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Mannose Binding Lectin Gene Polymorphism in Azarian Population of Iran

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Abstract: Mannose-Binding Lectin (MBL) is involved in first line defense by binding to pathogens through a pattern-recognition mode of detection and then initiating a range of host responses. In present study we analyzed MBL gene and promoter polymorphism in Azarian population and compare them with previous reports in other populations. Blood samples were obtained from 144 Azarian populations from Tabriz and surrounding areas from March 2004-July 2005. MBL genotypes were investigated by polymerase chain reaction and restriction fragment length polymorphism. Present study showed prevalence of Allele B and LXPA haplotype in Azarian population which can conclude to protection against intracellular pathogens such as leishmania or tuberculosis but in the contrary it can conclude to susceptibility to Extracellular pathogen and SLE and rheumatoid arthritis. Also, present finding showed genetic relation of Azarian population with other Eurasian population.

Key words: Azarian, population, genotype, mannose-binding lectin, gene, polymorphism

INTRODUCTION

Mannose-binding lectin is a member of the collectin family of proteins found in serum (Presanis *et al*., 2003), it binds to mannose and N-acetylglucosamine and activates the complement system independently of antibodies via two associated serin protease , mannose-binding lectin-associated serin protease 1 and 2 (Jack *et al*., 2001; Mass *et al*., 1998; Thiel *et al*., 1997). C1q and mannose binding lectin, as well as lung surfactant protein A, share the same phagocytic receptor, which is present on a variety of cells, including phagocytes, platelets and endothelial cells (Nepomuceno *et al*., 1997; Nepomoceno and Tenner, 1998).

Human mannose binding lectin is derived from a single gene on chromosome 10 (Sastry *et al*., 1989), the normal structural mannose binding lectin allele is named A, while the common designation for the 3 variant structural alleles B (mutation in codon 54, Gly to ASP), C (mutation in codon 57, Gly to Glu) and D (mutation in codon 52, Arg to Cys) are O (Hegele *et al*., 1999; Neth *et al*., 2001).

In general, individuals with a normal genotype (A/A) have mannose binding lectin concentration in serum that are 6-8 times higher that those in individuals heterozygous for one of the variant alleles (A/O: A/B, A/C or A/D), while individuals with a defective genotype (2 variant alleles B/B, C/C, D/D, B/C, B/D or C/D) have almost undetectable mannose binding lectin serum levels (Gradual *et al*., 2000; Dornelles *et al*., 2006).

Moreover, mannose binding lectin expression is influenced by polymorphic sites in the upstream part of the mannose binding lectin gene (Crosdale *et al*., 2001; I.P. *et al*., 1998) nucleotides substitutions at position-550, -221 and +4 give rise to H/L, Y/X and P/Q respectively and cause to different haplotypes, while LX haplotype is associated with low mannose binding lectin plasma levels (Santos *et al*., 2001; Soborg *et al*., 2003).

There is evidence that the B and C variant alleles arose independently in distinct populations (Lipcombe *et al*., 1992), the B allele has reached high frequencies in several Eurasian and (native) American population whereas the C allele is frequent in most Sub-Saharan population (Lipcombe *et al*., 1992; Lipscombe *et al*., 1996; Turner *et al*., 2000). Previous studies suggests that the distribution of the B allele arose after the putative migration of hominids out of Africa and subsequently spread to most of the non-African world (Lipcombe *et al*., 1992; Turner *et al*., 2000). Azari race is one of the biggest races in Iran which habitant mostly in northwest of Iran. There was of interest to investigate MBL structural gene mutation and promoter polymorphisms in this population.

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Also, in this study we tried to find prevalence of genotype polymorphism of MBL gene for detection probable susceptibility for infections and diseases in this population.

MATERIALS AND METHODS

Blood samples were obtained from one hundred and forty four Azarian populations randomly from Tabriz and surrounding areas from March 2004-July 2005. DNA was isolated from either granulocytes or mononuclear cells by the modified proteinase K, Sodium Dodecyl Sulfate (SDS), N-acetyl-N, N-trimethyl Ammonium Bromide (CTAB) (Asgharzadeh *et al*., 2007a).

PCR was performed in 20 to 100 μL volumes that contained 50-500ng of genomic DNA, 0.5 μm of specific primers (Asgharzadeh *et al*., 2007b) in the presence of 1.5 mM MgCl2, 100 μM of each dNTP, 50 mM KCl, 20 mM Tris-HCl, pH 8.4 and 1-2.5 unit recombinant DNA polymerase (Cinnagen, Iran). DNA was amplified by general PCR and sequence-specific primed polymerase chain reaction (SSP-PCR). All PCRs were initiated by a 4 min denaturizing step at 94°C and completed by a 7 min extension step at 72°C. The temperature cycles for different types of PCRs were as follows: 32 cycles of 40 second at 94°C, annealing temperature for 40s and 72°C for 55s.

Annealing temperatures which were used as bellow follow: 60, 63, 63, 62, 66, 63, 66, 67, 64, 67, 67,65, 65 and 66°C for codon 57 (wild type), 57 (mutant), codon 54 (wild type), 54 (mutant), codon 52 (wild type), 52(mutant), allele H , L , P , Q, haplotypes Hy , Ly , Lx and Hx amplification, respectively (Madsen *et al*., 1995; Crousdale *et al*., 2000; Sullivan *et al*., 1996).

In addition to SSP-PCR, B and C alleles were detected by Ban I and Mbo Ï restriction enzyme digestions of the 328-bp product amplified by the allele P and Q primers respectively (Asgharzadeh *et al*., 2007b), followed by a 2.5% agarose gel electrophoresis. Ban I cleaves the A allele into two fragments allele (245 and 83 bp) and leaves the B allele undigested, while Mbo II specifically cleaves the C allele into two fragments (266 and 62 bP) (Asgharzadeh *et al*., 2007c).

Statistical analyses were performed by χ^2 (Chi-square test). P-values below 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

The DNA from all 144 individuals was completely typed and results of typing presented in Table 1. As showed in Table 1 there was dominant prevalence of wild type (allele A) (79.2%) I comparing with other genotype alleles, but we had prevalence of codon 54 mutation (Gly to Asp) (13.2%) in our studied population.

Table 1: Prevalence of MBL gene alleles, promoter variants and position +4 and promoter haplotypes of MBL in study population

Structure origin	Number	Frequency (%)
Genotype frequency alleles		
Codon 54 mutation (B)	38	13.2
Codon 57 mutation (C)	6	2.1
Codon 52 mutation (D)	16	5.5
Wild type (A)	228	79.2
Promoter Variants and Position +4		
H	112	39
L	176	61
P	214	74
Q	74	26
Haplotypes		
HYPA	95	32.9
LYPA	5	1.7
LYQA	67	23.26
LXPA	60	20.8
LYPB	38	13.19
LYQC	6	2.08
HYPD	16	5.5
HXPA	1	0.3

Promoter variants and position +4 prevalence of MBL presented in Table 1. L and P were more frequent and in promoter haplotypes HYPA had highest prevalence (32.9%) and then LYQA, LXPA, LYQB, LYPD, LYQC, LYPA, HXPA, respectively had more frequent. Interestingly there was high prevalence of LXPA haplotype which accompanied with low MBL concentration in serum. In next step information of study combined to give complete haplotypes and heterozygosity for alleles and variants as presented in Table 2. As presented in Table 2, 62.53% of study population had genotypes of normal genotype of MBL which cause high serum MBL concentration, 33.3% had genotypes of MBL which cause law serum concentration of MBL and 4.17% had genotype of lack of MBL in serum.

In Table 3 we compared prevalence of MBL haplotypes in our study population with other populations previously reported. There was not any significant difference in our results and other Eurasian populations previously studied (Canary Island, Spain and Danish Caucasians and Japan) ($p>0.05$) but there was significant difference with African populations ($p<0.05$) (Table 3).

Three important finding revealed in present study. First, we had prevalence of codon 54 mutation (allele B) in Azarian population. Second, we had high prevalence of LXPA haplotype which cause to low concentration of MBL in serum of study population and third, presence of HYPA as a dominant haplotype (32.9%) in Azarian population. In the other hand only 62.53% of study population had a normal genotypes of MBL and 37.47%

Table 2: Genotype polymorphism of MBL gene in Azarian Population-Iran

Stractural genotype	Promoter	Complete genotype	Number	Percent under population (%)*	Percent in total Population (%)*
A/A	Hy/Hy	HYPA/HYPA	18	20	12.5
	LY/LY	LYPA/LYPA	0	0	0
		LYQA/LYQA	13	14.4	9.03
		LYQA/LYPA	1	1.1	0.7
	LX/LX	LXPA/LXPA	9	10	6.25
	HY/LY	HYPA/LYPA	1	1.1	0.7
		HYPA/LYQA	17	18.8	11.8
	HY/LX	HYPA/LXPA	17	18.8	11.8
	LX/LY	LXPA/LYPA	1	1.1	0.7
		LXPA/HYQA	12	13.3	8.3
	HX/LX	HXPA/LXPA	1	1.1	0.7
		TOTAL	90		
A/O	HY on A Haplotype	HYPA/LYPB	17	35.4	11.8
		HYPA/LYQC	2	4.2	4.1
		HYPA/HYPD	4	8.3	2.7
	LY on A haplotype	LYPA/LYPB	1	2.1	0.7
		LYQA/LYPB	7	14.6	4.9
		LYQA/HYPD	4	8.3	2.7
		LYQA/LYQC	2	4.2	1.4
		HYPA/HYPD	0	0	0
	LY on A Haplotype	LXPA/LYPB	5	10.4	3.5
		LXPA/LYPD	5	10.4	3.5
		LXPA/LYQC	1	2.1	0.7
		TOTAL	48		
O/O		LYPB/LYPB	3	50	2.1
		LYQC/HYPD	0	0	0
		HYPD/HYPD	1	16.6	0.7
		LYPB/HYPD	1	16.6	0.7
		LYQC/LYPB	1	16.6	0.7
		Total	6		

*. Percent under population contains percent included on structural genotype group and percent in total population contain percent in all study population

Table 3: Frequency of MBL allele (haplotypes) in Azarian population and from other population previously reported

			Allele						
Population	Ref.	No.	HYPA	LYQA	LYPA	LXPA	LYPB	LYQC	HYPD
Azarian	Present study	144	0.33	0.23	0.01	0.21	0.13	0.02	0.06
Canary Spain	Garcia 2001	344	0.24	0.22	0.08	0.19	0.17	0.03	0.03
Japan	Matsushita 1998	218	0.44	0.16	0.07	0.11	0.22	0	0
Eskimo	Madsen 1998	72	0.81	0	0.04	0.03	0.12	0	0
Kenia	Madsen 1998	61	0.08	0.25	0.13	0.24	0.02	0.24	0.04
Mozambik	Madsen 1998	154	0.06	0.27	0.30	0.13	0	0.24	0
Danish	Madsen 1998	250	0.31	0.19	0.04	0.26	0.11	0.03	0.06
South America	Madsen 1998	43	0.54	0.01	0.02	0.01	0.42	0	0
Walpiri australis	Turner 2000	190	0.75	0.01	0.23	0.01	0	0	0.003

of population had low or deficiency of MBL in their serum. This low concentration of MBL has some beneficiary and some disadvantages. Previous studies showed deficiency of MBL in serum confers protection against intracellular infections. In tuberculosis has been demonstrated that heterozygosity for MBL variant allele (XA/O), which encodes low serum MBL level is associated with protection against clinical turberculosis (Bellamy and Hill, 1998; Soborg *et al*., 2003) or a new study suggest a protective role for MBL deficiency against development of the most severe and multibacillary form of leprosy but not the tuberculoid form (Dornelles *et al*., 2006). It has proved lack of mannose-binding lectin enhances survival in a mouse model of acute septic peritonitis (Takahashi *et al*., 2002). Also in intracellular parasite like Leishmania increasing concentration of MBL cause increasing the release of TNF- and interleukin 6 from monocytes contaminated with parasite (Jack *et al*., 2001; Plmenta *et al*., 1991). MBL can promote the opsonization of microorganisms, thus intensify their attachment to phagocytic cells (Jack and Turner, 2003; Asgharzadeh *et al*., 2007d). In the other hand deficiency of MBL confer susceptibility to extracellular pathogens as our previous study in patients with kidney infections revealed this relation (Asgharzadeh *et al*., 2007c) or studies on MBL deficiency and infection with gram negative organisms and Aspergillosis showed MBL deficiency as a risk factor for Extracellular pathogens (Jack *et al*., 2001; Crosdale *et al*., 2001).

Also, new studies controversy revealed role of MBL deficiency in the pathogenesis of autoimmune disorders such as systemic lupus erythematosus and rheumatoid

arthritis (Asgharzadeh *et al*., 2007b; Turner *et al*., 1998; Sullivan *et al*., 1996; Gradual *et al*., 2000).

The MBL distribution of structural alleles in our population was similar to European populations previously reported (Table 3) (Crosdale *et al*., 2000; Madsen *et al*., 1998). HYPA was dominant haplotype in Azarian population. It was in agreement with suggest that the HYPA haplotype may have been the dominant haplotype 50000 year BP (before present) which supported by observations in indigenous Australians (Turner *et al*., 2000) and Eskimo population (Madsen *et al*., 1998). In agreement with present hypothesis, presence of codon 54 mutation in our population can confirm origin of our area as second migration of hominids to Eurasia after first migration from Africa in 150000 year BP (Turner *et al*., 2000).

CONCLUSION

In conclusion present study showed prevalence of Allele B and LXPA haplotype in Azarian population which can conclude to protection against intracellular pathogens such as leishmania or tuberculosis but in the contrary it can conclude to susceptibility to extracellular pathogen and SLE and rheumatoid arthritis. Present finding showed genetic relation of Azarian population with other Eurasian population. This work can be useful for future immunopathology studies in this population.

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