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Population Genetic Studies of the Philippine Negritos. III. Identification of the Carbonic Anhydrase-1 Variant with CA_1 Guam

KEIICHI OMOTO,[1] SHINTARO UEDA,[1] KAZUAKI GORIKI,[2]
NORIO TAKAHASHI,[2] SHOGO MISAWA,[3] AND ILDEFONSO G. PAGARAN[4]

SUMMARY

Investigation of blood samples from 277 Mamanwas of northeastern Mindanao, Philippines, confirmed the concentration of the variant carbonic anhydrase-1 (CA_1 3N) in this group. The frequency for the variant allele was estimated at .217 ± .017. It occurs also in the Manobos, the Mongoloid indigenous inhabitants of the same district, although the frequency is low (.019 ± .008). Survey of samples from other Philippine populations, including the Aeta and the Ifugao of Luzon, failed to find variants. This finding suggests different origins of the Aeta and the Mamanwa, although both are usually referred to as Negritos.

The CA_1 3N protein was purified by affinity chromatography using azosulfonamide and rechromatography on a DEAE-Sephadex column. The tryptic peptide pattern of CA_1 3N was similar to that of CA_1 Guam already reported. Furthermore, amino acid analyses of the tryptic peptides indicated that CA_1 3N is characterized by the substitution 253 Gly→Arg, confirming the identity of this variant with CA_1 Guam.

The widespread occurrence of CA_1 3 variants in the Western Pacific suggests that this variant was once common in an aboriginal population of this region, from which it was scattered by gene flow.

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[1] Department of Anthropology, Faculty of Science, The University of Tokyo, Japan.

[2] Department of Clinical Laboratories, Radiation Effects Research Foundation, Hiroshima, Japan.

[3] Department of Legal Medicine, Institute of Community Medicine, The University of Tsukuba, Ibaragi-Pref., Japan.

[4] Rural Health and Family Planning Center, Cabadbaran, Agusan del Norte, Philippines.

INTRODUCTION

Polymorphic occurrence of the variant carbonic anhydrase-1 (CA_1) among the aboriginal inhabitants of northeastern Mindanao was recently reported [1]. The data were based on a preliminary survey carried out in 1977 on 82 blood samples from the Mamanwas, who are regarded as one of the Negrito tribes. The variant isozyme was electrophoretically indistinguishable from CA_1 Guam (CA I Guam or CA_1 3Guam), and the variant allele was designated $CA_1^{3Negrito}$ (CA_1^{3N})*. The frequency estimated for the variant allele was remarkably high (.256), although it was unknown whether this variant occurred in other tribal groups of the Philippines [1].

A more extensive field survey was carried out in 1978 in the provinces of Agusan del Norte and Surigao del Norte, Mindanao [2, 3]. Blood samples were obtained not only from Mamanwas, but also from the Manobos, who are one of the slash-and-burn agriculturists of Mongoloid descent living adjacent to the Mamanwa group. Moreover, blood specimens were obtained from two Mamanwa subjects who had been known to be homozygous and heterozygous, respectively, for the CA_1 variant, on which peptide analysis was performed in 1979. The result indicating the identity of the "Negrito" variant with the "Guam" variant (253 Gly→Arg) has been partly reported [3, 4]. In the present study, we are concerned with the details of the peptide analysis and further studies on the distribution of the CA_1 variant in various Philippine tribes.

MATERIALS AND METHODS

Blood Samples

Blood samples for population studies were obtained from 277 Mamanwas and 134 Manobos in the 1978 field survey at Santiago and Kitcharao, Agusan del Norte, and at Urbistondo, Surigao del Norte, northeastern Mindanao. They were kept cool with wet ice for up to 7 days and then transported by air to Tokyo, where red cells were separated and preserved in liquid nitrogen. Blood samples of 50 ml each were obtained in acid citrate dextrose (ACD) solution from the Mamanwa subjects known to be either homozygous or heterozygous for the CA_1 variant, and transported to Tokyo within 3 days. The separated red cells were washed three times with cold saline and preserved in liquid nitrogen as packed red cells.

Further blood samples used in the screening of CA_1 variants in this study had been obtained since 1975 from various ethnic groups of the Philippines: 283 Aetas (Negritos of west-central Luzon), 97 Ifugaos (aboriginal inhabitants of Mountain Province, northern Luzon), 194 Tagalogs from Manila, and 125 Visayans from Bacolod, Negros Island [5, 6]. All these are "quasi-random" samples, since no exact relationships among subjects could be known.

Starch Gel Electrophoresis

Screening for CA_1 variants was carried out by means of horizontal starch gel electrophoresis using a Tris-EDTA-borate buffer, pH 8.6, and 4-methylumbelliferyl acetate as substrate [7]. Comparison of electrophoretic mobility of purified CA_1 proteins (fig. 1) was made using the vertical starch gel electrophoresis with Tris-EDTA-borate as the buffer, pH 8.6, and protein stain with Amido Black [8].

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* Dr. R. E. Tashian proposed the name CA I Mindanao, which he thought more appropriate for this variant (personal communication, 1980).

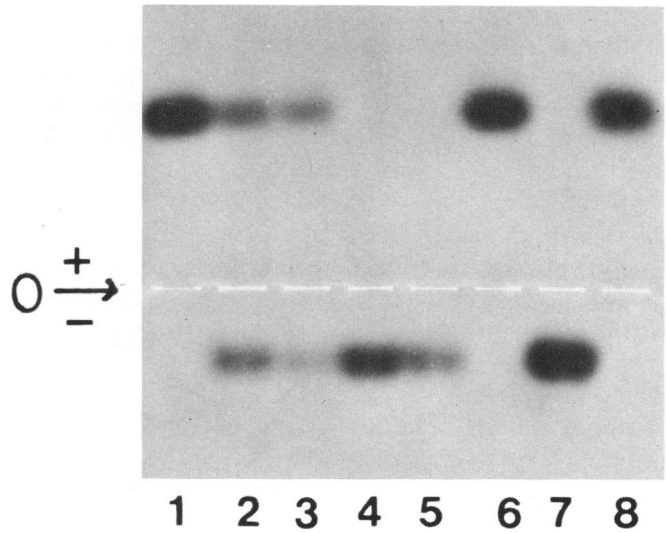

FIG. 1.—Carbonic anhydrase-1 patterns stained with Amido Black (protein stain) after vertical starch gel electrophoresis for 18 hrs at 8 V/cm, pH 8.6. *1*, CA_1 1; *2*, CA_1 1-HIR 1; *3*, CA_1 3N; *4* and *5*, CA_1 3N (homozygote); *6*, CA_1 1; *7*, purified CA_1 3N; *8*, purified CA_1 1.

Purification of CA_1

The normal and variant CA_1 isozymes were isolated from 25 ml packed red cells obtained from an individual of type CA_1 1-3N (heterozygote). The two CA forms (CA I and CA II) were initially separated by affinity chromatography on a sulfonamide-bound Sephadex column according to the method of Osborne and Tashian [9]. Further purification was achieved by chromatography on a DEAE-Sephadex column [10]. The concentration of the carbonic anhydrase was determined using a molar extinction coefficient at 280 nm of 4.9×10^4 liter · $mole^{-1}$ · cm^{-1} [9]. About 13.2 mg of CA_1 1 and 11.1 mg of CA_1 3N were separated.

Peptide Analyses

Tryptic digestion and peptide mapping of the purified CA_1 were carried out as described [11]. About 3.0 mg each of CA_1 1 and CA_1 3N proteins were digested with 2% trypsin for 90 min at 37° C. The digest was then centrifuged for 10 min at 2,000 *g* to separate the undigested core. Tryptic digests were applied to each sheet of Whatman 3MM filter paper. Descending chromatography was carried out in butanol-pyridine-acetic acid-water (15:10:13:12), and electrophoresed in pyridine-acetic acid-water (2.5:1:250), pH 6.5, for 80 min at 2,000 V. The peptides were eluted from the paper with 25% acetic acid. Amino acid composition of the eluted peptides was determined with an amino acid analyzer after hydrolysis for 21 hrs in 6 N HCl at 105°C. Purification and peptide analyses were carried out in the Department of Clinical Laboratories, Radiation Effects Research Foundation, Hiroshima, by K. G. and N. T.

RESULTS

Population Studies

The distribution of CA_1 variants among the Negrito (Mamanwa) and the non-Negrito (Manobo) samples from northeastern Mindanao is shown in table 1. The

extraordinarily high frequency for the variant allele CA_1^{3N} in the Mamanwa group was confirmed. It was found that the variant also occurs in low but polymorphic frequency among the Manobos. However, screening in the samples from other ethnic groups of the Philippines, namely, 283 Aetas, 97 Ifugaos, 194 Tagalogs, and 125 Visayans, failed to detect the variant.

Electrophoresis of Purified CA_1

Figure 1 shows a comparison of purified proteins of CA_1 3N and CA_1 1 with unpurified samples of CA_1 1, Ca_1 1-3N, CA_1 3N (homozygote), and CA_1 1-HIR 1 (a Japanese variant [12]). It is seen that Ca_1 3N is electrophoretically indistinguishable from the CA_1 HIR 1 component.

Peptide Analysis

The tryptic peptide patterns of normal CA_1 (CA_1 1) and the CA_1 3N variant are compared in figure 2. CA_1 3N is characterized by the presence of two peptides, T_{1a} and T_{4a}, and the absence of T_4, which is present in CA_1 1. This pattern of tryptic peptides of CA_1 3N corresponds to that of CA_1 Guam reported by Tashian et al. [11].

The amino acid compositions of the eluates from the tryptic peptides of CA_1 1 (T_1, T_3, T_4, and T_5) and those of CA_1 3N (T_1, T_{1a}, T_3, and T_5) are shown in table 2.

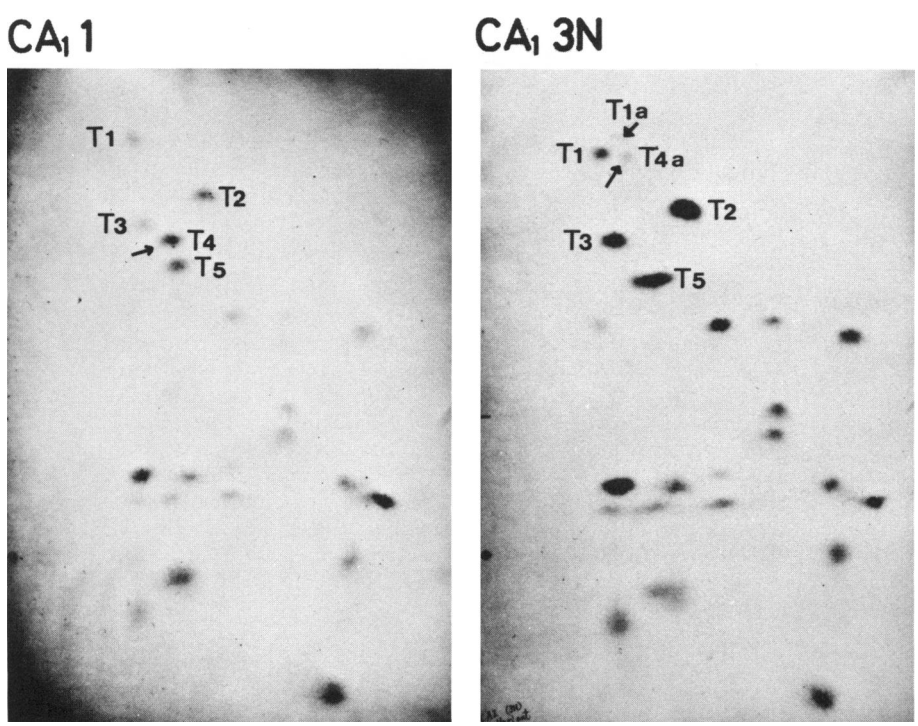

FIG. 2.—Tryptic peptide patterns of CA_1 I and CA_1 3N. *Arrows* point to the peptides that are different in the two patterns.

TABLE 1

DISTRIBUTION OF CA_1 VARIANT PHENOTYPES AMONG THE MAMANWA (NEGRITO) AND THE MANOBO (NON-NEGRITO) GROUPS IN NORTHEASTERN MINDANAO

POPULATION	NO.	CA_1 PHENOTYPES			ALLELE FREQUENCY (CA_1^{3N})	SE	χ^2
		1	1–3N	3N			
Mamanwa (Negrito)	277	165	104	8	.217	.017	3.13 ($.10 > P > .05$)
Manobo (non-Negrito).....	134	129	5	0	.019	.008	0.05 ($.90 > P > .80$)

The spot T_2 represents free arginine, for an arginine standard migrates to the T_2 position, and also arginine can be detected when the eluate from T_2 is analyzed without acid-hydrolysis treatment. The peptide T_{4a} has arginine only, although it is the dipeptide Arg-Arg. T_{1a} found in CA_1 3N is composed of lysine and arginine in equal amounts. This peptide has also been known to be present in the Guam variant [11]. It was concluded, therefore, that the peptide T_4 (Gly-Arg) corresponds to the residues at sites 253–254 in the normal CA I (CA_1 1) [13], of which glycine at site 253 has been substituted by an arginine residue in the CA_1 3N, and that CA_1 3N is identical to CA_1 Guam.

DISCUSSION

In this study, we have confirmed the occurrence in high frequency of the variant CA_1 among the Mamanwas of northeastern Mindanao. As shown in table 1, the frequency of the variant allele was estimated at $.217 \pm .017$, the value similar to $.256 \pm .034$ reported in the preliminary survey [1]. Since considerable intermarriage takes place between the Mamanwas and the Manobos, it was considered necessary to examine samples from the Manobos to determine the origin of the CA_1 polymorphism. The frequency for the variant CA_1 among the Manobos was much lower than in the Mamanwas, suggesting that the variant is inherent in the Mamanwa population rather than being introduced from alien populations through admixture.

TABLE 2

COMPARISON OF THE MOLAR RATIOS OF AMINO ACID RESIDUES IN TRYPTIC PEPTIDES OBTAINED FROM CA_1 1 AND CA_1 3N

AMINO ACID	CA_1 1				CA_1 3N			
	T_1	T_3	T_4	T_5	T_1	T_{1a}	T_3	T_5
Lysine	1.06 (1)	1.04 (1)	···	1.00 (1)	0.90 (1)	1.00 (1)	0.86 (1)	1.00 (1)
Arginine.....	1.18 (1)	···	1.17 (1)	···	0.92 (1)	1.10 (1)	···	···
Glycine.....	1.00 (1)	1.00 (1)	1.00 (1)	···	1.00 (1)	···	1.00 (1)	···
Threonine.....	···	···	···	1.04(1)	···	···	···	1.04 (1)

NOTE: The assumed no. residues is given in parentheses.

According to the electrophoretic mobility, this variant belongs to CA_I 3 (CA Ic), although in this study it was found also to be hardly distinguishable from CA_I HIR 1 (fig. 1). Of the CA_I 3 variants, only CA_I Guam has hitherto been characterized chemically (253 Gly→Arg) [11]. In the present study, it was possible to identify the "Negrito" variant with the "Guam" variant by means of peptide and amino acid analyses as shown in figure 2 and table 2.

The wide geographic distribution of this variant is unique among CA_I variants [8, 14]. It has been recorded in Chamorros of Guam and Saipan, Mariana Islanders, Filipino residents in the United States, and probably also Malaysians and Indonesians. The frequencies of the variant allele in these populations ranged from .002 to .011 [14]. The allele frequency found in the Manobos in the present study falls within this range. Therefore, the frequency found in the Mamanwas is by far the highest among the values thus far obtained, suggesting that this variant was once common in an aboriginal population of the Western Pacific, from which it was scattered by gene flow. It is likely that the Mamanwa is derived from such an aboriginal population. That this variant seems to be absent in the Aeta, the Negrito group of Luzon, raises a possibility that the Aeta and the Mamanwa have different genetic origins, representing distinct migrations to the Philippines. Although they are usually referred to as the Negrito, there are considerable differences in stature (our unpublished data, 1978) and in the distribution of several genetic markers, including HLA antigens [2, 3].

The failure to find the variant among samples from Tagalog and Visayan groups should not be taken as evidence for complete absence, since Lie-Injo found two individuals who were probably heterozygous for CA_I Guam variant among 120 Filipinos residing in the United States [15]. However, it is unlikely that the CA_I polymorphism with such a high allele frequency as found in the Mamanwa occurs among the majority Filipino group of Tagalogs and Visayans.

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