Original Article

The frequency of human leukocyte antigen-DRB1 alleles, using sequence-based genotyping in 68 parents-child trios study in Iranian subjects

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Abstract

Background: The human leukocyte antigen-DRB1 (HLA-DRB1) locus is one of the most polymorphic human loci and has a crucial role in the immune system. Assessing the allelic frequencies of HLA-DRB1 locus would be a fundamental factor in defining the origin of populations, relationships with other populations, disease association studies and the constitution of unrelated bone marrow donor registries. In the current study HLA-DRB1 alleles and their frequencies are determined in a family-based study by DNA sequencing-based typing high-resolution (2 field) level of typing.

Materials and Methods: Genomic DNA from 3 members of 68 unrelated families (a total of 204 individuals) was extracted. Exon 2 of DRB1 gene was amplified and sequenced and allele assignment was performed using Assign™ SBT v4.7sequence analysis software.

Results: We had DRB1*11:04 with frequency of 0.0931, DRB1*03:01 with 0.0882, DRB1*11:01 with 0.0735, DRB1*13:01 with 0.071 and also alleles DRB1*08:03, DRB1*13:42, DRB1*14:04 and DRB1*14:07 with frequency of 0.0024.

Conclusion: A total of 34 different alleles were found in the study subjects with DRB1*11:04, DRB1*03:01, DRB1*11:01 being the most frequent alleles respectively.

Keywords: Genotype, HLA-DRB1, Iran, HLA-typing

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Introduction

Human leukocyte antigen (HLA) genes are located in 6p21.3 region of major histocompatibility complex (MHC) which is the most polymorphic human locus (1-4). The diversity in the HLA region is generated and maintained by mutation, recombination, selection pressure, and genetic drift (5-7). HLA loci encode cell surface molecules which have an important role in presenting the antigens to T lymphocytes, and there is HLA-I receptors on natural killer cells, therefore playing a key role in

differentiating self from non-self and developing immune response (1, 8, 9).

Considering the central role that HLA has in the immune system, HLA typing is vital in matching the donor and acceptor in organ or cell transplantation (4, 8, 10, 11). Furthermore, many studies have demonstrated the association of specific HLA alleles with particular diseases; such studies are providing the basis of genetic risk assessment strategies to determine individual susceptibility to different diseases (1, 4, 10, 12-14).

Global investigations of HLA allele and haplotype frequencies show that there are considerable differences among different populations (3, 8, 11, 15-20). These differences can be used to define the origin of the populations, the relationships of different populations, and also migration patterns of humans from Africa to the other parts of the world (21-25). With regards to the original settling of populations in Iran it is considered that Aryans migrated south from southern Russia about 2000 years B.C. and forked into different branches, of which the proto-Iranian branch is considered to be the origin of the modern Iranians. The genetic diversity currently seen in Iran appears to have emerged through subsequent migration of populations of other ethnicities from neighboring regions. As Iran lies between Asia and Europe, migration from these regions has contributed greatly to Iranian genetic diversity (26-28).

The HLA-DRB1 locus encodes the Beta chain of the DR molecule and is highly polymorphic. As a result, accurate genotyping of this locus has represented a technical challenge. The aim of this study is to type HLA-DRB1 by DNA sequencing-based typing (SBT) of exon 2 and assess its allele frequency in Iranian population. A family-based approach, using parents and child trios, was applied here to facilitate accurate haplotype/allele assignment. All participants were recruited from Alborz hospital, rheumatology clinic. Whilst different methods are used to genotype this DRB1, SBT is considered the gold standard. SBT is able to type the alleles with high resolution and can detect the new alleles that have not been reported previously. This method is highly preferred in comparison to less accurate methods like PCR-SSCP and PCR-RFLP (29, 30). It has been demonstrated that sequencing the exon 2 in HLA-DRB1 is sufficiently able to define a considerable number of alleles in this locus (29, 31).

Methods

Study Subjects

A total of 68 unrelated trios (parents and child) were selected randomly from in house DNA bank (Department of Medical Genetics, Tehran University of Medical Sciences) containing trios with affected child to rheumatoid arthritis (RA) Parents were not affected by the disease (After obtaining informed consent, blood samples were taken from families). Genomic DNA was extracted from peripheral blood samples using a standard phenol-chloroform procedure.

Molecular analysis

Polymerase chain reaction (PCR) was performed to amplify exon 2 of the gene using seven forward primers, each specific for a particular allelic group, and a common reverse primer (32) as Table 1.

A hot-start PCR program was performed to amplify desired fragment. PCR conditions have been shown in Table 2. Primers were added to reactions after 1 minute from initiation of the first step.

The Quality of PCR products were verified with electrophoresis on 1.5% agarose gel. DNA sequencing of the products was carried out bidirectionally by ABI 3700 automated sequencer (Pishgam Biotech Company, Tehran, Iran) using the universal forward primer (M13R-pUC) and the reverse primer used in PCR (Table.1). The analysis of the acquired sequence was done with AssignTM SBT v4.7software (Conexio Genomics, Fremantle, Western Australia) to define the alleles of each individual with high resolution. Comparing the parental and child alleles in trios helped to confidently determine the genotypes (2field).

Statistical analysis

The HLA -DRB1 allele frequency was calculated using the equation; allele frequency = (n/2N) × 100, where n indicates the total counts of a particular allele and N indicates the total number of individuals.

Results

In 204 samples studied, all 408 alleles were typed at high resolution level and a total of 34 different DRB1 alleles were found. The most frequent allele was DRB1*11:04, with a frequency of 0.0931, followed by DRB1*03:01 (0.0882), DRB1*11:01 (0.0735) and DRB1*13:01 (0.071). Figure 1 shows the 34 alleles found in the studied population along with the frequencies of each allele. Frequency for each allele has been provided as a supplementary table.

Discussion

This study showed that the DRB1*11:04 and DRB1*03:01 alleles are the most frequent alleles in Iranian population. This finding is consistent with the previous report by Farjadian et al. (26). Their subjects had different Iranian ethnic background that was also the case in our study. In other words, our samples had Iranian mixed ethnic background.

The investigations on Korean and Japanese populations show that DRB1*09:01 allele is the most common, followed by DRB1*13:02 and DRB1*04:05 respectively (8, 15). In addition, Chinese studies have shown that DRB1*09:01 allele as the most frequent allele in Chinese population (33).

Considering the geographic locations of China, Korea and Japan, it is not unexpected to see the same allele as their most frequent. A similar scenario is seen in Iran and Turkey. These two countries share DRB1*03:01 as one of their frequent alleles (34). Another interesting example of sharing frequent alleles in neighboring populations/countries is seen among German, French and polish populations. In Germany DRB1*15:01 and DRB1*07:01 are the most frequent alleles (16). In France the most common alleles are DRB1*03:01 and DRB1*07:01 (35). On the other hand, in Poland the most common alleles are DRB1*03:01 and DRB1*15:01 (17). German population share one of its frequent alleles with one neighbor (French population) and the other one with another neighbor (Poland) on the other side of Germany. Similarities or dissimilarities in allele frequencies among different populations may reflect

Table 1: PCR primers. Underlined component: complementary to target alleles. Bold: to link first part to the third component (Italic; a site for universal sequencing primer, -21M13) and to guarantee that start site in sequencing reaction would be same for each allele group. "S" stands for G/C, "R" for A/G, "Y' for C/T and "K" for G/T.

Primer name	Allelic groups	Sequence of the primer
DRB1-01	DRB1*01	5' -*CAggAAACAgCTATgACC***TgAgAC**gCACgTTTCTTgTggSAgCTTAAgTT-3'
DRB1-04	DRB1*04	5' -*CAggAAACAgCTATgACC***TgAgAC**gCACgTTTCTTggAgCAggTTAAAC-3'
DRB1-07	DRB1*07	5' -*CAggAAACAgCTATgACC***TgAgACT**CACgTTTCCTgTggCAgggTAARTATA-3'
DRB1-09	DRB1*09	5' -*CAggAAACAgCTATgACC***TgAC**CAgCACgTTTCTTgAAgCAggATAAgTT-3'
DRB1-10	DRB1*10	5' -*CAggAAACAgCTATgACC***TgA**AgACCACgTTTCTTggAggAgg-3'
DRB1-15	DRB1*15, 16	5' -*CAggAAACAgCTATgACC***TgAgACT**CACgTTTCCTgTggCAgCCTAAgA-3'
DRB1-52	DRB1*03,11,12,13,14,08	5' -*CAggAAACAgCTATgAC*CCCCACAgCACgTTTCTTggAgTACYCTA-3'
DRB1-R	All alleles	5' -*TgTAAAACgACggCCAg*TgCTYACCTCgCCKCTgCAC-3'

Table 2: Hot-start PCR conditions.

First step	80 °C for 2.5 min
Second step	95 °C for 5 min
Third step	30 cycles (each cycle: 95 °C for 20 Sec, 62 °C for 10 Sec, 72 °C for 30 Sec)
Fourth step	72 °C for 5 min

Table 3: Comparing allele frequencies obtained in two studies in Iran.

Allele	**Frequencies**		**Number of alleses**	
	Present study	**Farjadian's study**	**Present study**	**Farjadian's study**
*11:04	0.0931	0.174	38	**284**
*03:01	0.0882	0.125	36	**204**
*11:01	0.0735	0.061	30	**100**
*13:01	0.071	0.062	29	**101**
*10:01	0.0661	0.013	27	**21**
*04:05	0.0612	0.004	25	**7**
*15:01	0.0588	0.051	24	**83**
*15:02	0.0563	0.050	23	**82**
*13:02	0.0416	0.023	17	**38**

the degree of racial/ethnical relationships between them.

Another issue that should be explored here is the possible effect of study design in the final results and weigh this against the advantages and disadvantages of different designs. Instead of a routine population-based study to obtain allele frequencies, we chose to use parents-child trio studies. The main benefit of such approach was the ability of obtaining genotypes with a much higher accuracy. Considering the difficult genotyping of HLA-DRB1, we found this approach very beneficial; helping for haplotype analysis and also avoiding population stratification error, give some advantages to trios study rather than case-control population-based studies.

On the other hand, our result was consistent with the previous report, obtained from population-based study, at least for common alleles. Although frequencies of some alleles in present study and Farjadian et al. study were very different, common alleles are the same in both studies (Table 3). This finding shows that trio approach not only overcomes many difficulties in HLA-typing but also results in to determine types of alleles and also common alleles present in the gene pool of the population.

Another issue that is important enough to be looked at, is having affected child with rheumatoid arthritis (RA) in the studied trios. This might have an effect on allele frequencies in studied group and creates different results to general population. But having the advantage of parental samples was a very important bonus to the study that we taught it worth to tolerate this issue as the shortage of the study. On the other hand it was very interesting that despite this shortage, our result is consistent with the previous reports presented above. Therefore, it is not reasonable to assume having samples from family with affected child with RA did not affect too much the allele frequencies in studies group.

Table 4: The report of DRB1 frequency studies in different populations

Population	Sample size	Total of different DRB1 alleles	Most common alleles	Allele frequency	Reference
Americans	558	42	*07:01	0.12455	**(36)**
			*15:01	0.11022	
Chinese	718	40	*09:01	0.1469	**(33)**
			*15:01	0.124	
French	42636	76	*03:01	0.0447	**(35)**
			*07:01	0.0283	
Japanese	371	27	*09:01	0.124	**(15)**
			*04:05	0.115	
Korean	485	31	*09:01	.00918	**(8)**
			*13:02	0.0866	
Macedonian	158	29	*16:01	0.149	**(37)**
			*11:04	0.139	
Iranian	816	41	*11:04/03	0.174	**(26)**
			*03:01	0.125	
Han population of China	618	122	*09:01	0.1715	**(10)**
			*07:01	0.0947	
Taiwanese	46915	53	*11:01	0.118	**(38)**
			*14:54	0.1159	
Polish	20653		*03:01	0.0602	**(17)**
			*15:01	0.0259	
Kazakhstan	157	37	*07:01	0.131	**(18)**
			*03:01	0.100	
German	8862	68	*15:01	0.1452	**(19)**
			*07:01	0.1213	
Turkey	250	36	*11:01	0.104	**(34)**
			*03:01	0.092	

The differences of DRB1 allele frequencies between distinct populations (Table 4), may partly explain the reason why they show different prevalence of specific diseases such as those affecting the immune system like autoimmune diseases.

Conflicts of Interest

There is no conflict of interest.

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