Proc. Natl. Acad. Sci. USA
Vol. 77, No. 9, pp. 5480–5482, September 1980
Medical Sciences

Albumin Naskapi variant in North American Indians and Eti Turks

(albumin B/albumin Mersin/albumin Adana/Central Asia)

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Contributed by Baruch S. Blumberg, June 28, 1980

ABSTRACT **Both conventional polyacrylamide gel electrophoresis and a new type of electrophoretic screening procedure indicate that the polymorphic albumin variants Naskapi, found chiefly in the Naskapi Indians of Quebec, and Mersin, found in the Eti Turks of southeastern Turkey, are molecularly identical or very similar and that the amino acid substitution site in these variants is located between residues 330 and 446. This discovery is consistent with a genetic relationship between the Eti Turks and American Indians. We also report a new variant found in the Eti Turks, albumin Adana, which migrates similarly to albumin B on conventional gels but which our new system shows to differ from the common albumin A and albumin B by a substitution between residues 549 and 585.**

Of the approximately two dozen known electrophoretic variants of human serum albumin, albumin Naskapi is one of the few that occur in polymorphic frequency (1, 2). It has been found, until now, exclusively in North American Indians, with the highest reported frequencies being in the Naskapi Indians of Quebec (1, 2). Albumin Mersin was recently discovered in the Eti Turks of southeastern Turkey.‡ We have found that it migrates identically to albumin Naskapi on polyacrylamide gel electrophoresis at pH 8.6 and occurs with high frequency in the Eti Turks; 8.8% of 397 Eti Turks in Mersin and 9.7% of 93 Eti Turks in Tarsus were heterozygotes for albumin Mersin. However, in two districts of Adana where Eti Turks are numerous in the population, albumin Mersin was not detected in the 442 subjects tested. All of these locations are in southeastern Turkey. By using a newly developed, more discriminating electrophoretic technique (3, 4), we have confirmed (within the limits of this technique) that albumins Naskapi and Mersin are identical. We also report a new variant (proposed name, albumin Adana) which migrates like albumin B on conventional polyacrylamide gel electrophoresis but which our new method has revealed to be different. The cyanogen bromide peptides containing the amino acid substitution sites have been identified for these variants.

MATERIALS AND METHODS

Sera. The albumin A and Naskapi heterozygote standards used in this study were from the Pima Indians of the Gila River Community in Arizona. The Naskapi homozygote standard was from the Naskapi Indians of the Ungava region of Quebec collected in Schefferville, Quebec. These samples had been previously classified by cellulose acetate electrophoresis (Tris citrate buffer, pH 5.4). The Eti Turk samples were from the sources mentioned in the text.

Variant Screening. Cyanogen bromide cleavage and iodoacetamide alkylation of the albumin samples were done as described (4). Polyacrylamide gel electrophoresis of native sera and of cleaved, alkylated sera was done as described earlier (4) and in the legends to Figs. 1 and 2.

RESULTS

Fig. 1 shows the electrophoretic mobilities of albumins A/A, A/Naskapi, Naskapi/Naskapi, A/Mersin, A/Adana, and A/B on a nondenaturing polyacrylamide gel at pH 8.6. Albumin A/Naskapi exhibited two bands of about equal intensity, one band comigrating with albumin A and the other migrating more rapidly towards the anode, whereas homozygotic albumin Naskapi had only the more rapidly migrating band. (The diffuse quality of the latter band is probably due to deamidations resulting from several years of storage.) Albumin A/Mersin migrated identically to A/Naskapi under these conditions, and albumin A/Adana migrated essentially the same as albumin A/B.

It is possible that electrophoretic variants arising from different substitutions or substitutions in different regions of the molecule may comigrate in conventional nondenaturing gels. Thus, it was necessary to devise a more discriminating screening system. The strategy we used was first to cleave the molecule at each of its six methionine residues with cyanogen bromide and then to alkylate the resulting fragments with iodoacetamide to prevent disulfide formation. The fragments were then analyzed on polyacrylamide gels containing acetic acid, urea, and the nonionic detergent Triton X-100 (5, 6). Amino acid substitutions can be localized to a specific cyanogen bromide fragment by this method. Deamidation or aggregation artifacts are not seen in this acidic, denaturing system. In addition, we have shown that the Triton gel system is capable of resolving proteins differing in neutral as well as charged amino acid substitutions (5, 6).

Fig. 2 illustrates the electrophoretic mobilities of the same samples as those of Fig. 1 after they were cleaved and alkylated as described above. When compared to albumin A, the albumin Naskapi heterozygote possesses an additional band, CNBr VN, migrating more slowly than the usual CNBr V, whereas the Naskapi homozygote possesses only the variant band. (A minor band, probably arising from cysteine oxidation or lysine alkylation of CNBr V, comigrated with CNBr VN. Note also that only those samples possessing the Naskapi-like fragment exhibited a modified counterpart of this band, VNm.) These results strongly suggest that fragment CNBr V (residues 330–446) is the region containing the Naskapi substitution. Albumin Mersin also possesses a variant CNBr VN fragment identical to that of albumin Naskapi whereas the remainder of the fragments migrate identically to those of albumin A. This

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‡ Bingöl, G. & Aydin, G. (1975) Bulletin of Abstracts Fifth Scientific Congress of the Scientific and Technical Organization of Turkey, Istanbul, Turkey.

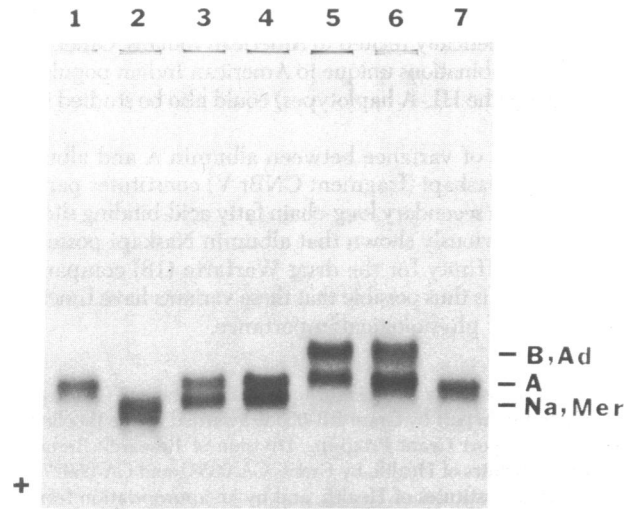

FIG. 1. Electrophoretic mobilities of native albumin variants. Tracks 1 and 7, A/A; track 2, Naskapi/Naskapi; track 3, A/Naskapi; track 4, A/Mersin; track 5, A/Adana; track 6, A/B. Gel was 8% polyacrylamide containing 50 mM Tris·HCl (pH 8.6). Ad, Adana; Na, Naskapi; Mer, Mersin. Stain was Coomassie brilliant blue R-250.

similarity greatly enhances the possibility that albumins Naskapi and Mersin are molecularly identical. Thus, we suggest that the precedent name Naskapi be used. Albumin Adana exhibited an additional band migrating ahead of fragment CNBr VI (CNBr VIAd), which suggests that this fragment (residues 447–548) contains the substitution site in this variant. Albumin A/B contains a very fast migrating counterpart of CNBr VII (residues 549–585), CNBr VIIB, which is consistent with its known lysine-glutamic acid substitution at position 570 (7). It is thus quite different from albumin Adana. Peptide mapping of CNBr VN from albumin Naskapi has shown the substitution site to be between residues 373 and 389.

DISCUSSION

Up to now, albumin variants reaching polymorphic frequencies have been found primarily among certain groups of North, Central, and South American Indians (2). Now two local populations of Eti Turks have also been shown to be polymorphic for a fast-migrating albumin variant which appears to be the same as albumin Naskapi. It is restricted to Eti Turks living in Mersin and Tarsus and is not found in Adana. This discontinuous distribution could result from ethnic diversity among the Eti or from inbreeding or genetic drift within local communities. The slow variant, albumin Adana, was discovered in a patient undergoing examination in a hospital, and its frequency among Eti Turks or the general Turkish population is unknown. It is possible that this variant also exhibits wider distribution but has been erroneously classified as albumin B.

The origins and affinities of American Indians have been questions of long-standing interest in population phylogeny. Skeletal (8), morphological (8), and genetic (9, 10) evidence clearly suggests that American Indians are descendants of Mongoloid populations that moved into the New World from Central and (or) Eastern Asia, most probably from the interior of Siberia. However, it is not known from what part of Asia the migrants came. An ancestral lineage can be constructed by identifying which contemporary Asian populations are most genetically similar to living American Indians. We have suggested that the restricted distribution of the Naskapi allele makes it particularly valuable in determining population affinities between Indian and Asian populations (11). Until now,

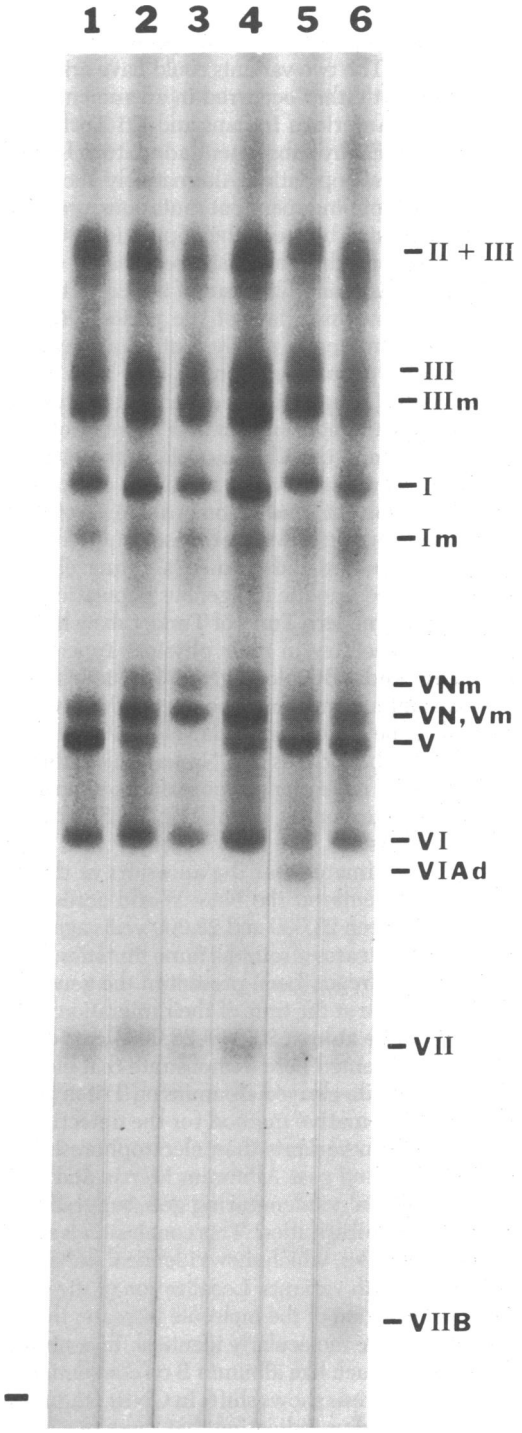

FIG. 2. Resolution of alkylated CNBr fragments of albumin variants. Gel 1, A/A; gel 2, A/Naskapi; gel 3, Naskapi/Naskapi; gel 4, A/Mersin; gel 5, A/Adana; gel 6, A/B. Gels were 12% polyacrylamide containing 5 M urea, 6 mM Triton X-100, and 5% (vol/vol) acetic acid. Stain was Amido Black 10B. CNBr fragments are numbered in accordance with their order in the known sequence of albumin A. N, Ad, and B refer to variant peptides found only in albumins Naskapi (Mersin), Adana, and B, respectively; m refers to modifications probably resulting from cysteine oxidation or lysine alkylation by iodoacetamide. The bands were originally identified by isolation on a preparative gel, $NaDodSO_4$ gel electrophoresis, amino acid analysis, and, in some cases, peptide mapping.

albumin Naskapi has been a genetic marker found exclusively among certain groups of North American Indians. Our discovery that albumin Naskapi may be molecularly identical to

an albumin variant (Mersin) in an Asian group is consistent with a genetic relationship between that specific population and American Indians. The two variants could have arisen from the same mutation that either occurred in an ancient population ancestral to both American Indians and Eti Turks or was introduced into the Eti by subsequent admixture from descendants of this ancestral population. Alternatively, the two variants may have arisen from independent mutations, and thus no direct relationship exists between the populations. On historical grounds population admixture is a reasonable explanation. Modern Turkey was in antiquity part of the Eastern (Byzantine) Roman Empire, and historians of the period have provided extensive documentation of nearly continuous invasion of the area by nomadic peoples of Central and East Asian stock. During the sixth through ninth centuries, the Byzantines alternately allied with and fought with a number of groups, including the Avars and the Khazars (12). In the 11th, 13th, and 15th centuries, Asia Minor was successively conquered by the Seljuks (13), Mongols (14), and Ottomans (15), all of whom were ultimately of Asian origin. Most of these conquerors were relatively few in number and formed a ruling elite that was eventually absorbed into the indigenous population. Coon (15) points out that the modern Turks of Turkey show little evidence of their Asiatic ancestry in their physical appearance, body measurements, and ABO blood group frequencies. The presence of a variant albumin may be one of the few detectable indicators of gene flow from Asia. However, to distinguish between the possible origins of a Naskapi-like albumin in the Eti Turks requires collection of more data on the albumins of geographically intervening populations in Siberia and other parts of Central Asia.

Stewart (16) estimates that the ancestors of the American Indians probably entered the New World across the Bering Land Bridge between 23,000 and 28,000 years ago. If albumins Naskapi and Mersin arose from the same mutation, the Naskapi allele must have already been present in the gene pool of this ancestral population at the time of their migration, and thus the mutation would be at least 23,000–28,000 years old.

The results presented here demonstrate that electrophoresis of cyanogen bromide-cleaved albumins on Triton gels is a more sensitive and informative method for the detection and comparison of albumin variants than electrophoresis on conventional nondenaturing gels. Albumins Mersin and Naskapi migrate identically on nondenaturing gels, suggesting that they contain the same substitution. This conclusion is supported by Triton electrophoresis, which shows identical shifts in the CNBr V fragment for both variants. Localization of the difference to a much smaller region of the molecule increases the probability that the variants are molecularly identical. In contrast, albumin Adana migrates much like albumin B on conventional gels, but Triton electrophoresis shows shifts in CNBr fragments VI and VII, respectively, thus indicating that these two variants have amino acid substitutions at different sites.

The ability of our screening procedure to discriminate between variants that possess similar mobilities in conventional systems but that arise from substitutions in different regions in the molecule provides a new tool for genetic and anthropological applications. Additional genetic studies, for example, of loci located on chromosome number 4 (the location of the albumin locus), can now be designed to test the hypothesis raised in this paper that the Eti Turk populations of Mersin and Tarsus are genetically related to American Indians. Other genes and gene combinations unique to American Indian populations (i.e., some of the HL-A haplotypes) could also be studied in the Eti Turks.

The region of variance between albumin A and albumins Mersin and Naskapi (fragment CNBr V) constitutes part of a primary and a secondary long-chain fatty acid-binding site (17). We have previously shown that albumin Naskapi possesses a diminished affinity for the drug Warfarin (18) compared to albumin A. It is thus possible that these variants have functional differences of physiological importance.

We thank Ms. Judy Mamas for technical assistance. This research was supported in part by Grant RR-05539 awarded by the Biochemical Research Support Grant Program, Division of Research Resources, National Institutes of Health, by Grants CA-06551 and CA-06927 from the National Institutes of Health, and by an appropriation from the Commonwealth of Pennsylvania.

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