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Population data on D6S2879 and D6S2806 markers located at HLA-DRB1 region in the Iranians: Identifying the signatures of balancing and directional selection

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Abstract
In this study, the genetic diversity and neutrality test for the MHC microsatellite loci, D6S2879 and D6S2806, located within the HLA-DRB1 gene region, were investigated. The genotyping data from 73 unrelated individuals were analyzed for Shannon index, the effective allele number of the markers and neutrality test by use of PyPop and Popgene32 programs. The Shannon index for D6S2879 and D6S2806 markers in the studied population was 1.0372 and 0.8601, respectively. The Fnd value computed for D6S2879 and D6S2806 markers were also estimated -0.8449 and 0.9904, respectively. The results obtained from Ewens-Watterson test indicated that D6S2879 and D6S2806 markers were under balancing and directional selection in the Iranian populations, respectively. The data suggested the presence of a selection force on HLA-DRB1 gene region in the Iranian populations.
Key words: HLA-DRB1 gene, Gene diversity, Ewens-Watterson test, MHC microsatellite marker, Selection

Introduction

The major histocompatibility complex (MHC) shows high allelic diversity in many vertebrates and it plays a unique role in the immune system and autoimmunity (Parham and Ohta, 1996; Gaudieri *et al*., 2000; Robinson *et al*., 2000). HLA-DRB1 belongs to MHC class II and encodes the most prevalent beta subunit of HLA DR beta chain. In the study on patients with chronic pancreatitis (CP), the HLA-DRB1*0401 allele was introduced as a susceptibility factor for CP patients (Cavestro *et al.*, 2003). A genetic association between HLA-DRB1*15 status and the risk of developing keloid following injury was reported in a study on a group of Caucasoid patients (Brown *et al*., 2008). Whereas a study on multiple sclerosis (MS) patients reported that the DRB1*0701, DRB1*04 sub-allele HLA-DRB1*0407 and HLA-DRB1*0901 may have been protective influence on MS susceptibility (Wu *et al.*, 2010). In fact, recent reports have indicated the association of particular alleles of HLA-DRB1 with resistance or susceptibility to different autoimmune

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and infectious diseases (Carrington *et al*., 1999; de Groot *et al*., 2002; Koo *et al*., 2003; Migita *et al*., 2006; Barnetche *et al*., 2008).

In view of the high polymorphic content of the microsatellite markers present in this region, analysis for inferring the level of polymorphism and the impact of selection force on MHC region has been the main focus of many investigations (Ammer *et al.*, 1992; Ellegren *et al*., 1993; Schwaiger *et al*., 1993; Schwaiger *et al*., 1994). According to previous studies, balancing selection, negative frequency- dependent selection and directional selection on MHC region seems to be evident. These selections could be used for interpretation of high allelic diversity on MHC region (Apanius *et al*., 1997; Hedrick, 1999; Hedrick, 2002; Bernatchez and Landry, 2003; Sommer, 2005; Piertney and Oliver, 2006).

Characterization of HLA-DRB1 region indicated the presence of several polymorphic microsatellite alleles (Marsh *et al*., 2002). Two MHC microsatellite markers, D6S2879 and D6S2806, were found in approximately 1 kbp downstream and 14 kbp upstream of HLA-DRB1 gene respectively (see http://www.ncbi.nlm.nih.gov/projects/gv/mhc/xslcgi.fcgi). In our recent study, characterization of D6S2879 and D6S2806 markers revealed a high variation in their allelic number (Vallian *et al*., 2010). Analysis of deviations from Hardy-Weinberg equilibrium (HWE) demonstrated that D6S2806 was in equilibrium ($P>0.05$). However, D6S2879 locus showed a significant deviation from HWE ($P<0.05$) (Vallian *et al*., 2010).

It has been suggested that all polymorphic markers were neutral and the changes of allele frequencies were rarely due to selection. A number of statistical tests of neutrality was devised that could be used to investigate neutral allele theory (Nielsen, 2001; Carlson *et al*., 2005). To determine whether these two MHC microsatellite markers in HLA-DRB1 gene region were subjected to selection in the Iranian populations, we investigated genetic diversity and neutrality test for these markers.

Material and Methods

Isolation of genomic DNA and Genotyping

The genotyping data used in this study were obtained from our previous studies on 73 healthy unrelated individuals form the Iranian populations (Vallian and Moeini, 2006; Vallian and Lahmi, 2009; Vallian *et al.,* 2010). To investigate the genotypes, total genomic DNA was isolated from peripheral blood leukocytes and genotyped using PCR amplification with specific primers followed by sequencing using an ABI 737 sequencer (Perkin Elmer/ABI) as described (Vallian *et al*., 2010).

Statistical Analysis

The genotype data of two MHC microsatellite markers, D6S2879 and D6S2806, were used to create input file. Slatkin (1994) implementation of the Ewens–Watterson homozygosity test of neutrality (Ewens, 1972; Watterson, 1977) was performed using PyPop (Lancaster *et al.,* 2003). The PyPop (Python for Population Genomics) is a computer program for performing population genetic analyses on genotype data. For each marker, the observed homozygosity (F), computed as the sum of the squared allele frequencies, the expected homozygosity and the normalized deviation of the homozygosity (Fnd), differences between the observed homozygosity and expected homozygosity, were estimated. The observed homozygosity (F) is computed on the basis of the actual data. In Ewens-Watterson test, the F value is compared to the expected homozygosity ($\hat{F}$) computed by simulation under neutrality/equilibrium expectations. If the difference

between the observed and expected homozygosity were larger or smaller than zero in the studied population, it could be inferred that this polymorphism was under directional and balancing selection, respectively (Nielsen, 2001). The Fnd is the difference between the observed homozygosity and expected homozygosity, divided by the square root of the variance of the expected homozygosity obtained by simulations. The data pertaining to 10 non-MHC microsatellite markers were used for the purpose of comparing the selection effect on MHC and non-MHC microsatellite in the Isfahan population. The data related to allele frequency of these non-MHC microsatellite markers were previously described (Vallian and Moeini, 2006; Vallian and Lahmi, 2009).

Ewens-Watterson test was also performed by use of Popgene32 software version 1.31 (available at http://www.ualberta.ca/~fyeh/download.htm) on the basis of algorithm given by Manly, (1985). This program is designed for many different types of analyses on a variety of molecular marker types. The observed homozygosity (F) and limit (upper and lower) at 95% confidence for the test were calculated for two MHC microsatellite markers, D6S2879 and D6S2806, using the genotyping data. The observed number of alleles, the effective number of alleles and Shannon's information index of the studied MHC microsatellite markers were also estimated using Popgene32. The allele frequency data of these 12 microsatellite markers, i.e. D6S2879, D6S2806, LPL, F13B, HUMvWA, HPRTB, HUMTPO, HUMTH01, HUMFES, D16S539, F13A01 and CSF1PO, were used to detect recent genetic bottlenecks in the Iranian populations. The bottleneck events were investigated using the homozygosity test implemented in BOTTLENECK 1.2.02 (Cornuet and Luikart, 1996). The homozygosity test was performed under the step-wise mutation model (SMM) and the two-phase mutation model (TPM). These two models were considered the most realistic mutation models for microsatellite markers (Ellegren, 2000). The sign test and Wilcoxon test were used to assess bottleneck in the studied population. The wilcoxon test provides relatively high power (Luikart and Cornuet, 1998) with as few as four polymorphic loci and any number of individuals (15-40 individuals and 10-15 polymorphic loci is recommend to achieve high power). The results of these statistical analyses could be used to determine evolutionary history of these microsatellite markers located on HLA-DRB1 gene region in the Iranian populations.

Results

Various measures of genetic diversity in terms of observed number of alleles, effective number of alleles and Shannon's information index are presented in Table 1. As shown, D6S2879 marker was observed in 4 different sizes in the Iranian populations. These alleles were located between 284-338 base pair (Vallian *et al.,* 2010). The effective number of the alleles for D6S2879 marker was estimated to be 2.2701. For D6S2806 marker, the observed and effective number of alleles was 6 and 1.6547, respectively. The observed alleles for D6S2806 marker in the Iranian populations were spaced between 312-338 base pair (Vallian *et al.,* 2010). As shown in Table 1, Shannon's information index of the D6S2879 and D6S2806 markers is 1.0372 and 0.8601, respectively. The neutrality of two MHC microsatellite markers was tested by use of Popgene32 software. As presented in Table 2, the F values (the observed homozygosity) for D6S2879 and D6S2806 were calculated 0.4405 and 0.6043, respectively. These obtained values lies inside the lower and upper limit of 95% confidence region of expected F value at both MHC microatellite markers. Table 3 shows the results of the homozygosity tests of neutrality for both D6S2879 and D6S2806 microsatellite markers of HLA-DRB1 gene region in the Iranian populations. The Fnd

value obtained for D6S2879 and D6S2806 markers were estimated to be -0.8449 and 0.9904, respectively. The negative Fnd value of all studied non-MHC microsatellite markers is significantly higher than 1. The p value obtained for all these non-MHC microsatellite markers were estimated to be less than 0.05, consistent with negative value of Fnd calculated for these markers. The data obtained from genetic bottleneck analysis is presented in Table 4. Under sign test, the expected numbers of loci with heterozygosity excess were 7.12 (TPM) and 7.08 (SMM), which were substantially lower than the observed numbers of loci 11 (TPM) and 10 (SMM) with heterozygosity excess. The probability values of 0.01709 (TPM) and 0.02124 (SMM) under Wilcoxon test were significant ($P<0.05$) in the Iranian populations.

Table 1: The observed number of alleles (na), effective number of alleles (ne) and Shannon's Information Index (I) for microsatellite markers, D6S2879 and D6S2806, at the HLA-DRB1 gene region in the Iranian populations.

Microsatellite marker	na	ne	I
D6S2879	4.0000	2.2701	1.0372
D6S2806	6.0000	1.6547	0.8601

Table 2: The Ewens-Watterson test for Neutrality at two microsatellite markers of HLA-DRB1 gene region by use of Popgene32 software in the Iranian populations.

MICROSATELLITE MARKER	K	F	L95	U95
D6S2879	4	0.4405	0.3167	0.9331
D6S2806	6	0.6043	0.2358	0.8087

k: the number of alleles; F: the sum of the squared allele frequencies; L95, U95: The 95% confidence interval upper and lower limit.

Table 3: The summarized results of Ewens-Watterson neutrality test applying to two MHC microsatellites and ten non-MHC microsatellites in the Iranian populations.

Marker		*F*	$\hat{F}$	Fnd	p-value of F
MHC microsatellite	D6S2879	0.4405	0.5931	-0.8449	0.2311
	D6S2806	0.6043	0.4523	0.9904	0.8251
non-MHC microsatellite	LPL	0.2001	0.5463	-1.9338	0.0000
	F13B	0.1925	0.4853	-1.7624	0.0008
	HUMvWA	0.1844	0.3865	-1.4042	0.0142
	HPRTB	0.2032	0.4368	-1.4930	0.0083
	HUMTPO	0.2125	0.4665	-1.5131	0.0093
	HUMTH01	0.2140	0.5142	-1.7051	0.0021
	HUMFES	0.2201	0.5142	-1.6706	0.0029
	D16S539	0.2118	0.4853	-1.6464	0.0028
	F13A01	0.2096	0.5466	-1.881	0.0001
	CSF1PO	0.1986	0.4368	-1.5227	0.0058

F: the observed homozygosity; $\hat{F}$: the expected homozygosity; Fnd: the normalized deviate of the homozygosity

Table 4: Bottleneck analysis of the Iranian populations using sign test and wilcoxon test under TPM and SMM.

Test	TPM		SMM	
	Expected	Observed	Expected	Observed
Sign Test: Number of loci with heterozygosity excess	7.12	11	7.08	10
Wilcoxon Test: Probability of heterozygosity excess	0.01709		0.02124	

Parameters for TPM: variance = 30.00, proportion of SMM= 70.00%, estimation based on 1000 replications.

Discussion

Two markers, D6S2879 and D6S2806, were presented in dbMHC web site as potential microsatellite markers in HLA-DRB1 gene region (Gourraud *et al.*, 2007). In this study, we used genotyping data of these two polymorphic markers and other previously studied markers in order to investigate the evolutionary history of the Iranian populations. According to Table 1, the highest number of alleles is observed for D6S2806 marker, but the highest effective number of alleles and the Shannon's information index are estimated for D6S2879 marker. Shannon index for D6S2879 marker is almost 1 and heterozygosity of this marker is high in the Iranian populations. It appears that genetic diversity of D6S2879 marker is higher than D6S2806 marker in the Iranian populations.

In Ewens-Watterson test of neutrality for these markers, F value (the observed homozygosity) lied inside the limit of 95% confidence region (Table 2). If the F value would have lied outside the lower and upper limit of 95% confidence region of expected F value, these markers were probability under genetic hitchhiking and associated with a selected allele at another gene. The results obtained from Ewens-Watterson test which were performed by use of Popgene32 software indicated that these two MHC microsatellite markers in HLA-DRB1 gene region were not under genetic hitchhiking. Therefore, selection operated on another locus could not influence allelic frequency and heterozygosity of these markers in the Iranian populations.

It has been reported that balancing selection could affect the evolution of a number of genes in the humans and plays an important role in maintenance variation responsible for long-term adaptation to the environment (Andrés *et al.*, 2009). In the present study, for each marker, the observed homozygosity (F), the expected homozygosity ($\hat{F}$) and the normalized deviate of the homozygosity (Fnd) by use of PyPop software were estimated. As shown in Table 3, the normalized deviate of the homozygosity (Fnd) for D6S2879 marker is negative. The observed homozygosity value is also lower than the expected homozygosity for this marker. Indeed, these results provided the first support for heterozygote advantage as a source of balancing selection at D6S2879 marker in the Iranian populations. It seemed that the alleles of this marker were actively maintained in the studied population, which could reflect the consequence of higher adaptive value of heterozygotes in comparison with homozygotes.

Positive value of Fnd and also $F > \hat{F}$ are evidence of directional selection at D6S2806 marker. Directional selection changes the frequency of an allele in a particular and constant direction. Under directional selection, the advantageous allele will increase in frequency and even might fix. Although, six alleles is observed for D6S2806 marker in the Iranian populations, the effective number of alleles is 1.6547 alleles. This data may suggest that directional selection could play an important role in decreasing the effective number of alleles for D6S2806 marker in the Iranian populations. The negative Fnd and that fact that the p-value was significantly less than 0.05 for all ten non-MHC microsatellites implied that these markers were under balancing selection in the Iranian populations (Table 3). Comparison of MHC microsatellites and non-MHC microsatellites indicated that the selection on the studied non-MHC microsatellites had more potent than two studied MHC microsatellites, i.e. D6S2879 and D6S2806 markers. These differences could be related to their physical position in MHC gene region. The studied non-MHC microsatellite markers are located closer to coding sequence than MHC microsatellite markers. Data obtained from bottleneck analysis of the Iranian populations indicated that the null hypothesis that the population was under mutation-drift equilibrium could not be supported. The results of sign

test and wilcoxon test showed that the studied population have undergone mild bottleneck.

Moreover, in this study, we reported balancing selection at almost 11 microsatellite markers in the Iranian populations. In our previous study, the balancing selection was observed in two markers of *PAH* gene (Fazeli and Vallian, 2010). Most of the studied markers in the Iranian populations showed observed heterozygosity higher than 50% (Fazeli and Vallian, 2009; Vallian and Moeini, 2006; Vallian and Lahmi, 2009). The high heterozyosity of studied markers implied that balancing selection probably counteract genetic bottleneck in the Iranian populations. Two studied MHC microsattelite markers, D6S2806 and D6S2879, showed low effective number of alleles (Table 1). It is likely that low gene diversity of these two MHC microsattelite markers could be the result of genetic bottleneck in the Iranian populations.

The high frequency of HLA-DRB1*15 and DRB1*04 has been found in the Iranian MS patients and the frequency of HLA-DRB1*07 and *11 has been shown a high increase in the Iranian optic neuritis (ON) patients (Amirzargar *et al.*, 2005). HLA-DRB1*1501 has been found significantly more frequent among MS patients, although no association was observed with clinical manifestation in the Iranian MS patients (Ghabaee *et al*.,2009). A significant positive association with AML for the HLA-DRB1*11allele was also reported in two studies performed on the Iranian populations (Sarafnejad *et al*., 2006; Khosravi *et al*., 2007). In the study on the Iranian non-Jewish patients with the Pemphigus vulgaris (PV), the HLA-DRB1*04 and DRB1*1401 alleles was reported as two major PV susceptibility factors (Shams *et al.*, 2009). The HLA-DRB1*07 was also found as the predisposing allele in the Iranian patients with pulmonary tuberculosis (Amirzargar *et al.*, 2004). The HLA-DRB1*13 allele was identified as an important factor in the protection against persisting hepatitis B infection in the Iranian populations (Ramezani *et al.*, 2008). As stated in the pervious study, the HLA-DRB1 region was under influence of selection force. Our results were also confirmed the presence of selection in this region. The evidence of balancing and directional selection at HLA-DRB1 gene region in the Iranian populations was found in the performed study. The results obtained from other researches could facilitate the interpretation of the polymorphism observed in the studied markers. It is highly probable that heterozygous individuals in D6S2879 marker could be more susceptible to a larger array of pathogens and autoimmune diseases than homozygous individuals. For D6S2806 marker, only one of the alleles could provide the most suitable role in immune response. In fact, it seems that this allele is in the process of being gradually fixed in the Iranian populations. Finally, in view of the unique role of HLA-DRB1 at response to pathogens, and susceptibility to autoimmune diseases, these results could make a novel contribution to the understanding of both the evolutionary history and the genetic diversity of this gene in the Iranian populations.

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اطلاعات جمعیتی مارکرهای D6S2879 و D6S2806 واقع در ناحیه HLA-DRB1 در جمعیت ایرانی: شناسایی اثرهایی از انتخاب طبیعی و جهت‌دار

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چکیده

در این مطالعه، تنوع ژنتیکی و آزمون neutrality برای جایگاه‌های ژنی ریزماهواره MHC، D6S2879 و D6S2806 واقع در ناحیه ژنی HLA-DRB1، بررسی شدند. اطلاعات تعیین ژنوتیپ ۷۳ فرد غیر خویشاوند برای شاخص شانون، تعداد آلل مؤثر مارکرها و آزمون neutrality با استفاده از برنامه‌های PyPop و Popgene32 بررسی شدند. شاخص شانون برای مارکرهای D6S2879 و D6S2806 در جمعیت مطالعه شده به ترتیب ۱/۰۳۷۲ و ۰/۸۶۰۱ بود. ارزش Fnd محاسبه شده برای مارکرهای D6S2879 و D6S2806 نیز به ترتیب ۰/۸۴۴۹- و ۰/۹۹۰۴ تخمین زده شدند. نتایج به دست آمده از آزمون Ewens-Watterson بیانگر آن است که مارکرهای D6S2879 و D6S2806 در جمعیت‌های ایرانی به ترتیب تحت انتخاب طبیعی و جهت‌دار هستند. این اطلاعات پیشنهاد کننده حضور یک فشار انتخابی بر روی ناحیه ژنی HLA-DRB1 در جمعیت‌های ایرانی است.

واژه‌های کلیدی: ژن HLA-DRB1، تنوع ژنی، آزمون Ewens-Watterson، مارکر ریز ماهواره MHC، انتخاب

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