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Heterogeneity of Taiwan's indigenous population: possible relation to prehistoric Mongoloid dispersals

Key words:

anthropology; HLA class I alleles; HLA class I haplotype; Taiwan indigenous groups; 'Taiwanese'

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Abstract: Taiwan's 9 indigenous tribes (Tsou, Bunun, Paiwan, Rukai, Atayal, Saisiat, Ami, Puyuma, Yami) are highly homogeneous within each tribe, but diversified among the different tribes due to long-term isolation, most probably since Taiwan became an island about 12,000 years ago. Homogeneity of each tribe is evidenced by many HLA-A,B,C alleles having the world's highest ever reported frequencies, e.g. A24 (86.3%), A26 (18.8%), Cw10 (36.8%), Cw7 (66%), Cw8 (32.1%), B13 (27.9%), B62 (37.4%), B75 (18%), B39 (53.5%), B60 (33.3%), and B48 (24%). Also, all of these tribes have HLA class I haplotype frequencies greater than 10%, with A24-Cw7-B39 in Saisiat (44.5%) being the highest, suggesting Taiwan's indigenous tribes are probably the most homogeneous (the "purest") population in the world. A24-Cw8-B48, A24-Cw10-B60 and A24-Cw9-B61 found common to many Taiwan indigenous tribes, have also been observed in Maori, Papua New Guinea Highlanders, Orochons, Mongolians, Inuit, Japanese, Man, Buryat, Yakut, Tlingit, Tibetans and Thais. These findings suggest Taiwan's indigenous groups are more or less genetically related to both northern and southern Asians. Principal component analysis and the phylogenetic tree (using the neighbor-joining method) showed close relationship between the indigenous groups and Oceanians. This relationship supports the hypothesis that Taiwan was probably on the route of prehistoric Mongoloid dispersals that most likely took place along the coastal lowland of the Asian continent (which is under the sea today). Cultural anthropology also suggests a relationship between Taiwan's indigenous tribes and southern Asians and to a lesser extent, northern Asians. However, the indigenous groups show little genetic relationship to current southern and northern Han Chinese.

Taiwan's population is heterogeneous and is made up of indigenous people (1.5%), Minnan and Hakka (91%), and mainland Chinese (7.5%) who arrived from China after World War II. Minnan and Hakka are also the so-called 'Taiwanese' who immigrated to Taiwan from southeast coast of China since 17th century. Physical and cultural anthropology and linguistic studies classified Taiwan's indigenous peoples into different groups (1). The geographical distributions of Taiwan 9 indigenous tribes (Tsou, Bunun, Paiwan, Rukai, Atayal, Saisiat, Ami, Puyuma and Yami) and Pazeh tribe (hybrid

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population) are shown in Fig. 1. Since the HLA system is a highly polymorphic genetic system, it has become an important tool to trace the migration of ancient populations. Due to the special ancient geographical situation of the Taiwan Island (2), and also the recent theory of Taiwan being the homeland of the Austronesians

(3), many biological anthropology studies have been done on Taiwan's indigenous people (4–10). Of the 3 HLA studies performed on Taiwan's indigenous peoples, only a limited number of tribes and individuals were tested (8, 10), and insufficient typing sera were used (9). For the past four years (1994–1998), we were able to submit samples from our 10 indigenous groups as panel cells at the Japanese Red Cross Central Block Histocompatibility Workshop (JRCW). These ten tribes comprise almost all available extant Taiwan indigenous groups except Thau tribe (a hybrid population of about 200 peoples). The HLA data of Minnan and Hakka ('Taiwanese') who have been interacted with indigenous tribes during last 4 centuries, are also included in this study for comparison. By analyzing the HLA data, coupled with our red cell blood group studies, we hope to be able to retrace the footprints of the ancestors of Taiwan's indigenous peoples.

Material and methods

HLA typing of the indigenous population

Blood samples were obtained from the following Taiwan indigenous groups : Tsou ($n=51$), Bunun ($n=88$), Paiwan ($n=51$), Rukai ($n=50$), Atayal ($n=50$), Saisiat ($n=57$), Ami ($n=50$), Puyuma ($n=50$), Yami ($n=64$) and Pazeh ($n=47$). All samples were from healthy unrelated individuals whose parents were also from the same tribe. Blood samples were collected in ACD tubes and transported within 24 h to the Immunohematology Reference Laboratory, Mackay Memorial Hospital. All samples were submitted as panel cells for the 1995, 1996, 1997 and 1998 JRCWs and were tested for HLA-A, B, C by serology (standard microlymphocytotoxicity technique). The typing sera distributed at these workshops were well-defined antisera collected and specified by the participating labs from Japan, Korea, Thailand, US, South Africa and Taiwan. The total number of typing sera used in the workshops were 560 HLA class I antisera for the 1995 and 1996 workshops, 509 antisera for 1997 and 420 antisera for 1998. About one-third of these samples were also tested by Terasaki Chinese HLA-ABC 72-Well Trays (lots 2, 3, 3A, 3B).

HLA typing of Minnan and Hakka ('Taiwanese')

There were 254 Minnan and Hakka individuals (parents were also Minnan or Hakka) enrolled for paternity testing during the period 1996–1998. These individuals were unrelated persons and were parents of inclusion trios and the mother of exclusion trios. Blood samples collected in ACD tubes were tested for HLA-A, B, C by the

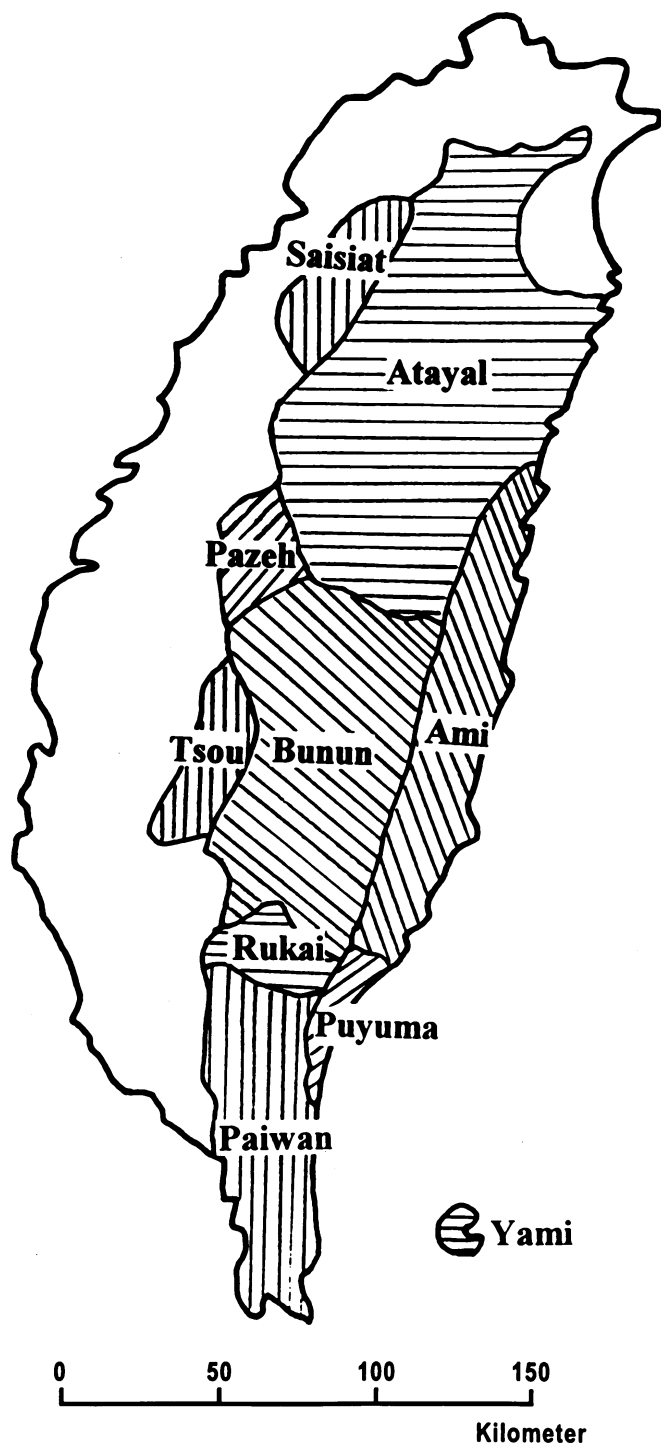


Fig. 1. Geographical distribution of Taiwan's indigenous tribes.

standard microlymphocytotoxicity technique using Terasaki Chinese HLA-ABC 72-Well Trays (lots 2, 3, 3A, 3B) and the latest Terasaki Special Monoclonal Tray-Asian HLA class I (lot 3).

Statistical analysis

Estimation of allele and haplotype frequencies for the HLA loci of Taiwan's indigenous groups was performed using the computer program with the maximum likelihood method developed for the 11th International Histocompatibility Workshop (11th IHW) (11). These frequencies were compared with those of other populations which were also reported at the 11th IHW (10). For the 'Taiwanese' group, we determined the HLA-A, B, C phenotypes of 254 unrelated parents. The allele and three loci haplotype frequencies were calculated by direct counting of the haplotypes of parents (inclusion trios) or mothers in the families (exclusion trios).

Principal component analysis was performed on 32 populations based on HLA-A, B, C gene frequencies by "SAS release 6.12". The modified Cavalli-Sforza distances (D_A) (12) between these populations were calculated by using "ODEN" (public domain software) according to the HLA-A,B,C gene frequencies of this report and data reported at the 11th IHW (10). Using these distances, a phylogenetic tree was constructed by the neighbor-joining (NJ) method (13).

Results

Indigenous population

The gene frequencies at the HLA-A, -B, and -C loci among 10 indigenous tribes in Taiwan are shown in Fig. 2. Except for Pazeh who are considered to be a mixed population (intermarriage with 'Taiwanese'), 9 tribes showed unique antigen distributions. A2, A1101, A1102, A24, A26 were commonly found in most tribes. All 9 tribes had a high A24 frequency (>56%) with Paiwan having the highest frequency (86.3%). A34 was found mainly in Ami (19%) and the neighboring Puyuma (4%). B48, B60 and B61 were found in all tribes, with the highest frequencies of B48 in Ami (24%), and B60 in Saisiat (33.3%). B13, B62, B39, B55, and B56 alleles were found in most tribes. The Rukai had the highest frequency of B13 (27.9%) and the Saisiat the highest frequency of B39 (53.5%). Cw7, Cw8, Cw9 and Cw10 were found in all tribes with the highest frequencies being 66% for Cw7 in Saisiat, 32.1% for Cw8 in Puyuma, 32.6% for Cw9 in Paiwan and 36.8% for Cw10 in Rukai. Ami was distinguished by high frequencies of A34 and B48; and Yami was different from the other 8 tribes by high frequencies of A1101, B62,

| | Tsou | Bunun | Paiwan | Rukai | Atayal | Saisiat | Ami | Puyuma | Yami | Pazeh | 'Taiwanese' ^a -MH |
|----------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|---------------------------------|
| N | 51 | 88 | 51 | 50 | 50 | 57 | 50 | 50 | 64 | 47 | 254 |
| A2 | 2.9 | 13.0 | 6.8 | 6.0 | 12.0 | 13.2 | 4.0 | 20.0 | 3.9 | 24.1 | 29.7 |
| A1101 | 10.8 | 9.7 | 2.0 | 4.0 | 9.0 | 13.2 | 3.0 | 2.0 | 36.7 | 25.9 | 28.3 |
| A1102 | 2.9 | 1.1 | | | 4.0 | 13.2 | 10.0 | 7.0 | 2.3 | 11.0 | 4.5 |
| A24 | 78.4 | 57.4 | 86.3 | 75.6 | 68.0 | 56.1 | 64.0 | 64.0 | 57.0 | 30.0 | 17.3 |
| A26 | 4.9 | 18.8 | 3.9 | 13.9 | 7.0 | 4.4 | | 3.0 | | 1.1 | 3.3 |
| A30 | | | | | | | | | | 1.1 | 1.4 |
| A31 | | | | | | | | | | 1.1 | 2.5 |
| A33 | | | | | | | | | | 3.2 | 11.6 |
| A34 | | | 1.0 | | | | 19.0 | 4.0 | | | |
| ABL | | | | 0.5 | | | | | | 2.7 | |
| A ^a | | | | | | | | | | | 0.8 |
| Cw1 | 11.2 | 11.7 | 5.0 | 7.0 | 9.5 | 1.8 | 21.7 | 5.1 | | 13.9 | 18.4 |
| Cw9 | 19.2 | 8.8 | 32.6 | 18.2 | 11.1 | 0.9 | 4.4 | 15.1 | 6.5 | 10.1 | 7.3 |
| Cw10 | 14.3 | 22.5 | 19.1 | 36.8 | 15.2 | 18.5 | 14.0 | 16.0 | 22.6 | 18.2 | 24.6 |
| Cw4 | | 13.9 | 1.0 | 5.2 | 6.0 | | 20.4 | 1.0 | | 1.1 | 3.6 |
| Cw6 | | | 2.0 | 1.0 | 1.0 | | | 1.0 | 1.6 | 1.1 | 3.4 |
| Cw7 | 24.9 | 16.2 | 10.3 | 16.5 | 30.1 | 66.0 | 14.0 | 9.0 | 8.3 | 17.6 | 20.7 |
| Cw8 | 10.2 | 13.3 | 7.0 | 7.0 | 22.4 | 7.0 | 21.7 | 32.1 | 11.7 | 10.1 | |
| Cw14 | | | | | | 1.9 | | | | 2.2 | |
| CBL | 20.2 | 13.1 | 23.0 | 8.3 | 4.6 | 3.9 | 3.9 | 20.6 | 49.3 | 25.9 | 21.2 |
| B51 | | | | | | 1.8 | | | | 1.1 | 5.3 |
| B13 | 16.4 | 24.4 | 25.5 | 27.9 | | 2.6 | | 17.0 | 3.1 | 13.8 | 9.1 |
| B62 | 4.9 | 6.8 | 4.9 | 12.8 | 1.0 | | 4.0 | 7.0 | 37.4 | 13.9 | 5.5 |
| B75 | 1.0 | | | | | | | 18.0 | 14.8 | 4.3 | 4.7 |
| B38 | | | | | | 0.9 | | 1.0 | 8.2 | 2.1 | 4.1 |
| B39 | 24.3 | 15.2 | 4.9 | 13.0 | 19.0 | 53.5 | 13.0 | 5.0 | | 2.1 | 2.8 |
| B58 | | | | | | | | | | 3.2 | 12.2 |
| B54 | | 1.7 | | | | | | | | 1.1 | 3.9 |
| B55 | 11.6 | 13.1 | 5.9 | 5.7 | 18.0 | 2.6 | 3.0 | 4.0 | | 2.1 | 4.1 |
| B56 | 2.0 | 3.1 | 1.0 | 1.0 | 1.0 | 0.9 | 18.0 | 4.0 | | 1.1 | 1.2 |
| B27 | 2.5 | | | | 6.0 | | 2.0 | 9.0 | 2.3 | 8.5 | 4.1 |
| B35 | | | 1.0 | | | | | | | 2.1 | 3.3 |
| B60 | 19.6 | 21.8 | 32.4 | 19.7 | 33.0 | 33.3 | 32.0 | 17.0 | 4.8 | 19.1 | 21.5 |
| B61 | 7.8 | 1.1 | 13.7 | 11.8 | 1.0 | 0.9 | 4.0 | 6.0 | 21.8 | 3.2 | 1.2 |
| B48 | 8.8 | 10.8 | 10.8 | 7.0 | 21.0 | 3.5 | 24.0 | 12.0 | 3.3 | 5.3 | 1.2 |
| B46 | | | | | | | | | | 11.7 | 11.4 |
| B67 | | | | | | | | | | 2.1 | |
| B81 | | | | | | | | | | 3.2 | |
| BBL | 1.1 | 1.8 | | 1.1 | | | | | 4.2 | | |
| B ^b | | | | | | | | | | | 3.4 |

^a included A1, A68, A29, A74.

^b included B52, B44, B7, B18, B22, B70, B71, B76, B67, B81.

Fig. 2. HLA-A,B,C gene frequencies (%) among 10 Taiwan's indigenous tribes and 'Taiwanese' (Minnan and Hakka). The black-letter numbers are either the world's highest ever reported frequencies or the frequencies close to the reported world highest frequencies.

B38 and B61. Pazeh a hybrid population, showed diverse allele distributions, having many 'Taiwanese' alleles as shown in Fig. 2.

The common three-locus haplotypes among each of the 10 indigenous tribes are shown in Fig. 3. Except for Pazeh, 9 tribes had haplotypes with frequencies higher than 10%, e.g. A24-Cw7-B39 (22.2%) and A24-Cw1-B55 (11.5%) in Tsou; A24-Cw10-B13 (13.9%) and A24-Cw8-B48 (10.2%) in Bunun; A24-CBL-B60 (18.1%), A24-Cw9-B13 (13.7%), A24-Cw10-B13 (11.8%) and A24-Cw9-B61 (11.8%) in Paiwan; A24-Cw10-B13 (15%), A24-Cw9-B61 (12%) and A24-Cw10-B60 (10.3%) in Rukai; A24-Cw10-B60 (13%) and A24-Cw7-B39 (13%) in Atayal; A24-Cw7-B39 (44.5%) in Saisiat; A24-Cw8-B48 (18.4%), A34-Cw1-B56 (18%), A24-Cw10-B60 (12%), A24-Cw7-B39 (11%) and A24-Cw4-B60 (10%) in Ami; A24-Cw8-B75 (13.7%) and A24-Cw10-B13 (12%) in Puyuma; A1101-CBL-B62 (33.5%) and A24-

Cw10-B61 (14.8%) in Yami. The haplotype with the highest frequency was A24-Cw7-B39 (44.5%) in Saisiat, followed by A1101-CBL-B62 (33.5%) in Yami, A24-Cw7-B39 (22%) in Tsou, A24-Cw8-B48 (18.4%) in Ami and A24-CBL-B60 (18.2%) in Paiwan. In 9 tribes the 7 most common haplotypes in each tribe comprised more than 52% of the total number of haplotypes, and in 5 tribes (Tsou, Paiwan, Saisiat, Ami and Yami) comprised more than 70% of the haplotypes. However, in Pazeh the highest haplotype frequency was 7.4% (A1102-CBL-B27) and the 7 most common haplotypes comprised only 31.1% of the total number of haplotypes. Heterogeneity between 9 tribes with different and distinctive haplotype distributions within each tribes are shown in Fig. 3, indicating that each tribe has their own

unique pattern. Some haplotypes shared by most or many tribes are also shown in Fig. 3. In Fig. 3, A24-Cw8-B48 was found in all tribes except Saisiat and Yami. A24-Cw10-B60, A24-Cw9-B61, A24-Cw7-B39 were also found in most tribes, while A24-CBL-B60, A24-Cw4-B60, A24-Cw1-B55, A24-CBL-B62,A1101-CBL-B62, A1102-CBL-B27, A34-Cw1-B56, A2-Cw8-B48,A26-Cw7-B39, A24-Cw7-B60 were shared by several tribes. A24-Cw9-B60, A24-Cw10-B61, A24-Cw7-B38, A2-Cw1-B55,A1101-Cw10-B60, A2-Cw7-B60 were each found only in individual tribes. All these haplotypes were also shared by other Asian populations as shown in Fig. 3, including Maori, PGN Highlanders, Orochons, Mongolians, Inuit, Japanese, Man, Buryat, Tlingit, Tibetans, Thai Chinese, Yakut, Thais, Javanese, Timor, Buyi, Miao, Singa-

| Haplotype | Population | | | | | | | | | | | | | | Others | | | | | |
|----------------|----------------|------|-------|--------|-------|--------|---------|------|--------|------|-------|-------|-------------|----------|--------|--------------------------------|-------|----------|-----|--|
| | N ^a | Tsou | Bunun | Paiwan | Rukai | Atayal | Saisiat | Ami | Puyuma | Yami | Pazeh | Maori | Highlanders | Orochons | | Mongolians | Inuit | Japanese | Man | Buryat ^b |
| A24-Cw8-B48 | 7.9 | 10.2 | 6.2 | 7.0 | 9.0 | 57 | 18.4 | 6.0 | 3.2 | 2.8 | 11.7 | 8.9 | 0.9 | 1.2 | 2.7 | Tibetans (99) ^c 2.0 | | | | |
| A24-Cw9-B60 | 9.8 | | | | | | | | | | 7.8 | 3.3 | 2.9 | | | | | | | |
| A24-Cw10-B60 | 3.9 | | | 10.3 | 13.0 | 4.8 | 12.0 | 4.4 | | | | 3.9 | 1.2 | | | | | | | |
| A24-CBL-B60 | | | 18.1 | | | 4.6 | | | | | | | | | 3.2 | | | | 6.8 | Thai Chinese (71) 3.0 |
| A24-Cw9-B61 | 4.9 | | 11.8 | 12.0 | | | 5.0 | 6.3 | | | | 3.4 | 2.1 | 10.0 | | | | | | Yakut ^d 3.4 |
| A24-Cw10-B61 | | | | | | | | 14.8 | | | | | 3.4 | 4.2 | 10.6 | 1.0 | 5.8 | | | |
| A24-Cw7-B39 | 22.2 | 5.7 | | 7.5 | 13.0 | 44.5 | 11.0 | 5.0 | | 8.5 | | | | | | | | | | |
| A24-Cw7-B38 | | | | | | | | 8.1 | | | | | | | | | | | | Thais (242) 1.4 |
| A24-Cw4-B60 | | 6.1 | | | | | 10.0 | | | | 4.1 | | 0.3 | | | | | | | |
| A24-Cw1-B55 | 11.5 | | | | 5.0 | | | | | | 7.5 | | 0.7 | | | | | | | |
| A24-CBL-B62 | | | | | | | 5.0 | 3.2 | | | 5.4 | | | | | | | | | |
| A24-CBL-BBL | | | | | | | | 7.5 | | | | | | 2.4 | 0.3 | 3.0 | | | | Javanese (68) 13.2; Timor (51)10.4 |
| A1101-CBL-B62 | 4.9 | | | | | | | 33.5 | 4.3 | | | | | | | 1.6 | | | | |
| A1102-CBL-B27 | | | | | 3.0 | | | | 7.4 | | 3.3 | | | | | | | | | |
| A34-Cw1-B56 | | | | | | | 18.0 | 4.0 | | | 2.1 | 8.1 | | | | | | | | |
| A2-Cw8-B48 | | | | | 7.0 | | | 5.0 | | | | | | | | | | | | |
| A26-Cw7-B39 | | 6.5 | | 5.5 | | | | | | | | | | | 0.3 | | | | | |
| A24-Cw7-B60 | | | 5.7 | | 6.3 | | | | | | | | | | | | | | | Buyi (69) 3.4; Miao (70) 2.8; 'Taiwanese' 2.6 |
| A2-Cw1-B55 | | 7.9 | | | | | | | | | 12.2 | | 0.6 | | | | | | | |
| A1101-Cw10-B60 | | | | | | 5.1 | | | | | | | | | 0.3 | | | | | Singapore Chinese (73) 2.7; 'Taiwanese' 1.2 |
| A2-Cw7-B60 | | | | | | 6.1 | | | | | | | | | | | | | | Buyi (69) 3.3; Thai Chinese 4.9; 'Taiwanese' 2.4 |
| A24-Cw10-B13 | 9.6 | 13.9 | 11.8 | 15 | | | | 12.0 | 6.3 | | | | | | | | | | | |
| A24-Cw9-B13 | | | 13.7 | | | | | 5.0 | | | | | | | | | | | | |
| A24-CBL-B13 | 4.3 | | | 6.6 | | | | | | | | | | | | | | | | |
| A24-Cw8-B75 | | | | | | | | 13.7 | 6.8 | | | | | | | | | | | |
| A24-Cw9-B55 | | | | | 9.0 | | | | | | | | | | | | | | | |
| A1102-Cw4-B60 | | | | | | | 5.0 | | | | | | | | | | | | | |

^a Except for Taiwan's indigenous tribes, numbers of tested individuals are from reference 10

^b Reference 14

^c The number of individuals tested (reference 10)

^d Reference 18

Fig. 3. The common HLA-A,B,C haplotypes and frequencies (%) of Taiwan's indigenous tribes and other Asian populations.

pore Chinese and 'Taiwanese'. However, no haplotypes were found to be shared by the indigenous groups and either Northern or Southern Han as reported at the 11th IHW (10). In addition to the above-mentioned haplotypes shared with other Asian populations, A24-Cw9-B13, A24-Cw10-B13, A24-CBL-B13, A24-Cw8-B75, A24-Cw9-B55 and A1102-Cw4-B60 were found exclusively in Taiwan's indigenous tribes, especially the former 3 haplotypes which were found only in those indigenous tribes who live in the southern and middle parts of Taiwan (Tsou, Bunun, Paiwan, Rukai and Puyuma). These haplotypes were not found in Atayal and Saisiat (northern tribes), Ami (east coast tribe), Yami (in Orchid Island) and Pazeh tribes.

Minnan and Hakka ('Taiwanese')

The HLA-A, -B and -C allele frequencies of 'Taiwanese' are shown in Fig. 2. In contrast to the indigenous groups, A2 and A1101 were the most common alleles in the HLA-A locus. A24 had a frequency of only 17.3%. At the B-locus, B60 showed the highest frequency of 21.5% which was similar to the indigenous groups. However, B58 (12.2%) and B46 (11.4%) were alleles not found in the indigenous groups except for the hybrid tribe (Pazeh). At the C locus, Cw10 showed a high frequency (24.6%), followed by Cw7 (20.7%) and Cw1 (18.4%).

HLA-A, B, C three-locus haplotypes and the frequencies determined by the direct counting method are shown in Fig. 4, only haplotypes with a haplotype count of more than 6 are listed in the figure. The 5 most common haplotypes among 'Taiwanese' were A33-Cw10-B58 (10%), A2-Cw1-B46 (7.3%), A1101-Cw7-B60 (4.7%), A1101-Cw10-B13 (3.3%) and A2-Cw7-B38 (3%). Among a total number (count) of 508 haplotypes, 42 different haplotypes had a haplotype count of more than 3 and these 42 comprised 355 counts of haplotypes. Of these 355 haplotypes, 45 counts of haplotypes were found mainly in the indigenous groups (e.g. 13 counts of A24-Cw7-B60, 11 counts of A24-Cw10-B60, 9 counts of A1102-CBL-B27, etc.) which makes an estimated 13% (45/355) of indigenous genes in the 'Taiwanese' gene pool.

Principal component analysis

The result of principal component analysis of 32 populations is shown in Fig. 5. Taiwan indigenous groups were found gathering in a cluster and locating near Maori and PNG Highlanders. Pazeh was found to have a genetic affinity with 'Taiwanese', and this close relationship between Pazeh and 'Taiwanese' correlated well with the past history of Taiwan.

| Haplotype | Count | HF |
|----------------|-------|------|
| A33-Cw10-B58 | 51 | 10.0 |
| A2-Cw1-B46 | 37 | 7.3 |
| A1101-Cw7-B60 | 24 | 4.7 |
| A1101-Cw10-B13 | 17 | 3.3 |
| A2-Cw7-B38 | 15 | 3.0 |
| A24-Cw7-B60 | 13 | 2.6 |
| A2-Cw7-B60 | 12 | 2.4 |
| A24-Cw1-B54 | 12 | 2.4 |
| A1101-CBL-B75 | 11 | 2.2 |
| A1101-Cw1-B46 | 11 | 2.2 |
| A2-Cw10-B60 | 11 | 2.2 |
| A24-Cw10-B60 | 11 | 2.2 |
| A1102-CBL-B27 | 9 | 1.8 |
| A2-Cw7-B39 | 7 | 1.4 |
| A30-Cw6-B13 | 7 | 1.4 |
| A1101-CBL-B51 | 6 | 1.2 |
| A1101-Cw1-B55 | 6 | 1.2 |
| A1101-Cw10-B60 | 6 | 1.2 |
| A2-CBL-B51 | 6 | 1.2 |
| A2-Cw9-B35 | 6 | 1.2 |

Fig. 4. HLA-A,B,C haplotype frequencies (%) among 'Taiwanese' by the direct counting method ($n=254$).

Phylogenetic analysis

Fig. 6 demonstrates a neighbor-joining tree for Asian, American Indian and Oceanian populations. Most indigenous tribes were found closely related to Oceanians, and Ami showing an affinity to PNG Highlanders, while Atayal, Saisiat, Bunun, Tsou, Paiwan and Rukai formed a cluster. Pazeh was found to have a close affinity to 'Taiwanese' as was shown in principal components analysis, supporting the notion that Pazeh is a hybrid population mixed with 'Taiwanese'. Although the genetic distance between Yami and Javanese is large and their affinity is uncertain, Yami is considered as having been isolated from the rest of Taiwan's indigenous groups for a long time.

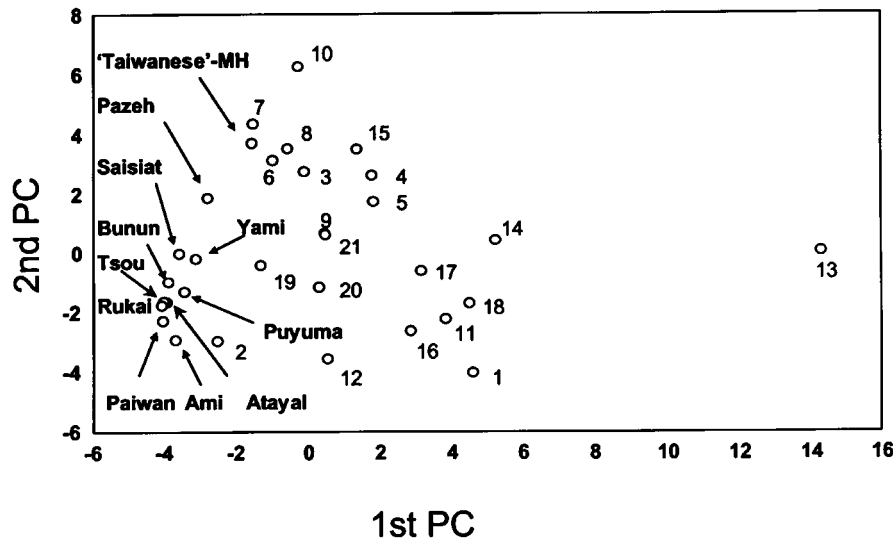


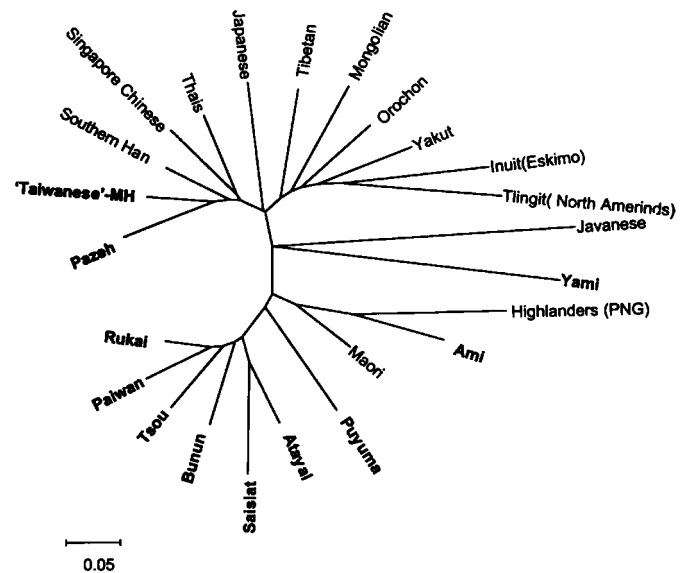
Fig. 5. Principal components analysis of Taiwan's indigenous groups and other populations based on HLA-A, B, C gene frequencies. Taiwan's indigenous groups were found clustering together and adjacent to Maori and PNG Highlanders. Pazeh showed affinity with 'Taiwanese'. Populations: 1. Australian Aborigines; 2. PNG Highlanders; 3. Japanese; 4. Korean; 5. Northern Han; 6. Southern Han; 7. Buyi; 8. Miao; 9. Javanese; 10. Singapore Chinese; 11. North American Indians; 12. Inuit Eskimo; 13. South African blacks; 14. Mongolians; 15. Thais; 16. Tlingit (North Ameinds); 17. Chilean and Colombian (South American Indians); 18. Yakut; 19. Maori; 20. Orochons; 21. Tibetans. Variance for 1st PC was 23%; for 2nd PC was 10.5%.

Discussion

Before the arrival of the Minnan and Hakka (the 'Taiwanese') from Fukien and Kwangton Provinces (southeast coast of China) in the 17th century, Taiwan was occupied by many indigenous tribes. They were later classified into plains people and mountain people (1). The plains people were originally comprised of 10 tribes that mostly occupied the large western plain of Taiwan, but these tribes disappeared gradually during a period of four centuries through wars and intermarriage with the 'Taiwanese'. The Pazeh tribe of this study is one of the remaining plains tribes. The HLA data support the notion that the tribe is a hybrid population mixed with 'Taiwanese'. The mountain people are classified into 9 different tribes according to linguistic and cultural anthropological studies, namely Tsou, Bunun, Paiwan, Rukai, Atayal, Saisiat, Ami, Puyuma and Yami tribes. The term "mountain" people is used for the "pure" indigenous groups who did not intermarry with the 'Taiwanese'. Hence, some of the mountain peoples, like the Puyuma and Ami tribes, actually live in the plains of the remote east coast of Taiwan surrounded by high mountains and the Pacific Ocean. Others such as the Yami live on Orchid Island off Taiwan's east coast. However, as shown in this study all 9 tribes are characterized by homogeneity within each tribe but differences between the tribes, they also have distinct cultures that exhibit different social and family structures, languages, and physical make-ups (Bunun are short with brown skin, and Tsou are tall with light colored skin) (1).

The high homogeneity at the HLA-A, -B and -C loci in the 9 "mountain" tribes of Taiwan's indigenous peoples is shown in Fig. 2. Each tribe has a specific and limited number of HLA-A, -B, and

-C alleles with high allele frequencies. Many alleles including A24, A26, Cw10, Cw7, Cw8, B13, B62, B75, B39, B60 and B48 were found to be highest in the world according to the reported data from the 11th IHW (10). In that report the highest frequency of A24 was found in PNG Highlanders (65.7%). However, in this study, Paiwan (86.3%), Tsou (78.4%), Rukai (75.6%) and Atayal (68%) showed



Figur 6. Neighbor-joining tree of Taiwan's indigenous peoples, Asians, North American Indians and Oceanians. Taiwan's indigenous groups were found to be closely related to Oceanians, Ami showing affinity with PNG Highlanders, and Atayal, Saisiat, Bunun, Tsou, Paiwan and Rukai forming a cluster. A close affinity between Pazeh and 'Taiwanese' was also shown. Yami was considered to be separated from other Taiwan indigenous groups.

much higher frequencies. Also A26 was highest in Japanese (10.9%) but much higher in Bunun (18.8%) and Rukai (13.9%); Cw10 was highest in Singapore Chinese (34.8%) but higher in Rukai (36.8%); Cw7 was highest in Australian Aborigine (38.8%) but higher in Saisiat (66%); Cw8 was highest in Iraqi Jews (31.9%) (14) but higher in Puyuma (32.1%); B13 was highest in Li (18.7%) but higher in Rukai (27.9%), Paiwan (25.5%) and Bunun (24.4%); B62 was highest in Timorese (32.4%) but higher in Yami (37.4%); B75 was highest in Miao (10%) but higher in Puyuma (18%) and Yami (14.8%); B39 was highest in Brazilian (18.3%) but higher in Saisiat (53.5%), Tsou (24.3%) and Atayal (19%); B60 was highest in Taiwan's indigenous people (32.3%), and in this study ranged from 4.8% in Yami to 33.3% in Saisiat; and B48 was highest in Orochons (19.8%) but higher in Ami (24%) and Atayal (21%). In addition to the above-mentioned highest allele frequencies, some other alleles in our indigenous groups also showed frequencies close to the highest frequencies reported at the 11th IHW: A34 was highest in Highlanders (19.1%) and in Ami 19%; Cw9 was highest in Inuit (33.1%) and in Paiwan 32.6%; B55 was highest in Maori (22.6%) and in Atayal 18%; B56 was highest in Inuit (22.5%) and in Ami 18%; and B61 was highest in Inuit (27.3%) and in Yami 21.8%. All these data agree with high homogeneity of Taiwan's indigenous peoples.

High homogeneity of the 9 tribes can also be seen in Fig. 3. Each tribe shows a unique and different haplotype distribution and having several high-frequency haplotypes, with the seven most common haplotypes comprising about 70% of the total number of haplotypes. Among the 27 haplotypes shared by the indigenous tribes in Taiwan as shown in Fig. 3, most of them are also found in other Asian populations, according to the reports of the 11th IHW (10), especially A24-Cw8-B48 (Cw8 was not detectable at the 11th IHW, so A24-CBL-B48 is supposed to be A24-Cw8-B48), A24-Cw9-B60 and A24-Cw10-B61 (15, 16). Because DNA studies have shown that each characteristic class I-class II haplotype has a unique organization of HLA genes and is composed of a specific set of HLA alleles (17, 18), each haplotype can be considered to have a single origin. And if the same haplotype is observed in different populations, these populations are thought to share at least in part a common ancestry (19). Since the present study was limited to class I genes analyzed by serological typing, the above discussion may not be completely true. Nevertheless, Taiwan's indigenous groups appear to be genetically related to northern Asians (Orochons, Mongolians, Inuit, Japanese, Man, Buryat and Yakut), Tibetans, Tlingit (North American Indians), Oceanians (Maori and Highlanders) and Thais because of substantial sharing of multiple class I haplotypes. Therefore, it is most likely that there have been many waves of migration of old world peoples who came through or settled in

Taiwan from Asian continents and mostly from the tropics as shown by principal component analysis and phylogenetic analysis, as Taiwan's indigenous groups are closely related to Oceanians. High homogeneity of HLA alleles and haplotypes also suggests that Taiwan's indigenous tribes were isolated from each other for a long time, most probably since Taiwan became an island about 12,000 years ago (2). Among these tribes, the Yami, who live in Orchid Island situated very far from Taiwan's east coast in very deep sea, have a different HLA pattern (high A1101 and B62, B61 and B38; low B60). This correlates with the linguistic findings that the Yami are closely related to the northern Philippines indigenous people (1). Saisiat is a homogenous population having the world's highest haplotype frequency for A24-Cw7-B39 of 44.5%. The next most common haplotypes A2-Cw7-B60 (6.1%) and A1101-Cw10-B60 (5.1%) were also found in 'Taiwanese', however, not in any other tribe, suggesting origination of these haplotypes from 'Taiwanese'. This correlates well with the fact that Hakka ('Taiwanese') used to live with Saisiat. Pazeh, the hybrid group, showed 57 haplotypes among 47 tested individuals with the highest frequency being only 7.4%. A1102-CBL-B27 and A24-Cw10-B13 appear to be their original specific (indigenous) haplotypes, with A1101-CBL-B75 and A1101-Cw7-B60 originating from the 'Taiwanese'. A24-Cw1-B46 (haplotype frequency of 3.5 with linkage disequilibrium 3) possibly represents a hybrid haplotype between the indigenous haplotype and A2 (A11)-Cw1-B46 of 'Taiwanese'.

Similar results were also shown in our previous red cell antigen study on 1,347 unrelated individuals from these tribes (17). The indigenous groups showed a diverse distribution of blood group antigens. The gene frequencies for the ABO groups were *A* gene ranging from 8% in Rukai to 32% in Yami; *B* gene from 8% in Tsou to 37% in Rukai and *O* gene from 47.4% in Pazeh to 80% in Tsou. The *Rⁱ* gene ranged from 60.5% in Rukai to 94% in Yami, and *Ms* gene (*Ms* plus *MsMiIII*) from 59% in Tsou to 83% in Ami. Because of the great diversity of blood groups among the different indigenous groups and also because a high *A* gene frequency has also been found in Polynesian while high *B* and *Ms* gene frequencies have been found in Southeast Asia and a high *O* gene frequency has been found in Buryat and American Indians, it appears likely that the origins of Taiwan's indigenous groups are different (20). There was also a marked difference between the Ami and its neighbor the Bunun with regards to their MiIII phenotype frequencies of 88.4% and 0%, respectively, suggest not only different origins for each of Taiwan's indigenous tribes but also long-term isolation from each other. Besides, the Di^a phenotype was only found in 5 individuals giving an overall frequency of 0.3% among the indigenous groups (Minnan 3.2%, Hakka 7%) (20) which is similar to that in Inuit and North American Indians (21).

The origin of Taiwan's indigenous people has been a mystery and a topic of interest since the late 19th century. Several theories about their origin, including a southern origin (1, 22, 23) and a northern origin (1, 23), have been promulgated. A recent theory states that Taiwan was the homeland of the Austronesians (3), and suggests that the Austronesians originated from Taiwan's indigenous people (4,000 B.C.), and subsequently expanded into the Philippines (3,000 B.C.), then to Timor, Micronesia, and Polynesia (3). However, archeological studies have shown that there are over a hundred archeological sites in Taiwan with cultures related to southern or northern Asia (23). The earliest sites in Taiwan are the Changpin caves (which have many sites with different datings) on the southeastern coast of Taiwan, dating from more than c. 15,000 to c. 4,715–3,250 B.C. and demonstrating a Changpin Culture of the post-Pleistocene Paleolithic period (24). The corresponding human remains (skull and teeth) found in southern Taiwan were fossils of *Homo sapiens* "Tso-chen Man", estimated by the fluorine and manganese methods to be from 20,000 to 30,000 B.P. (25). The earliest neolithic culture in Taiwan is Tapengkeng, which is not related to Changpin culture (26), and is found on the northwestern coast dating to c. 4,000–2,500 B.C. Moreover, archeological records strongly suggest that the earliest inhabitants of the island were the direct ancestors of the present indigenous people (23). Taiwan was geographically linked to the Asian mainland during the glacial period, and has been an island since 12,000 B.P. (2). Geographically Taiwan is at the edge of the South China continental shelf, located at the intersection between Island Southeast Asia and the Asian mainland. It also lies between a tropical and subtropical climate.

Most importantly, for nearly 20,000 years before Taiwan became an island, Taiwan was at the junction of two coastal lowland masses that are under the sea today (2). These two lands, the Tunghailand and Nanhailand (from the Gulf of Tonkin to Japan), were undoubtedly inhabited and were connected to the Sundaland (now the Gulf of Siam and the Java Sea) (27). From archeological findings, coupled with our recent HLA and our previous red cell blood groups studies, it is most likely that there have been many waves of migration of old world people coming to settle in Taiwan from the tropics or Asian continent before Taiwan became an island in the distant past, and their isolation from each other since then has resulted in the heterogeneous picture, but however, a homogeneous population, of the indigenous tribes today. Taiwan might also be on the route of ancient migrations, of the prehistoric Mongoloid dispersals, between the tropics and the Asian continent, and even the American continent, which most likely took place along the coastal low land which is under the sea today.

In this study, the finding that major haplotypes of the indigenous groups were found in 13% of the total 'Taiwanese' (Minnan and Hakka) HLA class I haplotypes suggests a low proportion of indigenous genes in the 'Taiwanese' gene pool. With the advent of DNA technology, high resolution DNA typing and sequence based typing of HLA class I-class II alleles have brought enormous new insights into population genetic relationships. Because of the uniqueness of Taiwan's indigenous population, the ongoing class I and class II DNA typing on the same samples is expected to supply additions to the interpretation on the HLA evolution and population relationships.

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