Polymorphism in Exon 2 of CD1 Genes in Southwest of Iran

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Abstract

Background: The CD1 family is less variable transmembrane antigen presenting molecules related to the MHC molecules. CD1a and CD1e genes are the most polymorphic ones associated with autoimmune diseases. The aim was to better clarify the map of CD1 genes in Southwest Iranian normal population for implications in vaccine design.
Methods: In this study we investigated the polymorphism of CD1a, CD1d and CD1e in 311 healthy individuals from Fars Province in Southwest of Iran by PCR-SSP method.
Results: Six of individuals had homozygote CD1a 01/01 genotype and 248 had homozygote CD1a 02/02 genotype. CD1d was found to be monomorphic with all tested individuals showing CD1d 01/01 genotype. Hundred and eleven individuals had homozygote CD1e 01/01 genotype and 48 had homozygote CD1e 02/02 genotype. The frequencies of CD1a 01 and CD1a 02 alleles were 11% and 89% while the frequencies of CD1e 01 and CD1e 02 alleles were 60.1% and 39.9%, respectively. Consistent with previous reports on other genes, a high degree of similarity in CD1a and CD1e allelic distribution was observed between Southwest Iranians and other Indo-European populations. However, the allelic frequency of the CD1a and CD1e alleles showed a significant difference from those of Chinese Han and She populations.
Conclusion: These data are notable in the light of relatively recent genetic admixture along the Silk Road. Considering the significance of CD1 alleles in some autoimmune and infectious diseases and with the admixed nature of Iranian population, mapping the distribution of CD1e alleles in different regions of Iran can be useful in future designing of preventive and therapeutic vaccines.

Keywords: CD1, Normal population, PCR-SSP, Iran

Introduction

CD1 genes, located on human chromosome 1, comprise a less polymorphic lineage of antigen presenting molecules related to MHC class I glycoproteins (1, 2). The genes encoding the five isoforms of CD1 molecule, namely CD1a, CD1b, CD1c, CD1d and CD1e, are believed to have emerged as a result of MHC genes duplication before the divergence of birds and mammals in some 300 million years ago (3-5). Further translocation of the CD1 genes in mammals around 180 million years ago seems to have resulted in the translocation of CD1 locus from its ancestral position in the MHC locus on chromosome 6 (6-8). CD1 genes continued to expand and diverge among eutherians by virtue of duplication and diversification. This idea is supported by different types and copies of CD1 isoforms with a considerable degree of sequence homology in higher mammalian species such as rat, mice, cattle and human (9-11). The functional importance of CD1 isoforms and their relative contribution to the immune response

has been under investigation during the past two decades (12-17). While CD1 molecules are all considered as self and non-self lipid antigen presenting molecules, it is now more evident that CD1d and CD1c are directly involved in presentation of pathogens' lipids to NKT and T cells (2, 18, 19). Therefore, this ancient locus which presents limited variation could have been under some degree of selection pressure by infectious pathogens. CD1d, the most conserved molecule of all CD1 isoforms, has been saved from deletions that affected other CD1 family members in rat and mice (6, 9, 15). Limited allelic variation exists in CD1d gene compared to CD1a with two known polymorphisms at positions 13 and 51 and CD1e with 6 known alleles (20-23). The limited diversity of CD1 genes, even for the most polymorphic gene, i.e. CD1e, points to the critical role of these lipid presenting molecules in the defense against pathogens. In this regard, accumulating evidence imply the possible role of CD1-restricted T cells in infectious, inflammatory, autoimmune, and malignant disorders (2). Accordingly, the role of CD1 molecules in pathogenesis of multiple sclerosis (MS), rheumatoid arthritis (RA), atherosclerosis as well as human malignancies has been reported (2, 24-27). In addition, while an association between CD1a and CD1e alleles with multiple sclerosis is suggested (28), the association of these CD1 alleles with other neuropathies remains controversial (29-31). Despite the considerable conservation of CD1 genes, a high degree of difference in the distribution of CD1 alleles in different populations is reported (28, 32-34). In a previous study we did not observe any difference between CD1a and CD1d alleles in cancer patients compared to their healthy sex-matched controls (34). In our data, we found a dominant frequency of CD1D*01 allele similar to other populations but a somewhat different frequency of CD1a alleles from South-western part of Iran compared to the other Asian populations.

In a curious attempt to better clarify the map of CD1 genes in our population, we studied the frequency of CD1a, CD1d and CD1e alleles in a group of randomly recruited healthy blood donors.

Materials and Methods

Subjects

In a cross-sectional study from 2008-2010, 311 healthy blood donors including 194 men and 117 women (mean age 46 ± 10; range= 17-65 years) from Fars Province in Southwest of Iran were chosen randomly. All of these individuals had no history of autoimmune diseases, diabetes, malignancies or any familial diseases among their relatives.

DNA Extraction

Approximately 10 ml of fresh PBMCs were taken in EDTA 1%. Genomic DNA was extracted using salting out method (35). DNA concentration was measured by specterophotometeric analysis and optimal DNA concentration (0.3 ng/ml) was prepared for PCR reaction.

PCR Reaction

Using PCR-SSP method, three SNPs in CD1a (622 T/C), CD1d (354 A/T) and CD1e (6129 A/G) genes were amplified. For each sample, the PCR reaction was carried out in two separate tubes each containing one specific primer and a common primer which was common in both tubes. The primer sequences for each gene are depicted in Table 1. Two forward and reverse primers of β-globin gene were used as the internal control.

Table 1: The primer sequences used for amplification of CD1a, d, e and β -globin genes

Genes	Primer sequences
CD1A (T622C)	F Primer: CCTCTCTCCTTCCATGTCAT R Primer: CCTCTCTCCTTCCATGTCAC Common primer: TTCAAACTGCAATT-CATGGGC
CD1D (A354T)	F Primer: GCTTCAGAGAGCGGACGGA R Primer: GCTTCAGAGAGCGGACGGT Common primer: TGAAGTCCCGCAAAGGCTTT
CD1E (A6129G)	F Primer: CCAGTTA-TACTTCCTAGTTTTATCCA R Primer: CCAGTTA-TACTTCCTAGTTTTATCCG Common primer: GTGTATATGGTGGAGTGTGGG
β-glubin	F primer: ACACAACTGTGTTCACTAGC R primer: CAACTTCATCCACGT TCA CC

PCR Condition

The PCR reaction was performed in a total volume of 25 μl including 13.5 μL DW, 2.5 μl of PCR buffer 10X, 0.75 μl MgCl$_2$ (50 mM), 0.75 μl dNTP (10 mM), 1 μl of each primer (20 pm), 1 μL of each internal control primers, 1 μl of genomic DNA (0.3 ng/ml), 0.5 μl Tween-20 and 2 μl Taq polymerase (1U). The PCR program was a touch-down method and included an initial denaturation at 94°C for 3 min followed by three loops; loop one included 5 cycles of 94°C for 25 sec, 70°C for 60 sec and 72°C for 60 sec, loop 2 included 21 cycles of 94°C for 25 sec, 64°C for 70 sec and 72 °C for 70 sec and loop 3 consisted 94°C for 25 sec, 55°C for 60 sec and 72°C for 120 sec and a final extension at 72°C for 5 min for CD1a gene and an initial denaturation at 94°C for 6 min followed by three loops; loop one included a 5 cycles of 94°C for 30 sec, 70°C for 90 sec and 72°C for 60 sec, loop 2 included 26 cycles of 94°C for 30 sec, 63.5°C for 35 sec and 72°C for 60 sec and loop 3 consisted of 94°C for 30 sec, 55°C for 50 sec and 72°C for 60 sec and a final extension at 72°C for 5 min for CD1d and CD1e genes. Then, 8 μl of each PCR product was run in ethidium bromide-stained agarose gel and visualized under UV light.

Statistical Analysis

Data were analyzed by SPSS 11.5 and Epi-info 2002 software. The Hardy-Weinberg equilibrium was checked using chi-square *t*-test. *P*-values less than 0.05 were considered significant.

Results

The electrophoresis results of the PCR products of CD1a (255 bp) and CD1e (241 bp) are shown in Fig. 1. The frequency of CD1a 01 and CD1a 02 alleles were 11% and 89% while the frequency of CD1e 01 and CD1e 02 alleles were 60.1% and 39.9%, respectively. Table 2 illustrates the genotype and allele frequencies of CD1a, CD1d and CD1e genes in the current study.

Table 2: Genotype and allele frequency of CD1a, d and e genes in Iranian normal population

Genes	Genotypes	Frequency	Alleles	Frequency
CD1a	01/01	6 (1.9%)	01	69 (11%)
	01/02	57 (18.3%)	02	553 (89 %)
	02/02	248 (79.8%)		
CD1d	01/01	311 (100%)	01	622 (100%)
	01/02	0 (0%)	02	0 (0%)
	02/02	0 (0%)		
CD1e	01/01	111 (35.7%)	01	374 (60.1%)
	01/02	152 (48.9%)	02	248 (39.9%)
	02/02	48 (15.4%)		
Total		311 (100%)		

Table 3: Comparison of CD1a, d and e allele frequency in different populations

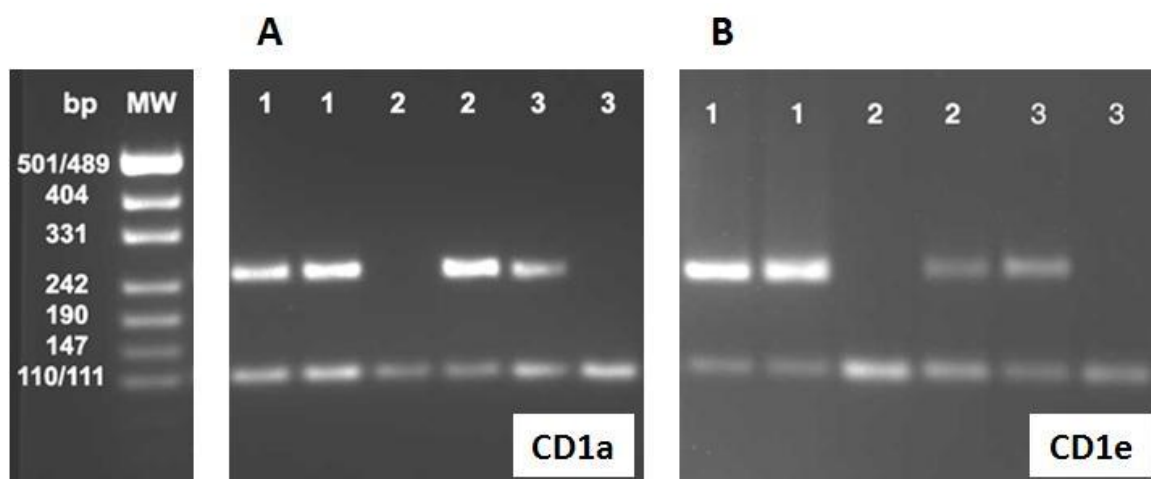| Alleles	Frequency in our population (n=311)	Italian population (n=100)(36)	Italian population (n=132)(30)	North American diverse ethnic group (n=110)(20)	Chinese Han (n=160) and She (n=260) population (51)	Dutch population (n=212)(31)	UK population (n=342)(38)	Italian Abbruzzo population (n=114)(37)
CD1A01	11%	11%	11%	13%	0%	6.4%	5%	1%
CD1A02	89%	89%	89%	87%	100%	93.6%	95%	99%
P-value		1	1	0.6	-	0.2	0.1	*0.002
CD1D01	100%	100%	-	99%	100%	-	100%	-
CD1D02	0%	0%	-	1%	0%	-	0%	-
P-value								
CD1E01	60.1%	61%	62%	49%	38%	66.5%	67%	74%
CD1E02	39.9%	39%	38%	51%	62%	33.5%	33%	26%
P-value		0.9	0.8	0.1	*0.002	0.3	0.3	*0.03

Fig.1: Gel electrophoresis of PCR products of CD1a and CD1e alleles. A. CD1a alleles amplification: The result of amplification for Isoleucine/Threonine (CT) heterozygote (lanes 1 and 1), Threonine (37) homozygote (lanes 2 and 2) genotypes and Isoleucine (TT) homozygote (lanes 3 and 3) are indicated. The reactions in both lanes 1 (Isoleucine/Threonine) indicate that the first individual is heterozygote, while lack of reaction in the first lanes 2 (Isoleucine) and the existence of PCR-amplified Threonine band in the second lane indicate that individual 2 is Threonine homozygote. The band in the first lane 3 (Isoleucine) and lack of reaction in the second lane 3 indicates that individual 3 is Isoleucine homozygote. B. CD1e alleles amplification: The result of amplification for Glutamine/Arginine heterozygote (lanes 1 and 1), Glutamine (52) homozygote (lanes 2 and 2) genotypes and Arginine (GG) homozygote (lanes 3 and 3) are indicated. The reactions in both lanes 1 (Glutamine/Arginine) indicate that the first individual is heterozygote, while lack of reaction in the first lanes 2 (Arginine) and the existence of PCR-amplified Glutamine band in the second lane indicate that individual 2 is Glutamine homozygote. The band in the first lane 3 (Arginine) and lack of reaction in the second lane 3 indicates that individual 3 is Arginine homozygote.

A comparison of the CD1 allele frequencies between different populations is shown in Table 3. As indicated, the frequencies of CD1a and CD1e alleles were significantly different from that of Chinese Han (P=0.002) and Italian Abbruzzo (P=0.03) populations (33, 37). It is worth mentioning that all the selected individuals were in Hardy-Weinberg equilibrium for the three polymorphisms.

Discussion

In present study, we investigated the polymorphisms in CD1a, CD1d and CD1e genes in 311 healthy individuals from Southwest of Iran. The results on CD1a and CD1d were very similar to the frequencies we obtained from another group of healthy individuals who were recruited and selected based on specific criteria in a previous study (34). Investigation of CD1e gene polymorphism in the current study revealed that the allelic frequency of these genes in our population was similar to some Italian, Dutch and British normal populations but far from that of Chinese Han population (P= 0.002, Table 3) (20, 30, 31, 33, 36, 37). The frequency of CD1a alleles in our population and in some Italian, Dutch and British populations were also found to be similar (Table 3) (31, 37, 38). Thus, it seems that in addition to CD1a, the CD1e alleles are also distributed very closely in our population and Italian population except Italian Abbruzzo and Chinese Han and She populations (Table 3) (37). These results are in accordance with the previous studies which showed a high degree of genetic relation between Southwestern Iranians and Italian population (39-41).

Our findings also indicate that the CD1a and CD1e gene polymorphisms in exon 2 are largely different among various ethnic groups in Asia. Current Iranian population is living in one of the oldest areas inhabited by modern humans (*Homo sapiens sapiens*). Despite the various ethnic groups and multiple languages spoken in Iran, investigation of the genetic structure of Iranian population is quite recent. The general consensus is that the Aryan tribes who first inhabited the South-western of Iran made a huge contribution to the genetic pool of current Iranians (42). However, a degree of genetic admixture has happened over the centuries of population movements and migrations from eastern and western neighboring lands (43). As such, the war and trade-related population movements along the Silk Road may have increased the diversity of the genetic pool in Iran (44-46). Previous studies have shown that despite this relatively recent genetic admixture, the footprints of Indo-Aryan alleles and mitochondrial DNA markers are still well preserved among Iranians (39, 40, 42). The extent of this preservation, however, varies in different areas of Iran owing to the extent of admixture with different populations and also inside-ethnicity breeding (42, 47).

It is suggested that the extent of genetic admixture can be studied by alleles that show a high frequency difference in different populations (48, 49). The high level of difference in allelic frequency allows scientists to estimate the extent of contribution of ancestral alleles in the genetic pool of an admixed population. This is especially important in the view of the associations between some alleles and diseases. Two recent reports with relatively large sample sizes from Iran and Switzerland point to the association of CD1a and CD1e alleles with susceptibility to multiple sclerosis (MS) (28, 50). Therefore, considering the relatively high difference in the frequency of CD1e and CD1a in our population and those from eastern Asian populations, investigation of other predisposing genes and environmental factors in Iranian and other Asian MS patients could be interesting (28, 33, 34). Moreover, given the importance of CD1 alleles in autoimmune diseases and neuropathies, tracing CD1a and CD1e alleles in admixed populations can be used in preventive and therapeutic planning, especially in designing efficient lipid and glycolipid vaccines. In this regard, the polymorphism of CD1 gene in other ethnic groups along with the functional consequences of such genetic variation should also be taken into account.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc) have been completely observed by the authors.

Acknowledgement

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