Polymorphisms of Complement Component I and C1R Subcomponent of C1 in Nine Aboriginal Taiwanese Populations

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Abstract Complement component I (IF) and C1R subcomponent of C1 (C1R) types were determined by isoelectric focusing and subsequent immunoblotting techniques for 658 individuals from nine aboriginal Taiwanese populations. The frequency of the *IF*A* allele ranges from 0.075 (Bunun) to 0.430 (Saisiat), and a new variant allele *IF*B2* was found to have polymorphic frequency in the Atayal. The frequency of the *C1R*1* allele ranges from 0.410 (Yami) to 0.650 (Atayal), and the frequency of the *C1R*2* allele ranges from 0.265 (Atayal) to 0.586 (Saisiat). The *C1R*5* allele was found in five populations (Atayal, Bunun, Ami, Puyuma, Yami), and the *C1R*9* allele was found in two populations (Tsou, Puyuma). The results indicate a remarkable degree of genetic variability among these populations. The variability may reflect long-term genetic and geographic isolation of each population.

The aboriginal populations of Taiwan (Kaoshan, Mountain Highlander) in 1985 consisted of about 320,000 people, making up 1.5% of the total population of Taiwan (Dai 1988). Nine aboriginal tribes are commonly recognized as linguistically and culturally distinct groups. Polymorphisms using various genetic markers have been analyzed in some of these tribes (Huang 1964; Ikemoto et al. 1966; Nakajima and Ohkura 1971; Nakajima et al. 1971; Chen et al. 1985), and it is inferred that they belong to the southern group of Mongoloids. However, the precise genetic origin of each tribe and their mutual relationships are not fully

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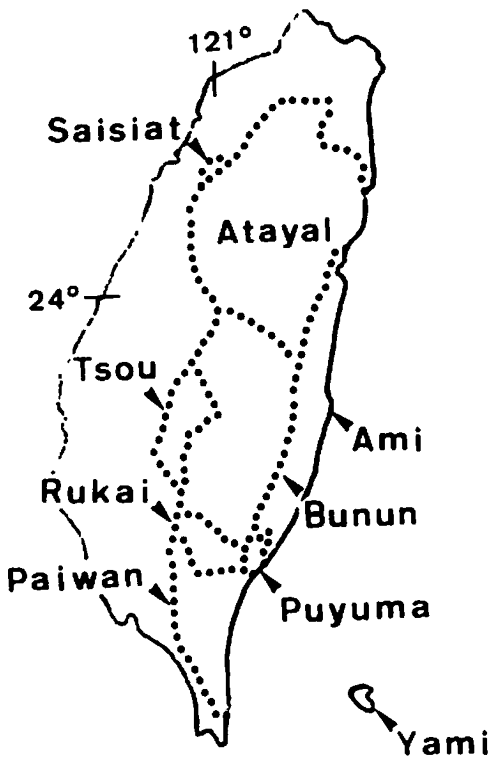

Figure 1. Geographic distribution of nine aboriginal Taiwanese populations.

resolved. To elucidate these problems, we collected blood samples from all nine aboriginal populations in 1990 and 1991. Here, we report a pilot study of polymorphisms of two serum proteins in these nine tribes.

The systems chosen were complement component I (IF) and the C1R subcomponent of the first complement component (C1R), described by Nakamura and Abe (1985) and Kamboh and Ferrell (1986), respectively. Only limited population studies have been performed with these genetic markers so far.

Materials and Methods

Blood samples were collected from 658 unrelated healthy individuals in the following aboriginal Taiwanese populations: Atayal (100), Saisiat (64), Bunun (87), Tsou (80), Ami (72), Puyuma (63), Rukai (54), Paiwan (60), and Yami (78). Their geographic locations are shown in Figure 1.

Plasma samples were treated with fourfold volumes of sialidase (0.5 U/ml; type V, Sigma, St. Louis, Missouri; pH 5.0) at room temperature overnight. The IF typing was carried out by agarose gel isoelectric focusing (IEF) followed by immunoblotting, as described elsewhere (Ding et al. 1991).

For C1R typing polyacrylamide gels (5% total acrylamide concentration, 3% cross-linker ratio, 220 × 120 × 0.3 mm) were prepared containing 2.4% Ampholine (pH 5–8) (Pharmacia, Uppsala, Sweden), 6 *M* urea, and 10% glycerol. The anodic and cathodic solutions were 1 *M* H_3PO_4 and 1 *N* NaOH, respectively. The gel was prerun for 30 min at 12°C and at a constant power supply of 5 W. Once samples (4 μl) had been applied, focusing was continued for 2.5 hr at a constant power supply of 10 W. After IEF, proteins were passively transferred to a Immobilon-P membrane (Millipore, Bedford, Massachusetts). After 60 min of blotting, the filters were removed, treated with a quenching buffer [phosphate-buffered saline (PBS) containing 3% skim milk and 0.01% Tween 20] for 20 min, and incubated for 60 min with peroxidase-conjugated anti-human C1R goat serum diluted 1:200 in PBS. The goat anti-human C1R serum (Binding site, Birmingham, England) was coupled with horseradish peroxidase (type I-C; Toyobo, Osaka, Japan) according to the method of Nakane (1975). The membranes were washed 3 times for 5 min with PBS containing 0.01% Tween 20. The immune complexes formed on the membranes were stained with diaminobenzidine.

Results

The distribution of IF phenotypes is shown in Table 1. Three common phenotypes were observed, which could be explained by two alleles, *IF*A* and *IF*B*. The *IF*A* allele frequency varied from 0.075 (Bunun) to 0.430 (Saisiat) among the nine aboriginal Taiwanese populations (see Table 2). The frequency of the *IF*A* allele in the Saisiat was the highest among all populations studied so far, whereas the frequency in the Bunun was one of the lowest levels in Mongoloids (Table 2). In addition to the common phenotypes, a new variant was encountered in 5 of 100 Atayal samples and was designated as IF B2. The variant band migrated slightly cathodal to the IF B band (Figure 2). The observed phenotypic distribution was in good agreement with Hardy-Weinberg expectation.

The distribution of C1R phenotypes is summarized in Table 3. Seven different phenotypic patterns explained by four alleles were observed. The symbols of these alleles were based on the nomenclature proposed by Kamboh et al. (1989) and Kido et al. (1991). In the nine populations the observed numbers were in accordance with the numbers expected under Hardy-Weinberg equilibrium. The frequency of the *C1R*1* allele

Table 1. Distribution of IF Phenotypes in Nine Aboriginal Taiwanese Populations[a]

Phenotype	*Atayal*	*Saisiat*	*Bunun*	*Tsou*	*Ami*	*Puyuma*	*Rukai*	*Paiwan*	*Yami*
IF A	3	11	1	3	2	1	1	3	2
	(2.9)	(11.8)	(0.5)	(2.8)	(2.2)	(2.5)	(2.9)	(5.7)	(3.5)
IF A,B	28	33	11	24	21	23	23	31	29
	(27.4)	(31.4)	(12.0)	(24.4)	(20.7)	(20.0)	(19.2)	(25.6)	(26.0)
IF B	64	20	75	53	49	39	30	26	47
	(64.8)	(20.8)	(74.5)	(52.8)	(49.2)	(40.5)	(31.9)	(28.7)	(48.5)
IF B,B2	5	0	0	0	0	0	0	0	0
	(4.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)
Other	0	0	0	0	0	0	0	0	0
	(0.9)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)
Total	100	64	87	80	72	63	54	60	78
	(100.0)	(64.0)	(87.0)	(80.0)	(72.0)	(63.0)	(54.0)	(60.0)	(78.0)
χ^2	1.177	0.173	0.636	0.019	0.020	1.375	2.098	2.680	1.024
d.f.	3	1	1	1	1	1	1	1	1
p	>0.7	>0.5	>0.3	>0.8	>0.8	>0.2	>0.1	>0.1	>0.3

a. Expected numbers are shown in parentheses.

Table 2. IF Allele Frequencies in Various Populations

Population	*n*	Allele Frequency *IF*A*	*IF*B*	*IF*B2*	*Other*	References
Aboriginal Taiwanese						
Atayal	100	0.170	0.805	0.025	–	This study
Saisiat	64	0.430	0.570	–	–	This study
Bunun	87	0.075	0.925	–	–	This study
Tsou	80	0.188	0.812	–	–	This study
Ami	72	0.174	0.826	–	–	This study
Puyuma	63	0.198	0.802	–	–	This study
Rukai	54	0.231	0.769	–	–	This study
Paiwan	60	0.308	0.692	–	–	This study
Yami	78	0.212	0.788	–	–	This study
Taiwanese (Han)	198	0.071	0.929	–	–	Yuasa et al. (1988)
Chinese	282	0.099	0.901	–	–	Ding et al. (1991)
Korean	239	0.092	0.908	–	–	Ding et al. (1991)
Japanese (Tokyo)	435	0.107	0.893	–	–	Nakamura and Abe (1985)
Japanese (Okinawa)	363	0.079	0.922	–	–	Yuasa et al. (1988)
Brazilian Indian	393	–	0.977	–	0.023	Salzano et al. (1990)
French	240	0.006	0.994	–	–	Yuasa et al. (1988)
Canadian	288	–	0.998	–	0.002	Zhou and Larsen (1989)

n: sample size.

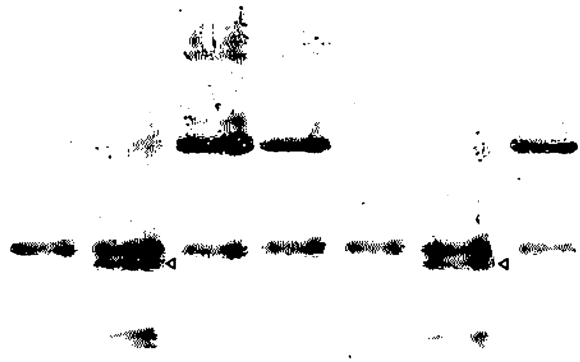

Figure 2. Immunoblotted band patterns of IF phenotypes. From left to right: IF B, IF B,B2, IF A, IF A,B, IF B, IF B,B2, and IF A,B. Anode is at the top. Open triangle indicates major variant band.

ranges from 0.410 (Yami) to 0.650 (Atayal), and that of the *C1R*2* allele ranges from 0.265 (Atayal) to 0.586 (Saisiat). The frequency of the *C1R*5* allele was highest (0.085) in the Atayal, but that allele was not observed in the Saisiat, Tsou, Rukai, and Paiwan. A variant allele, *C1R*9*, was found in the Tsou and Puyuma with frequencies of 0.013 and 0.008, respectively. The *C1R* allele frequencies in various populations are listed in Table 4. Although the *C1R*5* allele is widely distributed in blacks and Mongoloids, the *C1R*9* allele has been observed so far only among Japanese (Nakamura et al. 1988; Kido et al. 1991).

Discussion

The gene diversity (G_{ST}) between populations (Nei 1987) was estimated to be 0.0343 based on the present data. This large G_{ST} value indicates that a significant degree of genetic variability exists among the nine tribes, although only two protein polymorphisms of the complement system were analyzed in this study. Such a great diversity in native populations in Taiwan suggests that there were several migrations to Taiwan from different populations of neighboring regions, such as mainland China and Southeast Asia. If those migrations had occurred a relatively long time ago, low levels of gene flow between populations might also have been a factor producing such large genetic differentiation between these aboriginal Taiwanese populations. In any case, further examination of many more genetic polymorphisms is necessary to test this hypothesis.

Table 3. Distribution of C1R Phenotypes in Nine Aboriginal Taiwanese Populations[a]

Phenotype	*Atayal*	*Saisiat*	*Bunun*	*Tsou*	*Ami*	*Puyuma*	*Rukai*	*Paiwan*	*Yami*
C1R 1	43	8	28	34	25	13	11	23	14
	(42.3)	(11.0)	(26.5)	(31.9)	(25.1)	(15.2)	(11.6)	(22.2)	(13.1)
C1R 1,2	32	37	39	32	32	35	28	27	31
	(34.5)	(31.1)	(41.9)	(36.0)	(33.1)	(30.0)	(26.9)	(28.6)	(33.6)
C1R 2	8	19	18	12	12	12	15	10	23
	(7.0)	(22.0)	(16.6)	(10.2)	(10.9)	(14.8)	(15.6)	(9.2)	(21.6)
C1R 1,5	12	0	1		3	0	0	0	5
	(11.1)	(0.0)	(1.1)	(0.0)	(1.8)	(1.0)	(0.0)	(0.0)	(4.1)
C1R 2,5	5	0	1	0	0	2	0	0	5
	(4.5)	(0.0)	(0.9)	(0.0)	(1.2)	(1.0)	(0.0)	(0.0)	(5.3)
C1R 1,9	0	0	0	1	0	1	0	0	0
	(0.0)	(0.0)	(0.0)	(1.3)	(0.0)	(0.5)	(0.0)	(0.0)	(0.0)
C1R 2,9	0	0	0	1	0	0	0	0	0
	(0.0)	(0.0)	(0.0)	(0.7)	(0.0)	(0.5)	(0.0)	(0.0)	(0.0)
Other	0	0	0	0	0	0	0	0	0
	(0.7)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.3)
Total	100	64	87	80	72	63	54	60	78
	(100.0)	(64.0)	(87.0)	(80.0)	(72.0)	(63.0)	(54.0)	(60.0)	(78.0)
χ^2	1.182	2.346	0.450	1.101	2.198	4.806	0.099	0.186	0.892
d.f.	3	1	3	3	3	6	1	1	3
p	>0.7	>0.1	>0.9	>0.7	>0.5	>0.5	>0.7	>0.5	>0.8

a. Expected numbers are shown in parentheses.

Table 4. C1R Allele Frequencies in Various Populations

Population	*n*	Allele Frequency *C1R*1*	*C1R*2*	*C1R*5*	*C1R*9*	*Other*	*Reference*
Aboriginal Taiwanese							
Atayal	100	0.650	0.265	0.085	–	–	This study
Saisiat	64	0.414	0.586	–	–	–	This study
Bunun	87	0.552	0.437	0.011	–	–	This study
Tsou	80	0.631	0.356	–	0.013	–	This study
Ami	72	0.590	0.389	0.021	–	–	This study
Puyuma	63	0.492	0.484	0.016	0.008	–	This study
Rukai	54	0.463	0.537	–	–	–	This study
Paiwan	60	0.608	0.392	–	–	–	This study
Yami	78	0.410	0.526	0.064	–	–	This study
Japanese (Aomori)	256	0.459	0.357	0.168	–	0.016	Harada et al. (1992)
Japanese (Tokyo)	440	0.422	0.360	0.207	0.001	0.010	Nakamura et al. (1988)
Japanese (Yamanashi)	1000	0.476	0.326	0.194	0.001	0.007	Kido et al. (1991)
Mayan Indians	137	0.945	0.029	0.026	–	–	Kamboh et al. (1989)
Aleuts	187	0.893	0.067	0.040	–	–	Kamboh et al. (1989)
St. Lawrence Island Inuit	101	0.708	0.139	0.153	–	–	Kamboh et al. (1989)
US whites	201	0.908	0.090	–	–	–	Kamboh et al. (1989)
Nigerian blacks	247	0.816	0.164	0.016	–	0.004	Kamboh et al. (1989)

n: sample size.

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