both in starch zone electrophoresis at pH 5.6 and in cellulose acetate electrophoresis at pH 8.6.

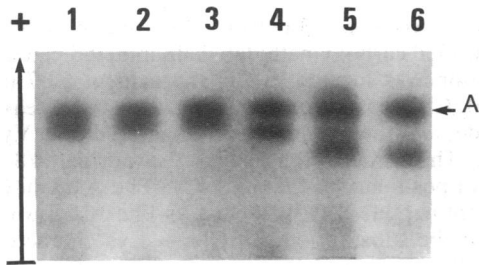

FIG. 1. Starch gel electrophoresis of albumin variants at pH 5.6: A/Mexico (lane 1), A/Nag-1 (lane 2), A/Hir-1 (lane 3), A/Hir-2 (lane 4), A/B Lübeck (lane 5), and A/proalbumin Pollibauer (lane 6).

Albumin Hir-1 (354 Glu → Lys). Hir-1 is a slow (+2) variant encountered in heterozygous condition in 5 of the 15,581 unrelated children in the RERF survey with the allele frequency of 0.00016 (12). The specimen used (plasma 950-417) was from one of the five children. Previous study of the CNBr digest by isoelectric focusing had indicated the amino acid substitution was in CB5, a large CNBr fragment (residues 330–446). However, despite extensive analysis the variant peptide was not identified in the tryptic peptide profile produced by the automated tandem HPLC system (9, 12). In the present work the total albumin (albumin A and albumin Hir-1, denoted A/Hir-1) was purified by the two-step procedure (13), carboxymethylated, and digested with CNBr. Isoelectric focusing confirmed that the substitution was in CB5. Two cycles of HPLC with a Vydac C_{18} column (4.6 × 250 mm) (The Nest Group, Southboro, MA) were required to purify the variant CB5 fragment because it was eluted together with normal CB3 in the first cycle. When a tryptic digest of the variant CB5 fragment was separated by HPLC with a Vydac C_{18} column, the normal peptide T48 was missing but two new peptides, T48A* and T48B*, were isolated (Fig. 2). These were identified by sequence analysis, thereby establishing the previously unreported substitution 354 Glu → Lys (Fig. 3). This explains the electrophoretic mobility of Hir-1 and accords with a single nucleotide change to *AAA* in the codon *GAA* for position 354 (22).

Albumin Hir-2 (382 Glu → Lys). In our previous work (12) albumin Hir-2 was not available for structural study. This rare slow (+2) variant was identified in only one child in the RERF survey and was present with an allele frequency of 0.00003, but inheritance was proven (12). Purification of the variant albumin from plasma 332-782, which was obtained from the father of the child, was done on an analytical scale by HPLC with a TSK DEAE 5PW column (7.5 × 75 mm) (Tosoh, Tokyo) with selection of the faster eluting shoulder of the albumin peak for study. Isoelectric focusing of a CNBr digest of the carboxymethylated albumin showed that the substitution was in CB5. Preparative purification of the total albumin (A/Hir-2) by the two-step method followed by isoelectric focusing of the CNBr digest confirmed that the substitution was in CB5. However, the variant CB5 was eluted together with normal CB3 by HPLC with a Vydac C_{18} column. A similar result was obtained with two specimens of Tochigi albumin, which were studied in parallel with Hir-2 because of their similar mobility and our identification of CB5 as the variant fragment in all three cases. After considerable experimentation with albumin Tochigi, two specimens of which were available in greater quantity, a two-step procedure was developed to purify the variant CB5. HPLC with a TSK G3000SW column (7.5 × 600 mm) (Tosoh) was used as the first step, which yielded a mixture of the variant CB5 and normal CB5 and CB6. In the second step the variant CB5 was purified by HPLC with a Vydac C_{18} column. However, to ensure adequate separation, multiple consecutive runs (5–15 runs) had to be used in each step (about 2 mg of CNBr digest per run); the final yield of purified variant CB5 was about 2 mg (≈200 nmol). Use of HPLC with a Vydac C_{18} column to separate a tryptic digest of the variant CB5 from Hir-2 yielded peptides T50A† and T50B† as shown by amino acid analysis (Fig. 2). Sequence analysis of both T50A† and T50B† proved the substitution 382 Glu → Lys, which had not previously been reported (Fig. 3). This exchange accords with the +2 slow mobility of Hir-2 and corresponds to a single base change in the codon *GAA* to *AAA*.

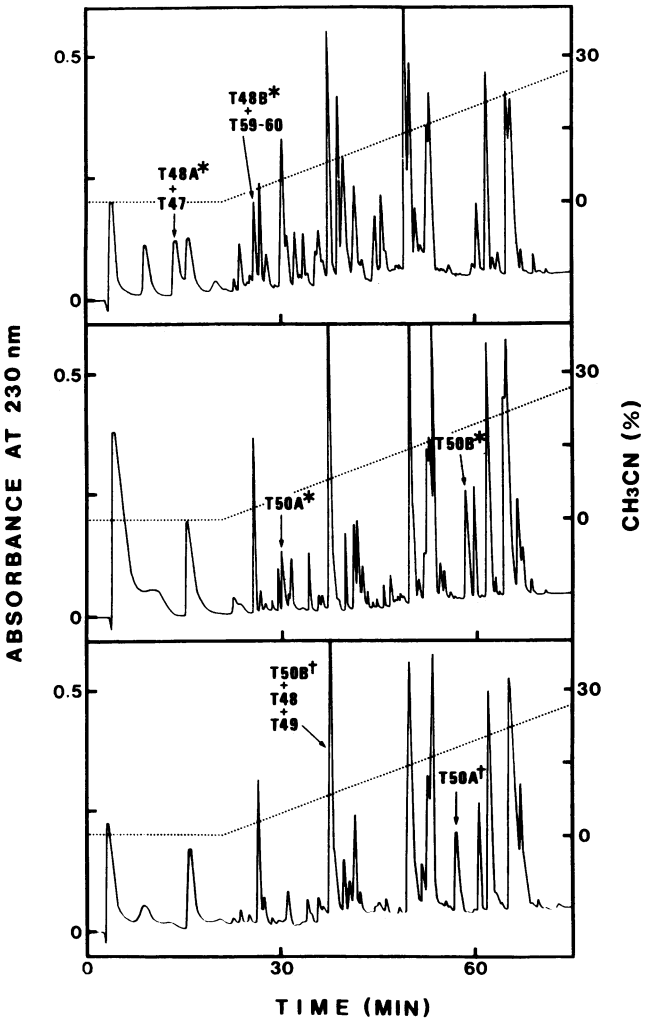

FIG. 2. HPLC elution profile on a Vydac C_{18} column of a tryptic digest of the variant CNBr fragment CB5 from albumin Hir-1 (*Top*), Tochigi (*Middle*), and Hir-2 (*Bottom*). The lyophilized tryptic digest was dissolved in 0.1% trifluoroacetic acid (buffer A) and eluted at a flow rate of 1 ml/min over 100 min with a linear gradient (dotted line) from 0% to 50% buffer B (acetonitrile/0.1% trifluoroacetic acid). Tryptic peptides are given the prefix T and are numbered consecutively in their order in the protein sequence (9). T48A* and T48B* denote the two new (variant) peptides containing the amino acid substitution in albumin Hir-1; T50A* and T50B* are the variant peptides for albumin Tochigi, and T50A† and T50B† are the variant peptides for albumin Hir-2. Fig. 3 gives the amino acid sequences of these peptides.

Albumin Tochigi (376 Glu → Lys). Two sera with similar albumin variants were discovered by I.S. during clinical electrophoresis. These sera were obtained from unrelated individuals (S and O) living in Tochigi prefecture, and the variant albumins were designated Tochigi from the place of origin. Inheritance was established in one case (S) but not the other. We had no initial evidence that the two Tochigi alloalbumins were identical. The two-step procedure described above for Hir-2 was carried out for purification of the variant CB5 fragment from both Tochigi specimens. The HPLC tryptic peptide profile of the variant CB5 fragments from the two Tochigi specimens was nearly identical but differed from that for Hir-2. Two new peptides, T50A* and T50B*, were isolated for each Tochigi specimen (Fig. 2). Amino acid analysis and sequence analysis of both peptides for both specimens showed that the previously unreported substitution 376 Glu → Lys (Fig. 3), so both were designated Tochigi. This exchange fits with the +2 slow mobility and corresponds to a single base change in the codon *GAA* to *AAA*.

Albumin Nag-1 (269 Asp → Gly). This alloalbumin had the highest frequency of any of the five variants discovered in the RERF Biochemical Genetics Study. It was present in 35 of 15,581 unrelated children and exhibited an allele frequency of 0.00112 (12), which is much below the level regarded as polymorphic (1). Although designated Nag-1 because it was the first alloalbumin discovered in the RERF Study and was first detected in Nagasaki (3), this alloalbumin is also present in families in Hiroshima. The allele frequencies in Hiroshima and Nagasaki are 0.00067 and 0.00174, respectively. In fact, the specimen studied in this and in our previous work (12) is from a member of a Hiroshima family.

In our previous study (12) the structural change in Nag-1 (plasma 952-626P) was not identified by tandem HPLC mapping and amino acid analysis of almost 40 tryptic peptides or by sequence analysis of the entire CB2 fragment and of several candidate tryptic peptides. In the present work we reexamined the tryptic peptides from the automated tandem HPLC profile of Nag-1, which had been stored. One peptide peak that appeared significant on the basis of position and amino acid composition was submitted to sequence analysis. This gave a double sequence; the major sequence was for peptide T38 (residues 263–274) but with the substitution 269 Asp → Gly. In further study the CB3 fragment (residues 124–298) was purified by HPLC and digested with trypsin; the tryptic peptides were separated by HPLC. Sequence analysis of one peptide peak gave a double sequence that confirmed the 269 Asp → Gly substitution in peptide T38. Sugita *et al.* (21) had reported that the molecular abnormality in albumin Niigata is the exchange 269 Asp → Gly, the same as we found in Nag-1. Albumin Niigata was detected by clinical electrophoresis in a single patient in Niigata, a city on the northwestern coast of Honshu, the main island of Japan. Inasmuch as albumin Nag-1 was reported in an extensive biochemical genetics study prior to the report of a single case of albumin Niigata (3), we propose that Nagasaki-1 be retained as the precedent name of this alloalbumin type.

Albumin Saitama-1 (B Type, 570 Glu → Lys). Albumins of the B type with the substitution 570 Glu → Lys had long appeared to be restricted to people of Caucasian ancestry. However, we recently reported that the 570 Glu → Lys exchange is present in an albumin variant occurring in two unrelated Japanese and one Cambodian (15). The two type B albumins from Japan were designated Tokyo-1 and Shinanomachi-1, and the one from Cambodia was designated Phnom Penh. All three had been identified by clinical electrophoresis. Tokyo-1 had been provided by I.S., who later detected another +2 slow albumin (Saitama-1) by clinical electrophoresis in a patient from Saitama in the environs of Tokyo. The carriers of Tokyo-1 and Saitama-1 were unrelated, but no information on inheritance of the variant has yet been obtained.

Structural study of albumin Saitama-1 was done essentially by the procedure described by Arai *et al.* for B albumins (15) except that the sequence was determined with the Applied Biosystems sequencer. Isoelectric focusing of a CNBr digest of the purified carboxymethylated albumin indicated that the substitution was located in CNBr fragment CB7 (residues 549–585). CB7 was purified by HPLC, a V8 protease digest was made, and the peptides were separated on a Vydac C_{18} column. This procedure yielded the normal V8 peptide S58-59 for positions 566–571 (Thr-Cys-Phe-Ala-Glu-Glu) and the variant peptide S58-59* (Thr-Cys-Phe-Ala-*Lys*-Glu); the latter has the substitution 570 Glu → Lys characteristic of albumin B.

Thus, we have demonstrated the presence of albumin B in three unrelated Japanese and also in a Cambodian and in six unrelated individuals of five different European descents

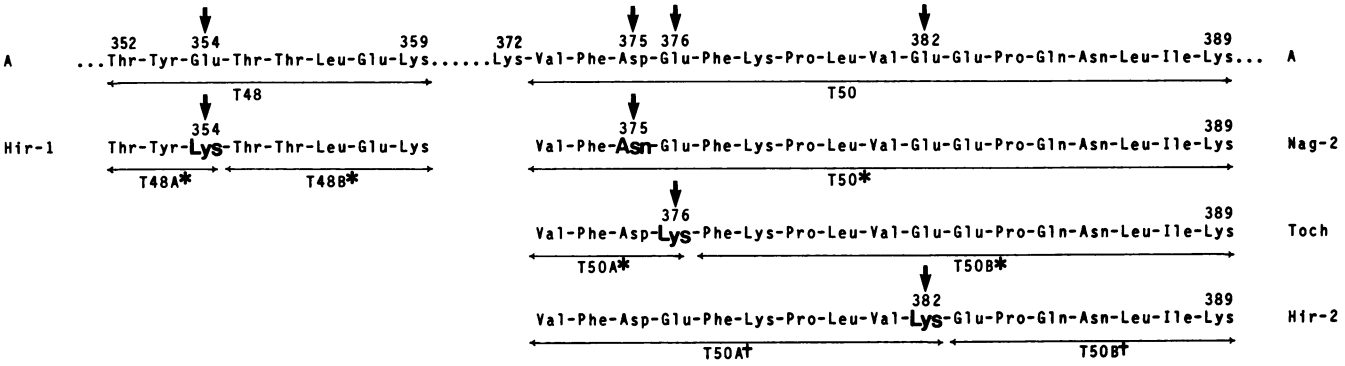

FIG. 3. Amino acid sequence analysis of variant tryptic peptides from the Japanese alloalbumins Hir-1, Tochigi (Toch), and Hir-2, which are described in the text, and for Nag-2, which was previously reported (12). The vertical arrows indicate the position of the substitution, which is given in boldface type. Asterisks and daggers identify variant peptides illustrated in Fig. 2 and referred to in the text. Additional substitutions for non-Japanese alloalbumins in the sequence from position 352 through 389 are given in Fig. 4.

(15). This indicates that the albumin B mutation has occurred a number of times in different populations, and it suggests that the site either is hypermutable or is subject to selection.

Proalbumin Shizuoka. A bisalbuminemic serum from a single individual from Shizuoka prefecture was supplied by I.S. No other information was available. The variant albumin had a slow +2 mobility at pH 8.6. This, together with its susceptibility to limited digestion with trypsin (11), suggested that this variant was a proalbumin. Purification of the proalbumin component was accomplished by use of ion-exchange HPLC (15). The purified proalbumin was applied directly to the Beckman protein sequencer and analyzed for 10 cycles. An unambiguous sequence characteristic of the Christchurch type of proalbumin was obtained: Arg-Gly-Val-Phe-Arg-*Gln*-Asp-Ala-His-Lys. In this sequence glutamine replaces the last arginine in the proalbumin hexapeptide amino-terminal sequence (−1 Arg → Gln) (11, 16). This accords with the single-base mutation C*G*A to C*A*A.

This is the second instance of a Christchurch type of proalbumin that we have identified in Japan, the other being proalbumin Fukuoka-3 (15). The two prefectures Shizuoka and Fukuoka are 500 miles apart by air. The two carriers of this proalbumin have different names; it is unlikely that they are closely related, but common ancestry cannot be excluded.

Relationship of the Genetic Polymorphism to the Genomic Sequence. Including the results reported here, a total of 18 different sites of substitution of a single amino acid have been recorded within the mature human albumin molecule, which contains 585 amino acid residues (4, 10–15, 17–21); all can be explained by change of a single base in the corresponding codon. Three similar point mutations at two sites occur in the hexapeptide propeptide at the amino terminus (11, 15, 16) as well as one frame shift at the carboxyl terminus (18). Of the 18 point mutation sites in the mature albumin molecule, only 5 occur in the first 353 residues, whereas 7 are in the sequence of 29 amino acids from position 354 through 382 illustrated in Fig. 4. There are also 5 mutation sites in a sequence of 34 residues in the carboxyl terminus (15). Thus, to the present, most of the point mutations in the albumin molecule have been identified in three segments: the propeptide and amino terminus (11, 15), the carboxyl terminus (15), and the short sequence illustrated in Fig. 4.

Clustering of substitutions may reflect vulnerability of certain regions of the albumin gene to mutation or the ease of accommodation to structural changes in the affected areas of the protein molecule, for example, at surface sites. The seven mutation sites illustrated in Fig. 4 are at the 3′ end of exon 9 and the 5′ end of exon 10 in the genomic sequence of Minghetti *et al.* (22). Three of the codons are close to the exon–exon juncture corresponding to the joining of residues 373 and 374 in the protein sequence. None of the 18 mutation sites identified thus far in the mature albumin molecule corresponds with any of the 13 codons listed by Minghetti *et al.* (22) as involved in silent or apparently effective DNA polymorphism as indicated by comparison of the genomic sequence with two cDNA sequences. Nor do any of the 18 mutated sites correspond with possible sequence-specific sites for the restriction enzymes used in the study of albumin genetic polymorphism (22, 24). Thus, examination of the genomic sequence of albumin has not yet offered an explanation of the apparent clustering of amino acid substitutions in the protein sequence.

Protein Structure in the Region of Clustered Amino Acid Replacements. Although a crystallographic structure of serum albumin is not yet available, much is known or has been hypothesized about its three-dimensional structure (23, 25). Brown and Shockley (23) have summarized this in a structural model that they have proposed. This depicts a largely helical repeated pattern of nine loops joined by 17 disulfide bridges to form three homologous domains of about 195

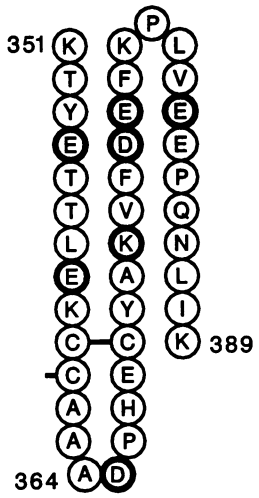

FIG. 4. Amino acid sequence in the one-letter code for the segment of human serum albumin illustrated in Fig. 3. This is the carboxyl-terminal segment of the large double-loop 6 at the end of domain 2 in the Brown and Shockley structural model (23). Boldface circles mark single-point amino acid exchanges in albumin genetic variants: 354 Glu → Lys, albumin Hir-1; 358 Glu → Lys, albumins Porto Alegre I and Coari I from Brazil (4); 365 Asp → His, albumin Parklands from New Zealand (17); 372 Lys → Glu, albumins Naskapi (North American Indians) and Mersin (Eti Turks) (10); 375 Asp → Asn, albumin Nag-2 (12); 376 Glu → Lys, albumin Tochigi; 382 Glu → Lys, albumin Hir-2. References and geographic or ethnic origin are given for non-Japanese variants not studied in this work.

residues each. The segment shown in Fig. 4 is at the end of loop 6 and is the end of the second domain. It appears to be a connecting segment between the second and third domains. Enzymatic cleavage of undenatured human serum albumin occurs after positions 380, 387, and 389 (25), indicating that the end of domain 2 is exposed. In general, amino acid replacements are more readily tolerated at the surface of a protein; for example, nearly all substitutions on the surface of the hemoglobin molecule are harmless (26).

Similar Substitutions May Produce Variants of Different Electrophoretic Mobility. Four of the seven substitutions in Fig. 4 consist of a replacement of a glutamic acid by a lysine. This produces a variant that has a slower electrophoretic mobility at pH 5.6 and 8.6 than normal A and has a nominal change in charge of +2. Yet, the ordering of mobility in starch gel electrophoresis at pH 5.6 of the three Glu → Lys variants compared in Fig. 1 is A > Hir-1 > Hir-2 > B. We have observed a similar ordering of the mobility of Glu → Lys variants at other positions (4). This variation in mobility probably reflects a difference in the pKa of the glutamic acid and lysine residues in different regions of the secondary structure and also electrostatic interaction with other amino acids in the protein. This is the factor that permits electrophoretic differentiation of many albumin variants even though there are only four steps in the charge differences that may result from a point mutation: +2, +1, 0, −1, −2.

Geographical Distribution of Japanese Albumin Variants. The Japanese albumin variants studied thus far appear to have two types of distribution. One set seems to be restricted to Japanese living in certain regions of Japan. This includes the five variants from Nagasaki and Hiroshima, albumin Tochigi, proalbumin Takefu, and several others still under study by us. The second set consists of proalbumins Christchurch and Lille and also albumin B (15, 20). Structural study and electrophoretic typing have documented that the latter three variants—though rare in all populations—do occur in Caucasians of various Western European descents and have a cumulative frequency of about 1:3000 (8). Albumin B has also been identified in a Cambodian (15) and proalbumin Lille in a Chinese (15). It is noteworthy that neither albumin B nor proalbumins were encountered in the RERF study of nearly 16,000 children in Hiroshima and Nagasaki, although the methods used were capable of detecting them (Fig. 1).

Those alloalbumins that have been encountered thus far only in Japanese also appear to be rather restricted geographically within Japan, as if their occurrence could be attributed to a founder principle. We have received more than 20 bisalbuminemic plasmas or sera from unrelated Japanese residents in Japan; these represent about a dozen different cities or prefectures. We also have the only two bisalbuminemic sera encountered in a study of some 15,000 Japanese living in Hawaii (6). Although our studies are incomplete, it is clear that none of our specimens received from outside the seaport cities of Hiroshima and Nagasaki have the substitutions manifested in the latter group. To be sure, a single individual in the coastal city of Niigata had the Nag-1 trait (21), but this could be attributed to migration. Albumin Tochigi was not encountered in the RERF study, and so far it appears to be unique. So also do several mutant albumins from Nagoya, Izumi, and Fukuoka that are in our collection (unpublished experiments of F.W.P., K.A., and J.M.). Prior to World War II the Japanese people usually lived in the same locale as did their ancestors. Thus, rare genetic variants such as alloalbumins were probably largely confined to the locale in which they arose. In contrast, alloalbumins such as the proalbumins and albumin B, which are encountered in diverse populations and widely separated regions, probably arose from independent mutations.

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1. Neel, J. V. (1978) *Am. J. Hum. Genet.* **30,** 564–590.
2. Neel, J. V., Satoh, C., Goriki, K., Asakawa, J., Fujita, M., Takahashi, N., Kageoka, T. & Hazama, R. (1988) *Am. J. Hum. Genet.* **42,** 663–676.
3. Ferrell, R. E., Ueda, N., Satoh, C., Tanis, R. J., Neel, J. V., Hamilton, H. B., Inamizu, T. & Baba, K. (1977) *Ann. Hum. Genet.* **40,** 407–418.
4. Arai, K., Huss, K., Madison, J., Putnam, F. W., Salzano, F. M., Franco, M. H. L. P., Santos, S. E. B. & Freitas, M. J. M. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 1821–1825.
5. Weitkamp, L. R., McDermid, E. M., Neel, J. V., Fine, J. M., Petrini, C., Bonazzi, L., Ortali, V., Porta, F., Tanis, R., Harris, D. J., Peters, T., Ruffini, G. & Johnson, E. (1973) *Ann. Hum. Genet.* **37,** 219–226.
6. Fukunaga, F. H. & Glober, G. A. (1973) *Am. J. Clin. Pathol.* **60,** 867–870.
7. Tárnoky, A. L. (1980) *Adv. Clin. Chem.* **21,** 101–146.
8. Fine, J. M., Marneux, M. & Rochu, D. (1987) *Am. J. Hum. Genet.* **40,** 278–286.
9. Takahashi, N., Takahashi, Y., Ishioka, N., Blumberg, B. S. & Putnam, F. W. (1986) *J. Chromatogr.* **359,** 181–191.
10. Takahashi, N., Takahashi, Y., Blumberg, B. S. & Putnam, F. W. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 4413–4417.
11. Takahashi, N., Takahashi, Y. & Putnam, F. W. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 7403–7407.
12. Takahashi, N., Takahashi, Y., Isobe, T., Putnam, F. W., Fujita, M., Satoh, C. & Neel, J. V. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 8001–8005.
13. Huss, K., Putnam, F. W., Takahashi, N., Takahashi, Y., Weaver, G. A. & Peters, T., Jr. (1988) *Clin. Chem.* **34,** 183–187.
14. Huss, K., Madison, J., Ishioka, N., Takahashi, N., Arai, K. & Putnam, F. W. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 6692–6696.
15. Arai, K., Ishioka, N., Huss, K., Madison, J. & Putnam, F. W. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 434–438.
16. Brennan, S. O. & Carrell, R. W. (1978) *Nature (London)* **274,** 908–909.
17. Brennan, S. O. (1985) *Biochim. Biophys. Acta* **830,** 320–324.
18. Galliano, M., Minchiotti, L., Iadarola, P., Zapponi, M. C., Ferri, G. & Castellani, A. A. (1986) *J. Biol. Chem.* **261,** 4283–4287.
19. Minchiotti, L., Galliano, M., Iadarola, P., Stoppini, M., Ferri, G. & Castellani, A. A. (1987) *Biochim. Biophys. Acta* **916,** 411–418.
20. Matsuda, Y., Ogushi, F., Ogawa, K. & Katunuma, N. (1986) *J. Biochem.* **100,** 375–379.
21. Sugita, O., Endo, N., Yamada, T., Yakata, M. & Odani, S. (1987) *Clin. Chim. Acta* **164,** 251–259.
22. Minghetti, P. P., Ruffner, D. E., Kuang, W.-J., Dennison, O. E., Hawkins, J. W., Beattie, W. G. & Dugaiczyk, A. (1986) *J. Biol. Chem.* **261,** 6747–6757.
23. Brown, J. R. & Shockley, P. (1982) in *Lipid-Protein Interactions*, eds. Jost, P. & Griffith, O. H. (Wiley, New York), Vol. 1, pp. 25–68.
24. Murray, J. C., Mills, K. A., Demopulos, C. M., Hornung, S. & Motulsky, A. G. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 3486–3490.
25. Peters, T., Jr. (1985) *Adv. Protein Chem.* **27,** 161–245.
26. Perutz, M. F. & Lehmann, H. (1968) *Nature (London)* **219,** 902–909.