HLA-B27 and Its Subtypes in 4 Taiwanese Aborigine Tribes: A Comparison to Han Chinese Patients with Ankylosing Spondylitis

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ABSTRACT. ***Objective.*** We surveyed B27 and its subtypes in 5 ethnic groups in Taiwan.

Methods. Blood was obtained from 281 Aborigine people of the Atayal tribe (I-Lan), 141 Paiwan (Ping-Ton), 38 Rukai (Ping-Ton), and 40 Yami (Orchid Island), and also 47 B27+ healthy Han Chinese subjects and 82 B27+ Han patients with ankylosing spondylitis (AS). HLA-B27 and its subtypes were determined by standard methods.

Results. A much higher prevalence of B27 was found among the Atayal Aborigines (9.2%), which was significantly different from a lower prevalence in the Paiwan (2.1%; p = 0.004) and even higher than that of Han Chinese (5.59%; p = 0.04). No blood sample from the 38 Rukai Aborigines showed any B27. B2704 was the only subtype (100%) found in the 28 healthy Aborigines and 2 Aborigine patients with AS. However, in Chinese subjects, 40 of 47 (85%) B27+ healthy subjects were B2704, and 7 of 47 (15%) were B2705. In Chinese B27+ AS patients, 77 of 82 (94%) were B2704 and 5 of 82 (6%) were B2705. No other subtypes were found. Only the Aborigines without AS carrying B2704 showed a significant difference from the Chinese without AS carrying B2704 (p = 0.041).

Conclusion. The different prevalence of B27, but similar frequency of the B2704 subtype, between Aborigines and Han Chinese suggests Aborigines are a unique population and may originate from an Asian country, possibly mainland China. (J Rheumatol 2003;30:321–5)

Key Indexing Terms:
HLA-B27 SUBTYPES ANKYLOSING SPONDYLITIS
POLYMERASE CHAIN REACTION TAIWAN ABORIGINES
SEQUENCE-SPECIFIC OLIGONUCLEOTIDE POLYMORPHISM

Ankylosing spondylitis (AS) is a genetically determined disease. Since a high prevalence of HLA-B27 occurs in different populations with AS, except for African people[1-5], B27 is currently considered a disease marker of AS. In Chinese subjects, the frequency of HLA-B27 in the general population is reportedly from 4% to 8%, which is lower compared to Native American people (10–50%), but higher compared to Japanese (1%)[3,6,7]. The prevalence of AS in Taiwan Chinese is roughly 0.3%[8], which is similar to that of Caucasians in Northern European countries[3]. There is a variation in distribution of AS among different ethnic groups. In general, the prevalence of AS is correlated with the B27 frequency in each population[2-4]. However, Nasution, *et al* reported that Indonesian Chinese people had a lower prevalence of B27, but a higher prevalence of AS compared to the native Indonesian people[9]. Thus study of the distribution of HLA-B27 and AS in ethnic populations might provide tools for identification of arthritis-causing factors.

In addition to the Taiwanese who migrated from mainland China's southern provinces 400 years ago and the mainlanders who migrated from different areas of mainland China after the civil war of 1949, there are ethnic minorities (about 1.5% of Taiwan's total population) — the Taiwan Aborigines, a branch of the Austronesians, who probably originated from Southeast Asia and migrated to Taiwan island 4000 years ago[10-12]. This unique population can be divided into 10 distinct indigenous groups or tribes, according to their language, geographic distribution, and cultures[10]. In 1996, the population of Taiwan Aborigines was 369,251, which is 1.71% of the total Taiwan population. Among the Aborigines, the Ami were the largest group, with a population of 138,646, followed by the Atayal (85,028), Paiwan (66,322), Bunun (41,044), Rukai (11,149), Puyuma (9830), Tsou (6732), Saisiat (6567), and Yami (3924). The geographic distribution of each tribe is shown in Figure 1; the Atayal and Saisiat live in the northern mountains, and they have similar clothes and facial tatooing; the Bunun and Tsou live in the central mountains; they have a system whereby the entire clan of individuals live together; the Rukai and Paiwan live in the southern mountains and they

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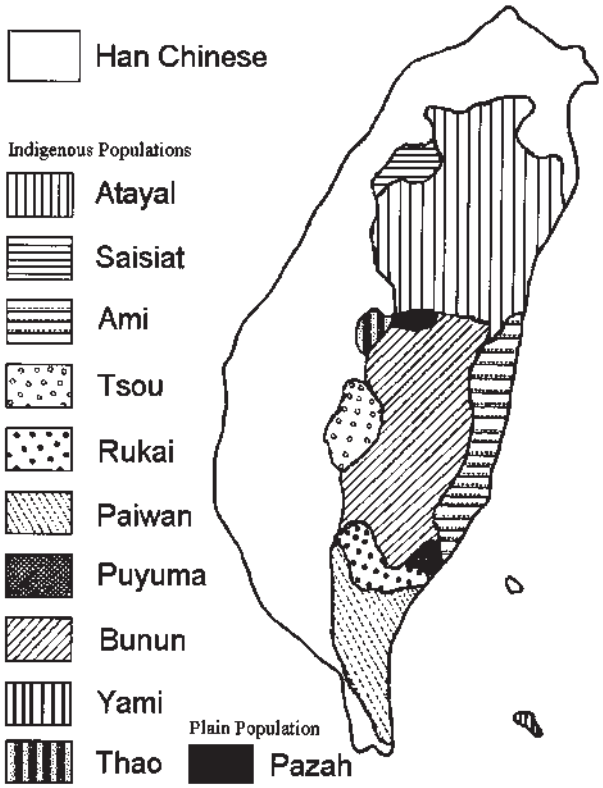

Figure 1. The geographic distribution of Taiwanese indigenous populations.

have a strictly hierarchical society; the Yami still preserve their original culture and lifestyle; they live on Orchid Island near Taiwan's east coast. The Puyuma live along the eastern coast and have a matrilateral-extended family pattern and age-graded social system. The people of 9 tribes (Atayal, Paiwan, Rukai, Yami, Tsou, Saisiat, Ami, Bunun, and Puyuma) are relatively isolated and live in high mountain areas.

Because of difficulty of access and cultural barriers, only limited studies concerning the prevalence of B27 in Taiwan's indigenous people have been reported[12-15]. The prevalence of B27 in Atayal Aborigines from different reports was higher and variable (6.0–17.9%) by comparison to the prevalence of B27 in the Paiwan, Rukai, Bunun, Yami, or Tsou (0–2.5%)[12-16]. However, the sample sizes that were previously tested for HLA in different indigenous people were too small (30–60) to be subjected to meaningful statistical comparisons. In addition, there has been no evaluation of the distribution of B27 subtypes among these Aborigines. The role of B27 subtypes in AS has been studied by many investigators[17-23]. Currently, more than 20 B27 subtypes have been reported. Two subtypes, including B2706 in people in Thailand and B2709 in Sardinia, were allegedly negatively associated with AS[20]. Since AS is allegedly not a common disease in Taiwan's indigenous people (estimated prevalence was less than 0.05%)[16], the determination of B27 subtypes in B27 positive Aborigines may also be helpful in understanding the differences between the native Taiwan Aborigines and the Han Chinese with and without AS.

MATERIALS AND METHODS

Cases among the Han people. From 1999 to 2001, we collected blood samples from 82 AS patients and 47 B27 positive healthy subjects. The 129 individuals in this study came from rheumatology outpatient clinic of Veterans General Hospital-Taipei after we screened for AS in 2000 patients with chronic low back pain. The diagnosis of AS was in accord with the modified New York criteria[24].

Cases among the indigenous population. Blood samples were collected by staff from the Buddhist Tzu Chi General Hospital-Hualien, Mackay Hospital-Taitung, and Christian Hospital-Ping Tung. Aborigines from 4 different tribes (Atayal, Paiwan, Rukai, Yami) agreed to participate. For the identification of AS, pelvic radiographs and blood tests (erythrocyte sedimentation rate, C-reactive protein, and HLA-B27, etc.) were performed for any subject who had chronic low back pain with stiffness for more than 3 months. Finally, only 2 subjects were found to have AS, and they were both from the Paiwan tribe.

HLA typing. Determination of HLA-B27[25]. Blood was collected by venepuncture into a sterile EDTA or heparin blood collection tube. Fifty microliters of blood were mixed with 30 µl of FITC-labeled anti-HLA-B27 [clone GS 145.2 (IgG1, kappa)], vortexed thoroughly at low speed for 3 s, and incubated 15 min at room temperature in the dark. For each sample, 2 ml of lysing solution was added, immediately vortexed thoroughly at low speed for 3 s, and incubated 10 min. After incubation, each tube was centrifuged at 200 × g for 5 min. After 2 washes in phosphate buffered saline (PBS), the supernatant was removed and the tube was vortexed thoroughly at low speed to resuspend the cell pellets in the residual fluid. Finally, 0.25 ml of 1% paraformaldehyde was added to each tube. After mixture with the fixing solution, samples were analyzed on a flow cytometer (Becton Dickinson). In this study, samples with a median fluorescence 1 channel result greater than or equal to the decision marker (144) were reported as B27 positive. Samples with a median channel result lower than the decision marker were reported as B27 negative.

Determination of HLA-B27 subtype[20]. Genomic DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen Inc., Valencia, CA, USA). HLA-B27 allele was determined using an Olerap SSP HLA-B27 typing kit, which contains 5' and 3' primer for identifying the HLA-B2701 to HLA-B2723 alleles (Qiagen/Genovision Inc., West Chester, PA, USA) in accord with the manufacturer's instructions. The polymerase chain reaction (PCR) reaction mixture contained 60 ng of extracted DNA, 200 µM of each dNTP, 0.4 U of Taq polymerase in 1× reaction buffer (50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl, pH 8.3, 0.001% w/v gelatin) with a final volume of 10 µl; PCR cycling parameters were initial step of 94°C for 2 min, 10 cycles of 94°C for 10 s, 65°C for 60 s, ending with 20 cycles of 94°C for 10 s, 61°C for 50 s, and 72°C for 30 s. Amplification was performed using a Perkin-Elmer GeneAmp PCR System 9600 DNA thermal cycler (Perkin-Elmer, Norwalk, CT, USA). The resulting DNA fragments were analyzed with 2% agarose gel electrophoresis in 0.5 × TBE buffer.

Statistical analysis. The chi-square test was used for comparison of the frequency of B27 and B27 subtypes in different groups.

RESULTS

Blood samples were obtained from 281 Atayal Aborigines, 141 from Paiwan, 38 from Rukai, and 40 from the Yami. The B27 positive rate was found to vary in the different indigenous populations (Table 1). In these 4 indigenous

Table 1. The frequency of B27 in different indigenous populations and Han Chinses.

	Tribes					
	Atayal	Paiwan	Rukai	Yami	Total	Han Chinese
No. in samples analyzed	281	141	38	40	500	877*
(total population)	(85,028)	(66,322)	(11,149)	(3924)	(166,423)	(23,000,000)
No. of B27 positive (%)	26 (9.2)	3 (2.1)	0 (0)	1 (2.5)	30 (6)	49 (5.59)

* Data from Taiwan Everlight Trading Co., Taipei, Taiwan.

groups, the frequency of B27 was significantly higher in the Atayal group (9.2%) versus the Paiwan (2.1%) (p = 0.004). It was also lower in the Yami (2.5%), but did not reach a statistically significant difference between Atayal and Yami, probably because of the small number of Yami samples (p = 0.055). None of the 38 blood samples from the Rukai were B27 positive. The average B27 frequency in the 4 aboriginal tribes was 6%. For the Han Chinese, we had a large number of samples from the previous analysis, which was done by Taiwan Everlight Trading Co. using the PCR-sequence-specific polymorphism method. The HLA-B27 frequency was 5.59% (unpublished data), which was significantly different from the Atayal group in this study (p = 0.042), but not statistically different from the other 3 tribes. No cases were suspected of having AS in the indigenous populations we studied after review of clinical histories and radiographic examination, except for 2 cases with AS in the Paiwan tribe. These 2 cases from the Paiwan were also positive for HLA-B27.

The frequencies of B27 subtypes in Aborigines and Han Chinese are given in Table 2. B2704 was the only subtype (100%) that we found in the 28 healthy Aborigines and 2 Aborigines with AS. It is comparatively higher in Aborigines (100%) with or without AS than in Chinese with AS (94%), and significantly higher in Aborigines without AS than in Chinese without AS (85%) (p = 0.041). In contrast, B2705 was not found in Aborigines, but appeared in 6% of Chinese with AS and 15% of Chinese without AS. There was no significant difference for B2705 between each group. No B2706 or other subtypes were observed in either Aborigines or Chinese with or without AS.

DISCUSSION

Previous HLA studies in Taiwan Aborigines showed that some alleles presented as the highest frequencies in the world, e.g., A24 (80–90%), A26, B13, B39, B60, and B62[12,13,16,26]. Among them, the most frequent allele of the A locus is A24, which is as high as in populations in Oceania; and for the B locus it is B60 in most tribes (40–50%), except for the Yami (11%). The Bunun and Tsou reveal relatively higher frequencies of B13 (20–30%) than B60. The Yami tribe reveals the highest frequency of B62 around the world. The tribe was thought to have migrated from Batan Island in the Philippines, because they have the same linguistic and sociocultural system. Apart from these 10 indigenous tribes, 2 other tribes living in the plains areas (the Thao and Pazeh) were originally interrelated with Han Chinese and their HLA patterns (high frequency of A2 and A11 and lower frequency of B39) are similar to the Mongoloid and Han Chinese[12,14,26].

In this minority population, previous studies showed hyperuricemia and gout to be prevalent[11]. In contrast, rheumatoid arthritis was uncommon and the prevalence was lower than 0.05%[16], which was significantly decreased compared to the Han Chinese in Taiwan (0.65%)[8].

Of note in this study, the reported rate of AS was also low

Table 2. Distribution of B27 alleles in Aborigines and Chinese with and without AS.

	Aborigines, n = 30						Han Chinese, n = 129	
	With AS, n = 2	Without AS, n = 28					With AS, n = 82	Without AS, n = 47
Subtype	Paiwan, n = 141	Atayal, n = 281	Paiwan, n = 141	Rukai, n = 38	Yami, n = 40	Total		
B2701	0	0	0	0	0	0	0	0
B2702	0	0	0	0	0	0	0	0
B2703	0	0	0	0	0	0	0	0
B2704	2 (100%)	26 (10%)	1 (100%)	0 (0%)	1 (100%)	28 (100%)*	77 (93.9%)	40 (85.1%)*
B2705	0	0	0	0	0	0	5 (6.1%)	7 (14.9%)
B2706	0	0	0	0	0	0	0	0
B2707	0	0	0	0	0	0	0	0
B2708	0	0	0	0	0	0	0	0
B2709	0	0	0	0	0	0	0	0

* p = 0.041 (< 0.05).

among these Aborigines (less than 0.05%)[16]. The first question we address is whether the low prevalence of AS is due to a low prevalence of HLA-B27. The answer is negative for the Atayal tribe. Their prevalence of HLA-B27 was similar to that of the Han people (6.0% or 9.2% vs 6.4% or 5.5%, respectively). We also extended the study to 3 other tribes, 2 (Paiwan, Rukai) from mountain regions in southern Taiwan, and one (Yami) from the very small island off the eastern coast of Taiwan island. The B27 frequency was lower in the Paiwan, Rukai, and Yami populations compared to the Atayal tribe.

According to the previous theory, most indigenous tribes are believed to be closely related to populations in Oceania[12,16]. The variation of B27 frequency between the Atayal and the other 3 groups was possibly due to a long period of isolation and a "bottleneck" effect.

The second question we address is whether the subtypes of HLA-B27 in these tribes are different from the Han Chinese. Except for the strong association between HLA-B27 and AS, the relationship between the HLA-B27 subtypes and AS was also studied for different ethnic populations[3,17-22,27-29]. A large survey of HLA-B27 subtypes in different ethnic groups (Caucasian, Asian, Polynesian, African, Amerindian, etc.) was done by Gonzales-Roces, *et al*[20]. In that study, the predominant B27 subtype was B2704 (81%) in healthy Chinese, which was slightly lower compared to our study (93.1%), and B2705 was the only subtype observed in both groups[20]. For the Taiwan indigenous people, B2704 was the only subtype (100%) that we could detect, and unlike Caucasians, the majority of B27 subtypes were either B2705 or B2702[3,28,29]. The finding for the B2704 subtype in Taiwan Aborigines differs from the results of Polynesian and Maori people, among whom the B2705 is even more common than B2704 (58% vs 42%)[3]. Both Taiwan Aborigines and Maori originated from Micronesia, and they share a high prevalence of hyperuricemia and gout[11]. It is unclear why there is a difference in B2704 and B2705 in these 2 populations. For other aboriginal population from the Chukot Peninsula (Eskimo and Chukchi) and the Mestizo population of Mexico, the predominant subtype was B2705 (> 80%)[3,20]. Possibly, B2704 shows an Asian characteristic. For 2 Aborigines with AS, B2704 was the only subtype that we found. In the Han Chinese with AS, B2704 was also dominant (93.9%) and the rest were B2705, which does not differ from the conclusion of Wei, *et al*[6], who report a frequency for B2704 of 96% and for B2705 of 4%). However, the B2704 finding is higher than the 54.8% reported by Lin, *et al* in Chinese patients with AS[27]. The discrepancy in the frequency of B2704 between Lin's and our study may be due to the different ethnic groups among Chinese.

The only subtype of B2704 in Taiwan Aborigines, which is different from other ethnic groups (Euro-Caucasoids, North America Natives, Jews, Africans, etc.), suggests that they originated from an Asian country, possibly mainland China, and previously were not an admixture of Caucasian or African people.

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