# Role of *CYP1A2* polymorphisms in breast cancer risk in women

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**Abstract.** Cytochrome *P4501A2* (*CYP1A2*) is a key enzyme in the etiology of breast cancer (BC). It is involved in breast carcinogen activation [aromatic (AAs) and heterocyclic amines (HAs), polycyclic aromatic hydrocarbons (PAHs)], in the production of beneficial oestrogen [2-hydroxyestrone (2-OHE1)] and in converting arachidonic acid (AAc) to epoxyeicosatrienoic acids (EETs), which have anti-inflammatory properties. Within a hospital-based case-control study, the effect of functional CYP1A2 variants [*-3860G/A* (rs2069514), *-2467T/delT* (rs3569413), *-163C/A* (rs762551)] and their interactions with environmental factors in BC risk was investigated. The study population included 125 BC cases and 43 non-cancer controls. Genotyping was performed in RT-PCR using Taqman assays. The gene-environment interaction was appraised using a case-only study design. We found that the *-3860A* variant, independently from environmental factors, as well as by interacting with fried foods (p=0.025) and indoor exposure to pollutants (p=0.050), reduced the risk of BC (p=0.025), whereas its interaction with coffee (p=0.045) increased the BC risk. This is the first study indicating that the *-3860A* variant, by decreasing CYP1A2 activity, modifies BC risk by interacting with environmental factors, thereby supporting the hypothesis that reduced CYP1A2 activity contributes to BC risk in different ways, for example, it may be protective by reducing the activation of pro-carcinogens such as AAs, HAs and PAHs, but would increase risk by reducing the beneficial formation of 2-OHE1 and EETs.

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*Abbreviations*: AAc, arachidonic acid; AA, aromatic amines; CYP1A2, cytochrome P4501A2; EETs, epoxyeicosatrienoic acids; HA, heterocyclic aromatic amine; PAH, polycyclic aromatic hydrocarbon

*Key words:* breast cancer, CYP1A2 polymorphism, gene-environment interaction, CYP1A2 inducibility, oestrogen hydroxylation, coffee intake, fried food, indoor exposure, breast inflammation

## Introduction

As in all ethnic groups, breast cancer (BC) is the most common cancer in women from Tunisia, although inflammatory BC is a Tunisian peculiarity (1). An increased BC incidence (age-standardized rate per 100,000 women) was observed: 16.7 in 1994 (2) to 29.2 in 2007 (3), which however, remains lower than that of developed countries (approximately 80) (2,3).

Human cytochrome P4501A2 (CYP1A2) is one of the major CYPs in the human liver and is a key enzyme, not only in the activation of the main suspected breast carcinogens, such as aromatic (AAs) and heterocyclic amines (HAs) and polycyclic aromatic hydrocarbons (PAHs), present in cigarette smoke and in fried and grilled meat (4,5), but also in hydroxylation of oestrogens (6,7) and in the metabolism of arachidonic acid (AAc) (8). AAc is involved in inflammation and breast carcinogenesis (9). Therefore, modulation of CYP1A2 activity may be important in the aetiology of BC.

Environmental and genetic factors influence the activity of CYP1A2. Tobacco smoking and consumption of fried and grilled food, coffee and cruciferous vegetables (i.e., broccoli-family) (10-15) increases CYP1A2 activity in humans. However, intake of apiaceous-like vegetables and the use of oral contraceptives decrease this activity (13). CYP1A2 activity is also modulated by specific polymorphisms in the *CYP1A2* gene (16-21). The *CYP1A2* polymorphisms located in the 5'-non-coding promoter region [*-3860G/A* (rs2069514), *-2467T/delT* (rs3569413)] and in intron 1 [*-163C/A* (rs762551)] of the *CYP1A2* gene modified CYP1A2 activity of smokers, measured by the urinary caffeine metabolic ratio (CMR) (16-21). The *-2467delT* polymorphism was also found by our group to be a significant risk modifier of smoking-induced lung and bladder cancer (22,23). A number of studies have reported the association between the *-164A/C* polymorphism and BC risk (24), however the results were inconclusive. To the best of our knowledge, no molecular epidemiological study has been published on the involvement of the other functional *CYP1A2* polymorphisms, such as *-3860G/A* and *-2467T/delT*, on the risk of BC.

In this study, we examined the role of functional *CYP1A2* polymorphisms (*-3860G/A*, *2467T/delT*, *-163C/A*) in modulating the relationship between exposure to environmental factors and risk for BC in Tunisian women. The aim was to identify new genetic characteristics that contribute to individual susceptibility to BC. The interaction was appraised by using a case-only study design (25,26).

## Materials and methods

*Subjects*. Study subjects included 125 women with histologically confirmed BC. All cases of BC were recruited from October 2007 until the end of 2008 at the Centre Hospitalier Universitaire 'Farhat Hached'. The control group collected in the same period comprised 43 ethnically and gender-matched healthy subjects. Controls were recruited from the occupational medicine service during their annual check-up. Trained interviewers informed all participants of the study objectives and collected personal data including coffee and tea consumption, alcohol drinking, job type, possible elevated non-occupational genotoxic-exposures, including smoking, diet, indoor and outdoor exposure, and consumption of vegetables by means of a structured questionnaire, as previously described (22,23). All of the information regarding participants was rendered anonymous following collection of data and blood samples. At recruitment, written informed consent was obtained from each study participant prior to interview and blood collection for genetic analyses. The Ethics Committee of the Hospital of Sousse approved the research study and the study has therefore been performed in accordance with the ethical standards of the 1964 Declaration of Helsinki. Individuals with dietary intake of genotoxins were those who reported consumption of grilled (PAHs) and fried meat (HAs); individuals with indoor exposure to pollutants were those who reported at least 1 of several exposure sources, including use of fireplace, coal or wood-stove as heating at home; or passive exposure to tobacco smoke, as previously described (22,23). Individuals with high vegetable consumption were those who reported a daily intake of tomatoes, onions and peppers, typical vegetables in a Mediterranean diet.

*Blood and DNA collection*. A sample (5 ml) of whole blood was collected from each subject in a K3ETDA vacutainer tube (violet cap). DNA was extracted from cells using protocols for genomic DNA isolation with the Promega Wizard genomic DNA purification kit (Promega, Italy). The extracted DNA was dissolved in 300 $\mu$l of TE buffer, divided in two aliquots and stored at -20˚C until shipping to the Department of Cardiological, Thoracic and Vascular Sciences, University of Padova (Italy), where quality and quantity control and genotype analyses were performed. DNA was free of RNA or protein contamination, as confirmed by the 260/230 and 260/280 nm absorbance ratios of DNA, which were always approximately 2.3 and 1.7, respectively, as previously described (20).

*Genotyping*. Genotyping was performed using commercially available Taqman drug metabolism genotyping assays: C__15859191_30 'rs2069514', C__60142977_10 'rs35694136', C___8881221_40 'rs762551' (Applied Biosystems, Foster City, CA, USA), as previously described (27). Briefly, reactions were set up according to the manufacturer's instructions and the samples were run on a Steponeplus Real-Time instrument (Applied Biosystems, Monza, Italy). Allelic discrimination was performed using the SDS software v2.3 (Applied Biosystems). The 25 $\mu$l reactions in 96-well plates included 12.5 $\mu$l TaqMan Universal PCR Master Mix, No AmpErase UNG (2X), 1.25 $\mu$l Drug Metabolism Genotyping Assay Mix (20X) and DNA 11.25 $\mu$l (1 ng/$\mu$l). Quality-control measures included validation of the results by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) genotyping and the blind repeat of 10% of samples for CYP1A2 polymorphic sites (*-3860 G/A*, *-2467T/delT* and *-163C/A*), as previously described (19). Briefly for the RFLP analysis, all PCR reactions (25 $\mu$l) were performed on a GeneAmp PCR System 9700 (Applied Biosystems, Monza, Italy), with each mastermix (Life Technologies, Monza, Italy) comprising 0.2 mM dNTPs, 1 unit of Taq polymerase, the appropriate concentration of $MgCl_2$ (1.75 and 1.25 mM) and 0.4 $\mu$M of each primer (Life Technologies). Variants -3860 G/A, -2467T/delT and -163C/A were identified using *Dde*I, *Nde*I and *Apa*I restriction enzymes purchased from New England Biolabs (Milan, Italy) (20).

*Statistical analysis*. Statistical comparisons were made between the cases and controls using Fisher's exact test. To investigate whether the genotype was in Hardy-Weinberg equilibrium, distribution of the observed and expected genotype frequencies were compared using a $\chi^2$ test. Pearson's $\chi^2$ test was used to test the association between cases and controls and genotype frequencies. The interaction of genotypes with the effects of environmental and dietary exposures on BC risk was evaluated by a case-only study approach and Pearson's $\chi^2$ test was used to test the association. Individuals with high vegetable consumption were those who reported a daily intake (≥20 servings/week) of tomatoes, onions and peppers, typical vegetables in a Mediterranean diet.

## Results

*Case-control study*. The characteristics of BC patients (n=125) and controls (n=43) are shown in Table I. All the subjects were non-smoking, non-alcohol-drinking and declared not to be exposed to passive smoke, therefore, these variables were not further considered. The variables considered were not homogeneously distributed in the study population (i.e., indoor and outdoor exposure to pollutants, intake of vegetables, fruits, daily coffee and tea, education). In particular, cancer cases were significantly older than the controls (P<0.0001), and consequently the majority of cases were postmenopausal (P<0.0001). Both cases and controls reported a high consumption of tomatoes, onions and peppers (from 5 to 7 or more servings of vegetables/week), while apiaceous (i.e., carrots) and cruciferous (i.e., cabbages) vegetables and fruits were consumed much less frequently. With the exception of one case, the women did not use oral contraceptives, therefore a potential factor influencing CYP1A2 activity (11) was absent.

Table II shows the observed and expected genotypes for *CYP1A2 -3860 G/A* (determined in 109 cases and 41 controls), *-2467T/delT* (determined in 117 cases and 42 controls) and *-163C/A* (determined in 108 cases and 38 controls). The expected genotype frequencies were not significantly different from the

Table I. Characteristics of the sample population.

| | Cases n=125 (%) | Controls n=43 (%) | P-value[a] |
|---|---|---|---|
| Gender | | | ND |
| Male | 0 (0) | 0 (0) | |
| Female | 125 (100) | 43 (100) | |
| Smoking status | | | ND |
| Current | 0 | 0 | |
| Never | 125 (100) | 43 (100) | |
| Alcohol drink | | | ND |
| Current | 0 | 0 | |
| Never | 125 | 43 | |
| Age (years) | | | <0.0001 |
| <40 | 22 | 28 | |
| 40-44 | 16 | 10 | |
| 45-49 | 15 | 1 | |
| 50-54 | 19 | 2 | |
| 55-59 | 17 | 2 | |
| 60-64 | 15 | 0 | |
| 65+ | 21 | 0 | |
| BMI | | | <0.0001 |
| Normal range (18.5-24.99 kg/m$^2$) | 46 | 21 | |
| Underweight (<18.50 kg/m$^2$) | 2 | 0 | |
| Overweight (25-29.99 kg/m$^2$) | 47 | 17 | |
| Obese (>29.99 kg/m$^2$) | 31 | 5 | |
| Menopausal status | | | <0.0001 |
| Yes | 108 | 0 | |
| No | 13 | 43 | |
| Fried food consumption | | | <0.0001 |
| Never | 0 | 0 | |
| <1 serving/week | 52 | 6 | |
| 1-3 servings/week | 73 | 23 | |
| >3 servings/week | 1 | 14 | |
| Grilled food consumption | | | <0.0001 |
| Never | 1 | 0 | |
| <1 servings/week | 78 | 43 | |
| 1-3 servings/week | 44 | 0 | |
| >3 servings/week | 1 | 0 | |
| Indoor exposure | | | ND |
| Passive smoke exposure | 125 | 43 | |
| Use of coal or wood-stove as heating at home | 29 | 0 | |
| Outdoor exposure | | | <0.0001 |
| Urban, light traffic | 28 | 1 | |
| Urban, normal traffic | 55 | 12 | |
| Urban, heavy traffic | 14 | 30 | |
| Rural | 29 | 0 | |
| Apiaceous (carrots) consumption | | | <0.0001 |
| <1 serving/month | 54 | 43 | |
| 1 serving/week | 71 | 0 | |
| Cruciferous consumption | | | <0.0001 |
| <1 serving/month | 80 | 0 | |
| 1 serving/week | 43 | 43 | |
| Tomato | | | <0.0001 |
| <7 servings/week | 82 | 0 | |
| >7 servings/week | 43 | 43 | |
| Onion | | | <0.0001 |
| <7 servings/week | 55 | 0 | |
| >7 servings/week | 70 | 43 | |
| Pepper | | | <0.0001 |
| <5 servings/week | 82 | 0 | |
| >5 servings/week | 43 | 43 | |
| Daily coffee consumption (cups) | | | <0.0001 |
| None | 31 | 0 | |
| 1 | 93 | 13 | |
| ≥2 | 2 | 30 | |
| Daily tea consumption (cups) | | | <0.0001 |
| None | 9 | 6 | |
| 1 | 83 | 26 | |
| 2-4 | 7 | 11 | |
| ≥ 5 | 27 | 0 | |
| Education level (years) | | | <0.0001 |
| 0, none | 64 | 0 | |
| 1-6, elementary school | 29 | 4 | |
| 7-14, secondary school | 28 | 20 | |
| >14, university | 5 | 19 | |

[a]Fisher's exact test. BMI, body mass index.

observed frequencies in BC cases and controls when separately considered, indicating that they were in Hardy-Weinberg equilibrium. The frequencies of *-3860A* (10%), *-2467delT* (18%) and *-163A* (61%) are similar to those found in our previous study on healthy Caucasian volunteers, in which the frequencies were 4, 24 and 67%, respectively (20). The incidence of *CYP1A2 -3860A* (Table III) was significantly lower among BC cases, indicating a decreased risk of BC associated with this *CYP1A2* genotype (odds ratio; 95% confidence interval: 0.35; 0.14-0.88, P=0.025). By contrast, distributions of the *CYP1A2 -2467T/delT* or *-2467delT/delT* and *-163A/C* or *A/A* genotypes among BC cases and controls were similar, indicating no overall effect of these polymorphisms on BC risk. However, the case-control comparison appears to be limited by the large differences in baseline characteristics, in particular differences of age.

*Case-only study.* We performed a case-only study analysis to appraise the interaction between genotypes and environmental exposure to risk factors (Table IV). All the significant associations involve the *-3860A/G* (rs2069514) polymorphism: the

Table II. *CYP1A2* genotype frequencies in BC cases and controls.

| | BC cases | | | Controls | | |
|---|---|---|---|---|---|---|
| *CYP1A2* genotype | N | Observed frequency | Expected frequency[a] | N | Observed frequency | Expected frequency[a] |
| *-3860 G/G* | 98 | 90 | 90 | 31 | 76 | 76 |
| *G/A* | 11 | 10 | 10 | 9 | 22 | 22 |
| *A/A* | 0 | | | 1 | 2 | 2 |
| *-163 C/C* | 17 | 14 | 15 | 4 | 9 | 11 |
| *C/A* | 58 | 50 | 48 | 20 | 48 | 44 |
| *A/A* | 42 | 36 | 37 | 18 | 43 | 45 |
| *-2467 T/T* | 74 | 68 | 66 | 25 | 66 | 67 |
| *T/del T* | 29 | 27 | 30 | 13 | 34 | 30 |
| *delT/delT* | 5 | 5 | 4 | | | 3 |

[a]According to Hardy-Weinberg. CYP1A2, cytochrome P4501A2; BC, breast cancer.

Table III. Risk of BC associated with the *CYP1A2* genotypes.

| *CYP1A2* genotype | Cases | Controls | Pearson $\chi^2$ | P-value |
|---|---|---|---|---|
| *-3860 G/G* | 98 | 31 | | |
| *G/ A* | 11 | 10 | 5.0591 | 0.025 |
| *-163 C/C* | 17 | 4 | | |
| *C/A A/A* | 38 | 100 | 1.0072 | 0.604 |
| *-2467 T/T* | 74 | 25 | | |
| *T/delT delT/delT* | 34 | 13 | 0.0959 | 0.757 |

CYP1A2, cytochrome P4501A2; BC, breast cancer.

inducible A variant interacted mainly with coffee (P=0.045) to increase the number of BC cases (there were more BC cases with a high consumption of coffee). However, we observed a significant interaction of the A variant with fried food intake (P=0.026) and indoor exposure (P=0.050) and a borderline interaction with grilled food (P=0.075) to decrease the number of BC cases. Subjects with the A variant present the variants *-163A* and *-2467delT*. No interaction was found with BMI, vegetable and tea consumption, nor with other *-2467T/delT* and *-163C/A* variants (Table IV).

## Discussion

This study shows that the *-3860A* variant, by decreasing CYP1A2 activity (16,19), modified BC risk by interacting with environmental exposures, in particular with dietary habits. The *-3860A* variant reduced the risk of BC both independently from environmental factors and by interacting with fried foods and indoor polyaromatic exposure, whereas its interaction with a high intake of coffee increased BC risk. To the best of our knowledge, this is the first study reporting such an interaction and is in agreement with the fact that this point mutation significantly decreases CYP1A2 activity (16,19).

A number of studies have reported the association between the *CYP1A2 -164A/C* polymorphism and BC risk (24,28-36), however, the results remain inconclusive even in the meta-analysis study (24). The variant *-163C* is located at intron 1, in a position not directly involved in the *CYP1A2* induction mechanism (37). The *-3860G/A* is in the regulatory region of the gene (-2964 position in the gene flanking region), near the binding region [positions -2495 and -2000 (37)] of the xenobiotic responsive element (XRE), which is involved in the CYP1A2 induction mechanism. In agreement with Nakajima *et al* (19), we suggest that the *-3860G/A* polymorphism affects the binding of the activator to the XRE regulating the expression of CYP1A2. Specifically, in the nucleus the ligand AhR is activated and forms a heterodimer with the Ah receptor nuclear translocator (Arnt) (38), as a consequence of the binding with polyaromatic chemicals (37,39). This heterodimer formation is required to activate the transcription of *CYP1A2* through binding the XRE (37-39). Our results suggest that the *-3860G/A* polymorphism, by interacting with polyaromatic chemicals, such as those present in fried food (15), coffee (10) and indoor exposure (40), may modify this binding site (e.g., chromatin structure) (37), thereby decreasing CYP1A2 expression. Given the role of the CYP1A2 in the activation of procarcinogens present in fried food and indoor pollutants (HAs and PAHs) and the modulating (decreasing) effect of the *-3860G/A* polymorphism on CYP1A2 activity (16-19), our results suggest that the ensuing decreased carcinogen activation may be protective for BC development. However, the consequent lower CYP1A2 activity may lead to other conditions of risk for BC development. These conditions include a reduced formation of the beneficial 2-hydroxyestrone (2-OHE1) that mutually allows the formation of the carcinogenic 16α-hydroxyestrone (16α-OHE1) (41). A high CYP1A2 level in fact has a protective effect on BC risk by increasing the 2-hydroxyestrone (OHE)/16α-OHE1 ratio (42,43). By contrast, an increased formation of 16α-HE has been associated with an elevated risk of BC (44), since 16α-HE binds to DNA, creating adducts, which may subsequently induce gene mutations (45,46). Additionally, CYP1A2 metabolizes AAc to epoxyeicosatrienoic acids (EETs), which

Table IV. Interaction of *CYP1A2* genotype and environmental exposure on the risk of BC.

| | *-3860 G/G* | *G/A* | *-2467 T/T* | *T/delT delT/delT* | *-163 C/C* | *C/A A/A* |
|---|---|---|---|---|---|---|
| Coffee (servings/week) | | | | | | |
| 0 | 27 | 0 | 24 | 6 | 3 | 26 |
| ≥1 | 71 | 11 | 50 | 28 | 14 | 74 |
| Pearson $\chi^2$/P-value | **4.03/0.045** | | 2.54/0.111 | | 0.54/0.320 | |
| Fried food (servings/week) | | | | | | |
| <1 | 37 | 8 | 30 | 15 | 8 | 41 |
| ≥1 | 61 | 3 | 43 | 19 | 9 | 58 |
| Pearson $\chi^2$/P-value | **4.99/0.0255** | | 0.09/0.768 | | 0.18/0.460 | |
| Indoor (servings/week) | | | | | | |
| 0 | 71 | 11 | 53 | 27 | 13 | 76 |
| 2 | 26 | 0 | 21 | 7 | 4 | 24 |
| Pearson $\chi^2$/P-value | **3.88/0.050** | | 0.74/0.391 | | 0.002/0.671 | |
| Tomatoes+onions+peppers (servings/week) | | | | | | |
| <20 | 65 | 8 | 54 | 24 | 11 | 68 |
| ≥20 | 33 | 3 | 19 | 10 | 6 | 44 |
| Pearson $\chi^2$/P-value | 0.73/0.477 | | 0.134/0.714 | | 0.099/0.753 | |
| BMI (kg/m$^2$) | | | | | | |
| ≤29.99 | 69 | 8 | 57 | 24 | 12 | 75 |
| >29.99 | 29 | 2 | 17 | 10 | 5 | 25 |
| Pearson $\chi^2$/P-value | 0.41/0.52 | | 0.52/0.473 | | 0.15/0.700 | |
| Tea (servings/week) | | | | | | |
| <6 | 73 | 10 | 54 | 28 | 14 | 78 |
| ≥6 | 25 | 1 | 20 | 6 | 3 | 22 |
| Pearson $\chi^2$/P-value | 1.47/0.226 | | 1.12/0.289 | | 0.16/0.686 | |
| Grilled food (servings/week) | | | | | | |
| <3 | 63 | 10 | 52 | 20 | 11 | 64 |
| ≥3 | 35 | 1 | 21 | 14 | 6 | 36 |
| Pearson $\chi^2$/P-value | 3.17/0.075 | | 1.62/0.203 | | 0.003/0.956 | |

Bold, statistically significant comparisons. CYP1A2, cytochrome P4501A2; BC, breast cancer; BMI, body mass index.

have anti-inflammatory and anti-apoptotic functions (8,9). Therefore, decreased CYP1A2 activity may contribute to a decrease in EET production and generate an inflammatory microenvironment, all suitable conditions for the development and progression of BC (8).

The strengths of this study include the case-only study design, the discovery of a new genetic polymorphism that modulates susceptibility to BC by interacting with personal behaviors, and the good characterization of the study population with the thorough and reliable collection of several personal, occupational and environmental variables. The case-only study design is a powerful method for studying gene-environment interactions, as it achieves greater statistical power than a case-control study of the same sample size (24,25). We have previously used the same design in studies of lung and bladder cancer (21,22). The weaknesses of the study include that only three variants were considered in relation to BC risk and due to the low sample size no haplotype analysis could be performed. Additionally, the case-control comparison appears to be limited by large differences in the baseline characteristics of our study population. However, the significant association with variant *-3860A* was also found in the case-only study. We observed that all the subjects with the *-3860A* variant present the variants *-163A* and *-2467delT*. Moreover, the studied *CYP1A2* variants are common in the human population and BC is a frequent cancer in women. Similarly, consumption of coffee and meals with fried food, as well as indoor exposure to polyaromatic compounds are prevalent not only among Tunisian women, and CYP1A2 is involved in their metabolism. Therefore, these variants may have an impact in public health.

In conclusion, this is the first study indicating that the *-3860A* variant modifies BC risk by interacting with environmental exposures. The decreasing CYP1A2 activity, deriving from the interaction of the *-3860A* variant with coffee, fried foods and indoor exposure to pollutants, supports the hypothesis that a reduced CYP1A2 activity contributes to BC risk in different ways that include: i) a reduced activation of procarcinogens (i.e., HAs and PAHs) which may be protective from BC development; whereas ii) a reduced beneficial

formation of 2-OHE1, which mutually allows the formation of the carcinogenic 16-OHE1, and iii) a decreased EETs production, which generates an inflammatory microenvironment, may be suitable conditions for the development and progression of BC.

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