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LMP2 and *LMP7* Gene Polymorphisms in the Southeastern Anatolia Population of Turkey

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ABSTRACT The low molecular weight polypeptide 2 (*LMP2*) and low molecular weight polypeptide 7 (*LMP7*) genes are located in the class II region of the Major Histocompatability Complex (MHC) locus on chromosome 6. These genes encode peptides forming the large components of the proteosome complex which degrades short-lived cytoplasmic proteins. Analysis of the spectrum of the peptides generated, transported and presented; disease association studies and population genetic studies are important for understanding the functional effects of genetic variations in *LMP* genes. In this study, *LMP2* and *LMP7* gene polymorphisms were analyzed in Southeastern Anatolia population of Turkey. A total of 110 healthy and unrelated individuals participated in this study. Polymorphism analyses were done by Polymerase Chain Reaction (PCR) – Restriction Fragment Length Polymorphism (RFLP) method and allele/genotype frequencies of *LMP2* and *LMP7* genes were determined. A deviation from the Hardy-Weinberg equilibrium ($p<0.05$) was found for the genotype distribution of *LMP2* gene polymorphism, while the *LMP7* genotypes found to be distributed in accordance with the Hardy-Weinberg equilibrium ($p>0.05$). Available allele frequency data for different populations were used to calculate genetic distances and to construct a neighbor-joining tree. Among the included populations, Nahuas (Mexico) population was found to have the lowest genetic distance from the Southeastern Anatolia-Turkey population.

INTRODUCTION

Antigen processing machinery restricted to the major histocompatibility complex (MHC) class-I is responsible for the presentation of intracellular antigens to cytotoxic T lymphocytes. The efficiency of peptide-Human Leukocyte Antigen (HLA) complex presentation on the cell surface depends on the production and processing of the peptide. Proteasomes and immunoproteasomes are responsible for the processing of intracellular proteins into peptides, while selection and transport is carried out by the heterodimeric transporter associated with antigen processing (*TAP*) (Basler et al. 2013). The 20S proteasome is a 700-kDa, barrel-shaped protease composed of 28 subunits arranged as four axially stacked rings. Among these subunits, low molecular weight polypeptide 2 (*LMP2*; *PSMB9*) and low molecular weight polypeptide 7 (*LMP7*; *PSMB8*) are catalytic subunits which can be induced with interferon γ resulting in distinct subunit composition and altered catalytic characteristics, called the "immunoproteasome" (Griffin et al. 1998).

TAP and *LMP* genes are localized in MHC class II region with an essential role in antigen presentation by class I MHC molecules. Although these genes are less polymorphic than other MHC genes, *LMP2*, *LMP7*, *TAP1* and *TAP2* gene polymorphisms may have significant implications on the analysis of the spectrum of peptides generated, transported and presented; disease association studies and population genetic studies (Rueda Faucz et al. 2000). It is generally assumed that the function of antigen processing and transport pathway might be influenced by structural differences encoded by *TAP* and *LMP* alleles and immunoproteosome improves quality and quantity of generated class-I ligands.

Due to the significant role of *LMP* products in antigen presentation, these genes can be accepted as strong candidates of susceptibility factors for different diseases. Polymorphisms in the *LMP2* and *LMP7* genes have been reported and the modifying effects of these polymorphisms have been investigated in many disorders; such as, hypersensitivity pneumonitis (Camarena et al. 2010), colorectal carcinoma (Fellerhoff et al. 2011), tuberculosis (Wang et al. 2012), hepatitis C (Sugimoto et al. 2002),

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Alzheimer (Mishto et al. 2006), autoimmune thyroid disease (Ding et al. 2002), spondyloarthritis (Vargas-Alarcon et al. 2004).

Functional studies concerning *LMP2* indicated that the highest chymotrypsin-like activity of proteasome was observed in heterozygotes (Arg/His) and lowest in homozygotes His/His, so it was stated that *LMP2* allelic polymorphism impact peptidase activity of immunoproteasome (Dosenko et al. 2005).

Population genetic studies can also contribute to understanding of the possible role of *LMP* gene polymorphisms. However population studies of *LMP* gene polymorphisms are very rare and limited to mainly Brazilian and Mexican Amerindians and Caucasoids (Rueda-Faucz et al. 2000; Vargas-Alarcon et al. 2002). Turkish populations have not been investigated so far. Anatolia has been occupied by modern humans in lower Paleolithic times and it has experienced numerous human migrations throughout the history (Kuhn 2002). Southeastern Anatolia region covers 9.7 percent of lands in Turkey and it is localized in so called "Upper Mesopotamia". It is in between Eastern Anatolia and Mediterranean Regions and has a border with Syria and Iraq.

The aim of this study was to determine the allele and genotype frequencies of the *LMP2* and *LMP7* gene polymorphisms in Southeastern Anatolia population and to compare these with the frequencies in other populations previously reported.

MATERIAL AND METHODS

Study Population

Blood samples from 110 unrelated, healthy volunteers representing the Southeastern Anatolian population were obtained with their written consent. The individuals were unrelated, they had different surnames and they defined themselves as belonging to a lineage residing in the area from at least three generations. Samples were collected in all provinces of the region; Gaziantep, Kilis, Adýyaman, Mardin, Diyarbakýr, Sanliurfa, Batman, Siirt and Sirnak.. The subjects were from the urban areas and between 17 and 58 years of age with the male/female ratio of 1:1.2. Blood samples (10 ml) were collected into ethylenediaminetetraacetic acid (EDTA) tubes. Genomic deoxyribonucleic acid (DNA) was isolated using a standard salting-out procedure (Miller et al. 1988). DNA was stored in Tris EDTA buffer at -80ºC until use.

Genotyping

PCR-RFLP method was used to analyze *LMP2* and *LMP7* polymorphisms. PCR primers and restriction enzymes used for these experiments are as previously described (Sugimoto et al. 2002). Genomic DNA samples (1 μg) were amplified in 25 μl reaction mixtures containing 1 μmol/l of each primer, 200 μM dNTPs, 1xTaq DNA polymerase buffer, 1.5 mmol/l $MgCl_2$ and 0.5 units of Taq DNA polymerase (Fermentas, Lithuania) using a thermal cycler (Takara PCR Thermal Cycler Dice, Otsu, Shiga, Japan). Reaction conditions were as follows: 95ºC for 5 min, 35 cycles of 95ºC for 30 seconds, 53ºC for 30 seconds, 72ºC for 30 seconds and a final extension at 72ºC for 5 min. Reaction products were electrophoresed on a 2% agarose gel containing ethidium bromide for visualization.

The amplified fragments were digested with appropriate restriction enzymes (4-4.5 h) and digestion products were separated on a 3% agarose gel.

Statistical Analysis

Allele and genotype frequencies were calculated by direct counting and Hardy-Weinberg equilibrium was tested by chi-square test. By using previously reported allele frequencies of other populations, a phylogenetic tree was constructed by the neighbor-joining method (Saitou and Nei 1987) with standard genetic distances using DISPAN software containing the programs GNKDST and TREEVIEW (Ota 1993).

RESULTS

Genotype and allele frequencies of *LMP2* and *LMP7* genes are given in Table 1. The *LMP2* genotype frequencies were found as 44% for R/R, 33% for R/H and 33% for H/H. *LMP2* - R allele frequency was found to be 0.55 while the frequency was 0.45 for H allele. A deviation from the Hardy-Weinberg equilibrium in Southeastern Anatolia population was found for *LMP2* genotypes (χ^2=17.97, p<0.001). On the other hand, genotype frequencies of *LMP7* gene were calculated as 97% for Q/Q, 13% for Q/K

and 0% for K/K. Allele frequencies of *LMP7* gene polymorphism were determined as 0.94 for Q allele and 0.06 for K allele. Observed and expected genotype frequencies were compared by chi-square test and it was noticed that genotypes for *LMP7* gene polymorphism distributed according to the Hardy-Weinberg equilibrium in the population (χ^2=0.43, p>0.5) (Table 1). The differences of the allele frequencies between different demographic groups were not evaluated due to the small population size.

Table 1: Genotype and allele frequencies of *LMP2* and *LMP7* gene polymorphisms in Southeastern Anatolia-Turkey population

Polymorphism	*n=110*	*%*	*÷²*	*P*
LMP2-60				
Genotypes				
R/R	44	40	17.97*	0.000
R/H	33	30		
H/H	33	30		
Alleles				
R	121	0.55		
H	99	0.45		
LMP7-145				
Genotypes				
Q/Q	97	88	0.43	0.81
Q/K	13	12		
K/K	0	0		
Alleles				
Q	207	0.94		
K	13	0.06		

In order to compare the *LMP2* and *LMP7* allele frequencies among populations, previously reported data were used (Table 2). It can be seen from the Table 2 that the Southeastern Anatolia population has the highest frequency for *LMP2*-H allele as 0.45 while the lowest H allele frequency is seen in Guarani population (0.011).

In case of *LMP7* gene, the Mazetecans (Mexico) has the highest frequency for *LMP7*-Q allele (0.942) which is actually very close to that of Southeastern Anatolia population (0.94). On the other hand, the lowest frequency of *LMP7*-Q allele is seen in Caucasian (USA) population (0.444).

From the allele frequencies of *LMP2* and *LMP7* genes, genetic distances between populations were determined (Table 3). It is clearly seen that genetic distances between Southeastern Anatolia population and Mexican populations are very low. In fact, the lowest genetic distance was calculated between Southeastern Anatolia population and Nahuas as 0.0059. Among the analyzed populations, Caucasian (USA) population has a highest genetic distance (0.2483) to Southeastern population followed by Kaingang (Brazil) population (0.2346).

By using the genetic distances between populations, a neighbor-joining tree was constructed (Fig. 1). It also clearly indicates the closeness of Mexican populations to Southeastern Anatolia population (Nahuas as the closest population).

The average heterozygosity of the populations according to the *LMP2* and *LMP7* gene polymorphisms were also calculated (Table 4). Average heterozygosity level ranged between 0.11 (Guarani-Brazil) and 0.44 (Caucasian-USA), where Anatolian population has an average heterozygosity level of 0.30. The Gst value for all loci was calculated as 0.12 while Ht was 0.35 and Hs was 0.31. Gst, Ht and Hs are the values representing the level of differentiation between populations.

DISCUSSION

Since *LMP* gene products have an important role in the antigen presentation, they can be accepted as candidate genes for susceptibility to many disorders. There have been many dis-

Table 2: Allele frequencies of *LMP2* and *LMP7* gene polymorphisms in different populations

Populations	*n*	*LMP2-H*	*LMP2-R*	*n*	*LMP7-Q*	*LMP7-K*	*Reference*
Mestizos (Mexico)	190	0.269	0.731	190	0.894	0.106	Vargas- Alarcón et al. 2002
Nahuas (Mexico)	96	0.344	0.656	96	0.916	0.084	Vargas- Alarcón et al. 2002
Mazetecans (Mexico)	112	0.232	0.767	52	0.942	0.058	Vargas- Alarcón et al. 2002
Teenek (Mexico)	100	0.28	0.72	100	0.91	0.09	Vargas- Alarcón et al. 2002
Mayos (Mexico)	126	0.349	0.651	126	0.873	0.127	Vargas- Alarcón et al. 2002
Guarani (Brazil)	184	0.011	0.989	93	0.892	0.108	Rueda-Faucz et al. 2000
Kaingang (Brazil)	478	0.195	0.805	241	0.485	0.515	Rueda-Faucz et al. 2000
Caucasian (USA)	372	0.26	0.74	372	0.444	0.556	Deng et al. 1995
Asian (Japan)	106	0.25	0.75	90	0.612	0.388	Kawaguchi et al. 1994
Caucasian (Spain)	204	0.216	0.784	102	0.883	0.117	Vinasco et al. 1998
Southeastern Anatolia (Turkey)	220	0.45	0.55	220	0.94	0.06	Present study

Table 3: The genetic distances between Southeastern Anatolia population and other populations calculated by using *LMP2* and *LMP7* allele frequencies

	Mestizos (Mexico)	*Nahuas (Mexico)*	*Mazete-cans (Mexico)*	*Teenek (Mexico)*	*Mayos (Mexico)*	*Guarani (Brazil)*	*Kaing-ang (Brazil)*	*Cauca-sian (USA)*	*Asian (Japan)*	*Cauca-sian (Spain)*
Nahuas(Mexico)	0.0009									
Mazetecans(Mexico)	-0.0011	0.0038								
Teenek(Mexico)	-0.0028	-0.0015	-0.0021							
Mayos(Mexico)	0.0013	-0.0033	0.0064	-0.0004						
Guarani(Brazil)	0.0344	0.0625	0.0266	0.0379	0.0631					
Kaingang(Brazil)	0.1366	0.1694	0.1573	0.1476	0.1452	0.1224				
Caucasian (USA)	0.1649	0.1924	0.1926	0.1762	0.1643	0.1712	0.0030			
Asian (Japan)	0.0550	0.0733	0.0702	0.0612	0.0578	0.0698	0.0130	0.0214		
Caucasian (Spain)	-0.0001	0.0089	-0.0005	0.0004	0.0088	0.0198	0.1213	0.1534	0.0485	
Southeastern Anatolia (Turkey)	0.0243	0.0059	0.0304	0.0194	0.0082	0.1250	0.2346	0.2483	0.1169	0.0411

Table 4: The average heterozygosity level of the populations according to the *LMP2* and *LMP7* genes

Population		*Average heterozygosity*	*Standard deviation*
Mestizos (Mexico)		0.29	0.10
Nahuas (Mexico)		0.30	0.15
Mazetecans (Mexico)		0.24	0.12
Teenek (Mexico)		0.28	0.12
Mayos (Mexico)		0.34	0.11
Guarani (Brazil)		0.11	0.08
Kaingang (Brazil)		0.41	0.09
Caucasian (USA)		0.44	0.05
Asian (Japan)		0.43	0.05
Caucasian (Spain)		0.27	0.07
Southeastern Anatolia (Turkey)		0.30	0.19
All loci	Gst = 0.12	Ht = 0.35	Hs = 0.31

ease association studies focused on *LMP2* and *LMP7* genes. However, several reports show lack of association or state that the association might be secondary to linkage disequilibrium with HLA genes primarily related to the disease (Kobayashi et al. 1998; Undilien et al. 1997). The actual disease-conferring alleles might be determined if the confounding effects of linkage disequilibrium can be removed by increasing the number of disease association studies in ethnic groups. Population genetic studies can be helpful to understand the role of *LMP* genes as genetic markers but they are actually very rare. In the present study, *LMP2* and *LMP7* gene polymorphisms were investigated in Southeast-

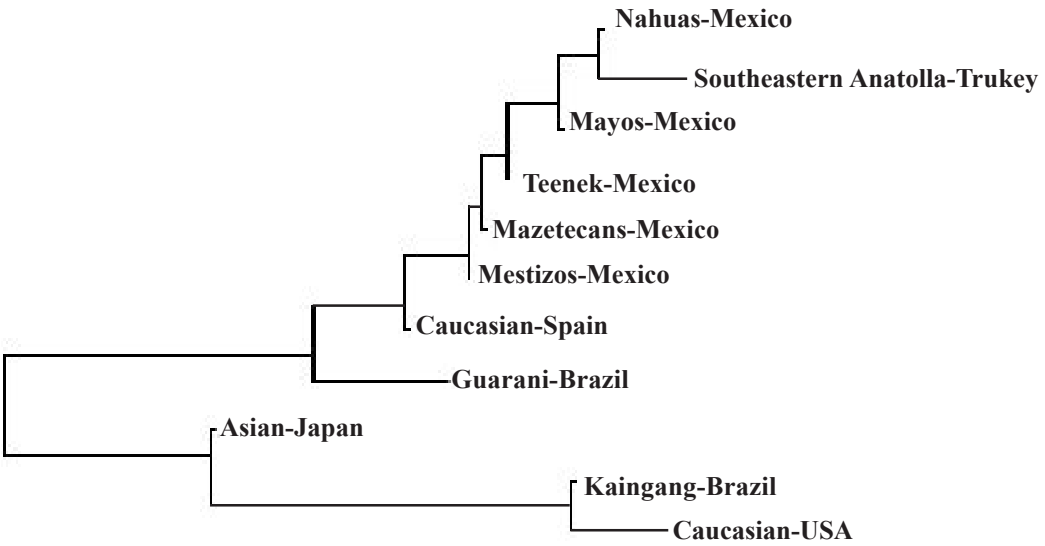

Fig.1. Neighbor-joining tree constructed by using the genetic distance between Southeastern Anatolia population and the other populations

ern Anatolia population. Anatolia was populated by various civilizations, such as Hattians, the Hurries, the Hittites, the Phrygians, Sassanids, the Byzantines, the Seljuk Turks and the Ottomans (Tambets et al. 2000). It acted as a bridge between the West and the East, so it has been subject to migrations from multiple regions throughout time. Southeastern Anatolia Region is found in Upper Mesopotamia which is widely known as the cradle of civilization.

Previous studies using different molecular markers such as proteins (Brega et al. 1998), mitochondrial DNA (mtDNA) (Calafell et al. 1996; Comas et al. 1996, 1998), and Y-chromosomal markers (Wells et al. 2001; Cinnioglu et al. 2004) have shown that Anatolian populations are more closely related to European populations than Central Asian populations. So, this suggests that migrations of Central Asian populations to Anatolia had a little genetic effect on the present day Turkish gene pool.

HLA alleles and haplotypes were also used to investigate the genetic relatedness of Turkish population to other populations (Arnaiz – Villena et al. 2001; Uyar et al. 2004). In addition, *TAP1* and *TAP2* gene polymorphisms were studied in the Anatolian population (Ozbas-Gerceker and Ozguc 2003) and it was stated that the Anatolian population is genetically close to European populations. *TAP1* and *TAP2* genes are localized in MHC class II region together with the *LMP2* and *LMP7* genes but there is no data in the literature on the *LMP2* and *LMP7* genes in the Anatolian population. This was the first study analyzing the polymorphisms of these genes in Southeastern Anatolia population. Since there is not much population data for *LMP2* and *LMP7* gene polymorphisms, neighboring populations and all European populations could not be included to this study. Data from Mexican populations (Mestizos and Amerindians) and Brazilian Amerindians and Caucasoids were used to investigate the genetic relatedness to Southeastern Anatolia.

When *LMP7* gene is considered it is clearly seen that the frequency of *LMP7*-Q allele in Southeastern Anatolia population is very close to that of the Mexican populations (Mazatecans, Nahuas, Teenek). It was reported that K/K genotype is observed only in one individual in Mazetecans among the Mexican populations (Vargas-Alarcon et al. 2002). The low frequency of *LMP7*-K allele in Native Americans was interpreted as that it has been acquired more recently by genetic admixture. It should be emphasized that K/K genotype was not observed in the Southeastern Anatolia population, suggesting a very recent contribution to the gene pool in this population too.

Neighbor-joining tree constructed by using genetic distances between populations indicated that among studied populations Nahuas population is the closest while Caucasian (USA) and Kaingang (Brazil) is the most distant population to Southeastern Anatolia population. It was interesting to see that geographically very distant populations were found genetically close. It can be concluded that the contribution time of these polymorphisms to the gene pool of these populations by genetic admixture could be very close. Further comprehensive studies are necessary to speculate if this similarity is a result of a common founder for these two populations or a potential example of convergent evolution.

The average heterozygosity level of Southeastern Anatolia population was found to be 0.30. A high level of heterozygosity is an indicative of high level of genetic diversity. Since Southeastern Anatolia Region has been subjected to many civilizations throughout time, a high value of heterozygosity could be expected for this population.

It can be concluded that, more studies using different types of genetic markers are needed to clarify the filogenetic relationships of Southeastern Anatolia population with other populations and also the number of population studies on *LMP2* and *LMP7* genes should be increased to understand their effects as a genetic marker.

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