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The distribution of HLA alleles revealed a founder effect in the geographically isolated Chinese population, Drung

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Abstract

The Drung ethnic minority is one of the smallest ethnic groups of China, geographically isolated by mountains and rivers. Before 1949, Drung society maintained many vestiges of the primitive commune system. The origin and migration of the Drung and their genetic background are still unknown because of limited records about this population. Here, we for the first time demonstrated the unique distribution of HLA alleles in the Drung by high-resolution sequence-based typing (SBT) method. Number of alleles detected is obviously less than expected and only a few alleles with a high homozygosity in each locus are predominant in this minority. The characteristics of HLA allele distribution in the Drung could reflect founder effects, suggesting the Drung probably descended from very few ancestors. The statistical analysis based on allele frequencies indicated that the Drung was an isolated ethnic group, but it also provided the clue that the Drung was genetically related to Chinese southwestern ethnic groups. Significant reduced allelic diversity and genetic isolate in the Drung make it an ideal homogeneous population and very useful model to study the evolution of HLA and the origin and migration of Chinese ethnic groups. The research paved a way to elucidate the genetic background of this mysterious minority and disease predisposition.

Keywords: HLA; Allele frequency; Drung; Founder effect

1. Introduction

Human leukocyte antigen (HLA) is the most polymorphic genetic system of human being and plays a key role in response to diseases in human. The high diversity of HLA alleles and haplotypes has made it a very useful genetic marker for anthropological studies and disease association (Riley and Olerup, 1992). The characterization of the HLA profile of a population, with high throughput techniques as sequence-based typing (SBT), is a helpful mechanism to trace genetic relationships between neighboring populations. Previous studies have shown that haplotype frequencies are characteristic of particular populations and even certain alleles are exclusively found in some ethnic groups (Arnaiz-Villena et al., 1999; Clayton et al., 1997).

The Drung ethnic minority is one of the smallest ethnic groups of China. The Drung has a total population of 5884 people based on census in 2000 (http://www.mw.yn.gov.cn), and is aboriginal in the Gongshan Drung and Nu Autonomous County in northwestern Yunnan province of China. The Drung are geographically isolated by living in the Dulong River valley which extends 150 km from north to south, flanked on the east by Mt. Gaoligong, 5000 m above sea level, and on the west by Mt. Dandanglika, 4000 m above sea level. The tops of both mountains are covered with snow all year round. This unusual geographical environment restricts communication of the Drung with the outside world. They can only leave the valley between June and October every year after iced-snow thaws partially for exchanging foods and other necessaries of life, and come back to the valley within few days. It usually takes 3 days of walking/climbing to get into the valley from the nearest town.

The earliest record of the Drung can be found in Da Yuan Yi Tong Zhi (roughly translated as China's Unification under

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the Great Yuan Dynasty, a famous work of geographical studies compiled under the auspices of the Yuan court; the great Mongolia Empire, 1271–1368). According to the record, the Drung were aboriginals who wear animal leathers and eat raw meats. Before the founding of the People's Republic of China in 1949, Drung society maintained many vestiges of the primitive commune system, with no calendar, medicine, metallurgy, even writing character. Traditionally, records were made and messages transmitted by engraving notches in wood and tying knots. As a result, a few of records about this ethnic are available.

In the present work, we investigated the allelic and haplotypic frequencies of HLA class I and class II genes for the Drung ethnic minority group by using high-resolution sequencing-based typing (SBT) to pave the way for studying their genetic background, thus elucidating the origin of the Drung people, and possible associations of their HLA with diseases.

2. Materials and methods

2.1. Population samples and HLA typing

A total of 105 healthy unrelated individuals of Drung ethnic minority were enrolled in this study. Genomic DNA was extracted from whole peripheral blood with ACD anticoagulant by the modified salting out method standardized by International Histocompatibility Work Group (IHWG) (http://www.ihwg.org/protocols). Sequencing based typing was performed for HLA-DRB1 (Jia et al., 2002; Kotsch et al., 1999), DQB1 (van der Zwan et al., 1997) and DPB1 loci (Versluis et al., 1993; Liu et al., 2001). Polymerase chain reaction using sequence-specific primers (PCR-SSP) and cloning were used to resolve the ambiguities. HLA-A, HLA-B, and HLA-C alleles were typed by directly sequencing exons 2 and 3 of HLA class I genes (Hong et al., 2005). The remaining infrequent genotypic ambiguities were resolved by cloning/sequencing individual alleles. Because polymorphisms outside exons 2 and 3 were not analyzed, some groups of alleles with sequence differences only in the other exons could not be distinguished in this study. In such case, an abbreviation of some allele group was given (Hong et al., 2005), which is based on "The IMGT/HLA Ambiguous Allele Combinations File" (release 2.11.0; 7 October 2005). The sequencing reactions were performed using ABI PRISM™ BigDye Terminator Cycle Sequencing Kit (The Perkin-Elmer Co., Foster City, CA, USA). HLA-DRB1, DQB1 and DPB1 alleles were typed in 70 Drung and HLA-A, -B and -C alleles were typed in 86 Drung, because of failure to amplify some loci for some samples. HLA allele frequencies were calculated by direct counting and compared to those of other Chinese and worldwide populations (http://www.ncbi.nlm.nih.gov/mhc).

2.2. Statistical analysis

Statistical analysis was performed using Arlequin 3.0 (Excoffier et al., 2005). Haplotype frequencies were estimated using the expectation-maximization (EM) algorithm. The homozygosity *F*-test for neutrality was applied using the Ewens–Watterson test and *P*-values at either extreme of the dis-

Table 1
HLA-A, -B, -C, -DRB1, -DQB1 and -DPB1 allele frequencies in the Drung population

A ($n=86$)	Frequency	C ($n=86$)	Frequency	B ($n=86$)	Frequency	DRB1 ($n=70$)	Frequency	DQB1 ($n=70$)	Frequency	DPB1 ($n=70$)	Frequency
020301	0.016	010201/010202[a]	0.036	070201	0.011	040301	0.006	030101/0309[a]	0.201	020102	0.033
0207	0.005	030401/030403[a]	0.061	150101G1[b]	0.043	040501	0.006	0302	0.078	030101	0.266
02G1[b]	0.560	040401	0.010	1502	0.049	0406	0.030	0401	0.019	0401	0.011
110101	0.198	04G1[b]	0.061	1512/1519[a]	0.005	080302	0.006	050201	0.675	0402	0.304
1103	0.038	070201G1[b]	0.770	35G1[b]	0.033	110101	0.006	050301	0.006	0501	0.245
1119	0.011	080101	0.051	380201	0.005	1111	0.006	060101	0.019	1401	0.005
24G1[b]	0.082	120201/120202[a]	0.005	40G1[b]	0.066	1117	0.006			1601	0.005
2601	0.055	140201	0.005	4601	0.005	120101/1206[a]	0.024			200101	0.038
310102	0.033			4801/4809[a]	0.005	120201	0.018			2101	0.005
				51G1[b]	0.005	140101	0.799			2201	0.011
				520101	0.016	140102	0.006			2801	0.033
				5502	0.022	1403	0.047			3601	0.005
				5601	0.732	1406	0.006			5701	0.011
						140701	0.006			5901	0.005
						1408	0.006			8001	0.022
						150101	0.006				
						150201	0.018				

[a] The sequences of exon 2 (for class II) or exons 2 and 3 (for class I) in these alleles are identity, and cannot be distinguished in this study.
[b] Allele groups based on "The IMGT/HLA Ambiguous Allele Combinations File" (release 2.11.0).