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The Frequency of "Rare" Protein Variants in Marshall Islanders and Other Micronesians

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Recently, in connection with an ongoing medical study of Marshall Islanders who were exposed to radioactive fallout in the Bikini incident [1], we had the opportunity to examine blood samples from these Islanders for the occurrence of polymorphisms and rare variants of serum proteins and erythrocyte enzymes. The results, combined with the findings of others on Micronesians, suggest that there may be a lower frequency of rare variants in this group than in Caucasians or South American Indians. Because of the problems inherent in comparisons of groups sampled in different ways, the usual statistical contrasts were not possible.

THE POPULATION

The study population is composed of individuals from Ebeye, Rongelap, Utirik, and Majuro Islands. Because they were often related, the number of independent genomes in the sample was considerably less than the number of persons. Approximately half of the children in the sample were born to parents inadvertently radiated as a result of fallout from a nuclear explosion at Bikini in 1954. However, the question of a radiation effect did not arise in any substantial manner.

METHODS

Samples were collected in 12 ml vacutainers (Becton-Dickinson, Rutherford, N.J.) containing ACD as anticoagulant on two different occasions, March–April 1974 and 1975. The samples were shipped by air, on ice, from Kwajalein Atoll, Marshall Islands to Honolulu, Hawaii for transshipment to Ann Arbor. Washed red blood cells and plasma were stored at −70° C prior to typing.

The 23 systems for which we performed typings are listed in table 1, together with their standard abbreviations. Our conditions for electrophoresis and identification of the types of ADA, AK_1, ICD_S, LDH, MDH_S, PEPA, PEPB, PGM_2 6PGD, PHI, Alb, Cp, Hp, Hb A, Hb A_2, and Tf have been described previously [2]. Electrophoresis of PGM_1 and TPI and staining of PGM_1 employed the method of Spencer et al. [3]; staining for TPI employed the positive staining method reported by Peters et al. [4]. ALD was determined by the method of Hopkinson et al. [5], CA_1 by the method of Tashian [6], DPGM by the method of Chen et al. [7], GALT by the method of Weitkamp [8], NP by the method of Edwards et al. [9], and Alb by the methods of Weitkamp et al. [10] and Tanis et al. [11]. Acid phosphatase (ACP) and group specific component (Gc) were determined as described previously [2].

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TABLE 1

SERUM PROTEINS AND RED CELL ENZYME DETERMINATIONS IN MICRONESIAN POPULATIONS

System	1974		1975		Present Investigation		Other Investigations		References	Total		
	Tested	Variants	Tested	Variants	Tested	Variants	Tested	Variants		Tested	Variants	Variants/1,000 Determinations
Adenosine deaminase (ADA)	185	0	187	0	372	0	382	0	[13]	754	0	0.0
Adenylate kinase (AK_1)	186	0	186	0	372	0	1387	0	[13, 20]	1759	0	0.0
Aldolase (ALD_A)	180	0	186	0	366	0	0	0	...	366	0	0
Carbonic anhydrase I (CA_1)	184	0	167	0	351	0	640	4	[21, 22]	991	4	4.0
2,3-Diphosphoglyceromutase (DPGM)	183	0	184	0	367	0	0	0	...	367	0	0.0
Galactose-1-phosphate uridylyl-transferase (GALT)	178	0	187	1	365	1	0	0	...	365	1	2.7
Indophenol "oxidase"	0	0	0	0	0	0	382	0	...	382	0	0.0
Isocitrate dehydrogenase (ICD_n) ..	169	0	180	0	349	0	0	0	...	349	0	0.0
Lactate dehydrogenase (LDH) ...	184	0	188	4	372	4	382	1	[13]	754	5	6.6
Malate dehydrogenase (MDH_S) ..	187	0	188	0	375	0	382	0	...	757	0	0.0
Nucleoside phosphorylase (NP) ..	185	0	186	0	371	0	0	0	...	371	0	0.0
Peptidase A (PEPA)	186	0	187	0	373	0	382	0	...	755	0	0.0
Peptidase B (PEPB)	187	0	188	0	375	0	382	0	...	757	0	0.0
Phosphoglucomutase-1 (PGM_1) ..	187	0	187	0	374	0	1387	0	[13, 20]	1761	0	0.0
Phosphoglucomutase-2 (PGM_2) ..	187	0	187	0	374	0	1387	0	[13, 20]	1761	0	0.0
6-Phosphogluconate dehydrogenase (6PGD)	185	0	187	0	372	0	1387	0	[13, 20]	1759	0	0.0
Phosphoglycerate kinase (PGK) ..	0	0	0	0	0	0	380	0	[13]	380	0	0.0
Phosphohexose isomerase (PHI) .	186	0	187	0	373	0	382	0	[13]	755	0	0.0
Triosephosphate isomerase (TPI)..	183	0	187	0	370	0	0	0	...	370	0	0.0
Albumin (Alb)	185	1	187	0	372	1	0	0	...	372	1	2.7
Ceruloplasmin (Cp)	183	0	187	0	370	0	0	0	...	370	0	0.0
Haptoglobin (Hp)	185	0	183	0	368	0	2283	0	[13–16] [17–19]	2651	0	0.0
Hemoglobin A (Hb A)	187	0	188	0	375	0	378	0	[15, 16]	753	0	0.0
Hemoglobin A_2 (Hb A_2)	187	0	188	0	375	0	0	0	...	375	0	0.0
Transferrin (Tf)	185	0	187	3	372	3	774	8	[14–16]	1146	11	9.6
Total	4,234	1	4,269	8	8503	9	12,677	13	...	21,180	22	1.03

RESULTS

Polymorphisms

Genetic polymorphisms were observed in six of the systems: haptoglobin, phosphoglucomutase-1, adenosine deaminase, acid phosphatase, 6-phosphoglucose dehydrogenase, and group specific component. Phenotype and allele frequencies are given in table 2. Because of extensive inter-island population movement since the

TABLE 2

GENE FREQUENCIES FOR SIX GENETIC POLYMORPHISMS IN THE MARSHALL ISLANDS

System		Phenotype 1	2-1	2	Total	Gene Frequency
Adenosine deaminase	1974	167	18	0		
	1975	157	30	0		
Total		324	48	0	372	$ADA^1 = .935$
Group specific component	1974	114	69	2		
	1975	76	81	20		
Total		190	150	22	362	$Gc^1 = .732$
Haptoglobin*	1974	56	94	31		
	1975	43	87	53		
Total		99	181	84	364	$Hp^1 = .521$
Phosphoglucomutase-1†	1974	158	25	1		
	1975	137	46	4		
Total		295	71	5	371	$PGM_1^1 = .891$
		A	AB	B		
Acid phosphatase	1974	101	73	11		
	1975	120	55	12		
Total		221	128	23	372	$ACP^A = .766$
6-Phosphogluconate dehydrogenase	1974	164	21	0		
	1975	180	7	0		
Total		344	28	0	372	$6PGD^A = .962$

* The Hp^0 type was observed in four individuals.
† Two examples of the PGM phenotype 2-7 and a single phenotype 1-7 were observed.

Bikini incident, results are not presented separately by island. Varying numbers of determinations for the systems listed in tables 1 and 2 are due to a considerable time lapse between collection of the samples and their receipt in the laboratory. As a consequence, either no pattern or an unclear pattern (even after repetition) was occasionally obtained for a given trait. Three persons had the PGM_1 7 phenotype. Because of the occurrence of this variant in polymorphic proportions elsewhere in

Micronesia (see below), we tabulated it with the polymorphisms. These three individuals were siblings; one parent was normal, the other was not tested.

Rare Variants

Four rare variants, affecting nine persons, were observed in a total of 8,520 determinations: (1) A fast albumin variant was detected by only one of the three screening systems in use for albumin, namely, the pH 5.0 sodium acetate buffer system of Weitkamp et al. [10] (see fig. 1). It occurred in a 5-year-old female

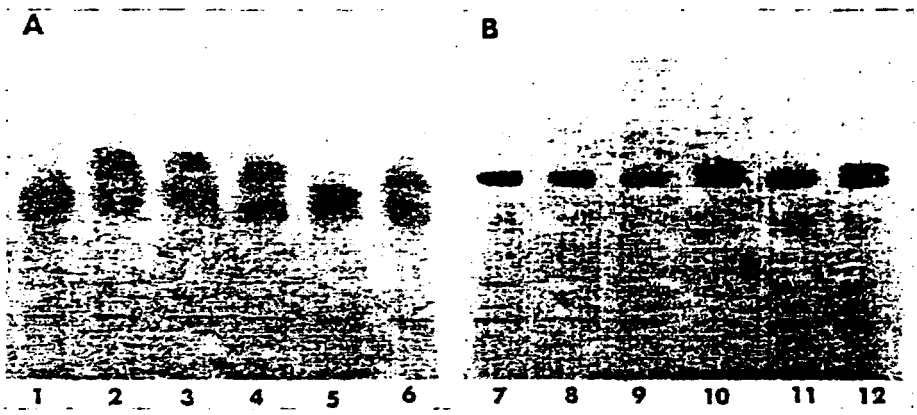

FIG. 1.—Albumin variant starch gel patterns using the following buffer systems: *A*: sodium acetate, pH 5.0; *B*: tris-EDTA-borate, pH 6.9. Albumin samples shown are normal serum 1, 5, 7, 8, and 11; Makiritare-2, 7; Marshall Island variant 3, 9; Makiritare-3, 4; Wapishana-1, 6, 12; Naskapi 10. Normal samples include reference serum from our laboratory and serum from normal Marshall Island samples. All variants, except the presently reported Marshall Island variant, have been previously compared by Tanis et al. [2].

whose mother was normal (and not exposed to fallout), and whose alleged father was also normal, but there was paternity exclusion on the basis of the MN blood group antigens. This variant was first observed in 1974 and confirmed in a sample from the same individual collected in 1975. (2) An unusual lactate dehydrogenase pattern was observed in four, apparently unrelated, individuals. This pattern, shown in figure 2*A*, is similar to the pattern seen in the LDH "Calcutta-1" variant reported by Ananthakrishnan et al. [12] and previously observed in the Western Caroline Islands by Blake et al. [13]. For three of these individuals neither parent was available. Both parents and two siblings of the fourth individual were normal; there was no parental exclusion with any of 12 polymorphic systems. The occurrence in this population of three other persons with the same defect strongly militates against considering this a mutation. (3) A single individual was found to have a galactose-1-phosphate uridylyltransferase phenotype which was indistinguishable from the "Duarte" variant common in Caucasian populations [8]. Neither parent of this individual was sampled. (4) The transferrin CB phenotype was observed in three unrelated individuals (fig. 2*B*). Although Hainline et al. [14]

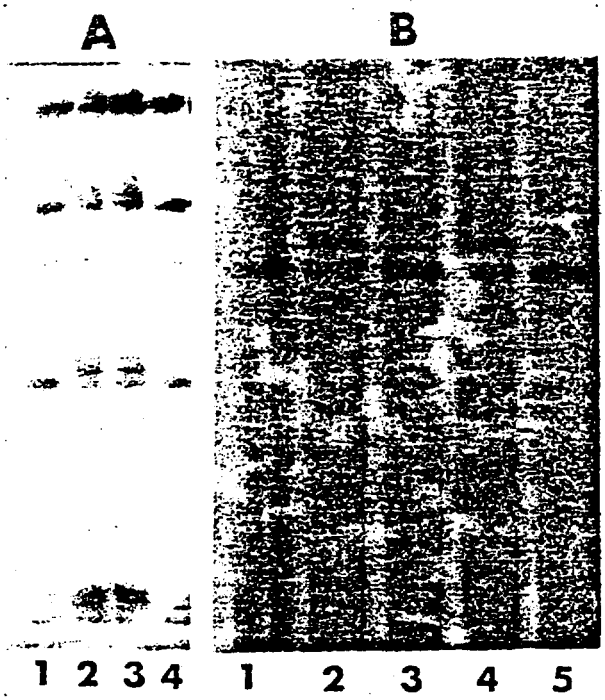

FIG. 2.—*A*, Starch gel patterns of isozymes of lactate dehydrogenase from Micronesian samples showing the normal (*slots 1 and 4*) and "Calcutta" like phenotype (*slots 2 and 3*). *B*, Polyacrylamide gel electrophoresis of serum transferrin from normal individuals (*slots 1, 3, and 5*) and individuals of the transferrin CB phenotype (*slots 2 and 4*).

have reported the transferrin CD phenotype in Micronesian populations, the CB phenotype has not been previously observed. For none of these three individuals were the parents available.

DISCUSSION

Gene frequencies for the 6 polymorphisms encountered all fell within the range reported in other studies of Micronesians [13–20].

In table 1 we summarize not only our own findings but also the results of all the other studies of rare variants of 25 systems in Micronesians which we were able to locate in the literature. To date, 27 islands have been sampled. Any effort to treat "rare" variants involves some arbitrary decisions; no approach is apt to find universal acceptance at this time. We excluded from this summary any variant which, *for the totality of the representatives of the population studied to date,* occurs in more than 2% of the group (one of the conventional definitions of a polymorphism). By this definition of rare variant, we excluded from the tabulation the polymorphisms involving types 3 and 7 of the PGM_1 system reported by Blake et al. [13] and the polymorphism for the type 2 of phosphoglycerate kinase reported by these same authors. The final column of table 1 presents the frequency

per 1,000 variants for each system, and the *unweighted* average of all the systems, employed to prevent an extensive study of a single system from dominating the picture. By this approach, the average frequency with which variants are encountered in the systems under consideration, on the basis of 21,180 determinations, is 1/1,000 observations.

Reports on the frequency of rare variants in a variety of populations are accumulating rapidly. For instance, among South American Indians, we find, based on ADA, AK_1, ICD_S, LDH, MDH_S, PEPA, PEPB, PGM_1, PGM_2, 6PGD, PHI, Alb, Cp, Hb A, and Tf of table 1, an unweighted rare variant frequency of 3.2/1,000 determinations [2]. On the other hand, among Caucasians living in the British Isles, Western Europe, and the United States, a study of ADA, AK_1, CA_1, ICD_S LDH, MDH_S NP, PEPA, PEPB, PGM_1, PGM_2, 6PGD, PHI, TPI, Cp, Hb A, and Tf of table 1 reveals an unweighted variant frequency of 2.9/1000 [6, 21–25]. This latter figure is greatly influenced by the relatively high frequency of ceruloplasmin variants in the two small series on Caucasians [22, 23]. Since the samples of Micronesians and South American Indians contain many relatives and the West European sample does not, the usual (χ^2) contrast is inappropriate. Caution in this contrast is also indicated because the mix of systems varies according to the population, the dividing line between "rare" and polymorphic is arbitrary, and the determinations originate in a variety of laboratories. Taken simply at face value, however, the samples of Micronesians and South American Indians differ by a factor of three in the frequency of rare variants; we suspect this is a biologically meaningful difference.

The frequency of such rare variants is determined by a complex interplay between mutation, selection, and random loss: the latter is highly related to population structure [26, 27]. Assuming for the moment the validity of the observed difference between Micronesians and Amerindians, both the data and our knowledge of these populations are still insufficient to permit conclusions concerning the factors responsible for the difference. However, we believe that there will be situations wherein differences exist, and the populations are more appropriately matched and sampled, as in studies on Japanese and English, for which it may be possible to reach valid inferences concerning the factors responsible for the difference [28].

SUMMARY

Blood specimens from a sample of 373 Marshall Islanders were studied with reference to variants of 23 serum proteins and erythrocyte enzymes. Six of the traits studied exhibited genetic polymorphisms (adenosine deaminase, phosphoglucomutase$_1$, acid phosphatase, 6-phosphogluconate dehydrogenase, haptoglobin, and group specific component). There were in addition four "rare" variants (albumin, transferrin, lactate dehydrogenase, and galactose-1-phosphate uridylyltransferase) involving nine persons, among 8,503 determinations. The frequency of rare variants in Micronesians was compared with the frequencies in West European Caucasians and Amerindians. There are many difficulties in such comparisons, and although the observed values for the three ethnic groups differ by a factor of three

(the Micronesians exhibiting the lowest frequency), it is felt that no firm conclusions concerning differences between ethnic groups can be drawn at this time.

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Continuing Medical Education

Radiation in Childhood and Thyroid Carcinoma

The association of thyroid malignancy with irradiation of the thymus and other structures in the neck area has been known for at least a quarter of a century. [1] Recent publications suggest a continuing incidence of such malignancies despite the discontinuance of irradiation for benign conditions for many years. In fact, the authors of these articles suggest an increasing prevalence of thyroid carcinoma with time in the exposed population. [2,3] In 100 unselected patients, fifteen patients were operated upon and seven carcinomas were found.

At the Michael Reese Hospital in Chicago, 1452 individuals with a history of prior irradiation to the neck region for benign conditions, 18-35 years ago, were evaluated by history, physical examination and thyroid scintigraphy. [4] Twenty-one percent (301) of the group had abnormalities on examination. Of these, 56 were found only on scintigraphy. One hundred and ninety-three were operated upon and 56 malignancies were found. This represented 29% of those operated upon, 18.6% of those with detectable abnormalities and 3.7% of the entire group. Four of 22 glands normal to palpation but with abnormal scintigraphy were positive for malignancy.

Public awareness of this problem has been heightened by a recent television program. This has resulted in many inquiries to physicians for guidance concerning management of this situation in the population at risk.

The question of what to do for these individuals is not easily resolved. Certainly each person should receive a thorough physical examination and then be followed by annual physical examination thereafter. Repeated thyroid scintigraphy does not appear to be a practical approach but perhaps a baseline study with technetium-99m pertechnetate would be of value. If functioning nodules are noted on the pertechnetate image it should be repeated with radioiodine, preferably iodine-123. Individuals with non-functioning nodules should be carefully selected for surgery. In the Michael Reese Hospital series the incidence of malignancy in glands with a single nodule and with multiple nodules was the same.

Prophylactic treatment with thyroid extract or l-thyroxin does not appear warranted on a routine basis. There is no evidence that this will prevent the occurrence of thyroid carcinoma.

While these malignancies are not, for the most part especially aggressive, they do require treatment. They will probably represent a continuing problem for many years to come. A concensus concerning management of this problem does not exist at this time but careful observation of the population at risk may help in evolving a logical plan of treatment in the future.

Harold L. Atkins, M.D.

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