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BRIEF COMMUNICATION

HLA-DPB1 polymorphism in Blang and Puyi ethnic groups of Southwest China inferred from sequence-based typing

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Abstract

In the present study, DNA typing for HLA-DPB1 was performed using polymerase chain reaction (PCR)–sequence-based typing method in two isolated Chinese populations: the Blangs ($n = 94$) in Shuangjiang County and the Puyis ($n = 76$) in Luoping County from Yunnan province of Southwest China. These two populations exhibited certain similarity in their allelic distributions of the HLA-DPB1 gene. A total of 11 and 12 alleles at the DPB1 locus were found in the Blang and Puyi groups, respectively. In the Blang group, the most frequent alleles were DPB1*0501 (51.0%) and DPB1*1301 (17.0%). DPB1*030101 was also common with a frequency of 6.4%. In the Puyi group, the most frequent allele was also DPB1*0501 with a frequency of 47.5%, followed by DPB1*1301 (21.1%). Two alleles DPB1*2101 and DPB1*0202 followed, with frequencies ranging between 5% and 8%. The alleles DPB1*4101, DPB1*3301, DPB1*6801 and DPB1*8401 were found for the first time in Chinese populations. A dendrogram constructed by neighbor-joining method showed that the Blang and Puyi ethnic minorities, which had the closest relationship belonged to the southern Chinese.

Introduction

The Blangs has a population of 91,882, who mostly live in Mount Blang, Xiding, Bada, Mengman and Daluo areas of Menghai County in Xishuangbanna Dai Autonomous Prefecture of Yunnan province of Southwest China. Some Blangs live in Simao, Lincang and Baoshan areas. The Blang villages usually spread out in mountain areas to the south of 25°N latitude, 1500–2300 m high above sea level, where, under the typical semitropical climate, there are mountains up and down, and the forests endless. According to historical records (1), the Blangs were once called 'Baoman', 'Minpu' in the Han Dynasty and 'Puman' in the Yuan Dynasty. The Blangs have been officially called 'Blang' since the founding of the new China. The Puyis' population is 2,971,460 currently. They mainly live in the South Guizhou Puyi and Miao Autonomous Prefecture and the southwest Guizhou Puyi and Miao Autonomous Prefecture, and some are also interspersed in Luoping County in Yunnan province. The Puyi area is located in the subtropical Karst highland in the southwest of China. Based on historical literatures (2), Puyi people had different names during various dynasties of history, such as 'Puyue' or 'Puyi' at the time before the Qin and Han Dynasty, 'Liao' in the time of Wei Jin and Southern and Northern Dynasties, 'Fanyi' in Tang and Song Dynasties and later 'Zhongjia', 'Nongjia', 'Bulong', and so on. In 1953, it was named 'Puyi' according to their will. Owing to their vague ancestries, the Blangs and the Puyis are of great interest from an anthropological perspective. Until now, a number of studies on the basis of different genetic markers have been performed to elucidate the origins and evolutionary histories of these ethnic minorities (3–5).

Human leukocyte antigen (HLA) genes are the most polymorphic in the human genome. They have been thoroughly studied among various populations, race, and

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ethnic groups (6, 7). HLA gene frequencies correlate with geographically related populations. The genetic polymorphism of HLA genes can be used for anthropological studies. Because of the remarkably dissimilar distribution patterns of DPB1 alleles in different populations, the DPB1 locus may be used to trace back the origin and the migration of a distinct population (8, 9). The aim of this study was to investigate the HLA-DPB1 polymorphism in Blang and Puyi ethnic groups and evolutionary relationships of them with other populations.

The present study population consisted of a total of 170 healthy unrelated ethnic individuals living in Yunnan province of southwestern China: Blang ethnic minority ($n = 94$) and Puyi ethnic minority ($n = 76$). The families of all these Blang and Puyi volunteers were known to have lived in the Shuangjiang and Luoping Counties, respectively, for at least three generations without admixture outside their own minority. Peripheral blood cells samples were drawn after informed consent was obtained. Genomic DNA was extracted from peripheral blood leukocytes by standard phenol/chloroform extraction and ethanol precipitation.

Exon 2 of HLA-DPB1 gene was amplified and sequenced according to International Histocompatibility Working Group (IHWG) technical manual (http://www.ihwg.org). Polymerase chain reaction (PCR) and unlabeled sequencing primers and PCR conditions were used as described by Versluis et al. (10). PCR products were pureed using the *Exo*I-shrimp alkaline phosphatase (SAP) protocol and were sequenced using the Big Dye terminator sequencing as described above. Genotypic ambiguities were resolved by PCR–sequence-specific primer. The detailed procedures were outlined previously (11).

The statistical analysis of molecular polymorphisms at the HLA-DPB1 locus within populations was performed using Arlequin 3.0 population genetics software (12). The exact test of Guo and Thompson (13) was used to evaluate deviation from expected Hardy–Weinberg genotypic proportions. The allelic frequencies were calculated by the direct counting method and the homozygosity *F*-test for neutrality was applied using the Ewens–Watterson (14). For phylogenetic analysis, data on HLA-DPB1 allele frequencies of other 16 populations are quoted from anthropology/allele frequencies of IHWG projects (http://www.ncbi.nlm.nih.gov/mhc). Genetic distances were measured according to the method of Nei (15). Phylogenetic trees based on the allelic frequency of the DPB1 gene were constructed by the neighbor-joining (NJ) method with MEGA2 software (16) and PHYLIP software (version 3.5) (17).

The DPB1 allele frequencies of the two populations were reported in Table 1. A total of 11 and 12 alleles at the DPB1 locus were detected in Blang and Puyi groups, respectively. In the Blang group, the most frequent alleles were DPB1*0501 (51.0%) and DPB1*1301 (17.0%).

Table 1 Allele frequencies of HLA-DPB1 gene in Blang and Puyi ethnic groups

Alleles	Blang ($n = 94$)	Puyi ($n = 76$)
DPB1*010101	0.043	0.000
DPB1*020102	0.021	0.026
DPB1*0202	0.043	0.053
DPB1*030101	0.064	0.026
DPB1*040101	0.043	0.026
DPB1*0402	0.043	0.000
DPB1*0501	0.510	0.475
DPB1*1301	0.170	0.211
DPB1*1401	0.000	0.026
DPB1*2101	0.000	0.079
DPB1*3101	0.021	0.000
DPB1*3301	0.000	0.026
DPB1*4101	0.021	0.000
DPB1*4801	0.021	0.000
DPB1*6301	0.000	0.026
DPB1*6801	0.000	0.013
DPB1*8401	0.000	0.013

DPB1*030101 was also common with a frequency of 6.4%. They accounted for 74.4% of DPB1 allele frequency. In the Puyi group, the most frequent allele was DPB1*0501 with a frequency of 47.5% followed by DPB1*1301 (21.1%). Two alleles DPB1*2101 and DPB1*0202, followed with frequencies ranging between 5% and 8%. They were highly predominant and amount to 81.8% of all DPB1 alleles. Each population exhibited some specific variants (Blang: DPB1*010101, DPB1*0402, DPB1*3101, DPB1*4101 and DPB1*4801; Puyi: DPB1*1401, DPB1*2101, DPB1*3301, DPB1*6301, DPB1*6801 and DPB1*8401), none of which exceeds 5%. In addition, some rare DPB1 alleles were detected for first time in Chinese populations (Blang: DPB1*4101; Puyi: DPB1*3301, DPB1*6801 and DPB1*8401). The expected and observed genotype frequency values for the DPB1 locus did not differ significantly, and both the populations were in Hardy–Weinberg equilibrium (Blang, $P = 0.098$; Puyi, $P = 0.161$). Generally speaking, the DPB1*0501 is the characteristic allele of the people of East Asia and mainly restricted geographically to Asian and Oceania population, almost absent in Caucasians and Africans (6). In terms of Chinese, its frequency exhibits higher in southern populations than in northern populations. If the global distribution of the DPB1*0501 is considered, it is observed that the distribution of the DPB1*0501 shows a trend decreasing gradually from East to West. For DPB1*1301, it is also common in Southeast Asian populations. The frequency of the DPB1*1301 in Chinese populations is also higher in southern populations than in northern populations. Our research results are consistent with previous reports (18). It was worthy to note that in this study, the DPB1*4101, DPB1*3301, DPB1*6801 and DPB1*8401, were found for the first time in Chinese

populations and also rare in Caucasians and Africans, probably because the resolution of typing methods used in previous genotyping of Chinese populations were lower than that used in our study.

In order to detect if the DPB1 locus in the both populations is under evolutionary selective forces, we compared the observed homozygosity F values with those expected under the Ewens–Watterson neutral model. There was no significant difference between the observed and the expected F value (Blang: observed $F = 0.303$, expected $F = 0.245$, $P = 0.804$; Puyi: observed $F = 0.288$, expected $F = 0.211$, $P = 0.860$), so the selective neutrality in the HLA-DPB1 gene of the both groups could not be rejected. The majority of studies have shown that DPB1 locus is not under balancing selection. The present study showed that homozygosity for DPB1 locus does not differ significantly from the value expected under neutrality, which is in concordance with other previously published results (19).

The dendrogram (Figure 1) based on allelic frequencies of the HLA-DPB1 gene of 18 representative populations all over the world by the NJ method displayed that the two main clusters are African and Asian/European with the latter divided into Asian group and European group. The Blang population and the Puyi population, closer together, were clustered into the Asian group, and they had the most close relationship with the Li, Jing, and Dai ethnic groups, which are all representative southern Chinese populations. This result was consistent with previous studies (20), supporting that the Blang and Puyi ethnic groups originated from the a common ancestor of southern China. Furthermore, our finding is also supported by other genetic markers (4, 5). Our results suggest that the Blang and Puyi ethnic groups also belong to the Southern group. Another dendrogram (figure not shown) based on the same data was also constructed by the maximum likelihood method calculated using PHYLIP (version 3.5), which was consistent with that using the NJ method. Based on the analyses of the allelic frequencies and the phylogenetic tree, the Blang and Puyi ethnic groups can belong to the southern group of Chinese people. Thus, combining previous history studies with this present analysis of the DPB1 gene, it can be proposed that the early ancestors of the Blang and Puyi ethnic people originally lived in Southern China.

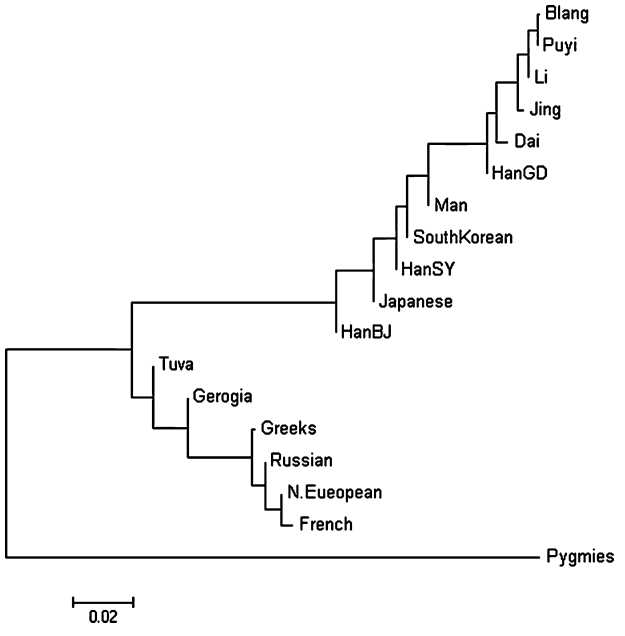

Figure 1 Dendrogram constructed by the neighbor-joining method showing the relationship among 18 populations based on the allele frequencies of HLA-DPB1 gene.

In conclusion, in this report, we presented for the first time the distributions of HLA-DPB1 alleles in the Yunnan Blang and Puyi ethnic minorities of Southwest China with a sequence-based typing method, and then conducted a preliminary investigation of the genetic relationship between these two ethnic minorities and other populations. Our results are consistent with previous historical records that place the origin of the Blang and Puyi people in southern China. Noticeably, anthropological studies such as in this study are also fundamental for subsequent epidemiological work aimed at elucidating the association between DPB1 and related diseases in members of these two ethnic groups.

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