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Mutations in genetic variants of human serum albumin found in Italy

(alloalbumins/proalbumins/genetic polymorphism/point mutations)

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ABSTRACT **A long-term electrophoretic survey of genetic variants of serum albumin has identified an alloalbumin in 589 unrelated individuals in Italy. The alloalbumins were classified electrophoretically into 17 types. The number of unrelated carriers for each type varied from 1 for several variants reported here to 103 for albumin B. The structural change in 8 of these types has previously been determined, and the amino acid substitutions in 3 additional types are reported here. Albumin Varese has a substitution, −2 arginine to histidine (−2 Arg → His), the same as that reported for proalbumin Lille; albumin Torino has the substitution 60 Glu → Lys; and albumin Vibo Valentia has the substitution 82 Glu → Lys. The ability to distinguish so many alloalbumin types by electrophoresis at several pH values indicates that similar substitutions at different sites produce variants with different electrophoretic mobilities. Except for chain terminations in two Italian variants, all the mutations thus far determined for alloalbumins are attributable to a single-base change in the structural gene, and there is a preponderance of transitions and purine mutations. Seven alloalbumins for which the structural change has been established have been ascertained only in Italy. Several of these are clustered in specific geographic regions of Italy, which suggests an origin through a founder individual. Other variants that occur worldwide are nonetheless clustered in geographic regions within Italy. In these cases an independent mutation probably occurred at a hypermutable site such as a CpG dinucleotide.**

Since the discovery in 1957 of an inherited variant of human serum albumin (alloalbumin), more than 100 named variants have been identified in surveys of population genetics or by routine electrophoresis in clinical laboratories (1–5), and more than 30 structural changes have been ascertained (6–22). A large number of alloalbumins has been discovered in Italy (2, 6–9, 11–13, 22) and Japan (4, 15–17) because of continuing extensive population surveys carried on in these countries: in Italy by the Italian Committee for Standardization of Electrophoretic Laboratory Methods (CISMEL) (2) and in Japan by the Biochemical Genetics Study of the Radiation Effects Research Foundation (4). Alloalbuminemia is rare with a frequency of only 1:3000 to 1:10,000 in most populations and is generally expressed in heterozygous form, without any apparent effect on the function of the protein (3, 5). Therefore, unlike lethal mutations, such as may occur for hemoglobin and coagulation factors, the benign trait of alloalbuminemia is transmitted as a genetic marker and provides a model for study of neutral molecular evolution. Structural study of alloalbumins carried out in several laboratories (6–22) has shown that the most common change is a single amino acid substitution, probably due to a point mutation in the structural gene: 25 amino acid substitutions are located within the mature polypeptide chain (6–9, 11, 15–20), and 5 are clustered in the Arg-Arg propeptide sequence required for posttranslational processing (10, 14, 20–22). The latter give rise to circulating proalbumins. Two Italian slow variants arise from an extensive modification of the COOH-terminal region (12, 13), but their genomic defects have not been determined.

In this paper we report the results of a continuing electrophoretic survey for albumin variants in the sera of patients from various regions of Italy and discuss their geographical distribution. To date, this survey, begun in 1971, has resulted in the ascertainment of a variant albumin in 589 unrelated individuals. The variants were classified electrophoretically into 17 types. In more than 500 instances the variant was demonstrated in other family members (2). A homozygous carrier was identified for four subjects and three alloalbumins. The structural change in 8 types of variants has been determined, including one proalbumin, five point substitutions in the mature albumin molecule, and two chain-termination mutants (6–9, 11–13, 22). Of these, three point substitutions and the two chain-terminations are so far apparently unique to Italy. Here we also report the structural characterization of three other slow types, albumins Torino, Varese, and Vibo Valentia. Albumin Varese was shown to be identical to proalbumin Lille (−2 Arg → His) (3), whereas substitutions that to our knowledge were previously unreported were found in the other two alloalbumins. Both have a replacement of glutamic acid by lysine (Glu → Lys) but at different positions of the primary structure. Albumin Torino is modified at residue 60 and albumin Vibo Valentia at residue 82.

MATERIALS AND METHODS

Electrophoretic Survey. The screening of the variants was performed by comparative cellulose acetate electrophoresis with the Chemetron apparatus (Chemetron Chimica, Milan) at two pH values: pH 5.0 (0.031 M sodium acetate/0.004 M EDTA) and pH 8.6 (0.004 M sodium barbital/0.0075 M barbital/0.0012 M calcium lactate). Each variant was named according to its geographical origin. Normal (common) albumin is designated albumin A. Fresh specimens of serum from individuals with the albumin Torino, Vibo Valentia, and Varese traits were supplied by E. Zepponi (Tagliacozzo, Abruzzo), E. Guagnellini (Cinisello Balsamo, Lombardia), and F.P., respectively. In all cases the donors were heterozygous and had a variant albumin with a slow mobility. A family study was possible only for albumin Varese, which was shown to be inherited.

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Structural Studies. The normal and variant albumins were separated on a DEAE-Sephadex column (1 × 100 cm), eluted with 0.11 M sodium phosphate (pH 5.75), and the purity of the proteins was checked by SDS/PAGE (9). The reduced and carboxymethylated albumins were cleaved with CNBr, and the CNBr digests were compared by analytical isoelectric focusing (pH 2.5–8) on PAGE in 8 M urea (12). For all three alloalbumins described here the variant CNBr fragment was identified as CB1 (residues 1–87). Limited tryptic digestion and isolation of the proteolytic products were performed on the three variants as reported (10). Automated sequence analysis was carried out on the NH_2-terminal region of albumin Varese in a Beckman 890M sequencer (10). The variant CB1 from albumins Torino and Vibo Valentia was isolated preparatively by gel filtration on a Toyo Soda TSK 3000 column (10 μm, 7.5 mm × 60 cm; Altex) and isocratic elution with 35% (vol/vol) CH_3CN/0.1% trifluoroacetic acid. The purified CB1 fragments from both alloalbumins were digested with *Staphylococcus aureus* V8 protease (Miles) (19), and CB1 from Torino was also digested with trypsin (20). The V8 and tryptic digests were mapped by reversed-phase HPLC on a Beckman apparatus equipped with a Vydac C_{18} column (4.6 × 250 mm; Anspec, Ann Arbor, MI) (19, 20). Tryptic and V8 peptides are given the prefixes T and S, respectively, and are numbered consecutively by their order in the sequence (15).

Amino acid analysis was done with a Beckman model 121M amino acid analyzer, and variant peptides were sequenced with the Applied Biosystems model 477B sequencer. The NH_2-terminal regions of the whole variant albumins Torino and Vibo Valentia were also submitted to automated sequence analysis.

RESULTS AND DISCUSSION

Electrophoretic Survey. The survey of the Committee for Standardization of Electrophoretic Laboratory Methods based on electrophoretic screening carried out since 1971 in clinical laboratories has uncovered 589 instances of inherited bisalbuminemia in unrelated individuals and about 100 examples of transient bisalbuminemia induced by pancreatic disease or penicillin therapy. Also, 4 cases of analbuminemia (0–0.2 g of albumin per 100 ml) were encountered in unrelated patients, presumably homozygotes. No evidence for a fresh mutation producing an alloalbumin was obtained. There was one instance of a double heterozygote who had both the Catania and Tagliocozzo traits. In this study the three slow albumins Torino, Varese, and Vibo Valentia were compared with the other alloalbumins found in Italy by cellulose acetate electrophoresis at pH 5.0 and pH 8.6. The use of the two buffer systems allowed the identification of 12 other Italian variants in addition to the 5 initially listed in the classification of Weitkamp *et al.* (1), which was based on starch-gel electrophoresis at several pH values.

Fig. 1 shows the pattern obtained for the 17 types of Italian mutants; 6 are fast and 11 are slow migrating. The structural changes in 8 of these had been determined (6–13); 3 additional types are reported here. This screening clearly showed that albumins Torino, Varese, and Vibo Valentia possess a slow electrophoretic behavior and exhibit different combinations of mobilities at the two pH values.

Isolation of Alloalbumins and Screening of CNBr Fragments. As expected on the basis of their electrophoretic behavior, the alloalbumins were eluted ahead of albumin A during ion-exchange chromatography. The ratio in serum of albumins A and Torino was 50:50 in the chromatographic pattern and in the densitometric scan of the electrophoretic pattern, but the ratio of A to Vibo Valentia and Varese was 60:40 in both methods.

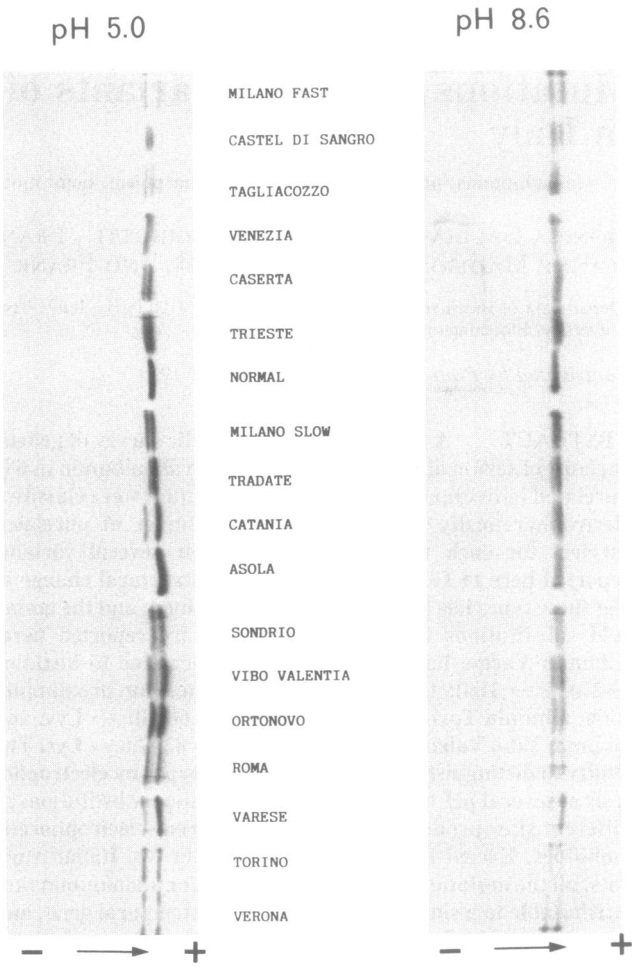

FIG. 1. Cellulose acetate electrophoretic screening at pH 8.6 and 5.0 of the sera containing the albumin variants found in Italy. Electrophoresis of the serum samples was performed for 60 min on Chemegel strips (5.7 × 14 cm) at 200 V (pH 8.6) or 220 V (pH 5.0).

The analytical isoelectric focusing of the CNBr digests showed that the CB1 bands for the three variants were shifted to a higher pH than the normal CB1, while all the other CNBr bands were identical. The pI value of the variant CB1 for albumin Vibo Valentia differed from that of normal CB1 by about 0.2 pH units; this suggested a double charge (+2) substitution. The pI value of the variant CB1 of albumin Torino was somewhat lower, corresponding to a difference in charge from normal between +1 and +2. On the other hand, the pI value of CB1 fragment of albumin Varese was higher, suggesting a difference in charge from normal between +2 and +3. Limited tryptic digestion was thus performed on the variants; this cleavage converted albumin Varese to albumin A, whereas the other two mutants were unaffected.

Albumin Varese. The amino acid composition of albumin Varese revealed that the slow variant differs from the normal protein by its higher arginine, glycine, histidine, phenylalanine, and valine content. This result and also the susceptibility to limited tryptic cleavage and the pI value of the variant CB1 fragment strongly suggested that Varese is in fact a proalbumin variant. The purified protein was thus analyzed—without prior carboxymethylation—for 10 cycles in the Beckman amino acid sequencer. This gave an unambiguous sequence for a proalbumin with the substitution −2 Arg → His, which has been reported for albumin Lille (3): Arg-Gly-Val-Phe-His-Arg-Asp-Ala-His-Lys. . . . This substitution explains the slow mobility of the variant and its CB1 fragment and is attributable to the point mutation CGT → CAT.

Structural Studies of Alloalbumins Torino and Vibo Valentia and of Their CNBr Fragments. In SDS/PAGE the purified variants had a molecular mass of 66.5 kDa, the same as albumin A, and amino acid analysis revealed no significant differences in the mutants compared to the normal albumin. Automated sequence analysis for 30 cycles showed that both variants had the normal NH_2-terminal primary structure. For both alloalbumins amino acid analysis of the purified CB1 fragments showed the presence of an additional lysine residue and the absence of one glutamic acid residue compared to the normal CB1. The amino acid composition and the pI values of the CB1 fragments indicated that both variants had a Glu → Lys substitution but at different locations.

Albumin Torino. The HPLC profiles of the V8 protease digests of CB1 from the normal and Torino albumins appeared similar in their major peaks and differed only in the position of one small peak (Fig. 2). Hence, amino acid analysis was done for all the V8 protease peptides of the CB1 fragments of the normal and variant albumins. The results revealed that, in addition to cleavage at the expected V8 protease-susceptible sites, an unexpected partial digestion occurred at the bond between Gly-71 and Asp-72. In the normal profile this gave rise to two peptides S9a (residues 61–71) and S9b (residues 72–82) . However, the amino acid composition and sequence analysis of a peak in the variant profile referred to as S8*-S9 indicated that it was a peptide composed of S8 and S9 (residues 58–82) but with a Glu → Lys substitution at position 60. This result was confirmed by amino acid and sequence analysis performed on a small aberrant peak that corresponded to S8*-S9a and arises from the same replacement (Fig. 3).

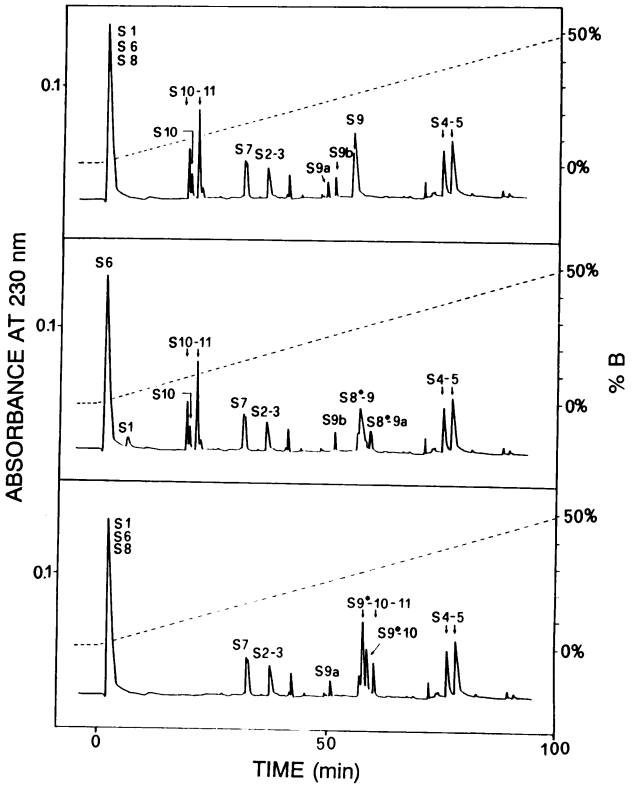

FIG. 2. HPLC elution profile on a Vydac C_{18} column of a V8 protease digest of fragment CB1 from albumin A (*Top*), Torino (*Middle*), and Vibo Valentia (*Bottom*). The digest was dissolved in 0.1% trifluoroacetic acid (buffer A) and eluted at a flow rate of 1 ml/min over 100 min with a linear gradient (dotted line) from 0% to 50% buffer B (acetonitrile/0.1% trifluoroacetic acid). V8 peptides are given the prefix S and are numbered consecutively by their order in the protein sequence (15). S8*-9 and S8*-9a denote the two variant peptides resulting from the amino acid substitution in albumin Torino; S9*-10 and S9*-10-11 are the variant peptides for albumin Vibo Valentia. Fig. 3 gives the amino acid sequences of these peptides.

Comparison of the elution profiles of the tryptic digests obtained from the normal and Torino CB1 fragments showed the disappearance of the peak corresponding to peptide T7 (residues 52–64). In this case the 60 Glu → Lys substitution gives rise to two new peptides (T7a* and T7b*) from the presence of an additional tryptic cleavage site (Fig. 3).

Albumin Vibo Valentia. The V8 protease digest of CB1 from albumin Vibo Valentia was also mapped by reversed-phase HPLC (Fig. 2). The profile clearly showed the absence of the peaks corresponding to normal peptides S9 and S10, and also of S10-11, which results from incomplete cleavage between Glu-86 and the COOH-terminal homoserine; however, several new peaks were eluted close to the position of the normal S9. Amino acid composition and sequence analysis of the new peptides (S9*-10 and S9*-10-11) showed that they extended from Asn-61 through Glu-86 and through homoserine-87, respectively, because of a Glu → Lys substitution at position 82 (Fig. 3).

Similar Substitutions Produce Variants with Different Electrophoretic Mobility. The ability to distinguish and classify so many alloalbumin types by electrophoresis at two pH values (Fig. 1) depends on the fact that similar substitutions may produce variants with different combinations of mobilities. The Glu → Lys substitution found in albumins Torino and Vibo Valentia produces a nominal net increase in charge of +2 that gives rise to a slower electrophoretic mobility than normal albumin at pH 5.0 and pH 8.6. However, the same amino acid replacement produces different electrophoretic mobilities in albumins Torino and Vibo Valentia and also in two other Italian Glu → Lys mutants, albumins Roma and Verona (Table 1) (8, 9). In fact, the ordering of mobilities at pH 8.6 of these alloalbumins is Vibo Valentia > Roma > Torino > Verona (Fig. 1). This variation in mobility may reflect a difference in the pK_a of the residues involved, electrostatic interactions with other charged amino acids, and conformational changes due to the substitution.

Also, as described earlier, the variant CB1 fragments of albumins Vibo Valentia and Torino exhibit different isoelectric points in the presence of 8 M urea; this suggests that large peptides of albumin may to some extent retain their three-dimensional structure under denaturing conditions.

Nucleotide Mutations that Result in Point Substitutions in the Albumin Molecule. The amino acid substitutions found in albumins Torino and Vibo Valentia are consistent with a point mutation in the structural gene when compared to the genomic sequence of Minghetti *et al.* (23). In codons 60 and 82, GAA in the normal albumin gene must be changed to AAA, encoding lysine in the variants. Excluding the chain-termination mutants Venezia and Catania, all the amino acid substitutions thus far determined in Italian alloalbumins can be accounted for by a single-base change in the structural gene (Table 1). Eight of these are transitions, and one is a transversion (albumin Tagliacozzo). Of even greater interest is the fact that in all the cases examined except proalbumin Tradate, the base that is changed is a purine (6 guanines and 2 adenines) (Table 1). These findings are not unique to the Italian variants. Among the 32 point mutations thus far reported in alloalbumins from all over the world, 23 are transitions and 9 are transversions; in 29 cases a purine is mutated (21 guanines and 8 adenines) compared to only 3 cases in which a pyrimidine is mutated. Additional study is required to ascertain whether the seemingly high frequency of mutations involving purines reflects greater susceptibility to mutation of these bases, particularly guanine, or whether the apparent disparity simply reflects the fact that purine mutations unlike pyrimidine mutations may result in amino acid substitutions that have a double-charge change and, therefore, are easy to detect electrophoretically.

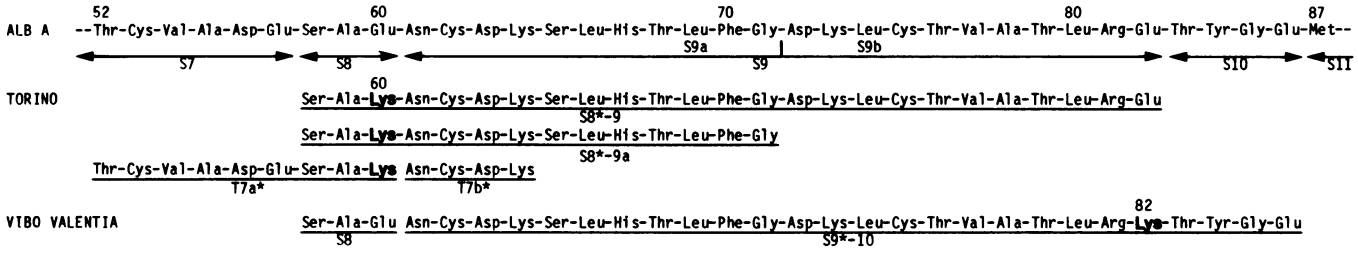

FIG. 3. Amino acid sequence analysis of variant peptides from albumins Torino and Vibo Valentia compared to the sequence of normal albumin (ALB A). The tryptic (T) and V8 protease (S) peptide designations are defined in the text, and the V8 protease peptide profiles are given in Fig. 2. The amino acid substitutions in the variant peptides are shown in boldface letters.

Are Certain Alloalbumins Restricted to a Particular Ethnic Group or Geographic Region? Because of the large number of possible alloalbumins and the difficulty of procuring and cross-checking all reported variants, electrophoretic behavior is obviously an insufficient criterion for ascertaining whether particular variants are largely restricted to a specific ethnic group or geographical region. However, structural studies—though small in number and difficult to perform—do provide an unambiguous determinant of identity or difference. Thus far, seven alloalbumins for which the structural change has been established have surfaced in Italy but have not yet been reported elsewhere: Torino, Vibo Valentia, Roma, Castel di Sangro, Milano Fast, Catania, and Venezia (Table 1). Among them, Venezia, Catania, and Milano Fast have been detected in a number of unrelated subjects, which allows discussion of their geographic distribution. These variants are almost entirely segregated to specific regions (Table 1). This geographic restriction very likely has some basis in the relative immobility of these population groups in the city–state political divisions of Italy until recent times. In fact, two homozygotes were detected for the Venezia variant and one each for Catania and Milano Fast. The finding of homozygotes suggests a founder principle or genetic drift within a restricted population. Indeed, homozygotes for alloalbumins are rare except in certain North American Indian tribes that exhibit a polymorphism (i.e., an allele frequency greater than 1%) (18) or in the minimally admixed South American Indian tribes that have "private variants" (15). Three Amerindian mutants, Yanomama, Maku, and Mexico, characterized by structural study, have not been detected in other racial groups. Also, nine other albumin variants have been detected in Japanese but not yet in other ethnic groups (see table 1 of ref. 16). Because of the extensive electrophoretic surveys with several pH systems in both Japan and Italy, we conclude that at least in these countries there is no evidence for overlapping in the incidence of these two sets of alloalbumins.

Alloalbumins Present in Diverse Ethnic Groups May Result from Independent Mutations. Several alloalbumins that appear to be clustered in certain Italian regions have also been found in diverse ethnic groups in other countries and, therefore, may have resulted from independent mutations. These include albumins B and Tagliacozzo and also certain proalbumins. Albumin B (Verona) is the alloalbumin most frequently encountered in France and Germany and elsewhere in populations of European descent, and we found it in 103 unrelated individuals in Italy. Almost all carriers had the same geographic origin—namely, the Veneto region and nearby Lombardy. However, albumin B has also been detected in other racial groups—e.g., in a number of unrelated Japanese and in a Cambodian (16, 17, 20). Another example is albumin Tagliacozzo (313 Lys → Asn) (7); this was present in 19 unrelated families in the Abruzzo region of central Italy. However, this alloalbumin has been found elsewhere in other unrelated families of Caucasian descent (e.g., Canterbury in New Zealand and Cooperstown in the United States); more important, it also is present in indigenes of New Guinea (16).

Table 1. Italian genetic variants of human serum albumin: Amino acid substitutions, nucleotide changes, and geographic distribution

Type of variant	Structural change	Genomic change: Codon in albumin A	Genomic change: Minimum change	Genomic change: New codon	Number found	Region	Ref.
Point substitution							
Proalbumin							
Varese	−2 Arg → His	CGT	G → A	CAT	8		
Tradate	−2 Arg → Cys	CGT	C → T	TGT	5		22
Albumin							
Torino	60 Glu → Lys	GAA	G → A	AAA	1		
Vibo Valentia	82 Glu → Lys	GAA	G → A	AAA	2		
Tagliacozzo	313 Lys → Asn	AAG	G → Y	AAY	49	Abruzzo	7
Roma	321 Glu → Lys	GAG	G → A	AAG	25	Unrestricted	8
Castel di Sangro	536 Lys → Glu	AAG	A → G	GAG	1		11
Verona B	570 Glu → Lys	GAG	G → A	AAG	103	Veneto	9
Milano Fast*	573 Lys → Glu	AAA	A → G	GAA	80	Lombardy	6
COOH terminus							
Catania*	576-Val-Ala-Ala-Ser-Lys-Leu-Pro†				62	Sicily	12
Venezia*	571-Glu-Pro-Thr-Met-Arg-Ile-Arg-Glu‡				105	Veneto	13

Alloalbumins Varese, Torino, and Vibo Valentia are described in the text. Albumin codons are from the genomic sequence of Minghetti *et al.* (23). Nucleotides exchanged in the codons are underlined. Y is either T or C. Amino acids changed in COOH-terminal sequence are underlined. Number found is the number of ascertainments in unrelated individuals regardless of region. The region is specified only if 10 or more unrelated carriers were identified in the region.

*Homozygote identified.

†Frame-shift mutation.

‡Exon-deletion mutation.

Only two of the five known proalbumins (3, 10, 14, 21, 22) have so far been identified in Italy; these are proalbumins Varese (−2 Arg → His) and Tradate (−2 Arg → Cys). The Varese proalbumin, present in eight unrelated subjects with random geographical distribution in Italy, is of the Lille type that occurs in several European countries (3) and also in Japan and Taiwan (16, 17). A polymorphic frequency (1:50) of this proalbumin has been reported for the Somali population of Africa (24). Structural studies performed at Pavia showed that two slow variants RS1 and RS2 earlier reported to be present in Somalia (1) both possess the −2 Arg → His substitution but differ because of instability of the proalbumin (25). Thus, a proalbumin with the −2 Arg → His mutation occurs in Caucasians, Somalis, Japanese, and Chinese. However, the proalbumin (−2 Arg → Cys) has thus far been detected only in Caucasians (22). Proalbumin Tradate, discovered in five families in northern Italy, has the same amino acid replacement (−2 Arg → Cys) as the most common Swedish alloalbumin (Malmö I) and also the albumin variant Kaikoura that is present in a family of Scottish descent in New Zealand (22).

It is noteworthy that three of the four alloalbumins that occur both in Italy and in diverse ethnic groups elsewhere have point mutations in CpG dinucleotides—namely, albumin B and the two proalbumins. Hypermutability of CpG dinucleotides has been invoked to account for the relative frequency of these mutations (22). However, the other proalbumin type that results from a CpG mutation (Christchurch, −1 Arg → Gln) has not yet been detected in Italy although it is one of the more frequent variants in France (3) and has been found in New Zealand (21), Japan (17), and the United States (14). In summary, it would appear that a few alloalbumins (such as albumin B and certain proalbumins) are widely distributed ethnically and geographically because they reflect identical but independent mutations at CpG dinucleotide sites. On the other hand, other alloalbumins, such as those clustered in and largely restricted to particular regions of Italy, probably originated as a fresh mutation in a founder individual as long ago as medieval times.

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