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Original Research Article

Association of *CYP1A1* and *CYP2E1* gene polymorphisms with prostate cancer in a Lebanese population

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The present study investigates the association of the genetic polymorphisms of cytochromes P450 (CYP) 1A1 and 2E1 with prostate cancer (PCa), which is among the most common cancers in Lebanon and many countries. Prostate cancer is a multifactor disease where genetic polymorphisms of genes involved in carcinogen metabolism such as *CYP1A1* and *CYP2E1* could play a key role. Regarding the association of these genes polymorphisms with PCa, case-control studies as well as meta-analyzes investigations reported conflicting results in diverse populations. We conducted the present case-control study to assess whether the alleles *CYP1A1*2A* (6235T>C) and *CYP2E1*6* (7632T>A) are associated with PCa in Lebanon and to compare with polymorphism in other populations. Our results showed that *CYP1A1*2A* allele was associated with PCa (*P*= 0.016) and that individuals carrying at least one *CYP1A1*2A* allele present 2.7-fold increase of PCa risk (*P*= 0.038). On the contrary, the *CYP2E1*6* allele was not significantly associated with this cancer in the studied population despite a clear difference of genotype distribution between patients and controls. We conclude that *CYP1A1*2A* is among the genetic risk factors that contribute to PCa occurrence in Lebanon.

Key words: Case-control study, cytochromes P450, human, genetic polymorphism, prostate cancer, RFLP.

INTRODUCTION

Prostate cancer (PCa) is among the most common cancers that affect men in the world (Simard et al., 2002). It is the most common cancer in men in the United States (Edwards et al., 2010). In Lebanon, PCa is the most frequent malignancy among men (Lebanese Ministry of Public Health-Epidemiological Surveillance Program, 2010).

Despite PCa high morbidity, its etiology remains poorly understood (Hsing et al., 2006). Prostate carcinogenesis is a complex, multistep and multifactor process where many factors are involved. Aging, ethnic origin, and family history of PCa are the only established risk factors (Lesko et al., 1996). The roles of environmental and genetic factors in the etiology of this disease were documented (Coughlin et al., 2002). It is estimated that as much as 42% of PCa risk involves genetic influences (Lichtenstein et al., 2000). Genetic polymorphisms of many genes were associated with PCa such as the genes of estrogen receptors (ER)α and ERβ (Safarinejad et al., 2012), the gene of the nucleotide excision repair (XPC) (Mandal et al., 2012), cytochrome P450 (CYP) *3A4*1B* and the glutathione S-transferase *(GST) T1* null (Rodrigues et al., 2010), the allele of *CYP1B1* (4326C>G, rs1056836)(Yang et al., 2012) and A453G (Li et al., 2015), the endothelial nitric oxide synthase gene (Safarinejad et al., 2012), and the prostate cancer gene 3 (PCA3) (Fontenete et al., 2012).

Among the important polymorphisms that could be associated with PCa are those of key phase I genes such as *CYP1A1* which bioactivates many polycyclic aromatic hydrocarbons (PAH), e.g. benzo(a)pyrene (BaP) (Gelboin et al.,1980; Bartsch et al., 2009) leading to the highly reactive intermediate BaP-diol epoxide (BPDE). A point mutation in exon 7 downstream of the poly-A signal of *CYP1A1* gene (rs4646903, 6235T>C) creating the allele *CYP1A1*2A* (also known as , m1 allele) was associated with a high mRNA

inducibility and enzymatic activity (Petersen et al., 1991; Landi et al., 1994) thereby leading to increased risk of carcinogen bioactivation. *CYP2E1* is another key phase I enzyme of interest which bioactivates the carcinogen diethylnitrosamine (Tanaka et al., 2000), and whose mutation in intron 6 (rs6413432, 7632T>A) creates the *CYP2E1*6*. The effect of this mutation on enzyme activity remains undefined. However, it was reported that the patients carrying the mutant *CYP2E1*6* allele show lower chlorzoxazone hydroxylation activity (a CYP2E1-catalyzed reaction) compared to the healthy wild-type subjects (Haufroid et al., 2002).

Numerous studies have examined the association between various genetic polymorphisms of *CYP1A1* and *CYP2E1* and the incidence of different types of cancer. For instance, polymorphisms of *CYP1A1* such as the m2 allele (Ile462Val, 2455A>G) and *CYP1A1*2A* (6235T>C, m1 allele, rs4646903) increase the risk of lung cancer in Asian (Lee et al., 2008) and Kashmir populations (Shaffi et al., 2009). Moreover, *CYP1A1*2A* (6235T>C) increases the risk of breast cancer among Koreans (Shin et al., 2007) and Indians (Syamala et al., 2010), and of colon cancer in Japan (Sivaraman et al., 1994). Similarly, the m2 allele of *CYP1A1* (Ile462Val, 2455A>G) increases the risk of breast cancer among Caucasian (Sergentanis et al., 2010) and Indian populations (Surekha et al., 2009).

*Rsa*I polymorphism of *CYP2E1* increases the risk of lung cancer in USA (El-Zein et al., 1997), esophageal cancer in China (Lin et al., 1998), oral cancer in Taiwan (Hung et al., 1997), and stomach cancer among Chinese population (Cai et al., 2005; Gao et al., 2007). Other *CYP2E1* genetic polymorphisms increase the risk of stomach cancer in Taiwan (*CYP2E1*7B*: -71G>T) (Neuhaus et al., 2004) and among alcoholics in Italy (*CYP2E1*6*:7632T>A) (Boccia et al., 2007) and cancer of the esophagus (*CYP2E1*6*: 7632T>A) (Li et al., 2005). Moreover, there is an association between the genetic polymorphism of *CYP2E1Rsa*I and stomach cancer in Korea (Nan et al., 2005) but not between *CYP2E1*6* (7632T>A) and cancer of the esophagus in the Caucasian population (Rossini et al., 2007). These reports clearly illustrate the population-dependent importance of the genetic factors in terms of cancer development.

PCa was associated with *CYP1A1* (3801T>C) (Mittal et al., 2007, Shaik et al., 2009) and with *CYP1A1*2A* (6235T>C) (Vijayalakshm et al., 2005) in the Indian population as well as in Chilean population (Acevedo et al., 2014). Association with PCa was also found for *CYP1A1*3*(4887C>A) (Aktas et al., 2004) in a Turkish population, and for *CYP2E1 Dra*I (7668T>A) in Portugual (Ferreira et al., 2003). However, other reports failed to demonstrate PCa association with *CYP2E1* and *CYP1A1* gene polymorphisms in Japan and Tunisia (Murata et al., 2001; Souiden et al., 2012). Recent meta-analyzes investigations showed population-dependent conflicting results regarding the association of PCa with *CYP1A1*2A* (3801T>C) (Li et al., 2012; Ding et al., 2013; He et al., 2013).

The objective of the present study was to evaluate the association between genetic polymorphisms of *CYP1A1*2A* (6235T>C) and *CYP2E1*6* (7632T>A) and PCa susceptibility in a Lebanese population where smoking, a source of carcinogens bioactivated by these two enzymes, is still a public health concern (Waked et al., 2012). Moreover, these two alleles are significantly present in the Lebanese population (Darazy et al., 2011; Zgheib et al., 2010). Investigation of the relationship between these two polymorphisms and PCa risk was never performed in Lebanon and yielded conflicting results in other populations. The present study would contribute to understand and compare the genetic risk factors determined by *CYP1A1*2A* and *CYP2E1*6* in PCa development among different populations.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals were purchased from BDH-England. The primers, the PCR reagents, the restriction enzymes and the DNA ladder were from Sigma-Germany.

Ethics statement

The present study was approved by the ethics committee of Rafic Al-Hariri Public Hospital (Beirut - Lebanon). Form of informed consent was filled by all participants anonymously and approved by the committee. The consent form explained the objective of our study.

Study design and population

The present study was designed as an observational cross-sectional case-control study. Forty histologically-confirmed PCa patients and 84 healthy controls were included in the study from diverse medical institutions of Lebanon. Both groups were comparable regarding age. Samples collection was conducted over three years between 2011 and 2013. Patients were recruited by their urologists at most a year after PCa diagnosis, and control individuals hadn't any symptom of urology-related diseases based on their medical history.

Collection of medical data

All participants were asked for standard parameters such as age, smoking habit, occupation and family history of PCa. The average age was 71± 10 years for the patients and 68± 11 years for controls. The smoking habit was not possible to be accurately defined for the patients and controls because many of the participants were exposed to tobacco smoke passively in their socio-professional life. Information about alcohol drinking habit was hard to be known accurately due to religious reasons. Therefore, these variables were not considered in our study although included in the initial survey.

Table 1. Statistical analysis and distribution of *CYP1A1*2A* genotypes (*Msp*I, 6235T>C, rs4646903) and allelic frequency (percentage) for controls and prostate cancer patients. N, number of cases; OR, odds ratio; p, Fisher's exact p; CI, confidence interval; wt, wild-type allele for the investigated polymorphism

CYP1A1 genotypes and alleles	Prostate cancer				Controls
	N(%)	OR	95% CI	P-value	N(%)
CYP1A1 wt/wt	27 (67.5%)	3.8	0.65-21.96	0.117	51 (85%)
CYP1A1 wt/*2A	9 (22.5%)	2.4	0.81-7.24	0.105	7 (11.7%)
CYP1A1*2A/*2A	4 (10%)	1.5	0.22-11.08	0.658	2 (3.3%)
CYP1A1 wt/*2A and*2A/*2A	13	2.7	1.04-7.19	0.038	9
CYP1A1 wt/wt and wt/*2A	36	3.2	0.56-18.49	0.169	58
CYP1A1*2A	17 (21.25%)	2.7	1.18-6.07	0.016	11 (9.2%)

Collection of blood samples and DNA extraction

Blood samples were collected in EDTA (ethylene diamine tetra acetic acid)-containing test tubes and transported on ice to the laboratory. The samples were conserved for 12 to 36 hours at 4°C before DNA extraction is performed. Genomic DNA was isolated from peripheral blood leukocytes as previously described (Darazy et al., 2011).

Genotyping assays

Genotyping for *CYP1A1*2A* allele (6235T>C; rs4646903) was performed by PCR-RFLP. A 340 bp DNA fragment corresponding to the 3'-flanking sequence and encompassing the polymorphic *Msp*I restriction site, was amplified using the forward primer 5'TAGGAGTCTTGTCTCATGCCT and the reverse one 5'CAGTGAAGAGGTGTAGCCGCT (Sivaraman et al., 1994). The PCR reaction contained 0.5 µM of each primer and 100 - 500 ng of the extracted genomic DNA. Cycling conditions were as follows: pre-incubation at 95°C for 5 min; 35 cycles consisting of denaturation at 94°C for 45 seconds, annealing at 58°C for 1 min, and extension at 72°C for 1 min, followed by a final extension at 72°C for 10 min. After amplification, the PCR products were sorted on 3% agarose gel (1x TBE) to check the PCR product size. Finally, 12.5µl of each PCR reaction were digested at 37°C for 16 hours with 20 units of the *Msp*I restriction enzyme. The products of digestion were identified by electrophoresis on a 3% agarose gel in 1x TBE and then stained with ethidium bromide.

*CYP2E1*6* allele (7632T>A; rs6413432) genotyping was performed by PCR-RFLP. A 327 bp DNA fragment corresponding to intron six of *CYP2E1* gene and containing the polymorphic *Dra*I restriction site was amplified using the pair of primers (5'CGACATGTGATGGATCCAGGG and 5'TCGTGATCGCCTGCCTCA, Darazy et al., 2011). The PCR reaction contained 0.5 µM of each primer and 100 - 500 ng of the extracted genomic DNA and the same PCR cycling program was applied as for *CYP1A1* except that annealing temperature was 62°C. After assessing the PCR amplicon size on aliquots using 3% agarose gel electrophoresis, 12.5µl of each amplification reaction were digested with 20 units of the *Dra*I restriction enzyme overnight at 37°C. The products of digestion were then sorted on a 3% agarose gel in 1x TBE and then stained with ethidium bromide. *CYP2E1* genotyping assay was conclusive for all patient and control samples while that of *CYP1A1* was conclusive for all patients and 60 control samples. The remaining non genotyped controls were excluded from the calculation of odds ratio in Table 1.

Remark: The wild type (wt) denotation in this article refers to the wild-type allele at the investigated polymorphic site only, regardless of the other unstudied polymorphic sequences for that particular gene. Positions of the mutant nucleotides for the diverse CYP1A1 polymorphic alleles were taken as given in the respective references despite the numbering discrepancy. For instance, the mutations 3801T>C and 6235T>C are both referred to as *CYP1A1*2A* (Wright et al., 2010; Ihsan et al., 2011).

Statistical Analysis

The Fisher's exact test was calculated to compare the percentages of the studied genetic polymorphisms in the PCa patients and controls. The odds ratio (OR) with 95% confidence interval (CI) were calculated to describe the strength of the association. The associations were considered to be statistically significant if the Fisher's exact P-value was less than 0.05 and if the 95% CI excluded the value 1. Logistic regression analysis was performed for age and resulted in $P>0.05$. All statistical calculations were done using the SPSS*17 (Statistical Product and Service Solutions*version 17).

RESULTS

CYP1A1 genotyping

Representative *CYP1A1* genotyping results are illustrated in Figure 1. Table 1 shows the distribution of *CYP1A1* genotypes and the allelic frequencies (percentages) among the tested patient and control subjects, it also illustrates the statistical analyses. The percentage of the *CYP1A1*2A* allele

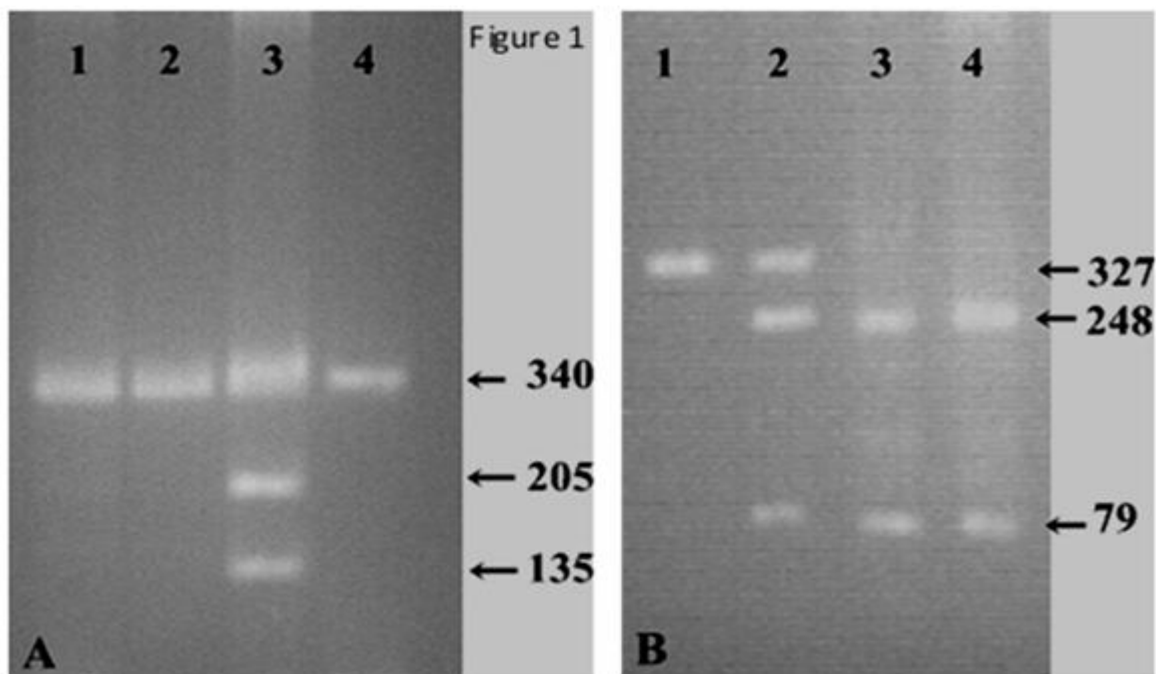

Figure 1: Representative genotyping results for the investigated genes. Amplification products (digested with the corresponding restriction enzyme as described in the experimental procedure) were sorted on a 3% agarose gel stained with ethidium bromide. **A:** Genotyping results of four samples for *CYP1A1*; the amplicon is subjected to digestion with *Msp*I restriction enzyme prior to electrophoresis. The 340 bp uncut amplicon reveals *CYP1A1wt/wt* homozygous wild type, homozygous mutant *CYP1A1*2A/*2A* should show two fragments (200 and 140 bp) while the heterozygous genotype *CYP1A1wt/*2A* presents three fragments: the uncut 340 bp fragment and two restriction fragments of 200 and 140 bp. **B:** genotyping results of four samples for *CYP2E1*. The amplicon (327 bp) is subjected to digestion with *Dra*I prior to electrophoresis. The homozygous normal genotype *CYP2E1wt/wt* presents two fragments 248 and 79 bp. The mutant heterozygous *CYP2E1wt/*6* shows three fragments (327, 248, 79bp). The *CYP2E1*6/*6* genotype shows only the 327 bp

Table 2. Statistical analysis and distribution of *CYP2E1*6* genotypes (*Dra*I, 7632t>A, rs6413432) and allelic frequency (percentage) for controls and prostate cancer patients. N, number of cases; OR, odds ratio; p, Fisher's exact p; CI, confidence interval; wt, wild-type allele for the investigated polymorphism.

CYP2E1 genotypes and alleles	Prostate cancer				Controls
	N (%)	OR	95% CI	P-value	N (%)
CYP2E1 wt/wt	38 (95%)	0.97	0.65-21.96	0.158	77 (91.7%)
CYP2E1 wt/*6	1 (2.5%)	0.29	0.03-2.44	0.227	7 (8.3%)
CYP2E1*6/*6	1 (2.5%)	0.97	0.93-1.02	0.158	0 (0%)
CYP2E1 wt/*6 and*6/*6	2	0.58	0.11-2.92	0.504	7
CYP2E1 wt/wt and wt/*6	39	0.97	0.93-1.02	0.146	84
CYP2E1*6	3 (3.8%)	0.89	0.23-3.56	0.876	7 (4.2%)

was significantly higher among patients than controls (21.25 % vs.9.2%, *P*=0.016, OR=2.7, 95% CI: 1.18-6.07). Therefore, the allele *CYP1A1*2A* increases the risk of PCa by 2.7-fold. However, the difference of *CYP1A1*2A/*2A* genotype distribution between cancer patients (10%) and healthy controls (3.3%) was not statistically significant (*P*= 0.658, OR=1.5, 95% CI: 0.22-11.08). Similarly, the distribution of the *CYP1A1*2A/wt* genotype in patients was not statistically different from that in controls (*P*= 0.105, 22.5% and 11.7%, respectively) with an OR of 2.4 (95% CI: 0.81-7.24). Nevertheless, the difference was significant when both *CYP1A1*2A/*2A* and *wt/*2A* genotypes were considered together as one group and compared to the *CYP1A1 wt/wt* (*P*=0.038, OR=2.7, 95% CI: 1.04-7.19) (Table 1).

CYP2E1 genotyping

Typical *CYP2E1* genotyping results are illustrated in Figure 1. The distribution of *CYP2E1* genotypes and allelic frequencies (percentages) among the tested subjects and the statistical analyses are detailed in Table 2. The percentage of the *CYP2E1*6* allele was 3.8% in patients and 4.2% in controls, this difference was not statistically

significant (*P*=0.876, OR=0.89, 95% CI:0.23-3.56). Only one individual was found to be homozygous for the variant allele (*CYP2E1*6/*6*) in this investigation. The distribution of *CYP2E1*6/wt* genotype was 2.5% in patients and 8.3 % in controls but this difference was not statistically significant (*P*=0.227, OR=0.29, 95% CI: 0.03-2.44). The difference remained non-significant when both genotypes *CYP2E1*6/wt* and *CYP2E1 wt/wt* were considered together as one group (*P*=0.146, OR= 0.97, 95% CI: 0.93-1.02).

Investigation of genotypes combination for *CYP1A1 and CYP2E1*

The genotype combinations were investigated for the two polymorphic sites in the two investigated genes. Only one control individual among the tested subjects carried the variant alleles (*CYP1A1*2A and CYP2E1*6*) for both genes simultaneously.

DISCUSSION

Although PCa is one of the most common cancers in Lebanon (Shamseddine et al., 2010), its etiology, particularly the genetic risk factors, have never been investigated in this country. The present study was focused on *CYP1A1* and *CYP2E1* that play key roles in carcinogen bioactivation (Bartsch et al., 2009), and whose polymorphisms were associated with several cancer types in diverse populations. Particularly, the alleles *CYP1A1*2A* and *CYP2E1*6* have been reported as risk factors in diverse cancers in several populations (Shin et al., 2007; Syamala et al., 2010; Sivaraman et al., 1994; Rossini et al., 2007). Lebanese are exposed to carcinogens bioactivated by these enzymes especially from smoking source (Waked et al., 2012).

Our results showed a statistically significant association of the allele *CYP1A1*2A* with PCa in the tested population (2.7 times higher risk to develop PCa). Genotype distribution comparison of the group enclosing heterozygous (*CYP1A1*2A/wt*) and homozygous mutant (*CYP1A1*2A/*2A*) with the group of homozygous normal (*CYP1A1 wt/wt*), showed that individuals carrying at least one allele *CYP1A1*2A* are at higher risk (2.7-fold, *P*= 0.038) to develop PCa (Table 1). However, no significant difference was found in the genotype distribution when each genotype is considered as one category. The association of *CYP1A1* m1 allele (6235T>C) with PCa reported here is in agreement with previously reported results in other countries such as Japan (Murata et al., 1998, Suzuki et al., 2003) and India (Shaik et al., 2009) as well as with results of a meta-analysis among Asians (Vijayalakshmi et al., 2005) and Chileans (Acevedo et al., 2014). However, our results disagree with those reported by the meta-analysis of Li et al., 2012.

Role of other CYP1A1 polymorphisms in PCa development was also evidenced by studies on a Chinese population where the m2 allele (Ile462Val, 2455A>G) increases the risk of smoking-associated risk of PCa (Yang et al., 2006). Therefore, our results are in agreement with the studies that confirm a significant role of *CYP1A1* gene polymorphism in PCa development (Holt et al., 2013 and others), and show a role for this gene in PCa etiology in Lebanon where PAH occurrence as environmental pollutants has been shown by several reports, especially from a smoking source (Waked et al., 2012; El-Zein et al., 2007; Monzer et al., 2008; Saade et al., 2010).

CYP1A1 is clearly among the key genes in cancer development. In fact, its genetic polymorphisms were associated with other types of cancer such as the association of *CYP1A1*2A* (3801T>C), *CYP1A1*2C* (2455A>G), and *CYP1A1*4* (2453C>A) with lung cancer in an Australian population (Wright et al., 2010), as well as in an Asian (*CYP1A1*2A*, 6235T>C) (Lee et al., 2008), African-American (*CYP1A1*2A*, 6235T>C) (Taioli et al., 1998), and Indian (*CYP1A1*2A*: 6235T>C) (Ihsan et al., 2011) populations. Another genetic polymorphism of *CYP1A1* (4887C>A) was associated with endometrial cancer in an Italian population (Esteller et al., 1997), and with cervical cancer in a Caucasian population (*CYP1A1*2A*, 6235T>C)(Sergentanis et al., 2012), and with colorectal cancer among Chinese (*CYP1A1*2C*: 2455A>G)(Zheng et al., 2012).

In disagreement with our finding, and despite the key role of *CYP1A1* in carcinogen bioactivation, a lack of association was reported between its polymorphism and PCa in Tunisia (Souiden et al., 2012). Moreover, *CYP1A1*2A* allele was neither associated with breast cancer in Canada (Ashley-Martin et al., 2012), nor with leukemia in India (Suneetha et al., 2011), nor with lung cancer in Brazil (Honma et al., 2009), nor with bladder cancer in India (Srivastava et al., 2008), nor with gastrointestinal cancer in Lebanon (Darazy et al., 2011). The lack of association in these studies could be due to an interaction among other genetic and/or environmental factors.

Regarding *CYP2E1*6*, the allelic frequency among the controls (4.2%) was lower than that previously reported (6.3%) (Zgheib et al., 2010). This discrepancy could be due to the difference in the analyzed population size. In the present study, no association was found between *CYP2E1*6* and PCa. The number of homozygous mutant individuals (*CYP2E1*6/*6)* was very low in the studied population (Table 2) which limited the possibility to conclude regarding the statistical significance despite a clear difference in the genotypes distribution. The lack of association with PCa reported here contradicts previous studies in Portugal (Ferreira et al., 2003) and China (*CYP2E1* 1259G>C)(Yang et al., 2006; Yang et al., 2009). However, our result is in agreement with that of (Murata et al., 2001) in a Japanese population (allele *CYP2E1* -1293G>C).

Several previous studies emphasized the important role of *CYP2E1* polymorphisms in cancer etiology such as esophageal cancer in Kazakh (*CYP2E1*, 677C>T) (Qin et al., 2008) and Kashmiri populations (*CYP2E1*, 1901C>T) (Malik et al., 2010), and colorectal cancer in China (Yang et al.,

2009). *CYP2E1*6* (7632T>A) was associated with gastric cancer (Kato et al., 2011) and liver cancer in Japan (Kato et al., 2003), with cancer of the mouth in China (Niu et al., 2012). However, *CYP2E1*6* (7632T>A) was not associated with gastrointestinal cancer in Lebanon (Darazy et al., 2011) nor with bladder cancer in Spain (Cantor et al., 2010). These conflicting results could be due to a potential interaction among the many genetic and environmental factors involving this gene which plays a key toxicological role.

In conclusion, the present work demonstrated an association between the *CYP1A1*2A* allele and PCa in Lebanon, but there were no significant association with the *CYP2E1*6* allele. Genetic polymorphisms of drug metabolizing enzymes are probably major factors involved in PCa etiology in Lebanon. Lack of significance for *CYP2E1*6* result could be due to the low number of patients. Increasing the patients number and investigating other genes should be done to better estimate the genetic risk factors of PCa.

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Conflict of interest

No conflict of interest exits in the submission of this manuscript.

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