

BRIEF COMMUNICATION

Polymorphism of HLA-DRB1, -DQB1 and -DPB1 genes in Bai ethnic group in southwestern China

W. Hu*, L. Tang*, J. Wang, B. Wang, S. Li, H. Yu, W. Tang, H. Li, S. Tan, W. Shou & C. Xiao

Key Laboratory of Bioresources Conservation and Utilization and Human Genetics Center, Yunnan University, Kunming, Yunnan, China

Key words

Bai ethnic minority; human leukocyte antigen class II genes; polymerase chain reaction–sequence-based typing; polymorphism

Correspondence

Prof. Chunjie Xiao
Key Laboratory of Bioresources Conservation and Utilization
Human Genetics Center
Yunnan University
2 N Cuihu, Kunming
Yunnan 650091
China
Tel/Fax: +86 871 503 4636
e-mail: cjxiao@public.km.yn.cn

Received 7 May 2008; revised 4 July 2008; accepted 28 July 2008

doi: 10.1111/j.1399-0039.2008.01120.x

Abstract

In this work, polymorphism of human leukocyte antigen (HLA)-DRB1, -DQB1 and -DPB1 genes was detected using polymerase chain reaction–sequence-based typing method in 128 healthy unrelated volunteers from the Bai ethnic group of Yunnan province of southwest China. Among all the 28 alleles detected for the DRB1 gene, the most common allele was DRB1*120201 with a frequency of 16.41%, followed by DRB1*090102, DRB1*080302, DRB1*1404, DRB1*150101, DRB1*140101 and DRB1*160201, with frequencies of 10.16%, 9.77%, 9.38%, 8.98%, 8.59% and 8.21%, respectively. Among 19 DQB1 alleles detected, the most frequent allele was DQB1*030101/0309 (35.94%), followed by DQB1*050201 (11.33%), DQB1*060101/060103 (10.54%) and DQB1*0401 (10.16%). For the DPB1 locus, the most common alleles were DPB1*0501 (42.19%), DPB1*1301 (13.28%), DPB1*020102 (10.93%) and DPB1*040101 (9.77%). The comparison of HLA class II allele frequencies of Bais with those of other Chinese populations suggested that the Bai ethnic group belonged to the southern group of Chinese.

The Bai ethnic group is one of the minority groups in China, which has a total of 1,858,063 people. About 80% of the Bai people live in communities in the Dali Bai Autonomous Prefecture in Yunnan province in southwest China. The rest are scattered in sides of Nujiang River and Lancang River, and Xichang in Sichuan province, Bijie in Guizhou province and Sangzhi in Hunan province. The Bai language belongs to the Tibetan–Myanmese group of the Chinese–Tibetan language family. However, the ancestor of the Bai people still remains contradictory. In a traditional view, the Bai were derived from a branch of the ancient Qiang group of northwestern China, which migrated to southwestern China to avoid the attack of the Qin kingdom in 380 B.C. (1). However, a study based on archeology suggested that the Bai people were aborigines around the Dianchi and Erhai areas in Yunnan as early as the Neolithic Age and Bronze Age (2). Furthermore, the hypothesis that the Bai ethnic

group were derived from a fusing population between a nomadic northern tribe migrated to south and a planted rice southern tribe migrated to north was proposed (3). Therefore, the earlier origins of the Bai people remain elusive.

Human leukocyte antigens (HLAs) are cell surface glycoproteins encoded by genes located in the short arm of chromosome 6 (6p21.3). HLA molecules function as receptors for processed peptides derived from intracellular and extracellular proteins, which are presented to CD8⁺ and CD4⁺ T lymphocytes to initiate a series of immune response. HLA genes are the most polymorphic coding loci known in human genome, which have been extensively used for anthropological studies for the fact that HLA allelic distribution is markedly different among different ethnic groups (4). In the present study, the allelic polymorphisms of HLA class II genes were first investigated in the Bai ethnic population of southwest China by the high-resolution sequence-based typing (SBT) method.

*These authors contributed equally to this work.

A total of 128 healthy unrelated Bai ethnic individuals living in Jianchuan County of the Dali Bai Autonomous Prefecture in Yunnan province from southwest China participated in this study. The ethnic background of the subjects was established by personal interview and informed consent was obtained. Peripheral blood cells were collected from participants and genomic DNA was isolated from peripheral blood leukocytes by standard phenol/chloroform extraction and ethanol precipitation. SBT was performed for HLA-DRB1, -DQB1 and -DPB1 to type all subjects as previously described (5–7). In brief, we typed all samples in HLA-DRB1 locus using an SBT protocol described by Kotsch *et al.* In fact, the SBT protocol includes two steps: first, pretyping, that is, 14 pairs of group-specific primers are used to amplify the HLA-DRB1 alleles corresponding to their serologically defined specificities DR1–DR14; and second, according to the information obtained in the pretyping, two primer mixes (one for each haplotype) are selected for polymerase chain reaction amplification and sequencing. Group-specific primers were used to resolve ambiguous allelic combinations in the DQB1

gene and DPB1 gene following Voorter *et al.* (8) and van der Zwan *et al.* (9).

HLA class II allele frequencies were calculated by direct counting. Linkage disequilibrium (LD) between pairs of loci was tested using a likelihood ratio test and maximum-likelihood haplotype frequencies were computed using the expectation–maximization algorithm with ARLEQUIN software version 3.0 (10).

The allelic frequencies of HLA-DRB1, -DQB1 and -DPB1 genes in the Bai population are summarized in Table 1. A total of 28 DRB1, 19 DQB1 and 18 DPB1 alleles were detected. The allelic distributions of these three loci in the Bai population showed good fitness to Hardy–Weinberg equilibrium using exact test of Guo and Thompson (11).

Significant LD was shown by the LD test between DRB1 and DQB1 ($P = 0.0000$), DRB1 and DPB1 ($P = 0.0027$), and DQB1 and DPB1 ($P = 0.0122$). Haplotypes of HLA-DRB1–DQB1–DPB1 with a frequency of more than 2% are shown in Table 2. The most frequent DRB1–DQB1–DPB1 haplotype was DRB1*120201–DQB1*030101/

Table 1 Allele frequencies of HLA-DRB1, -DQB1 and -DPB1 in the Bai ethnic group

DRB1 (2n = 256)		DQB1 (2n = 256)		DPB1 (2n = 256)	
Allele	Frequency (%)	Allele	Frequency (%)	Allele	Frequency (%)
010101	0.39	0201/0202 ^a	4.69	010101	0.78
030101	3.52	030101/0309 ^a	35.94	020102	10.93
030102	1.95	030201	7.03	0202	5.08
040101	0.39	030302	3.91	030101	6.25
040301	1.56	0401	10.16	040101	9.77
0404	0.39	0402	3.13	0402	1.17
040501	3.13	050101	1.95	0501	42.19
040601	2.34	050201	11.33	0601	2.74
070101	0.78	050301	5.47	1001	1.56
080201	0.39	060101/060103 ^a	10.54	1301	13.28
080302	9.77	0602	1.17	1401	1.57
090102	10.16	0603	1.17	1701	0.78
100101	0.78	060401	0.39	1801	0.39
110101	4.69	060501	0.39	2101	0.78
120101/1206/1210 ^a	1.95	0609	0.78	2301	1.17
120201	16.41	0610	0.39	2501	0.39
130101	0.39	061101	0.78	3801	0.39
130201	1.56	0617	0.39	6301	0.78
130301	0.39	0619	0.39		
1312	0.78				
140101	8.59				
1404	9.38				
1405	0.39				
140701	0.39				
150101	8.98				
150201	1.56				
1504	0.78				
160201	8.21				

^a These alleles could not be distinguished by the typing method used in this work.

Table 2 Human leukocyte antigen (HLA)-DRB1-DQB1-DPB1 haplotype frequencies in the Bai ethnic group

HLA-DRB1-DQB1-DPB1 haplotype			
DRB1*	DQB1*	DPB1*	Frequency (%)
120201	030101/0309	020102	8.2
080302	060101/060103	1301	6.2
140101	050201	030101	4.2
160201	050201	0501	3.9
1404	050301	0501	3.1
090102	030302	0501	2.7
110101	030101/0309	0501	2.7
030101	0201/0202	040101	2.3
120201	030101/0309	0501	2.3
090102	060101/060103	0501	2.0
080302	050301	0501	2.0
040501	0401	0501	2.0
150101	050201	0501	2.0

0309-DPB1*020102 with a frequency of 8.2%, followed by DRB1*080302-DQB1*060101/060103-DPB1*1301 (6.2%).

Among all the 28 alleles detected for the DRB1 gene, the most common allele was DRB1*120201 with a frequency of 16.41%, followed by DRB1*090102 (10.16%), DRB1*080302 (9.77%), DRB1*1404 (9.38%), DRB1*150101 (8.98%), DRB1*140101 (8.59%) and DRB1*160201 (8.21%). The DRB1*120201, which is the characteristic allele of the people of East Asia, was much more frequent among southern Chinese populations, with frequencies from 10.2% to 28.8% (12–17) than among northern Chinese populations, with frequencies from 1.2% to 6.7% (18–22). DRB1*090102 and DRB1*080302 were also the characteristic alleles of Mongoloid people. Likewise, the DRB1*150101 and DRB1*140101 were commonly observed among most Chinese populations. DRB1*1404 (9.38%) is not common in other Chinese populations except for Naxi (9.7%) (14) and Yi (8.7%) (23). In addition, DRB1*160201 was also frequently observed among southern Chinese groups such as Buyi (16.2%) (24), Dai (16%) (25), Miao (10.7%) (13), Jing (6.9%) (16), and Lahu (6.3%) (12), but less frequent among most northern Chinese groups (18–22). In contrast, DRB1*070101, which was rarely found in the Bais (0.78%), was widely observed in the northern Chinese groups with gene frequencies generally exceeding 10% but presented at markedly lower frequencies less than 5% in the southern Chinese groups.

In the DQB1 locus, DQB1*030101/0309 was the most common allele with a frequency of 35.94% in the Bai population, which was very common in many groups in the world including most Chinese populations. The second most frequent DQB1 allele in the Bai ethnic group was DQB1*050201 with a frequency of 11.33%. It was especially common among the southern Chinese groups, with frequencies ranging from 13.9% to 38% (12–17, 24, 25),

but obviously infrequent among most northern Chinese groups, with frequencies less than 5.7% (18–22). Furthermore, both DQB1*060101/060103 and DQB1*0401 alleles were commonly observed in the Bai ethnic group, with gene frequencies exceeding 10%, which were also frequently found in most Chinese populations. In contrast, the DQB1*0201/0202, DQB1*0501 and DQB1*0603, which were frequently detected among northern Chinese people, were found in Bais with frequencies 4.69%, 1.95% and 1.17%, respectively, and at relatively lower frequencies in many southern Chinese people as well.

As for the DPB1 locus, DPB1*0501 with a frequency of 42.19% was the most frequent allele in Bais. The DPB1*0501 is the characteristic allele of the people of East Asia and mainly restricted geographically to Asian and Oceania population. In terms of the Chinese, its frequency exhibits relatively high in southern populations than in northern populations. DPB1*1301, the second frequent allele in Bais (13.28%), was also very common among many Southeast Asian populations. Likewise, the frequency of the DPB1*1301 in Chinese populations is also higher in southern Chinese populations (10.1%–33.3%) (13–17, 24–29) than in northern Chinese populations like Kazak (1.2%) (19), Shenyang Han (1.8%) (18), Man (2.1%) (20), Uyгур (4.4%) (22) and Northern Han (5.1%) (21). DPB1*020101 and DPB1*040101, being the two widely observed alleles all over the world, were also frequently observed in Bai, with frequencies of 10.93% and 9.77%, respectively.

In conclusion, all the above comparisons showed that the Bai ethnic population possessed southern Chinese characters and suggested a close relationship between them. However, further analysis of HLA class I genes, together with mt-DNA and Y-chromosomal studies, will give us more information about the Bais' precise genetic background and evolutionary histories.

Acknowledgments

All the experiments comply with the regulations in China. This study was supported by grants (nos. 05JJD850007, 2006CB708502, 2006GP10, 30660076 and 07Z10778).

References

1. You Z. Ancient Ethnic of Southwestern China. Kunming: Yunnan People's Press, 1980.
2. Guo J, Duan YM, Yang FQ. *The General Survey of Yunnan Minority Nationalities*. Kunming: Yunnan Nationality Press, 1999.
3. Tawa C, Wang TZ. *China's Ethnic Groups*. Beijing: Nationality Press, 2004.
4. Mack SL, Bugawan TL, Moonsamy PV *et al.* Evolution of Pacific/Asian populations inferred from HLA class II allele frequency distributions. *Tissue Antigens* 2000; **55**: 383–400.

5. Kotsch K, Wehling J, Blasczyk R Sequencing of HLA class II genes based on the conserved diversity of the non-coding regions: sequencing based typing of HLA-DRB genes. *Tissue Antigens* 1999; **53**: 486–97.
6. van der Zwan A, Griffith B, Rozemuller E, Williams T, Tilanus MG. Sequence-based typing for HLA-DQB1 strategy for ABI sequencing equipment. <http://www.ihwg.org>
7. van der Zwan A-W, Rozemuller E, Tilanus MGJ. Sequence-based typing for HLA-DPB1. Strategy for ABI equipment. <http://www.ihwg.org>
8. Voorter CEM, Kik MC, van den Berg-Loonen EM. Sequence-based typing for HLA-DQB1. Strategy for ALF express. <http://www.ihwg.org>
9. van der Zwan A-W, Rozemuller E, Tilanus MGJ. DPB1 ambiguities resolved with the group specific amplification primer approach. <http://www.ihwg.org>
10. Excoffier L, Laval G, Schneider S. Arlequin version 3.0: an integrated software package for population genetics data analysis. *Evol Bioinform* 2005; **1**: 47–50.
11. Guo S, Thompson E Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics* 1992; **48**: 361–72.
12. Jia Z, Fu Y, Pan D *et al.* Determination of HLA-DRB1 gene polymorphism by PCR-SBT in Lahu ethnic group of Yunnan, China. *Hereditas (Beijing)* 2002; **24**: 131–6.
13. Liu Y, Liu Z, Fu Y, Jia Z, Chen S, Xu A Polymorphism of HLA class II genes in Miao and Yao nationalities of Southwest China. *Tissue Antigens* 2006; **67**: 157–9.
14. Fu YG, Liu ZH, Lin JH *et al.* HLA-DRB1, DQB1 and DPB1 polymorphism in the Naxi ethnic group of Southwestern China. *Tissue Antigens* 2003; **61**: 179–83.
15. Sun X, Li Q, Sun Y *et al.* The polymorphism of HLA class II alleles in Guangdong Han nationality. *Chin J Med Genet* 1997; **14**: 270–3.
16. Lin JH, Liu ZH, Lv FJ *et al.* Molecular analyses of HLA-DRB1, -DPB1, -DQB1 in Jing Ethnic Minority of Southwest China. *Hum Immunol* 2003; **64**: 830–4.
17. Pan S, Li C, Long G *et al.* The polymorphism of the HLA-DRB1 locus of the Guangxi Zhuang People and its comparison with the main surrounding ethnic groups. *J Microbiol Immunol* 2004; **2**: 201–4.
18. Yu G, Sun Y, Xu L *et al.* A study on HLA-DR, DQ, DP DNA typing in Shenyang Han populations. *Zhongguo Mian Yi Xue Za Zhi* 1995; **11**: 142–5.
19. Mizuki N, Ohno S, Ando H *et al.* Major histocompatibility complex class II alleles in Kazak and Han populations in the Silk Route of northwestern China. *Tissue Antigens* 1997; **50**: 527–34.
20. Geng L, Imanishi T, Tokunaga K *et al.* Determination of HLA class II alleles by genotyping in a Manchu population in the northern part of China and its relationship with Han and Japanese populations. *Tissue Antigens* 1995; **46**: 111–6.
21. Gao X, Sun Y, An J *et al.* DNA typing for HLA-DR, and -DP alleles in a Chinese population using the polymerase chain reaction (PCR) and oligonucleotide probes. *Tissue Antigens* 1991; **38**: 24–30.
22. Mizuki N, Ohno S, Ando H *et al.* Major histocompatibility complex class II alleles in an Uyghur population in the Silk Route of Northwest China. *Tissue Antigens* 1998; **51**: 287–92.
23. Fei H, Jiang W, Lu Y *et al.* The oligotyping of HLA D region in Yi Chinese. *Chung Hua Wei Sheng Wu Hsueh Ho Mieh I Hsueh Tsa Chih* 1993; **13**: 219–25.
24. Xu X, Wang C, Cao J Study on the DNA typing of HLA class II genes in Chinese Buyi nationality. *Chung Hua Wei Sheng Wu Hsueh Ho Mieh I Hsueh Tsa Chih* 1992; **12**: 285–9.
25. Fan L, Yang Y, Dong J *et al.* HLA class II oligo nucleotide typing of a Dai minority population in Xishuangbanna of China. *Zhongguo Mian Yi Xue Za Zhi* 1992; **8**: 287–90.
26. Hu WH, Lu J, Lei YP *et al.* HLA-DPB1 allelic frequency of the Lisu ethnic group in the Southwest China and evolutionary relationship of Lisu with other populations. *Tissue Antigens* 2005; **65**: 289–92.
27. Hu WH, Lu J, Dong YL *et al.* Polymorphism of the DPB1 locus in Hani ethnic group of south-western China. *Int J Immunogenet* 2005; **32**: 421–3.
28. Wang B, Hu WH, Wang JX *et al.* HLA-DPB1 polymorphism in Blang and Puyi ethnic groups of Southwest China inferred from sequence-based typing. *Tissue Antigens* 2008; **71**: 81–4.
29. Hu WH, Wang JX, Wang B *et al.* Sequencing-based analysis of the HLA-DPB1 polymorphism in Nu ethnic group of south-west China. *Int J Immunogenet* 2006; **33**: 397–400.