Association study of HLA-B alleles with idiopathic male infertility in Han population of China

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Abstract

Purpose To investigate the distributions of HLA-B alleles and estimate their associations with idiopathic male infertility in Chinese Han population.

Methods Polymerase chain reaction-sequence-based typing (PCR-SBT) method was used for DNA typing at HLA-B locus in 109 patients with idiopathic male infertility and 152 healthy controls in male Han population of Shaanxi Province, situated in northwestern China.

Capsule In this study, the distribution of HLA-B alleles was detected by polymerase chain reaction-sequence-based typing (PCR-SBT) method in idiopathic male infertile patients and controls. The result showed that HLA-B gene was unlikely a major risk factor of idiopathic male infertility in Han population of China.

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Results In total, we detected 45 HLA-B alleles in idiopathic infertile patients, 48 HLA-B alleles in control subjects. However, no significant differences of these allelic frequencies were found between the infertile patients and the controls.

Conclusion HLA-B gene was unlikely a major risk factor of idiopathic male infertility in this sample population. As different populations have different HLA polymorphisms, investigation of the relationship of other HLA genes and idiopathic male infertility with larger sample size, is warranted in the future.

Keywords Human leukocyte antigen (HLA)-B · Polymerase chain reaction-sequence-based typing (PCR-SBT) · Idiopathic male infertility

Introduction

Infertility affects 10–15% of couples worldwide, and male factors account for nearly half of all infertility cases [1]. Unfortunately, approximately 50% of male infertility cases are still unexplained or idiopathic for men [2]. Male infertility is a complex disease resulting from multiple interactions between genetic and environmental factors [3–5]. The genetic background of male infertility has long been a concern. Other than Y chromosome microdeletion, more recent studies have suggested that polymorphism of genes in autosomal chromosomes may also play an important role in male infertility [6, 7].

Human leukocyte antigen (HLA) genes are the most polymorphic genetic systems, which located on the chromosome 6p21.3. Currently, more than 6403 alleles were identified in HLA system (<http://www.ebi.ac.uk/imgt/hla/stats.html>). HLA genes in humans include three groups:

class I, class II and class III. HLA-A and -B are major genes in class II loci [8]. High polymorphism of HLA complex has made it valuable for disease-association studies. Studies have linked HLA antigens to this susceptibility to more than 100 diseases such as rheumatoid arthritis and diabetes mellitus etc. [9, 10].

Recently, there has been increased awareness of possible effects of HLA gene on male infertility. Multiple HLA genes have been confirmed to be prevalent in several populations with idiopathic male infertility. In a Japanese population, several studies showed a strong linkage of HLA-A33, -B13, -B44, -DR13 and -DRB1-1302 genes to the susceptibility of idiopathic male infertility in Japanese men. [11–14]. In Yugoslavia population, the study of Aleksovski D et al. demonstrated that higher frequency of HLA-A26, -A28, -B18 genes in idiopathic azoospermia and oligospermia patients than control group [15].

China is one of the largest and most populous areas in the world. Han nationality constitutes about 92% of population of China. However, to the best of our knowledge, there are few studies concerning the association of HLA genes and male infertility in Chinese Han population. We previously investigated the relationship between HLA-A gene and idiopathic male infertility in Chinese Han population and it showed no strong linkage of HLA-A alleles to the susceptibility of idiopathic male infertility [16]. HLA-B gene, as another major gene in class II loci, has the highest polymorphism in the whole human genome. Therefore, the current study aimed to investigate the potential association of HLA-B alleles with idiopathic male infertility in Chinese Han population using the polymerase chain reaction-sequence-based typing (PCR-SBT) method. These results will not only provide useful information for anthropological researches and population genetics, but also lay down a scientific basis for male reproduction studies.

Materials and methods

Subjects selection

The protocol was fully approved by the Institutional Medical Ethics Committee. All subjects were unrelated Chinese Han nationality men whose ancestors have lived in the Shaanxi Province for at least three generations. The risks and benefits of participating in the study were explained to all subjects.

We selected the infertile patients from couples attending the infertility clinic who had a history of infertility of ≥ 12 months. After obtaining the informed consent of patients, a detailed medical and reproductive

history was obtained from all subjects, including reproductive history and infertility evaluation of the female partner. A male-infertility specialist performed the genital examination of the male partner. Patients with normal female partners, that is normal reproductive history, normal ovulation (by follicular ultrasound scan, serum hormones levels, and endometrial biopsy), and tubal patency (hysterosalpingogram) were eligible for the study. Finally, 109 patients were selected as idiopathic infertile men (with abnormality in semen parameters on repeated analyses, normal genital examination and normal female partner). At the same time, healthy Han nationality married male volunteers who have at least one child and live in same place of residence were recruited as control group.

Blood samples and DNA extraction

After informed consent, 2 ml noncoagulated ethylenediaminetetraacetic acid (EDTA) blood samples were collected and stored at -20°C before DNA extraction. Genomic DNA was obtained from peripheral blood leukocytes and extracted by standard techniques [17].

Genotyping of HLA-B gene

All subjects were typed for HLA-B locus by using Polymerase chain reaction-sequence-based typing (PCR-SBT) method. Sequence-based typing of the HLA-B locus was performed on exon 2 and 3 (The HLA-B gene contains 8 exons. Exon 1 encodes the leader peptide, exon 2 and 3 encode the $\alpha 1$ and $\alpha 2$ domains, which both bind the peptide, exon 4 encodes the $\alpha 3$ domain, exon 5 encodes the transmembrane region and exons 6 and 7 encode the cytoplasmic tail. Polymorphisms within exon 2 and exon 3 are responsible for the peptide binding specificity of each class I molecule. Therefore, typing for these polymorphisms is routinely done for bone marrow, kidney transplantation and disease-association studies). Polymerase chain reaction amplifications were accomplished on GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA, USA), amplified DNA fragments were purified and sequenced using ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kits (Applied Biosystems, Foster City, CA, USA) in an ABI 3730XL DNA Sequencer (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Sequencing was always performed in forward and reverse directions and processed using Sequencing Analysis, Match Tools and Navigator software (Match Tools Allele Identification package, Applied Biosystems, Foster City, CA, USA). Ambiguous types were resolved

to four digits according to the updated database (IMGT release 2.21.0).

Statistical analysis

Hardy-Weinberg Equilibrium (HWE) of HLA-B locus was estimated using the Arlequin software package version 3.1 (Laurent Excoffier, CMPG, Zoological Institute, University of Bern, Switzerland), $P < 0.05$ was regarded as statistically significant. Genetic parameters including Homozygotes (Ho), Heterozygosity (He), Power of discrimination (PD), Polymorphism information content (PIC), and probability of paternity exclusion (PPE) were computed using the PowerStat Version 1.2 spreadsheet (Promega Corporation, USA). Allele frequencies of HLA-B locus were calculated by direct counting using SPSS13.0 software (SPSS Inc., Chicago, USA).

Finally, all analyses related to the case-control study were performed using SPSS13.0 software (SPSS Inc., Chicago, USA). The frequencies of HLA alleles were compared between controls and patients with idiopathic male infertility using the Chi-square test; Fisher's exact test was applied for expected numbers less than 5. Relative risk was calculated as an odds ratio (OR) with 95% confidence interval (CI) using Cornfield's approximation. To avoid type I errors, P values were corrected (P_c) for multiple testing using Bonferroni correction method. That is, P_c was corrected by multiplying the number of alleles observed. $P_c < 0.05$ was accepted as statistically significant.

Results

Subjects

Totally, 109 idiopathic male infertile patients (101 azoospermia and 8 oligozoospermia) and 152 healthy controls were selected. The mean age was no significantly different between infertile patients (30.50 ± 3.59) and controls (30.18 ± 3.40) ($P = 0.602$).

All idiopathic infertile patients ($P = 0.335826$) and controls ($P = 0.841174$) were in compliance with Hardy-Weinberg equilibrium at HLA-B locus.

Genetic parameters

Table 1 showed the values of genetic parameters of HLA-B locus in idiopathic infertile patients and controls.

HLA-B allelic frequencies

In total, we found 45 high-resolution HLA-B alleles in 109 idiopathic infertile patients. The allele frequencies of HLA-B locus were summarized in Table 2. At the

Table 1 Genetic parameters of HLA-B locus in idiopathic male infertile patients and controls

Locus	Group	Ho	He	PD	PIC	PPE
HLA-B	Patients	2.0%	98%	0.986	0.95	0.959
	Control	3.3%	96.7%	0.987	0.95	0.934

Ho Homozygotes, He Heterozygotes, PD power of discrimination, PIC polymorphism information content, PPE probability of paternity exclusion

HLA-B loci, the seven most common alleles with a frequency greater than 5% were HLA-B*4601(9.2%), B*5101(6.9%), B*4001(6.4%), B*1501(6%), B*1302 (5.5%), B*0702 (5%) and B*5201(5%) respectively.

On the other hand, we detected 48 high-resolution HLA-B alleles in 152 control subjects (Table 2). At the HLA-B loci, the six most common alleles with a frequency larger than 5% were HLA-B*4601 (8.2%), B*4001 (8.2%), B*5101 (7.6%), B*1302 (6.3%), B*1301 (5.6%) and B*1501(5.6%) respectively.

Association of HLA-B allele frequency with idiopathic male infertility

We compared the distributions of high-resolution HLA-B alleles between the idiopathic infertile patients and controls (Table 2), and found that HLA-B*4002 allele was significantly lower in patients than in the control group ($P = 0.018$). The Relative risk for the HLA-B*4002 allele for idiopathic male infertility was 0.123 (95% CI: 0.016–0.958). However, this association lost its significance after Bonferroni correction for multiple testing.

Since most previous association studies of HLA-B and male infertility were based on low-resolution (serotypes) of HLA. To be compatible with previous studies, we grouped our sequence-based high-resolution HLA genotype to their associated serotype according to the classification of Schreuder et al. [18]. The 54 high-resolution genotypes of our subjects could be grouped into 34 serotypes. We reanalyzed their association with idiopathic male infertility, no significant differences of the frequencies of these 34 serology-defined genotypes between infertile patients and controls were detected (Table 3).

As B13, B18 and B44 were ever reported to be associated with male infertility in previous population studies, we considered them as candidate alleles in this study. The results showed that no significant decrease of B13 in patients group, with an odds ratio (OR) 0.711 (95% CI: 0.396–1.276; $P = 0.251$). The frequency of B18 allele was only 1% in controls whereas it was not been detected in patients group. Moreover, B44 allele was slightly lower in patients than in the control group, with an odds ratio (OR) 0.423 (95%CI: 0.152–1.171; $P = 0.089$) (Table 3).

Table 2 Allelic distributions of HLA-B polymorphism in idiopathic male infertile patients and controls

HLA-B	Control(<i>n</i> =304 alleles)		Patients(<i>n</i> =218 alleles)		<i>P</i>	<i>P_c</i>	OR (95% CI)
	n	AF(%)	n	AF(%)			
B*0702	7	2.3	11	5.0	0.090	ns	2.255(0.860–5.913)
B*0801	4	1.3	4	1.8	0.725	ns	1.402(0.347–5.668)
B*1301	17	5.6	7	3.2	0.200	ns	0.560(0.228–1.375)
B*1302	19	6.3	12	5.5	0.722	ns	0.874(0.415–1.840)
B*1401	1	0.3	2	0.9	0.574	ns	2.806(0.253–31.137)
B*1402	2	0.7	2	0.9	1.000	ns	1.398(0.195–10.003)
B*1501	17	5.6	13	6.0	0.857	ns	1.071(0.509–2.253)
B*1502	10	3.3	5	2.3	0.502	ns	0.690(0.233–2.048)
B*1505	0	0	2	0.9	–	ns	–
B*1507	1	0.3	0	0	–	ns	–
B*1511	4	1.3	3	1.4	1.000	ns	1.047(0.232–4.724)
B*1512	0	0	1	0.5	–	ns	–
B*1517	0	0	1	0.5	–	ns	–
B*1518	2	0.7	3	1.4	0.654	ns	2.107(0.349–12.717)
B*1525	2	0.7	1	0.5	1.000	ns	0.696(0.063–7.723)
B*1527	2	0.7	1	0.5	1.000	ns	0.696(0.063–7.723)
B*1529	1	0.3	0	0	–	ns	–
B*1532	2	0.7	1	0.5	1.000	ns	0.696(0.063–7.723)
B*1546	1	0.3	0	0	–	ns	–
B*1558	1	0.3	0	0	–	ns	–
B*1801	3	1.0	0	0	–	ns	–
B*2704	1	0.3	2	0.9	0.574	ns	2.806(0.253–31.137)
B*2705	3	1.0	2	0.9	1.000	ns	0.929(0.154–5.607)
B*2707	1	0.3	1	0.5	1.000	ns	1.139(0.087–22.446)
B*2724	0	0	1	0.5	–	ns	–
B*3501	11	3.6	8	3.7	0.975	ns	1.015(0.401–2.566)
B*3503	7	2.3	5	2.3	0.995	ns	0.996(0.312–3.180)
B*3508	2	0.7	0	0	–	ns	–
B*3701	3	1.0	4	1.8	0.459	ns	1.875(0.415–8.465)
B*3801	1	0.3	2	0.9	0.574	ns	2.806(0.253–31.137)
B*3802	2	0.7	4	1.8	0.241	ns	2.822(0.512–15.549)
B*3901	3	1.0	5	2.3	0.288	ns	2.355(0.557–9.961)
B*3905	0	0	1	0.5	–	ns	–
B*4001	25	8.2	14	6.4	0.440	ns	0.766(0.389–1.510)
B*4002	11	3.6	1	0.5	0.018*	ns	0.123(0.016–0.958)
B*4003	1	0.3	0	0	–	ns	–
B*4006	12	3.9	8	3.7	0.871	ns	0.927(0.372–2.308)
B*4102	1	0.3	0	0	–	ns	–
B*4402	7	2.3	2	0.9	0.316	ns	0.393(0.081–1.910)
B*4403	9	3.0	3	1.4	0.234	ns	0.457(0.122–1.709)
B*4501	0	0	1	0.5	–	ns	–
B*4601	25	8.2	20	9.2	0.703	ns	1.127(0.609–2.086)
B*4701	1	0.3	1	0.5	1.000	ns	1.139(0.087–22.446)
B*4801	9	3.0	7	3.2	0.870	ns	1.087(0.399–2.966)
B*5001	4	1.3	3	1.4	1.000	ns	1.047(0.232–4.724)
B*5101	23	7.6	15	6.9	0.766	ns	0.903(0.406–1.773)
B*5102	2	0.7	3	1.4	0.654	ns	2.107(0.349–12.717)

Table 2 (continued)

HLA-B	Control(<i>n</i> =304 alleles)		Patients(<i>n</i> =218 alleles)		<i>P</i>	<i>P_c</i>	OR (95% CI)
	<i>n</i>	AF(%)	<i>n</i>	AF(%)			
B*5201	10	3.3	11	5.0	0.314	ns	1.562(0.652–3.746)
B*5401	10	3.3	8	3.7	0.814	ns	1.120(0.435–2.886)
B*5502	6	2.0	4	1.8	1.000	ns	0.928(0.259–3.330)
B*5601	1	0.3	0	0	–	ns	–
B*5701	6	2.0	3	1.4	0.741	ns	0.693(0.171–2.802)
B*5801	9	3.0	7	3.2	0.870	ns	1.087(0.399–2.966)
B*6701	2	0.7	3	1.4	0.654	ns	2.107(0.349–12.717)

AF Allele frequency, OR Odds ratio, CI Confidence interval, ns not significant; * $P < 0.05$

Discussion

This is the first study to investigate the association of HLA-B alleles with idiopathic male infertility in Chinese Han population by using the PCR-SBT method. In this study, we did not find an association of HLA-B alleles with idiopathic male infertility, indicating that this gene might not be a major risk gene of idiopathic male infertility in Chinese Han population.

Male infertility is a complex disease resulting from multiple interactions between genetic and environmental factors. Recent studies have suggested that polymorphism of genes in autosomal chromosomes may also play an important role in male infertility [6, 7]. As the most polymorphic genetic system of human genome, and high polymorphism of HLA complex has made it valuable for disease-association studies [19, 20].

The HLA-B gene was one of the most often studies genes in HLA class I genes. In our present study, we found the frequency of B*4002 was significant lower ($P=0.018$) in idiopathic infertile patients than in controls. However, this association lost their significance after correction for multiple testing. It would be interesting to examine the association of B*4002 with idiopathic male infertility with a larger sample size in the future study.

The association between HLA-B antigens and idiopathic male infertility has also been examined in several populations, however the results remain inconsistent. Miura et al. suggested that the HLA-B13, B44 was strongly linked to the susceptibility of idiopathic male infertility in Japanese men, suggesting that HLA-B antigens are important genetic markers involved in idiopathic male infertility [11]. Aleksovski et al. found association of HLA-B18 with idiopathic male infertility in Yugoslavia population [15]. However, other studies have reported no such association [21, 22]. These conflicting results might be attributed to differences in HLA typing methodology, sample size, ethnic background and geographic variations.

PCR-SBT method is considered the gold standard for high resolution definition of HLA [19, 20]. Previous HLA association studies in male infertility of other population have been done with low-resolution HLA serologic typing. In this study, we adopted the PCR-SBT method to detect the genotype of HLA. This method has much higher accuracy and reliability than simply serological typing methods, and also facilitates the standardization of HLA typing processes [20].

Sample size is an import factor affecting the result of case-control association studies [23]. In this study, all samples in idiopathic infertile group and control group were in Hardy-Weinberg equilibrium at HLA-B locus. Ho, He, PD, PIC, and PPE are genetic parameters for evaluating the polymorphisms of gene. A gene locus is considered highly polymorphic when its PIC is higher than 0.5, or PD value higher than 0.8, PPE value higher than 0.5 respectively [20]. In our present study, the PIC values (0.950 and 0.950), PD values (0.986 and 0.987), and PPE values (0.959 and 0.934) of HLA-B locus in two groups were higher than the cutoff values mentioned above. Therefore, the HLA-B data obtained from our sample population is highly polymorphism which was valuable for further genetic science research.

Moreover, post-hoc power analysis showed that with the total sample size of 522 chromosomes, this study had a power of 0.9 to detect an effect size (0.2) of the allelic distributions of all these 54 HLA-B alleles at the alpha level of 0.05. We still need a larger sample size to detect the small effect size of HLA-B allelic differences between patients and controls. Further investigation using a larger sample size to confirm the current finding is necessary.

Ethnic background and geographic variations are very import factors affecting the study results. The distributions of HLA alleles show high variation in different ethnic groups or even the same ethnic group living in different geographical areas [19, 20]. Now there are 56 officially identified ethnic groups in China [19], Han nationality

Table 3 Serotype (low-resolution typing) distributions of HLA-B in idiopathic male infertile patients and controls

Serotype	DNA-based genotype	Control (<i>n</i> =304 alleles)	Patients (<i>n</i> =218 alleles)	<i>P</i>	<i>P_c</i>	OR (95%CI)
B7	B*0702	7 (2.3%)	11 (5.0%)	0.090	ns	2.255(0.860–5.913)
B8	B*0801	4 (1.3%)	4 (1.8%)	0.725	ns	1.402(0.347–5.668)
B13	B*1301, B*1302	36 (11.8%)	19 (8.7%)	0.251	ns	0.711(0.396–1.276)
B18	B*1801	3 (1.0%)	0 (0%)	–	ns	–
B27	B*2704, B*2705, B*2707, B*2724	5 (1.6%)	6 (2.8%)	0.539	ns	1.692(0.510–5.618)
B35	B*3501, B*3503, B*3508	20 (6.6%)	13 (6.0%)	0.776	ns	0.900(0.438–1.852)
B37	B*3701	3 (1.0%)	4 (1.8%)	0.459	ns	1.875(0.415–8.465)
B38	B*3801, B*3802	3 (1.0%)	6 (2.8%)	0.174	ns	2.840(0.702–11.481)
B39	B*3901, B*3905	3 (1.0%)	6 (2.8%)	0.174	ns	2.840(0.702–11.481)
B41	B*4102	1 (0.3%)	0 (0%)	–	ns	–
B44	B*4402, B*4403	16 (5.3%)	5 (2.3%)	0.089	ns	0.423(0.152–1.171)
B45	B*4501	0 (0%)	1 (0.5%)	–	ns	–
B46	B*4601	25 (8.2%)	20 (9.2%)	0.703	ns	1.127(0.609–2.086)
B47	B*4701	1 (0.3%)	1 (0.5%)	1.000	ns	1.139(0.087–22.446)
B48	B*4801	9 (3.0%)	7 (3.2%)	0.870	ns	1.087(0.399–2.966)
B50	B*5001	4 (1.3%)	3 (1.4%)	1.000	ns	1.047(0.232–4.724)
B51	B*5101, B*5102	25 (8.2%)	18 (8.3%)	0.989	ns	1.004(0.534–1.891)
B54	B*5401	10 (3.3%)	8 (3.7%)	0.814	ns	1.120(0.435–2.886)
B55	B*5502	6 (2.0%)	4 (1.8%)	1.000	ns	0.928(0.259–3.330)
B56	B*5601	1 (0.3%)	0 (0%)	–	ns	–
B57	B*5701	6 (2.0%)	3 (1.4%)	0.741	ns	0.693(0.171–2.802)
B58	B*5801	9 (3.0%)	7 (3.2%)	0.870	ns	1.087(0.399–2.966)
B60	B*4001	25 (8.2%)	14 (6.4%)	0.440	ns	0.766(0.389–1.510)
B61	B*4002, B*4003, B*4006	24 (7.9%)	9 (4.1%)	0.081	ns	0.502(0.229–1.103)
B62	B*1501, B*1505, B*1507, B*1525, B*1527, B*1532, B*1558	25 (8.2%)	18 (8.3%)	0.989	ns	1.004(0.534–1.891)
B63	B*1517	0 (0%)	1 (0.5%)	–	ns	–
B64	B*1401	1 (0.3%)	9 (0.9%)	0.574	ns	2.806(0.253–31.137)
B65	B*1402	2 (0.7%)	2 (0.9%)	1.000	ns	1.398(0.195–10.003)
B67	B*6701	2 (0.7%)	3 (1.4%)	0.654	ns	2.107(0.349–12.717)
B70	B*1529	1 (0.3%)	0 (0%)	–	ns	–
B71	B*1518	2 (0.7%)	3 (1.4%)	0.654	ns	2.107(0.349–12.717)
B72	B*1546	1 (0.3%)	0 (0%)	–	ns	–
B75	B*1502, B*1511	14 (4.6%)	8 (3.7%)	0.600	ns	0.789(0.325–1.915)
B76	B*1512	0 (0%)	1 (0.5%)	–	ns	–

OR Odds ratio, CI Confidence interval, ns not significant

constitutes about 92% of population of China. Shaanxi province, located in the northwest of China, is a major Han population residual region. It is not only the birthplace of the Chinese nation, but also the birthplace of human civilization in Asia and the cultural center of prehistoric civilization. In our present study, we selected subjects from the same ethnic and geographic background to avoid the possible occurrence of sampling errors.

Our studies showed that HLA-B*4601 (9.2% in idiopathic patient and 8.2% in controls) is the most common HLA-B allele in Han population of Shaanxi Province; it

also has the highest prevalence in Han population in some regions of China [19, 20]. However, it is rarely seen in European populations [24] and never seen in African populations [25]. Therefore, the genetic association of HLA with male infertility may be different in different populations.

In conclusion, our present study investigated the association of the HLA-B gene with idiopathic male infertility in Chinese Han population using PCR-SBT high-resolution HLA genotyping method, which provides a better resolution than the other methods. Although the result

of this study is negative, it would provide useful information for anthropological researches and male reproduction studies.

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