The Impact of Single Nucleotide Polymorphisms in the Gene of Toll-Like Receptor-4 in Prostate Cancer

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Abstract

Background: Prostate cancer is one of the major medical problems in male population. Chronic inflammation has an important role in many human cancers and could be involved in the etiology of prostate cancer. Toll-like receptors are important tolls in innate immunity to pathogens. Many environmental factors could predispose for this cancer, however, genetic background of the population cannot be neglected.

Objective: To explore the hypothesis that genetic variation in *TLR4* gene can influence the individual's susceptibility to prostate cancer.

Patients and Methods: A total of 95 outpatient men with histologically-proven prostate cancer as well as 45 apparently healthy men were recruited for this study. Blood samples were taken from each participant from which genomic DNA was extracted and *TLR4* gene was PCR-amplified, using specific primers. PCR products were genotyped by direct sequencing.

Results: *Asp299gly* and *Thr399Ile* SNPs had only two genotypes; the distributions of these genotypes were statistically not significant between prostate cancer patients and controls. At allele levels, the control group had significantly higher frequency in the mutant allele of *Thr399Ile* SNP (allele T) than prostate cancer patients. On the other hand, the *rs11536889* had three genotypes with the mutant homozygous genotype (CC) and the mutant allele were more prevalent in PCa than controls with significant difference (OR=3.433, 95%CI=1.107-10.645, *P*= 0.033 and OR=2.730, 95%CI= 1.095-6.803, *P*=0.031 respectively).

Conclusion: Allele T of Thr399Ile SNP may have a protective role, while allele C of *rs11536889* SNP might increase the risk of prostate cancer.

Key words: prostate cancer, Genetic variation, *TLR4* gene, *Asp299gly*, *Thr399Ile*, *rs11536889*, Single Nucleotide Polymorphisms.

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Introduction

Cancer of the prostate ranks the first among all malignancies in men, and the second as leading cause of cancer-related death in USA in 2014 [1]. Many risk factors have been well documented to be associated with this malignancy. The most prominent of these are age, race, diet, and imbalance of sex hormones, obesity and urine influx [2].

A growing body of evidence suggested a major role of chronic inflammation in the occurrence of prostate cancer (PCa). Eicosanoid pathway activated by cyclooxygenase (COX-2) was proposed as one important link between chronic inflammation and PCa. Evidence for such a link was deduced from high expression of COX-2 in PCa tissue. Such inflammation not only induces carcinogenesis but also can promote neoplastic progression [3].

Toll like receptors (TLRs) are the most important class of pattern recognition receptors (PRR) that are involved in inflammation and defense against different pathogens [4]. They are type 1 trans-memebrane proteins, and triggered by pathogen-associated molecular patterns (PAMPs), that are the characteristics of various groups of pathogens [5]. Among of the most studied TLRs is TLR4. Structural analysis of TLR4 revealed that the receptor is consisted of 3 domains: an intracellular toll-interleukin-1 receptor (TIR) domain, a trans-membrane domain, and an extracellular leucine-rich-repeat (LRR) domain. The extracellular part of the receptor is involved in binding of lipopolysaccharide (LPS) and may be other ligands [6].

Arbour *et al.* (2000) identified single nucleotide polymorphisms (SNPs) in *TLR4* and co-segregating missense mutations. These SNPs are an A/C transition in exon 4 causing aspartic acid/glycine substitution at amino acid location *Asp299Gly* (rs4986790) and C/T transition causing a therionine/isoleucine switch at amino acid location *Thr399Ile* (rs4986791). These alterations in the amino acid sequence affected the extracellular domain and the ligand recognition area of the receptor [7]. Another novel polymorphism in *TLR4* gene was described in the 3' un-translated region (3'UTR) of the gene. This SNP is called *TLR4*+3725G/C (rs11536889), and was found to be functional in many diseases [8, 9].

In a meta-analysis, Jing *et al.* (2012) indicated a significant increase risk for *Thr399Ile* polymorphism and recommended more attention for this SNP as a most interesting *TLR4* polymorphism in many diseases [10]. However, Priyadarshini *et al.* (2013) found that *Asp299Gly* but not *Thr399Ile* SNP is associated with PCa in Indian men [11]. Zheng *et al.* (2004) reported an association between rs11536889 SNP and PCa [12]. These studies suggest that genetic variations in TLR4 gene may influence human malignant diseases [13].

So, this study was aimed to verify the hypothetical association of these three SNPs in *TLR4* gene (*Asp299Gly*, *Thr399Ile* and *TLR4*+3725G/C), and correlation with the risk of developing PCa.

Patients and Methods

Subjects and Samples

Ninety five out-patients male with histologically-confirmed PCa during the period from July 2013 to July 2014, were collected from the Hospital of Radiation and Nuclear Medicine, and Al-Kadhimyia Teaching Hospital-Baghdad were tested. Ethical approval to conduct the research was obtained from each hospital Institutional Review Board (IRB). 45 unrelated, cancer-free males from the college of medicine - Al-Nahrain university and Al-Kadhimyia teaching hospital were enrolled to be the control group. The ages of these patients and control ranged from 30-70 years. Control subjects and PCa patients with acute/chronic prostatitis, urinary tract infection or uncontrolled diabetes mellitus were excluded from the study. Informed consents were obtained from the patients and control which also included age, smoking, body mass index (BMI), residence and first relative family history of PCa and breast cancer (BCa).

Blood Samples

Five ml of venous blood was taken from each participant; 2 ml of which was kept in EDTA tube and the other 3ml in plane tube. The latter was undergone centrifugation where the serum was obtained and preserved at -20 ^{o}C till be used.

Estimation of prostate specific antigen (PSA) in control group

Prostate specific antigen test was conducted to confirm that controls have no PCa, because some individuals with PCa have no obvious clinical signs. The test was done according to the manufacturer's manual using commercial kit (ACON laboratories, Inc-USA). Out of 45 serum samples, 4 gave high level of serum PSA concentration which might indicates PCa. Therefore, another four samples were taken from age-matched different men, and PSA test was performed which gave negative results.

DNA Extraction and Genotyping

DNA was extracted from whole blood samples using (gSYNC^{TM} DNA Mini Kit Whole Blood Protocol-Geneaid- Korea) according to the manufacturer's manual. The nanodrop (UVS-99-ACTGene-USA) was used to estimate the concentration and purity of the DNA yield.

The primers sets used for *TLR4* gene amplification are demonstrated in table 1.

Table (1): Primer sets sequences and their corresponding genes.

Genes	Primers 5'→3'	Fragments
TLR4 Asp299Gl y	F:GATTAGCATACTTAGACTACTACCTCCATGR R:GATCAACTTCTGAAAAAGCATTCCCAC	249 bp
TLR2 Thr399Ile	F:GGTTCGTGTTCTCAAAGTGATTTTGGGAGAA R:ACCTAAGACTGGAGAGTGAGTTAAATGCT	406 bp
TLR4 rs11536889	F: TGGGATCCCTCCCCTGTACCCTTC R: CTGGATCCGTTTCTGAGGAGGCTGGATG	227 bp

Polymerase chain reaction products were sent to the Bioneer company-Korea for DNA sequencing. The obtained sequences are aligned by "Clustalw" software with normal sequence from GenBank (Gen Bank Accession No U93091 for *Asp299gly* and *Thr399Ile* SNPs and NC-000009.12 for rs11536889), and then were examined for the presence of mutations.

Statistical analysis

Statistical analysis was performed with the statistical package for social sciences (SPSS) 21.0 and Microsoft Excel 2013. Categorical data formulated as count and percentage. Fisher exact test was used to describe the association of these data, in addition to relative risk study (RR). Numerical data were described as mean, standard deviation of mean. Independent sample t-test was used for comparison between two groups while analysis of variance (ANOVA) was used for comparison among more than two groups. The lowest level of accepted statistical significant difference is bellow or equal to 0.05.

Results

Characteristics of the study groups

Table 2 shows the association of different risk factors with PCa. The study was initially intended to select age-matched control group for PCa patients. Accordingly there was no significant difference in the mean of age between the patients and control (64.6± 10.68 and 63.71±8.32 respectively, *P*=0.247). Our study also revealed no significant influence of family history of solid cancer, BMI and smoking status on the incidence of PCa (*P*= 0.266, 0.077 and 0.402 respectively). On the other hand, residency appeared to be the only risk factor that affects the incidence of PCa in

this study. Out of 95 PCa patients 53(55.8%) were of urban residence versus 42 (44.2%) of rural residence (OR= 2.287, 95% CI= 1.1-4.758, *P*= 0.025).

Table (2): Association of risk factors with the incidence of PCa**.**

Risk Factors	Cases N=95	Control N=45	*P*-value	OR(95%CI)
Mean age in years (SD)	64.6 (10.68)	63.71 (8.32)	0.247	1.198 (0.992- 1.134)
Family history				
No	79 (83.16%)	41 (91.11%)	0.266	1.0
Yes	16 (16.84%)	4 (8.89%)		1.922(0.599-6.164)
Mean BMI (SD)	26.44 (4.27)	24.71 (5.02)	0.077	1.108 (0.989- 1.243)
Smoking				
Never	64 (67.37%)	27 (60%)	0.402	1.0
Ex-smoker	15 (15.79%)	6 (13.33%)	0.197	1.778 (0.742-4.257)
Current smoker	16(16.84%)	12 (26.57%)	0.307	1.875(0.561-6.268)
Residency				
Rural	42(44.2%)	29 (64.44%)	0.025	1.0
Urban	53 (55.8%)	16 (35.56%)		2.287 (1.100- 4.758)

N: number, **SD:** standard deviation, **BMI:** body mass index, **CI:** confidence interval, **OR:** odds ratio.

Asp299gly* and *Thr399Ile

Each of *Asp299gly* and *Thr399Ile* SNPs appeared in two genotypes. For *Asp299gly*, these were AA and AG which represented 96.84% and 3.16% respectively in PCa patients and 91.11% and 4.89% respectively in controls with no significant difference (OR =0.334, 95%CI =0.072-1.561, *P* =0.164) figure1, table -3-. For *Thr399Ile* SNP, these genotypes were CC and CT which accounted for 98.42% and 1.58% respectively in PCa and 80 and 20% in controls with no significant difference (OR=0.176, 95%CI=0.051-0.607, *P*=0.06)**.** None of either patients or healthy control in this study has homozygous mutant genotype for both SNPs (Figure 2, Table 3).

Chi-square analysis was used for testing allele distribution, and the result indicated that the two SNPs met the Hardy-Weinberg equilibrium. Analysis of allele frequencies showed non-significant differences in the frequency of the G allele (the mutant allele of *Asp299gly* SNP) between PCa patients and control (1.58% and 4.44% respectively). However, frequency of T allele (the mutant allele of *Thr399Ile* SNP) was higher in controls (10%) compared to PCa patients (2.1%) with significant difference (OR = 0.194, 95%CI =0.058-0.647, *P* =0.008) (Table 3).

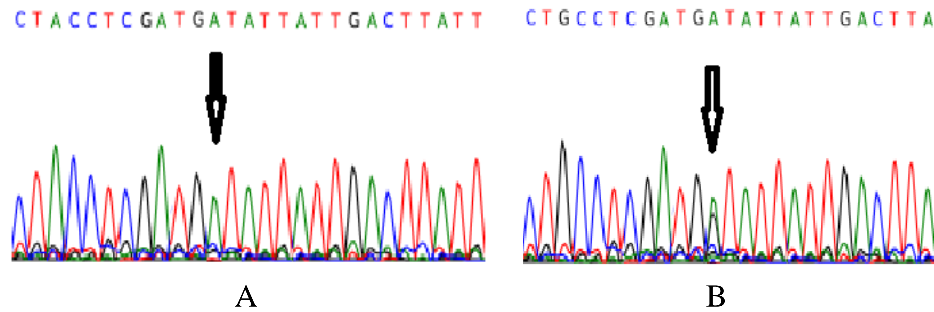

Figure (1): DNA sequencing for part of *TLR4* gene). The arrow indicates the position of the SNP *Asp299Gly*. A: represents the genotype AA, B: AG.

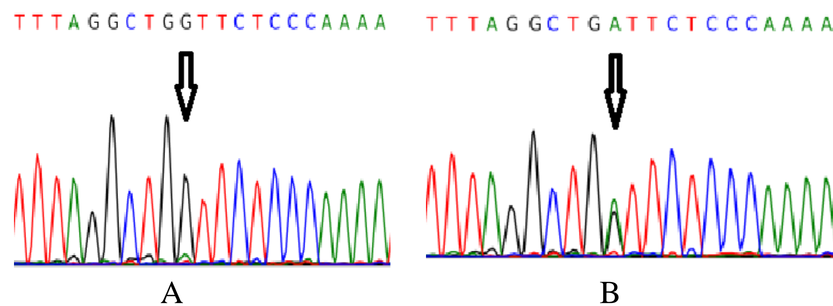

Figure (2): DNA sequencing for part of *TLR4* gene (reverse strand). The arrow shows the position of the SNP *Thr399Ile*. A: represents genotype CC, B: TC.

rs11536889

The rs11536889 SNP had three genotypes which were GG, CG and CC (Figure 3). These genotypes accounted for 72.63%, 22.1% and 5.26% respectively in PCa patients compared with 88.89%, 8.89% and 2.25% respectively in control. The homozygous mutant genotype (CC) showed significant difference between the patients and control (OR =3.433, 95%CI =1.107-10.645, *P* =0.033). At allele level, the frequency of mutant allele (C) was significantly higher (16.32%) in PCa patients than that of control (6.67%)(OR =2.730, 95%CI =1.095-6.803, *P* =0.031). (Table3).

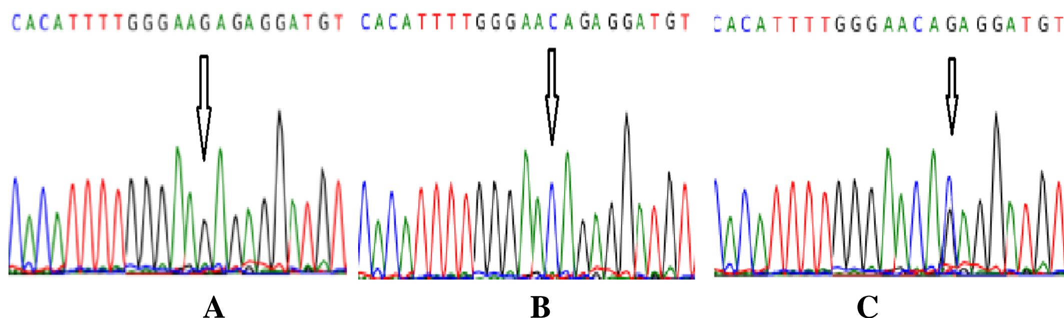

Figure (3): DNA sequencing for part of *TLR4* gene. The arrow shows the position of the SNP rs11536889 SNP. A: GG genotype, B: CC genotype and C: CG genotype.

Table (3): Genotypes and allele frequencies in the study groups.

Variables	patients N=95	Control N=45	*P*- value	OR (95%CI)
Asp299gly				
AA	92 (96.84%)	41(91.11%)	0.164	1.0
AG	3 (3.16%)	4(4.89%)		0.334(0.072-1.561)
GG	0	0		
Alleles				
A	187(98.42%)	86(95.56%)	0.169	1.0
G	3(1.58%)	4(4.44%)		0.345(0.575-1.575)
Thr399Ile				
CC	91(95.58%)	36 (80%)	0.06	1.0
CT	4 (4.21%)	9 (20%)		0.176(0.051-0.607)
TT	0	0		
Alleles				
C	186(97.89%)	81(90%)	0.008	1.0
T	4 (2.1%)	9 (10%)		0.194(0.058-0.647)
rs11536889				
GG	69 (72.63%)	40(88.89%)	0.01	1.0
CG	21 (22.1%)	4 (8.89%)	0.326	2.985(0.337-26.472)
CC	5 (5.26%)	1 (2.225%)	0.033	3.433(1.107-10.645)
Alleles				
G	159 (83.68%)	84(93.33%)	0.031	1.0
C	31 (16.32%)	6(6.67)		2.730(1.095-6.803)

Discussion

Globally, PCa is the second most common male malignancy and the sixth leading cause of male death, making a considerable global health burden [14]. Despite this high prevalence; the number of known risk factors is still limited, providing little insight in the elucidation which men would be susceptible to develop the disease.

Residency appeared to be the only environmental risk factors which had significant effect on the individual's susceptibility to PCa (OR=2.287). These results imply that urban residents have 2.287-fold greater risk of getting PCa than rural residents. However, such results may implement some bias because accession of health care centers and screening with PSA test is more available for urban residents. Consequently, more cases among those residents are recorded.

One of the most documented risk factor for PCa is family history of cancer. Not only first relative history of PCa could affect the individual's risk for this malignancy, but also the presence of PCa in such a relative could do so [15-17]. The mechanism accounts for such association could be related to SNPs in breast cancer (BCA1 and BCA2) genes [17]. However, our study did not find significant association between the family history of PCa or BCa and the risk of getting PCa may be due to the limited numbers of PCa patients involved in this study.

Results of the present study indicate that the allele T of *Thr399Ile* reversely associated with risk of PCa (subjects who carry this allele are 5.154-fold less likely to have PCa

than subjects who carry C allele). Most contemporary researches indicate either none such associations with PCa [18]. Positive association with PCa [11]. Or positive association with other malignancies such as noncardia gastric cancer [19]. And breast cancer [20]. The exact reasons for this discrepancy between our findings and that of these authors cannot be explained, but it might reflect the role of different geographical areas, different ethnics groups and different immune status. That is because in most of the instances; the association between SNPs and predisposition to certain diseases has been shown to depend on several factors among which ethnic background, and/or prior immune deficiencies [21]. For instance, several SNPs in *c-Myc* gene were found to reduce the risk of PCa in caucasians but not in african americans [22].

Immune system plays an important role, not only in the defense against microbial infections but also in controlling and surveillance of malignant tumors. Immune system cells can scan the tissues in order to remove newly formed malignant or transformed cells before they can turn into fully formed malignancy [23].

How can these two SNPs alter the structure and/or function of TLR4 is a question the exact answer of which is still a controversial issue. However, mutant allele can exploit one or more of three possible ways to influence TLR4 function; expression, signaling, or ligand binding. The majority of researches in this regard pointed out that expression of TLR4 is not affected by these SNPs [21, 24].

Accordingly, Wu, in 2011 hypothesized a disruption in the interaction between the mutant TLR4 and other components such as CD14, LBP, or MD-2 which are part of the functional response of TLR4. This disruption results from conformational changes in the receptor [24]. Henckaerts *et al* (2007), proposed saddle-like surface of extracellular domain of mutant TLR4 with the *Asp299Gly* and *Thr399Ile* amino acids which are positioned at the two opposite ends of the saddle, and the concavity that lies between the two amino acids suggesting a possible docking site for either the ligand or the co-receptor that may disrupt the normal function of the receptor. Threonine amino acid at a 399 position conserved the branched-side chain, but it increases the overall steric bulk in this region and might be precluding the ligand/cofactor docking [25].

Undoubtedly such conformational changes and the disruption in the ligand-docking will alter these signaling pathways of mutant *TLR4*. Davoodi *et al* (2012) demonstrated that the activity of NF-kB in the mutant *TLR4*-cells was higher than that of the wild type in response to LPS. In addition, there were high levels of interleukin-1 receptor-associated kinase (IRAK) that were accompanied with a rapid degradation of this factor upon treatment with LPS in wild type compared with the mutant *TLR4* [26]. This caused reduce in the signaling and reduced cytokine genes transcription because the degradation of IRAK serves as a negative feedback mechanism. Eventually, there will be less inflammatory response, and this may explain what is seemed as a protective role of *Thr399Ile* SNP against prostate cancer.

Our study revealed a significant importance of CC genotype of the rs11536889 SNP as a PCa risk factor. Individuals carrying this genotype have 3.433-fold risk of getting PCa those who carry GG genotype. These results are in accordance with a previous large study conducted in Sweden which analyzed eight SNPs in *TLR4* genes, in 1383 patients with PCa, and 780 age-matched controls. The study showed that GC or CC genotypes of rs11536889 increase the risk of PCa by 4.9% [12].

Unlike the previous two SNPs, this SNP is unlikely to affect the conformation of TLR4 protein because it is located in the 3'UTR, but not in the coding-region of *TLR4* gene. Accordingly, this may influence transcription or translation of this gene [27]. Sato *et al.* (2012) showed that there was no significant difference in the expression levels of *TLR4* mRNA on the peripheral blood mononuclear cells (PBMCs) between the different genotypes of this SNP [13]. However, protein levels of TLR4 were increased significantly, only in the CC genotype-carrying patients. This suggests that this variant affects the translation rather than the transcription.

Functionally, the mutant TLR4 protein from this SNP seems to be more active than the wild type protein in the secretion of the proinflammatory cytokines. Bihl *et al*. (2003) showed that PBMCs from CC patients produced slightly higher levels of TNF-α and IL-6 than cells from persons with other genotypes [28]. Later on, Sato *et al.* (2012) found significantly higher levels of IL-8 secreted from PBMCs belong to CC and GC subject in response to LPS than those cells from GG subjects [13]. These evidences suggested that up-regulation of TLR4 expression accompanied by increased TLR4 function may promote recognition of LPS in gram-negative bacteria in the prostate and increase the production of proinflammatory cytokines. Eventually, a chronic inflammation may evident which predispose to PCa.

It may be premature to reach such conclusions, and clearly there may be other possible interpretations for our findings. However, our results indicate a protective role of mutant allele of the *Thr399Ile* SNP (T allele) against the PCa, while mutant allele of rs11536889 increases the risk of this cancer may be through hyper function of mutated TLR4 and subsequent increase of pro-inflammatory cytokine production. This can create a chronic inflammation microenvironment which facilitates the transgenic process. We recommend further studies on larger sample size and other different mutations in TLR4 genes.

Conflict of Interest: Authors declare no conflict of interest.

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References

[1] Siegel R, Ma J, Zou Z, and Jemal A. Cancer statistics. CA Cancer J Clin. 2014; 64:9-29.

[2] Platz EA and Giovannuci E. The epidemiology of the sex steroid hormones and their signaling and metabolic pathways in the etiology of prostate cancer. J Steroid Biochem Mol Biol. 2004; 92:237-253.

[3] Balisteri CR, Candore G, Lio D and Carruba G. Prostate cancer from the pathophysiologic implications of some genetic risk factors to translation in personalized cancer treatment. Cancer Gene Ther. 2014; 21:2-11

[4] Akira S and Takeda K. Toll-like receptor signaling. Nat Rev Immunol. 2004;4:499-511.

[5] Iwasaki A and Medzhitov A. Toll-like receptor control of the adaptive immune response. Nat Immunol. 2004; 5:987-995.

[6] Ferwarda B, McCall B, Verheijen K, Kullberg B, van der Ven A, van der Meer J, and Netea MG. Functional consequences of *toll-like receptor 4* polymorphisms. Mol Med. 2008; 14:346-352.

[7] Arbour NC, Lorenz E, Schutte BC, Zabner, J, Kline JN, Jones M.*et al. TLR4* mutations are associated with endotoxin hyporesponsiveness in humans. Nat Genet. 2000; 25:187-191.

[8] Miedema KG, te Poele EM, Tissing WJ, Postma DS, Koppelman GH de Pagter AP. *Et al*. Association of polymorphisms in the

TLR4 gene with the risk of developing neutropenia in children with leukemia. Leukemia. 2011; 25:995-1000.

[9] Zhou L, Wei B, Xing C, Xie H, Yu X, Wu L. *et al.* Polymorphism in 3'-untranslated region of toll-like receptor 4 gene is associated with protection from hepatitis B virus recurrence after liver transplantation. Transpl Infect Dis. 2011; 13:250-258.

[10] Jing J J, Li M and Yuan Y. *Toll-like receptor 4 Asp299Gly* and *Thr399Ile* polymorphisms in cancer: a meta-analysis. Gene. 2012; 499:237-42.

[11] Priyadarshini A, Chakrsborti A, Mandal A and Singh S. *Asp299Gly* and *Thr399Ile* polymorphism of *TLR-4* gene in patients with prostate cancer from North India. Ind J Urol. 2013; 29:37-41.

[12] Zheng SL, Augustsson-Balter K, Chang B, Hedelin M, Li L, Adami HO *et al*. Sequence variants of toll-like receptor 4 are associated with prostate cancer risk: results from the Cancer Prostate in Sweden Study. Cancer es. 2004;64:2918-2922.

[13] Sato K, Yoshimura A, Kaneko T, Ukai T, Ozaki Y, Nakamura HA *et al*. single nucleotide polymorphism in 3'-untranslated region contributes to the regulation of Toll-like receptor 4 translation. J Biol Chem. 2012; 287:25163-25172.

[14] Baade PD, Youlden DR, and Krnjacki LJ. International epidemiology of prostate cancer: geographical distribution and secular trends. Mol Nutr Food Res. 2009;53:171-184.

[15] Cerhan JR, Parker AS, Putnam SD, Chiu BC, Lynch CF, Cohen MB *et al*. Family history and prostate cancer risk in a population-based cohort of Iowa men. Cancer Epidemiol Biomarkers Prev. 1999;1: 53-60.

[16] Makinen T, Tammela TL, Stenman UH, Maattanen L and Rannikko S. Family history and prostate cancer screening with prostate specific antigen. J Clin Oncol. 2002; 20:2658-2663.

[17] Chen Y, Page JH, Chen R and Giovannucci E. Family history of prostate and breast cancer and the risk of prostate cancer in the PSA era. Prostate. 2008; 68:1582-1591.

[18] Balistreri CR, Caruso C, Carruba C, Micelli V and Campisi I. A pilot study on prostate cancer risk and proinflammatory genotype: pathophysiology and therapeutic implications. Curr Pharm Des. 2010; 16:718-742.

[19] Hold GL, Rabkin CS, Chow WH, Smith MG, and Gammon MD. A functional polymorphism of *toll-like receptor 4* gene increases risk of gastric carcinoma and its precursors. Gastroenterol. 2007;132: 905-912.

[20] Theodoropoulos GE, Saridakis V, Karantanos T, Michalopoulos NV and Zagouri F. Toll-like receptor gene polymorphisms may confer increased susceptibility to breast cancer development. Breast. 2012;21:534-538.

[21] Figueroa L, Xiong Y, Song C, Piao W, Vogel SN and Medvedev AE. The *Asp299Gly* polymorphism alters TLR4 signaling by interfering with the recruitment of MyD88 and TRIF. J Immunol. 2012; 188:4506-4515.

[22] Shan J, Al-Rumaihi K, Rabah D, Al-Bozom I and Kizhakayil D. Genome scan study of prostate cancer in Arabs: identification of three genomic regions with multiple prostate cancer susceptibility loci in Tunisians. J Translational Med. 2013;11:121-128.

[23] Igney FH and Krammer PH. Immune escape of tumors: apoptosis resistance and tumor counterattack. J Leukocyte Biol. 2002; 71:907-920.

[24] Wu Y. The neuroimmunopharmacology of alcohol. Ph.D. thesis. Unversity of Adelaide, South Australia, 2011, pp 157-158.

[25] Henckaerts L, Pierick M, Joossens M, Ferrante, M, Rutgeerts P and Vermeire S. Mutations in pattern recognition receptor genes modulate seropositivity to microbial

antigens in patients with inflammatory bowel disease. Gut. 2007; 56:1536-1542.

[26] Davoodi H, Hashemi SR and Seow HF. Increased NF-kB activity in HCT116 colorectal cancer cell line harboring *TLR4 Asp299Gly* variant. Iran J Allergy Asthma Immunol. 2012; 11:121-132.

[27] Hesketh J. 3'-Untranslated regions are important in mRNA localization and translation. Lessons from selenium and metallothionein. Biochem Soc Trans. 2004; 32, 990–993.

[28] Bihl F, Salez L, Beaubier M, Torres D, Larivière L, Laroche L *et al*. Overexpression of Toll-like receptor 4 amplifies the host response to lipopolysaccharide and provides a survival advantage in transgenic mice. J Immunol. 2003; 170, 6141–6150.