RESEARCH ARTICLE

Lack of Association between *CYP1A1 M2* and *M4* Polymorphisms and Breast Carcinoma in Jordanian Women: a Case-Control Study

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Abstract

Background: *CYP1A1* is a candidate gene for low-penetrance breast cancer susceptibility, as it plays an important role in the metabolism of carcinogens and estrogens. Purpose: The objective of this study was to assess the association between *M2 (A2455G, Ile462Val)* and *M4 (C2453A, Thr461Asn)* polymorphisms in *CYP1A1* and breast cancer risk among Jordanian women and in subgroups stratified by menopausal status and smoking history. Materials and Methods: Blood samples were collected from 112 breast cancer female patients and 115 age-matched controls who underwent breast cancer screening with imaging and showed negative results (BI-RADS I or BI-RADS II). Genotyping was performed using the PCR-RFLP technique. Results: No statistically significant overall association was found between breast cancer risk and *CYP1A1 M2* genotypes (p= 0.55; OR = 0.77; 95% CI= 0.32 - 1.83) nor with the *M4* polymorphism (p= 0.95; OR= 0.95; 95% CI= 0.51- 1.88). Analysis of subgroups defined by menopausal status or smoking history also revealed no association with these polymorphisms. Furthermore, the four identified haplotypes (AC; AA; GC and GA) were equally distributed among cases and controls, and haplotype analysis showed a strong linkage disequilibrium of both studied loci in either cases or controls (D'=1). Conclusions: Based on the study results, *CYP1A1 M2* and *M4* polymorphisms do not seem to play a major role in breast cancer risk among Jordanian females.

Keywords: *CYP1A1*, breast cancer, genotype, genetic polymorphism, SNP, Jordanian women, menopause, smoking.

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Introduction

Breast cancer is the most common female malignancy worldwide. According to the latest statistics in 2010, breast cancer ranked first among cancers in Jordanian women accounting for 37.4% of all female cancers (Tarawneh et al., 2010). Breast cancer is both genetically and histopathologically heterogeneous and the mechanisms underlying breast cancer development remain poorly understood (Stuckey, 2011). Genetic studies have identified and confirmed four rare high-penetrance genes (*BRCA1, BRCA2, TP53 and PTEN*), four rare moderate-penetrance genes (*CHEK2, ATM, BRIP1 and PALB2*), and more than 20 common low-penetrance variants in 19 genes or loci that contribute to a woman's risk of breast cancer (Zhang et al., 2011; Huo et al., 2012). Low penetrance genes pose a low risk at the individual level but are more common in general population. Modulated by environmental exposure and lifestyle factors, these genes account for most sporadic breast cancer cases and are likely to explain the majority (90-95%) of all breast cancer cases (Weber and Nathanson, 2000). *CYP1A1* is a candidate gene for low-penetrance breast cancer susceptibility because it plays a dual role, both in the phase I metabolism of carcinogens such as polyaromatic hydrocarbons (PAHs) (Shimada and Fujii-Kuriyama, 2004; Hodek et al., 2013) and in the oxidative metabolism of estrogens (Tsuchiya et al., 2005). The reactive intermediates resulting from these reactions may bind to DNA and form DNA adducts that may eventually produce mutations and trigger carcinogenesis (Firozi et al., 2002; Henkler et al., 2012). PAHs have been found to cause mammary tumors in rodents (Cavalieri et al., 1988) and estrogen is a known causal factor in breast cancer (Clemons and Goss, 2001; Tworoger et al., 2014). Catechol metabolites of estrogen may be causal as well. In humans, cytochrome P450 (CYP) 1A1 is responsible for extrahepatic 2-hydroxyaltion of estradiol (Spink et al., 1992). The resulting metabolite is devoid of estrogenic activity and the 2-methoxy derivatives are shown to possess anti-proliferative and anti-carcinogenic properties (Zhu and Conney, 1998). *CYP1A1* gene is located on chromosome 15q22-q24; it spans 5,987 base pairs;

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comprises seven exons and six introns and encodes for a 512 amino acid protein (Kawajiri et al, 1986). *CYP1A1* is highly polymorphic in humans. Four polymorphisms have been extensively studied and differences in the distribution of these polymorphisms among the different ethnicities and populations have been reported (Cosma, et al., 1993). The *M1* polymorphism (T3801C) is located in the 3'-noncoding region giving rise to a *MspI* restriction site. The *M2* polymorphism is the replacement in position 2455, on the exon 7, of an adenine (A) by a guanine (G). This leads to the replacement of isoleucine (Ile) by valine (Val) on amino acid 462, in the heme binding region of the cytochrome P450 (Giri et al., 2012). The polymorphism *M3* (T3205C) is the creation of another site of restriction *MspI* in the 3'-non-coding region and is specific of African-Americans. The polymorphism *M4* has not been studied extensively. It is located in position 2453, adjacent to the *M2* polymorphism. The *M4* polymorphism corresponds to the substitution of a cytosine (C) by an adenine (A) which leads to the loss of restriction site BsaI and is translated in the protein sequence by a replacement of a threonine (Thr) by aspargine (Asn) on the codon 461 (Sergentanis and Economopoulos, 2009). It is conceivable that the change in the protein sequence associated with *M2* and *M4* polymorphisms could result in change in the enzyme activity (Cosma et al., 1993; Crofts et al., 1994). Functional significance of the different *CYP1A1* genotypes has been studied with inconclusive results. One study has found an increased inducibility of mRNA and 3 fold increase in enzyme activity associated with the *462Val* variant allele (Crofts et al., 1994). This latter has also been found to increase lymphocyte *CYP1A1* enzyme activity in two other studies (Cosma et al., 1993; Kiyohara et al., 1996). However, several studies showed no change in enzyme function (Zhang et al., 1996; Persson et al., 1997)

CYP1A1 polymorphisms and breast cancer association studies have raised conflicting results. A significant overall increased risk associated with *462Val* allele was observed among Asian (Chacko et al., 2005; Surekha et al., 2009; Saadatian et al., 2014), Caucasian (Zhang et al., 2004) and Mexican women (Martinez-Ramirez et al., 2013). Similarly, an increased risk in association with *461Asn* variant allele was observed among French-Canadian Caucasian women (Krajinovic et al., 2001). However, a reduced risk was observed among Asian women in several studies (Miyoshi et al., 2002; Boyapati et al., 2005; Shin et al., 2007; Singh et al., 2007) and Caucasian women (Hefler et al., 2004). While all these studies have shown strong association between *CYP1A1* polymorphisms and breast cancer risk, others have indicated no association (Rebbeck et al., 1994; Bailey et al., 1998; Basham et al., 2001; Sillanpaa el al., 2007; Kiruthiga et al., 2011; Petchkovskiy et al., 2014; Sun et al., 2015).

The aim of this work was to investigate the association between *M2* and *M4* polymorphisms of *CYP1A1* and in Jordanian women. A secondary objective was to assess the association between *M2* and *M4* polymorphisms in *CYP1A1* and breast cancer risk in subgroups stratified by menopausal and smoking status.

Materials and Methods

Subjects

This multi-center case-control study was conducted in three major hospitals in Amman, the capital of Jordan: Jordan University Hospital (JUH), King Hussein Cancer Center (KHCC) and Jordan Hospital (JH). The study was approved by the Institutional Review Board (IRB) of these centers and written consent form was obtained from each participant. One hundred and twelve consecutive breast cancer female patients who were on their active follow-up were recruited from both Hematology/Oncology and Breast Surgery Clinics at JUH, as well as Breast Surgery Clinic at KHCC. All Jordanian patients with breast cancer were considered eligible (newly diagnosed cases, cases diagnosed years back and recurrent cases). Patients were age-matched with 115 female controls with no previous or present cancer history. All control subjects were selected during breast cancer screening programs and therefore have undergone mammography with or without breast ultrasound and had negative results (BIRADS I and BIRADS II). Controls were recruited from Breast Imaging Departments at JUH, KHCC, and JH. All blood samples were collected in the period between July 2010 and February 2011 in a 3ml EDTA anti-coagulant vacutainer tube under sterile conditions and were stored at 4°C for one week at maximum or were frozen at -20°C until DNA extraction was performed. Since all women approached except 25 agreed to participate in the study, the response rate was 90%. During a face to face interview detailed information was obtained on demographic factors, reproductive and breastfeeding history, hormone use, age at breast cancer diagnosis if relevant, history of malignant diseases, dietary habits, physical activity, tobacco and alcohol use, and family history of breast cancer. Most of the participants had their waist and hip circumferences measured. The body mass index (BMI) was also recorded. Clinical history including the size of the tumor, presence of axillary lymph nodes, extent of metastasis (stage), histopathological type of the tumor, hormone receptor and HER-2 status was obtained from the patient file.

Genotyping

Genomic DNA specimens of patients and controls were extracted from buffy coat fractions (of fresh samples or samples stored at 4°C) using Wizard Genomic DNA purification Kit (Promega, USA) following the manufacturer's protocol. For frozen samples (almost 20 samples), extraction from the whole blood was performed using NucleoSpin® Blood DNA purification Kit (Macherey Nagel, Germany) according to the manufacturer's protocol. DNA samples were stored at -20°C. DNA quality and quantity were assessed using spectrophotometry. For determination of *M2* polymorphism in *CYP1A1* gene, a 204bp DNA fragment was amplified and restricted using PCR-RFLP based assay. The primers used were: *M2*F 5'-CTGTCTCCCTCTGGTTACAGGAAG-3'(NC_000015.10:74720794-74720771) and *M2*R 5'-TTCCACCCGTTGCAGCAGGATAGCC-3' (NC_000015.10:74720591-74720615) (Cascorbi et al., 1996). The PCR reactions were performed in a PTC- 100

Peltier thermal cycler (MJ Research Inc, USA) and target DNA was amplified in a 50μl of PCR mixture containing 2μl (of genomic DNA, 0.3μl Taq polymerase, 1μl dNTP Mix, 10μl 5x Taq polymerase green buffer, 1μl of each primer and 2μl $MgCl_2$ and finally Nuclease free water up to 50μl. The reaction mixture was initially denaturated at 94°C for 5 minutes followed by 40 cycles of 94°C for 30 seconds, 55°C for 1 minute and 72°C for 30 seconds. The reaction was completed by a final extension at 72°C for 5 minutes.

Digestion of PCR products (204 bp) with BsrDI (New England Biolabs, USA) at 65°C overnight, allowed the distinction between the fragments with wild-type allele (*462Ile*) by giving 2 fragments (149 bp and 55 bp) and the fragments with *462Val* variant allele which remains undigested. *M4* polymorphism could be determined from the same 204-bp fragment but using another restriction enzyme BsaI (New England Biolabs, USA) at 37°C overnight.

PCR products are digested into 2 fragments (139 bp and 65 bp) in case of wild-type allele (*461Thr*) and remain intact in case of *461Asn* variant allele. The restricted products were analyzed by electrophoresis in 4% agarose gel containing ethidium bromide.

Haplotype analysis

The interaction between genetic polymorphism at the two loci was assessed by haplotype analysis. We analyzed haplotype frequencies of the two SNPs for breast cancer cases and compared them with those of controls. Haplotype frequencies were calculated using Golden Helix Tree® software and linkage disequilibrium was represented by D prime (D'). The Golden Helix software enables to estimate haplotype frequencies even with some missing data of one or both SNPs. Similar findings were obtained utilizing Multiallelic Interallelic Disequilibrium Analysis Software (University of Southampton, Highfield, Southampton, UK) (PMID: 16643648).

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) software (SPSS Inc, USA) version 17.0.The association between *CYP1A1 M2* and *M4* polymorphisms and breast cancer risk was analyzed by calculating the crude odds ratios (OR) and 95% confidence intervals (95% CI) using Chi-square

Table 1. Selected Characteristics of Study Participants

Characteristics	Cases (N=112) %[a] or Mean±Sd	Controls (N=115) % or Mean ±Sd	*P* Value: Cases vs. Controls
Age	50.18±9.43	48.03±8.21	0.69
Educational level			
Illiterate / elementary school	18.7%	6.1%	0.009
Middle and High school	27.7%	38.3%	
College or higher	53.6%	55.6%	
Family monthly income			
<250JD[b]	25%	10.4%	0.004
>250JD	75%	89.6%	
Age at menarche			
<14	55.5%	66.7 %	0.76
≥14	44.5 %	33.3 %	
Regular period	83%	87%	0.39
Menopausal status			
Pre-menopausal	67.9 %	63.5%	0.88
Post-menopausal	32.1 %	36.5%	
Age at menopause	44.44±9.07	46.50±7.29	0.30
Parous	81.3%	88.7%	0.12
Age at first delivery (if parous)	24.55±5.42	23.35±5.14	0.12
Breastfeeding	77.7%	83.4%	0.27
Family history of breast cancer in the first degree relatives	14.3%	8.7%	0.19
Direct hit to the breast	17.9 %	8.7%	0.001
Body size/ Lifestyle			
BMI [c]	28.65±5.41	27.7±4.34	0.15
WHR[d]	0.87±0.07	0.85±0.10	0.07
Ever smokers	16.1%	25.2%	0.09
Number of pack-years (if smoker)e	11.94±7.62	9.52±8.87	0.34
Physical activity in the last 5 years[f]	40.2%	62.6%	0.001
Occasional alcohol intake	6.3%	11.3%	0.179
High fat intake	28.6%	21.7%	0.23
Exogenous hormone use			
Ever used OCs [g]	44.6%	49.6%	0.458
Years of OCs use among users	3.87 ±4.06	2.90±3.59	0.19
Years of HRT use among users	2.80±5.04	2.86±3.55	0.98

a: Valid percentage (There are missing data). **b**: Jordanian Dinar. **c**: Body mass index. **d**: waist to hip ratio. **e**: Number of pack years = (number of cigarettes smoked per day x number of years smoked)/20 (1 pack contains 20 cigarettes). **f**: Physical activity in the last 5 years for controls and the 5 years prior to diagnosis for patients. **g**: Oral contraceptives, ever users are defined as women who used oral contraceptives for a duration equal or longer than 1 month. Only *p* values for statistically significant differences are highlighted in bold

test. Individual demographic and clinical factors were also examined for their relations to breast cancer using Chi-square test for categorical variables. The independent-sample t-test was used for the continuous variables. Based on the data from previous studies, homozygotes for the *CYP1A1 462Ile* and *461Thr* alleles were chosen as the reference categories in all separate analyses for these loci,. In a separate analysis, we also combined *462ValVal* homozygotes with the *462Ile/Val* heterozygotes and *461Asn/Asn* homozygotes with *461Thr/Asn* heterozygotes to increase the statistical power. Subgroup analysis was carried out separately for premenopausal and postmenopausal women and for the ever smokers and never smokers, respectively, to explore the potential modifying effect of non-genetic factors in the *CYP1A1 M2* and *M4*-breast carcinoma association. Women were considered to be postmenopausal if they had reported natural menopause determined as cessation of menstruation for at least 12 months, or had undergone bilateral oophorectomy. Women that were hysterectomized with intact ovaries (ovary) or for whom details of the operations were unknown were classified as postmenopausal if they were no longer menstruating and were older than 51 years or had used hormone replacement therapy. All the rest were classified as premenopausal (Sillanpaa et al., 2007). In breast cancer patients, criteria for menopausal status were applied to the age of diagnosis rather than to the age at the encounter, i.e., if the patient was still menstruating at the time of diagnosis, she was considered to be pre-menopausal. The patient was considered to be post-menopausal if she had experienced menopause before the date of diagnosis, and the cessation of menstruation has been for one year or more. Age at menopause was set to age at which menstrual periods ended or age of first use of hormone therapy, whichever came first. We also evaluated deviation from Hardy-Weinberg equilibrium for each SNP.

Results

The demographic characteristics and the putative risk factors of breast cancer investigated in this study are listed in Table 1. The mean age of breast cancer patients (50 years) did not statistically differ from that of controls (48 years, p=0.69). No significant difference was observed between cases and controls in terms of reproductive factors, exogenous hormone intake, smoking history, alcohol and dietary fat intake and lifestyle factors. However, controls had a higher family income (p=0.004), a higher educational level (p=0.009) and tended to be more physically active (p=0.001) than patients. Moreover, women with breast carcinoma frequently reported direct hit (physical trauma) to the breast (p=0.001). About one-third of patients in both groups were postmenopausal (p=0.88). Most of the tumors were presented on the right breast (55.4%), with invasive ductal histology (75%), diagnosed at advanced stages (stage III-IV=50.4%), estrogen and progesterone receptor positive (66%), and HER-2 negative (54.3%).

The distribution of the *M2* and *M4* genotypes is shown in Table 2. All genotype frequencies were consistent with expectations from Hardy-Weinberg equilibrium in both: patients (χ^2=0.10; p=0.75) and controls (χ^2=0.087; p=0.77).

Association of M2 (Ile462Val) polymorphism with breast cancer risk

Frequencies of *M2 462Ile* and *462Val* alleles were 0.956 and 0.044, respectively, among cases and 0.944 and 0.056, respectively, among control subjects. Frequencies of *M2* (*462Ile/Ile*), (*462Ile/Val*), and (*462Val/Val*) for patients group (0.910, 0.893, 0 respectively) were not significantly different (p=0.55) from those in the control group (0.884, 0.104, and 0.008 respectively). When compared with *M2* (*462Ile/Ile*) homozygous genotype carriers, carriers of the variant allele (*M2* (*462Ile/Val*), and *M2* (*462Val/Val*)) had a non-significant 23% reduced risk of breast cancer (OR=0.77, 95% CI 0.32-1.83).When stratified by menopausal status, the distribution of *M2* genotypes in cases did not significantly vary from that of the control group (Table 3). Similarly, smoking history

Table 2. Association between *CYP1A1 M2* and *M4* genotypes and breast cancer risk

Genotype	Cases n (%)	Controls n (%)	OR (95% CI)	P value
CYP1A1 M2 (Ile462Val)				
Ile/Ile	102 (91.07)	102 (88.4)	1.00	0.55
Ile/Val	10 (8.93)	12 (10.43)	-	
Val/Val	0 (0)	1 (0.87)	-	
Ile/Val, +Val/Val	10 (8.93)	13 (11.6)	0.77 (0.32-1.83)	
CYP1A1 M4 (Thr461Asn)				
Thr/Thr	90 (80.4)	92 (80.0)	1.00	0.95
Thr/Asn	21 (18.7)	22 (19.1)	-	
Asn/Asn	1(0.9)	1 (0.9)	-	
Thr/Asn, + Asn/Asn	22 (19.6)	23 (20.0)	0.95 (0.51-1.88)	

Table 3. Association Between *CYP1A1 M2* and *M4* Genotypes and Breast Cancer According to Menopausal Status

Genotype	Premenopausal women n (%)		OR (95%CI)	Postmenopausal women n (%)		OR(95%CI)
	Cases	Controls		Cases	Controls	
CYP1A1 (Ile462Val)						
Ile/Ile	69 (88.5)	68 (86.1)	0.8 (0.31-2.06)	33 (97.1)	34 (94.4)	0.51 (0.04-5.96)
Ile/Val+Val/Val	9 (11.5)	11 (13.9)		1 (2.9)	2 (5.6)	
CYP1A1(Thr461Asn)						
Thr/Thr	63 (80.8)	66 (83.5)	1.21 (0.53-2.74)	27 (79.4)	26 (72.2)	0.67 (0.22-2.04)
Thr/Asn + Asn/Asn	15 (19.2)	13 (16.5)		7 (20.6)	10 (27.8)	

Table 4. Association between *CYP1A1 M2* and *M4* Genotypes and Breast Cancer According to Smoking History

Genotype	Never smokers n (%)		OR (95%CI)	Ever smokers n (%)		OR(95%CI)
	Cases	Controls		Cases	Controls	
CYP1A1 (Ile462Val)						
Ile/Ile	84 (89.4)	77 (89.5)	1.09 (0.39-2.64)	18 (100)	25 (86.2)	0
Ile/Val+Val/Val	10 (10.6)	9 (10.5)		0 (0)	4 (13.8)	
CYP1A1(Thr461Asn)						
Thr/Thr	74 (78.7)	71 (82.6)	1.28 (0.61-2.69)	16 (88.9)	21 (72.4)	0.33 (0.06-1.76)
Thr/Asn + Asn/Asn	20 (21.3)	15 (17.4)		2 (11.1)	8 (27.6)	

Table 5. Association between *CYP1A1 M2 / M4* Haplotypes and Breast Cancer Risk

M2/M4 Haplotype	Cases	Control
AC	161	162
AA	18	19
GC	8	10
GA	1	1
D'	1	1
r2	0.00535	0.00755

had no impact on the relation of *M2* genotypes to breast cancer (Table 4).

Association of M4 (Thr461Asn) polymorphism with breast cancer risk:

Frequencies of *461Thr* and *461Asn* alleles were 0.897 and 0.103, respectively, among cases and 0.896 and 0.104, respectively, among control subjects. Among 112 breast cancer 80.4%, 18.7% and 0.9% were homozygote wild type (*461Thr/Thr*), heterozygote variant type (46*Thr/Asn*), and homozygotes variant type (*461Asn/Asn*) respectively. The distribution of these genotypes among the 115 controls was 80.0%, 19.1% and 0.9% respectively (Table 2). No statistically significant association was observed between the *M4* (*Thr461Asn*) polymorphism and breast cancer risk (*p-val*ue=0.95, OR=0.95; 95% CI=0.51-1.88).

CYP1A1 haplotypes:

Four different haplotypes appeared in our analysis. The most frequent haplotypes were AC (*M2*-A and *M4*-C) (cases: 85.7%; controls: 84.1%), followed by AA (cases: 9.8%; controls: 9.8%) and GC (cases: 4%; controls: 5.5%), while the rarest haplotype was GA (cases: 0.46%; controls: 0.63%) (Table 5). Our results indicated that the two loci *M2* and *M4* show relatively strong linkage disequilibrium (Lewontin's coefficient [D']) (Controls: D'=1; Cases: D'=1). None of the haplotypes was associated with breast cancer risk.

Discussion

In the current study, we investigated the relation between the two genotypes of *CYP1A1*, *M2* (*Ile462Val*) and *M4* (*Thr461Asn*) and breast cancer risk in Jordanian female breast cancer patients compared to age-matched Jordanian female controls. To the best of our knowledge, this is the first study among Jordanian women assessing the interaction of both *M2* and *M4* genetic polymorphisms and breast cancer. This is also the first case-control study that used high accuracy in selecting control participants, as all of the controls have undergone mammography with or without breast ultrasound and have been proven to be breast malignancy-free. In addition, our study population is ethnically homogenous (Caucasians) which minimizes potential bias due to population stratification. The cooperation rate was 90%. Thus, the cases can be viewed as representing unselected typical breast cancer patients from Amman area. However, it should be kept in mind that people who agree to participate in a study tend to be different from the remaining population regarding their demographic and lifestyle factors (Sillanpaa et al., 2007).

A wide ethnic variation in the distribution of *M2* and *M4* polymorphisms was observed, with the *462Val* variant allele being more common in Asians (30%) (Huang et al., 1999) than in Caucasians (3-10%) (Ambrosone et al., 1995; Zhang et al., 2004; Hefler et al., 2004). An intermediate value was observed among African-Americans (21%) (Le Marchand et al., 2005). In the present study the frequency of the *462Val* variant allele (4.46% among cases and 5.67% among controls) is in accordance with that of Caucasians. Regarding the *461Asn* variant allele there is little data available on its distribution in different ethnic populations. Our results show a higher frequency (10.27 %, 10.43% among cases and controls respectively) compared with the other studies in Caucasians (2.0 - 7.52%) (Mrozikiewicz et al., 1997; Krajinovic et al., 2001; Sillanpaa et al., 2007).

The present study showed the lack of significant overall association between the *M2* and *M4* polymorphisms and breast cancer risk in Jordanian women. No association was also observed in premenopausal or postmenopausal subgroups. Our findings are in good agreement with several studies (Rebbeck et al., 1994; Bailey et al., 1998; Ishibe et al., 1998; Basham et al., 2001; Sillanpaa et al., 2007 and Kiruthiga et al., 2011; Sun et al., 2015). However, reduced risk of breast cancer was observed among postmenopausal Asian *462Val* allele carriers, including Japanese (OR=0.66; 95% CI=0.45-0.96) (Miyoshi et al., 2002), North Indian (OR=0.33; 95% CI=0.12-0.89) (Singh et al., 2007) Chinese women who are homozygous for both *CYP1A1 M1* and *M2* variant alleles (OR=0.43; 95 CI=0.19 - 0.99) (Boyapati et al., 2005), and, finally, Korean women only when the *M1* and *M2* variant alleles were combined together (OR =0.59; 95% CI =0.43- 0.80) (Shin et al., 2007). On the contrary, a significant increased risk of breast cancer was observed among Caucasian women with at least one *462Val* variant allele (OR=3.6; 95% CI =1.5 - 8.2) (Zhang et al., 2004), among Asian women in North India (OR=2.08; 95% CI=1.45-3.03) (Surekha et al., 2009) and

in South Indian premenopausal women (OR=3.7; 95% CI=1.5-9.1) (Chacko et al., 2005). A recent meta-analysis has also found that the *462Val* allele increases the risk of breast cancer among homozygous Caucasian participants (Sergentanis and Economopoulos, 2009).

When taking smoking into account, no association between the *CYP1A1M2* and *M4* polymorphisms and breast cancer risk was observed in either smokers or non-smokers, being consistent with the findings from the other studies among Caucasian women (Krajinovic, et al., 2001; Li et al., 2004) . However, among other Caucasian populations, a significant association between the breast cancer risk and *CYP1A1 M2* (Ambrosone et al., 1995) and *M1* polymorphisms has been observed among smokers compared to non-smokers (Ishibe et al., 1998; Li et al., 2004)

Mild to moderate linkage disequilibrium between the *CYP1A1 M1*, *M2* and *M4* variants has been reported (D': 0.19-0.37) (PMID: 24772967). Our haplotype analysis revealed strong linkage disequilibrium for the two SNPs (*M2* and *M4*). Very few studies evaluated haplotypes of *CYP1A1* in relation to breast cancer risk. Haplotype analysis of the two loci showed that the CA haplotype was associated with lowest risk of breast cancer among Korean women (Shin et al., 2007). In a study that was conducted in USA there was the suggestion of a difference in breast cancer risk by race in relation to the *CYP1A1* haplotype. However, the relation did not remain significant after adjustment for multiple testing (Reding, et al., 2012). In the current study, there were no significant differences in haplotype frequencies between controls and breast cancer cases.

The discrepancies in association of *CYP1A1M2* or *M4* variant genotypes and breast cancer risk in different studies may be explained by several factors including ethnic differences in the frequency of the genetic polymorphism, diet, environmental exposure and number of subjects included in each study.

In summary, there is no overall association between *CYP1A1 M2* and *M4* polymorphisms and breast cancer among Jordanian females, even after stratifying participants by menopausal status or smoking habits. Additional studies involving larger number of participants are needed to confirm or refute these findings.

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