Interaction between genetic polymorphisms of Cytochrome P450-1B1, sulfotransferase 1A1, cathecol-O-methyl transferase, and tobacco exposure on breast cancer risk

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INTERACTIONS BETWEEN GENETIC POLYMORPHISM OF CYTOCHROME P450-1B1, SULFOTRANSFERASE 1A1, CATECHOL-O-METHYLTRANSFERASE AND TOBACCO EXPOSURE IN BREAST CANCER RISK

Monique SAINTOT[1], Christian MALAVEILLE[2], Agnès HAUTEFEUILLE[2] and Mariette GERBER[1]*

[1]*Groupe d'Epidémiologie Métabolique, INSERM-CRLC, Montpellier, France*
[2]*International Agency for Research on Cancer, Lyon, France*

Genetic polymorphisms of enzymes involved in the metabolism of xenobiotics and estrogens might play a role in breast carcinogenesis related to environmental exposures. In a case-only study on 282 women with breast cancer, we studied the interaction effects (ORi) between smoking habits and the gene polymorphisms of Cytochrome P450 1B1 (Val432Leu CYP1B1), Phenol-sulfotransferase 1A1 (Arg213His SULT1A1) and Catechol-O-methyltransferase (Val158Met COMT). The smokers carrying the *Val CYP1B1* allele associated with a high hydroxylation activity had a higher risk of breast cancer than never smokers with the *Leu/Leu* genotype (ORi=2.32, 95%CI: 1.00–5.38). Also, the smokers carrying the *His SULT1A1* allele associated with a low sulfation activity had a 2-fold excess risk compared to never smokers carrying *Arg/Arg SULT1A1* common genotype (ORi= 2.55, 95%CI: 1.21–5.36). The *His SULT1A1* allele increased the risk only in premenopausal patients. The *Met COMT* allele with a lower methylation activity than *Val COMT* did not modify the risk among smokers. The excess risk due to joint effect could result from a higher exposure to activated tobacco-compounds for women homo/heterozygous for the *Val CYP1B1* allele. Also, a lower sulfation of the tobacco carcinogens among women with *His SULT1A1* could increase exposure to genotoxic compounds. Alternatively, the *Val* CYP1B1 or *His* SULT1A1 allele with modified ability to metabolize estrogens could increase the level of genotoxic catechol estrogen (*i.e.*, 4-hydroxy-estradiol) among smokers. Our study showed that gene polymorphisms of *CYP1B1* and *SULT1A1* induce an individual susceptibility to breast cancer among current smokers.
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Key words: *CYP1B1; SULT1A1; COMT; gene polymorphism; tobacco; breast cancer*

Little is known about genotoxic factors in breast cancer development, whereas promotional factors related to sexual hormones or growth factors are well identified.[1–3] Endogenous compounds such as catechol estrogens are believed to be genotoxic for mammary epithelium and thus are suspected to play a role in breast carcinogenesis.[4–7] Certain environmental factors have long been suspected of being genotoxic agents.[8] So far, ionizing radiation is the only known exogenous genotoxic risk factor for breast cancer. Tobacco smoke contain a variety of procarcinogens such as polycyclic aromatic hydrocarbons and heterocyclic aromatic amines,[9] which can be metabolized and activated by mammary cells and may be involved in breast carcinogenesis among tobacco-exposed women.[10–12] However, the relationship between tobacco smoke and breast carcinogenesis is complex.[13,14] It has been suggested that particular mainstream cigarette compounds display anti-estrogenic effects.[15,16] Some studies on passive smoking have shown a tendency towards an increased breast cancer risk,[17–20] although this was not observed in a prospective study.[21]

Most environmental carcinogens are metabolically activated and detoxified by polymorphic enzymes. Some of these enzymes are also involved in estrogen metabolism. Molecular biological studies on these polymorphisms show that they determine change in the functionality of enzymes and thus may affect the level of carcinogens exposure in the breast cells during life. Cytochrome P450 1B1 is a monoxygenase involved in the activation of polycyclic aromatic hydrocarbons and heterocyclic aromatic amines; it catalyses specifically the hydroxylation of estrogens into genotoxic catechol estrogen, *i.e.*, 4-hydroxy-estradiol.[7,22] Polymorphism of the *CYP1B1* gene encoding an amino acid change in codon 432 (*Val* to *Leu*) was shown to have a decreased catalytic efficiency for 4-hydroxylation of estradiol and some alterations in enzymatic activity towards others mammary procarcinogens .[23–26]

Phenol-sulfotransferase SULT1A1 conjugates carcinogens and estrogens by sulfate transfer to phenolic group, and it activates some hydroxylated metabolites of polycyclic aromatic hydrocarbons and aromatic amines.[11,27–30] This enzyme has a role in modulating the biovailability of estrogens, since sulfated estrogens are poor ligands for the estrogen receptor. A common polymorphism of *SULT1A1* at nucleotide 638 (G to A) of the *ST1A3* gene coding for SULT1A1 causes an amino-acid substitution at codon 213 (*Arg* to *His*).[31,32] Studies have shown that individuals with the homozygous *His SULT1A1* genotype had significantly lower phenol-sulfotransferase activity than individuals heterozygous or homozygous for the *Arg* common allele.[33,34] Catechol-O-methyltransferase (COMT) conjugates and thus inactivates catechol estrogens. The *COMT* (*Met/Met*) genotype induces a 3- to 4-fold decrease in methylation activity compared to the (*Val/Val*) genotype and this change may contribute to alter breast cancer susceptibility through hormone metabolism.[35,36]

It is well established that cigarette smoking can increase the expression of various mono-oxygenases and conjugating enzymes, possibly CYP1B1 and SULT1A1, thus modulating the metabolism of xenobiotics as well as endobiotics.[37] We hypothesized that these changes could modify the level of exposure to carcinogens. In this case-series of pre- and post-menopausal women with breast cancer, we have investigated whether tobacco smoking associated with the polymorphism of CYP1B1, or SULT1A1 or COMT induce an excess risk of breast cancer.

MATERIAL AND METHODS

Study design

A case-only study design does not provide odds ratio for exposure or genotype alone but permits screening for gene-environment interaction. Our study design has an higher statistical power to detect significant OR for interaction than a case-control study, and it avoids bias due to differential ascertainment of risk factors between cases and controls.[38] A valid interpretation of the interaction parameter (ORi) requires the assumption of independence

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*Correspondence to: Groupe d'Epidémiologie Métabolique, Centre de Recherche en Cancérologie INSERM-CRLC, 34298 Montpellier cedex 5, France. Fax: +33-04-67-61-37-87.
E-mail: marietger@valdoren.fn.clcc.fr

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between gene polymorphism and exposure in the population.[39,40] We verified the validity of this requirement by estimating gene-exposure association (OR) in Caucasian controls from the cross-products of a 2×2 table with available data published in 2 reports.[41,42]

The study protocol involved visiting the patient, explaining the study and seeking her agreement to participate, in accordance with the recommendations of the ethics committees (institutional and regional committees). Once the patient had agreed to participate, fasting blood was collected for genotyping and a 50 to 60 min interview was conducted to elicit information on the environmental risk factors, among them tobacco use and on potential confounding factors.

Subjects

All breast cancer patients admitted to the surgical wards of the Cancer Centre in Montpellier (Southern France) were invited to participate in the study. The Cancer Centre is a regional hospital that draws more than 80% of the breast cancer patients in the region Languedoc-Roussillon, but not completely representative of the socio-economic classes of the region. The objective was to recruit similar sample of pre- and post-menopausal cases because of different effects of genetic polymorphisms in pre- and post-menopausal cases.[43] We recruited 295 women (149 pre- and 146 post-menopausal) between 1998 and 2001, 263 were at first diagnosis and 32 were hospitalized for relapse of breast cancer; the exclusion of these prevalent cases did not modify the results. A questionnaire elicited information on sociodemographic characteristics, reproductive events and medical and familial histories. The smoking information included past and/or present smoking status, number of cigarettes smoked per day, smoking duration and age at start of smoking. Subjects smoking at the time of diagnosis were considered current smokers; those who had ever smoked but had stopped at least 1 year before diagnosis were considered former smokers. The same information was also obtained with regard to passive exposure to tobacco smoke at home and/or in the workplace. The final sample include 282 patients with a complete set of data.

DNA isolation, PCR amplification and genotyping

DNA solutions were prepared from whole blood using the method described by Spurr *et al*.[44] The solutions were cooled and stored at −30°C. The *G to C* polymorphism at position 1640 of *CYP1B1* ,which causes a *Val to Leu* change at codon 432, was analyzed using the PCR-RFLP method described by Bailey *et al*.[25] The base substitution at 638 (*G* to *A*) of the *ST1A3* gene coding for SULT1A1, which causes an amino-acid change at 213 (*Arg* to *His*), was detected using the PCR-RFLP method described by Ozawa *et al*.[45] The *Val* to *Met* transition at amino acid 158 of COMT, due to *G* to *A* polymorphism, which results is a low activity form of the enzyme, was examined using an amplification refractory mutation system assay as described by Hoda *et al*.[46]

Statistical analysis

Under assumption of independence between gene and exposure in the population, departures from multiplicative joint effect of genotype and tobacco exposure were measured by the OR of interaction (ORi) which was obtained from the case-only 2×2 cross tabulation of the genotype at risk (+/−) and the environmental exposure at risk (+/−). An interaction parameter of more than 1 indicates a greater than multiplicative effect between the 2 factors.

In preliminary analysis, smoking exposures were categorized as follows: never exposed, ever passive-exposed, former smokers and current smokers. The group of ever passive-exposed include the never-smoker women who were exposed to environmental tobacco smoke for 5 years at least. For study on active smoking habit, never smokers and passive-exposed were included in the reference group. Smoking characteristics were categorized as follows: age at initiation (≤20, >20 years old), duration of smoking (≤20, >20 years), daily cigarettes consumption (≤5, >5) and pack-years (the product of intensity and duration, ≤10, >10). These 2 last cut-points were selected because the sample was only made of light to moderate smokers.

The genetic polymorphisms of enzymes were categorized as follows: homozygous *Leu/Leu* CYP1B1 (low enzymatic activity) against heterozygous any *Val* carriers, homozygous *Arg/Arg* SULT1A1 (high enzymatic activity) against heterozygous *His* carriers, and *Val/Val* COMT (high enzymatic activity) against heterozygous *Met* carriers.

We used Fisher's exact test (2-sided) to examine the relation between genotypes enzymes or smoker status and potential confounding factors related to reproductive histories and hormonal supplies: age at menarche (≤12, >12 years old), age at first full term pregnancy (≤20, >20 years old), parity (≤1, >1 children), use of oral contraceptives (yes, no), use of hormone-replacement therapy (yes, no), age at menopause (≤50, >50 years old) and body mass index (BMI) (<25, ≥25). Unconditional logistic regression analysis estimated the gene-exposure interaction (ORis and 95%CIs) with adjustment for these confounders.[38] All testing was performed by using 2-sided Wald test, the significant level was $p<0.05$. SAS version 8.02 was used for statistical analysis.

RESULTS

The mean age of cases was 52 years (38−87 years) and 59% of women had been at menopause for at least 1 year. All women were Caucasian. The characteristics of the patients stratified on smoking habit (never/ever smokers) are listed in Table I. With regard to tobacco exposure, 24% of patients were current smokers, 22% were former smokers who had stopped 1 year or more ago, 32% were exposed to environmental tobacco smoke and 22% were never exposed and never smokers. The independence between smoking status (never smoker/ever smoker) and gene polymorphisms was estimated by OR from the cross-products of 2×2 table with control data published in others studies.[41,42] The values of OR were not significantly different from unity for *CYP1B1* (*leuleu/anyVal*): OR=0.77, 95%CI: 0.19–3.10; for *COMT* (*ValVal/anyMet*): OR=0.90, 95%CI: 0.45–1.81; and for *SULT1A1*(*ArgArg/anyHis*): OR=0.72, 95%CI: 0.38–1.34.

We assessed the independence of exposures and genotypes from factors related to breast cancer: age at first diagnosis, age at menarche, age at first full term pregnancy, age at menopause, use of oral contraceptives or hormone replacement therapy, benign breast disease and family history of breast cancer. All the results of the Fisher's test were not significant, exposure and genotype are unlikely to be associated by the mean of a known risk factor of breast cancer.

Breast cancer was detected earlier in life in smokers than in never smokers (38% *vs*. 19% before 46 years of age, $p<0.01$) and the smokers used oral contraceptives more often than nonsmokers (78 % *vs*. 58%, $p<0.01$).

Table II shows the genetic polymorphisms frequencies of metabolizing enzymes (homozygous wild, heterozygous wild-variant and homozygous variant) and the frequency of allelic variant. The frequency of the *Leu CYP1B1* was similar between pre- and post-menopausal patients, whereas the frequency of the *His SULT1A1* allele was significantly higher in premenopausal than in postmenopausal patients (31.7% *vs*. 22.1%). For COMT, the frequency of the *Met* variant was slightly higher for pre- than for post-menopausal patients.

Table III shows the ORis between genetic polymorphism and tobacco exposure obtained by unconditional logistic regression analysis after adjustment for confounding factors. For CYP1B1, the reference group consisted of never exposed with the *Leu/Leu* genotype characterized by a lower catalytic efficiency for 4-hydroxylation of estrogens than the *Any Val* genotype. The current smokers carrying the *Val CYP1B1* allele had an increased breast cancer risk compared to the reference group ($p=0.05$). For the

TABLE I – BREAST CANCER CASE-ONLY STUDY: COMPARISON OF THE RISK FACTORS BETWEEN NEVER AND EVER SMOKERS WOMEN

Risk factors	Never smokers	Ever smokers	p^1
Age (mean ± SD, years)	54.7 ± 9.78	50.19 ± 7.68	<0.01
Age at menarche (mean ± SD, years)	12.65 ± 1.48	12.55 ± 1.77	0.4
Use of oral contraceptive (*n*,%)			
Yes	89 (58.2)	98 (76)	<0.01
No	64 (41.8)	31 (24)	
Age at first full term pregnancy (*n*,%)			
Nulliparous	13 (8.5)	14 (10.8)	0.7
≤ 25 years	98 (64)	78 (60.5)	
> 25 years	42 (27.5)	37 (28.7)	
Parity (*n*,%)			
Nulliparous	52 (34)	56 (43.4)	0.1
> 1	101 (66)	73 (56.6)	
Menopause (*n*,%)			
Yes	87 (56.8)	58 (45)	0.05
No	66 (43.2)	71 (55)	
Hormonal replacement therapy (*n*,%)			
Yes	46 (52.3)	26 (44.8)	0.4
No	42 (47.7)	32 (55.2)	
Family history of breast cancer (*n*,%)			
Yes	18 (11.5)	11 (8.4)	0.4
No	139 (88.5)	120 (91.6)	
BMI (mean ± SD, kg/m^2)	23.74 ± 4.13	23.23 ± 4.84	0.09

[1]*p* value calculated by Fisher's test for categorical variables and by the Mann-Whitney test for continuous variables.

TABLE II – GENETIC POLYMORPHISMS FREQUENCIES OF *CYP1B1, SULT1A1* AND *COMT* AMONG PRE- AND POST-MENOPAUSAL BREAST CANCER PATIENTS

Enzymes	Genotypes	Pre-menopause		Post-menopause		p^1
		n	%	*n*	%	
CYP 1B1	*Val Val*	27	19.7	23	15.9	
	Val Leu	72	52.6	70	48.3	
	Leu Leu	38	27.7	52	35.8	0.3
	Leu frequency		54.0		60.0	
SULT1A1	*Arg Arg*	64	46.7	86	59.3	
	Arg His	59	43.1	54	37.2	
	His His	14	10.2	5	3.5	0.02
	His frequency		31.7		22.1	
COMT	*Val Val*	34	24.8	53	36.6	
	Val Met	72	52.6	68	46.9	
	Met Met	31	22.6	24	16.5	0.08
	Met frequency		48.9		40.0	

[1]*p* value calculated by chi-square test.

former smokers, the ORi was higher than 1 but not significant. For SULT1A1, the current smokers carrying the *His* variant (homo- and heterozygous) with a low sulfotransferase activity had an increased risk compared to never exposed *Arg/Arg* homozygous ($p=0.01$). No significant interaction was observed between *COMT* polymorphism and tobacco smoking.

The genetic polymorphism did not modify the risk related to passive exposure; also for further analysis, the reference group included both never smokers and passive smokers. The tobacco habits of former and current smokers differed significantly with regard to intensity and duration of smoking: current smokers had started smoking earlier in life than former smokers (66% *vs*. 34% before 18 years old, $p<0.001$), the daily cigarette consumption of current smokers was higher than that of former smokers (62% versus 38% smoked more than 10 cigarettes/day, $p=0.01$) and the duration of smoking was longer for current smokers (77% of current smokers had been smoking for more than 15 years *vs*. 23% for former ones, $p=0.01$).

We investigated the interaction of *CYP1B1* and *SULT1A1* polymorphisms with different levels of tobacco exposure among ever smokers: number of cigarettes/day, duration of smoking, pack-years and age at smoking initiation. For CYP1B1, the results in Table IV show a significant excess risk for patients carrying the "high activity" *Val CYP1B1* variant (homo- or hetero-zygous), who had smoked either more than 5 cigarettes/day ($p<0.01$), or for more than 20 years ($p=0.01$) and had started smoking before 20 years of age ($p<0.01$). The stratified analysis on menopausal status did not shown any difference between pre- and post-menopausal patients.

For SULT1A1, the results in Table V show that premenopausal patients carrying the *His SULT1A1* allele had an increased risk for breast cancer when exposed to tobacco smoke, compared to *Arg SULT1A1* homozygous patients never exposed. The excess risks were significant for women who had smoked either more than 5 cigarettes/day ($p=0.05$) or for more than 20 years ($p=0.01$).

Since none significant positive association was shown for COMT genotype in Table III, we stratified the analysis on menopausal status. We found that the association between current smokers and *Met* COMT was higher for postmenopausal patients (ORi= 2.97, 95%CI 0.85–10.32) than for premenopausal ones (ORi= 0.70, 95%CI 0.23–2.14), but the OR is with large confidence intervals are not significant.

DISCUSSION

In this case-only study among breast cancer patients, we showed an excess of breast cancer risk in women who were moderate to heavy smokers and carrying *Val CYP1B1* allele or *His SULT1A1*

TABLE III – BREAST CANCER CASE-ONLY ANALYSIS OF THE EFFECT OF TOBACCO SMOKE EXPOSURE: ASSOCIATION WITH *CYP1B1, SULT1A1* AND *COMT* GENOTYPES

	Genetic polymorphism[2]	Never exposed	Passive exposed	Former smokers	Current smokers
CYP 1B1	Any *Val*	41	52	44	55
	Leu leu	21	39	17	13
	ORi (95%CI)[1]	1[3]	0.69 (0.35–1.37)	1.33 (0.59–2.96)	2.32 (1.00–5.38)
SULT1A1	*Arg Arg*	36	51	38	25
	Any *His*	26	40	23	43
	ORi (95%CI)[1]	1[3]	1.08 (0.55–2.11)	0.79 (0.37–1.68)	2.55 (1.21–5.36)
COMT	*Val Val*	22	26	19	18
	Any *Met*	40	65	42	50
	ORi (95%CI)[1]	1[3]	1.26 (0.62–2.57)	1.07 (0.49–2.35)	1.42 (0.65–3.13)

[1]ORi (95% CI) Odds ratios for gene-tobacco exposure interaction with confidence intervals, under assumption of independence gene-tobacco exposure in the controls. This parameter was adjusted for variables listed in Table I.–[2] Genotypes were dichotomized according to activities of allele-encoded isozymes: we compared "high" *vs*. "low" enzymatic activity for CYP1B1, "low" *vs*. "high" sulfation for SULT1A1 and "high" *vs*. "low" catechol-O-methylation (COMT).–[3] The reference group consisted of never exposed with the *Leu/Leu* genotype for CYP1B1, *Arg/Arg* for SULT1A1 or *Val/Val* for COMT enzymes.

TABLE IV – BREAST CANCER CASE-ONLY ANALYSIS OF THE EFFECT OF TOBACCO SMOKE EXPOSURE: ASSOCIATION WITH *CYP1B1* GENOTYPES

	CYP1B1 Any *Val/Leu Leu*[2] ORi (95%CI)[1]		
	All patients	Pre-menopause	Post-menopause
Cigarettes/day			
None	1[3]	1	1
≤ 5	1.72 (0.67–4.42)	3.09 (0.61–15.60)	1.37 (0.39–4.82)
> 5	2.32 (1.28–4.21)	2.00 (0.87–4.57)	3.56 (1.40–9.02)
Duration of smoking			
Never smokers	1	1	1
≤ 20 years	1.97 (0.92–4.22)	1.52 (0.53–4.29)	2.98 (0.92–9.62)
> 20 years	2.37 (1.24–4.51)	2.79 (1.06–7.33)	2.23 (0.90–5.52)
Pack-years			
None	1	1	1
≤ 10	2.01 (0.97–4.15)	2.03 (0.70–5.87)	2.05 (0.74–5.73)
> 10	2.38 (1.23–4.63)	2.22 (0.86–5.70)	2.81 (1.07–7.43)
Age at smoking initiation			
Never smokers	1	1	1
≤ 20 years	2.81 (1.46–5.41)	3.25 (1.28–8.25)	2.67 (1.00–7.18)
> 20 years	1.45 (0.67–3.15)	0.89 (0.26–3.03)	2.25 (0.79–6.43)

[1]ORi (95% CI) Odds ratios for gene-tobacco exposure interaction with confidence intervals, under assumption of independence gene-tobacco exposure in the controls. This parameter was adjusted for variables listed in Table I.–[2] Genotypes were dichotomized according to activities of allele-encoded isozymes: we compared the "high" *vs*. the "low" catalytic efficiency of *CYP1B1* alleles.–[3] Reference group consisted of never smoker patients with the *Leu/Leu* genotype.

variant allele. The increased risk is significant for pre- and post-menopausal women carrying *Val CYP1B1* allele but significant only for the premenopausal women carrying *His SULT1A1* allele. Our primary hypothesis was mainly based on the effect modifier of the polymorphisms of xenobiotics metabolizing enzymes on the relationship between smoking and breast cancer. However, the results on the SULT1A1 polymorphism led us to consider a relationship with estrogen metabolism.

The use of case-only study is a convenient approach for evaluating the presence of gene-environment interaction (ORi) when exposure and genotype occur independently in the population. Such a design is exploratory; an interaction parameter significantly different from one reveals an inter-individual susceptibility to risk exposure depending on gene polymorphisms. The required assumptions of independence between the environmental exposure and gene polymorphisms have been verified with the data of controls described in case-control studies.[41,42] The prevalences of *Leu CYP1B1* allele (0.57), *His SULT1A1* allele (0.27) and *Met* COMT allele (0.44) are comparable with those found in other Caucasian samples.[25,30,41,47,48] No direct association between *CYP1B1* polymorphism and breast cancer risk has been found.[23,25,47] For SULT1A1, a study suggests that the *SULT1A1* genotype influences the age of onset among early-onset patients.[29] In a cohort of postmenopausal women, investigators found a significant association between the *His/His* genotype and breast cancer risk.[30] Some studies provided conflicting results on *COMT* polymorphism as a breast cancer risk factor.[35,36] The CYP1B1 expressed in breast cells catalyses selectively the 4-hydroxylation of estradiol, but it activates other procarcinogens.[22] Its expression is inducible in response to aryl hydrocarbon receptor agonists including polycyclic aromatic hydrocarbons found in tobacco smoke. Pharmacogenetics studies showed that polymorphisms in the *CYP1B1* gene caused some alterations in catalytic function.[24,26] The *Val* CYP1B1 allele has higher catalytic efficiency for 4-hydroxylation of 17β-estradiol than the *Leu* allele, for others procarcinogens this change in activity has not been demonstrated clearly. A recent study showed that the inheritance of *Val* CYP1B1 was insufficient to influence breast cancer risk in Caucasian women, but its expression was associated with lower levels of estradiol compared to *Leu* variant, which could result from an increased synthesis of catechol estrogens.[47]

One may suggest that the inheritance of *Val CYP1B1* associated with tobacco-induced expression increases the level of genotoxic 4-OH-estradiol in mammary cells and then enhances the susceptibility of women towards breast carcinogenesis. In agreement with our findings, a study on ovarian cancer, showed an increase risk among smokers carrying at least one *Val CYP1B1* allele.[41]

SULT1A1 is expressed in liver and recently detected in mammary cytosols. The functional polymorphism of SULT1A1 could

TABLE V – BREAST CANCER CASE-ONLY ANALYSIS OF THE EFFECT OF TOBACCO SMOKE EXPOSURE: ASSOCIATION WITH *SULT1A1* GENOTYPES

	SULT1A1 Any *His/Arg Arg*[2] ORi (95%CI)[1]		
	All patients	Pre-menopause	Post-menopause
Daily cigarette/day			
None	1[3]	1	1
≤ 5	0.54 (0.22–1.33)	0.67 (0.19–2.31)	0.40 (0.10–1.67)
> 5	1.65 (0.97–2.80)	2.11 (1.00–4.46)	1.50 (0.67–3.39)
Duration of smoking			
Never smokers	1	1	1
≤ 20 years	0.84 (0.42–1.66)	0.76 (0.30–1.97)	1.06 (0.37–3.04)
> 20 years	1.71 (0.97–3.03)	2.83 (1.23–6.54)	1.17 (0.49–2.76)
Pack-years			
None	1	1	1
≤ 10	1.00 (0.53–1.92)	1.44 (0.58–3.54)	0.70 (0.25–1.93)
> 10	1.68 (0.93–3.04)	1.89 (0.83–4.30)	1.59 (0.65–3.85)
Age at smoking initiation			
Never smokers	1	1	1
≤ 20 years	1.49 (0.85–2.60)	1.91 (0.91–4.04)	1.31 (0.50–3.39)
> 20 years	1.07 (0.52–2.22)	1.14 (0.35–3.66)	0.98 (0.38–2.57)

[1]ORi (95% CI) Odds ratios for gene-tobacco exposure interaction with confidence intervals, under assumption of independence gene-tobacco exposure in the controls. This parameter was adjusted for variables listed in Table I.–[2] Genotypes were dichotomized according to activities of allele-encoded isozymes: we compared the "low" *vs*. the "high" sulfation activity of *SULT1A1* alleles.–[3]Reference group consisted of never smoker patients with the *Arg/Arg* genotype.

influence the inactivation of estrogen and catechol-estrogen as well as mutagenic activation of xenobiotics in the breast.[49,50] Two biological pathways can be proposed to explain the positive interaction between tobacco smoking and *His* SULT1A1 variant found in premenopausal women. First, the detoxification of tobacco carcinogens by sulfotransferase would be inefficient among *His* SULT1A1 carriers and the electrophilic intermediates, and DNA adducts would be higher than in *Arg* SULT1A1 carriers. A lung cancer study showed that the *His SULT1A1* genotype was significantly associated with increased risk for current smokers.[42] A study on colorectal cancer suggested that the high-activity phenol sulfotransferase protect against environmental chemicals.[51] Second, since the interaction was significant in premenopausal women only, we could assume an indirect effect through estrogen metabolism. The enzymatic process of xenobiotics detoxification by SULT1A1 could be overloaded by a large supply of tobacco carcinogens, and hence the sulfation of estrogens be reduced. This process of inactivation should be lower with *His SULT1A1* allele. Consequently, the level of estrogen in breast tissue to be activated into catechol estrogen would be higher in women carrying *His SULT1A1* allele with low sulfation activity as compared to those with fast activity *i.e*., carriers of *Arg SULT1A1* allele. The pathway of O-methylation of catechol estrogen does not appear to be modified by metabolism of tobacco compounds since we found no association between smoking and COMT polymorphism.

Our results suggest that genetic polymorphisms of some enzymes, implicated both in metabolism of estrogens and of tobacco compounds, could have a role in individual susceptibility related to tobacco exposure with regard to breast cancer risk. They could explain the discrepancy among results found in the literature about the role of tobacco exposure in this cancer risk.[13] Because of the sensitivity of the case-only design to departures from independence gene-environment, we cannot rule out the possibility that these results may be induced by an uncontrolled confounding variable that would be related to both factors.[40] These findings require more investigation with case-control or cohort designs. Nevertheless, the recommendation "not to smoke" is to be emphasized especially among young women, since the prevalence of *His* SULT1A1 and *Val* CYP1B1 alleles are relatively important in Caucasian women.

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REFERENCES

1. Kelsey JL, Gammon MD, John EM. Reproductive and hormonal risk factors. Epidemiol Rev 1993;15:36–47.
2. Clavel-Chapelon F, Gerber M. Reproductive factors and breast cancer risk: do they differ according to age at diagnosis. Breast Cancer Res Treat 2002;72:107–15.
3. Kaaks R, Lukanova A. Energy balance and cancer: the role of insulin and insulin-like growth factor-I. Proc Nutrition Soc 2001;60:91–106.
4. Liehr JG. Dual role of oestrogens as hormones and procarcinogens: tumor initiation by metabolic activation of oestrogens. Eur J Cancer Prev 1996;6:3–10.
5. Cavalieri EL, Stack DE, Devanesan PD, Todorovic R, Dwivedy I, Higginbotham S, Johansson SL, Patil KD, Gross ML, Gooden JK, Ramanathan R, Cerny RL, et al. Molecular origin of cancer: catechol estrogen-3,4-quinones as endogenous tumor initiators. Proc Natl Acad Sci 1997;94:10937–42.
6. Zhu BT, Conney AH. Functional role of estrogen metabolism in target cells: review and perspectives. Carcinogenesis 1998;19:1–27.
7. Hayes CL, Spink DC, Spink BC, Cao JQ, Walker NJ, Sutter TR. 17β-estradiol hydroxylation catalysed by human cytochrome P4501B1. Proc Natl Acad Sci 1996;93:9776–81.
8. Krieger N. Exposure, susceptibility, and breast cancer risk. Breast Cancer Res Treat 1989;13:205–23.
9. IARC. Tobacco smoking. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans. Lyon, France: IARC, 1986. 38:1–397.
10. Palmer LR, Rosenberg L, Clarke EA, Stolley PD, Warshauer ME, Zauber AG, Shapiro S. Breast cancer and cigarette smoking: a hypothesis. Am J Epidemiol 1991;134:1–13.
11. Williams JA, Phillips DH. Mammary expression of xenobiotic metabolising enzymes and their potential role in breast cancer. Cancer Res 2000;60:4667–77.
12. Hecht SS. Tobacco smoke carcinogens and breast cancer. Environ Mol Mutagen 2002;39:119–26.
13. Terry PD, Rohan TE. Cigarette smoking and the risk of breast cancer in women: a review of the literature. Cancer Epidemiol Biomarkers Prev 2002;11:953–71.
14. Morabia A. Smoking (active and passive) and breast cancer: epide-

miologic evidence up to June 2001. Environ Mol Mutagen 2002;39: 89–95.
15. Meeks MD, Finch Gl. Diluted mainstream cigarette smoke condensates activate estrogen receptor and aryl hydrocarbon receptor-mediated gene transcription. Environ Res 1999;80:9–17.
16. Morabia A, Bernstein M, Ruiz J, Heritier S, Diebold Berger S, Borisch B. Relation of smoking to breast cancer by estrogen receptor status. Int J Cancer 1998;75:339–42.
17. Morabia A, Bernstein M, Heritier S, Khatchatrian N. Relation of breast cancer with passive and active exposure to tobacco smoke. Am J Epidemiol 1996;143:918–28.
18. Johnson KC, Hu J, Mao Y. Passive and active smoking and breast cancer risk in Canada, 1994–97. The Canadian Cancer Registries Epidemiology Research Group. Cancer Causes Control 2000;11:211–21.
19. Lash TL, Aschengrau, A. Active and passive cigarette smoking and the occurrence of breast cancer. Am J Epidemiol 1999;49:5–12.
20. Kropp S Chang-Claude J. Active and passive smoking and risk of breast cancer by age 50 years among German women. Am J Epidemiol 2002;156:616–26.
21. Egan KM, Stampfer MJ, Hunter D, Hankinson S, Rosner BA, Holmes M, Willet WC, Colditz GA. Active and passive smoking in breast cancer: prospective results from the Nurses' health study. Epidemiology 2002;13:138–45.
22. Shimada T, Hayes CL, Yamazaki H, Amin S, Hecht SS, Guengerich FP. Activation of chemically diverse procarcinogens by human cytochrome P-4501B1. Cancer Res 1996;56:2979–84.
23. Zheng W, Xie DW, Jin F, Cheng JR, Dai Q, Wen WQ, Shu XO, Gao YT. Genetic polymorphism of cytochrome P450-1B1 and risk of breast cancer. Cancer Epidemiol Biomarkers Prev 2000;9:147–50.
24. Shimada T, Watanabe J, Kawajiri K, Sutter TR, Guengerich FP, Gillam MJ, Inoue K. Catalytic properties of polymorphic human cytochrome P450 1B1 variants. Carcinogenesis 1999;20:1607–13.
25. Bailey LR, Roodi N, Dupont WD, Parl FF. Association of cytochrome P450 1B1 (CYP1B1): polymorphism with steroid receptor status in breast cancer. Cancer Res 1998;58:5038–41.
26. Hanna IH, Dawling S, Roodi N, Guengerich FP, Parl FF. Cytochrome P450 1B1 (CYP1B1) pharmacogenetics: association of polymorphisms with functional differences in estrogen hydroxylation activity. Cancer Res 2000;60:3440–44.
27. Glatt H, Engelke CE, Pabel U, Teubner W, Jones AL, Coughtrie MW, Andrae U, Falany CN, Meinl W. Sulfotransferases: genetics and role in toxicology. Toxicol. Lett 2000;15:341–48.
28. Falany JL, Falany CN. Expression of cytosolic sulfotransferases in normal mammary epithelial cells and breast cancer cell lines. Cancer Res 1996;56:1551–55.
29. Seth P, Lunetta KL, Bell DW, Gray H, Nasser SM, Rhei E, Kaelin CM, Iglehart DJ, Marks JR, Garber JE, Haber DA, Polyak K. Phenol-sulfotransferases: hormonal regulation, polymorphism, and age of onset of breast cancer. Cancer Res 2000;60:6859–63.
30. Zheng W, Xie D, Cerhan JR, Sellers TA, Wen W, Folsom AR. Sulfotransferase 1A1 polymorphism, endogenous estrogen exposure, well-done meat intake, and breast cancer risk. Cancer Epidemiol Biomark Prev 2001;10:89–94.
31. Coughtrie MWH, Gilissen RAH, Shek B, Strange RC, Fryer AA, Jones PW. Bamber DE. Phenol sulphotransferase SULT1A1 polymorphism: molecular diagnosis and allele frequencies in Caucasian and African populations. Biochem J 1999;337:45–9.
32. Nagata K, Yamazoe Y. Pharmacogenetics of sulfotransferase. Annu Rev Pharmacol Toxicol 2000;40:159–76.
33. Raftogianis RB, Wood TC, Otterness DM, Loon JAV, Weinshilboum RM. Phenol sulfotransferase pharmacogenetics in humans: association of common SULT1A1 alleles with TS PST phenotype. Biochem Biophys Res Comm 1997;239:298–304.
34. Nowell SA, Ambrosone CB, Ozawa S, Macleod SL, Mrackova G, Williams S, Plaxco J, Kadlubar FF, Lang NP. Relationship of phenol sulfotransferase activity (SULT1A1) genotype to sulfotransferase activity phenotype in platelet cytosol. Pharmacogenetics 2000;10:789–97.
35. Thompson PA, Shields PG, Freudenheim JL, Stone A, Vena JE, Marshall JR, Graham S, Laughlin R, Nemoto T, Kadlubar FF, Ambrosone CB. Genetic polymorphisms in catechol-O-methyltransferase, menopausal status, and breast cancer risk. Cancer Res 1998; 58:2107–10.
36. Mitrunen K, Jourenkova N, Kataja V, Eskelinen M, Kosma VM, Benhamou S. Polymorphic catechol-O-methyltransferase gene and breast cancer risk. Cancer Epidemiol Biomarkers Prev 2001;10:635–40.
37. Zevin S. Benowitz NL. Drug interactions with tobacco smoking: an update. Clin Pharmacokinet 1999;36:425–38.
38. Begg CB, Zhang ZF. Statistical analysis of molecular epidemiology studies employing case-series. Cancer Epidemiol Biomarkers Prev 1994;3:173–75.
39. Yang Q, Khoury MJ. Evolving methods in genetic epidemiology. III. Gene-environment interaction in epidemiologic research. Epidemiol Rev 1997;19:33–43.
40. Albert PS, Ratnasinghe D, Tangrea J, Wacholder S. Limitations of the case-only design for identifying gene-environment interactions. Am J Epidemiol 2001;154:687–93.
41. Goodman MT, Mcduffie K, Kolonel LN, Terada K, Donlon TA, Wilkens LR, Guo C, Le Marchand L. Case-control study of ovarian cancer and polymorphism in genes involved in catechol-estrogen formation and metabolism. Cancer Epidemiol Biomarkers Prev 2001; 10:209–16.
42. Wang Y, Spitz MR, Meng-Hsuan Tsou A, Zhang K, Makan N, Wu X. Sulfotransferase (SULT) 1A1 polymorphism as a predisposition factor for lung cancer: a case-control analysis. Lung Cancer 2002;35: 137–42.
43. Ambrosone CB, Freudenheim Jl, Graham S, Marshall JR, Vena JE, Brasure JR., Michalek AM., Laughlin R, Nemoto T, Gillenwater KA., Harrington AM, Shields PG. Cigarette smoking, N-acetyltransferase 2 genetic polymorphisms, and breast cancer risk. JAMA 1996;276: 1494–1501.
44. Spurr NK, Gough AC, Smith CAD, Wolf RC. Genetic analysis of cytochrome P450 system. In: Waterman MR, Johnson ER, eds. Methods in enzymology. New York: Academic Press, 1991 149–66.
45. Ozawa S, Tang YM, Yamazoe Y, Kato R, Lang NP, Kadubar FF. Genetic polymorphism in human liver phenol sulfotransferases involved in the bioactivation of N-hydroxy derivatives of carcinogenic arylamines and heterocyclic amines. Chem Biol Interact 1998;109: 237–48.
46. Hoda F, Nicholl D, Bennett P, Arranz M, Aitchinson KJ, Al-Chalabi A. No association between Parkinson' disease and low activity alleles of catechol-O-methyltransferase. Biochem Biophys Res Commun 1996;21:780–4.
47. De Vivo I, Hankinson SE, Li L, Colditz GA, Hunte, DJ. Association of CYP1B1 polymorphisms and breast cancer risk. Cancer Epidemiol Biomarkers Prev 2002;11:489–92.
48. Carlini EJ, Raftogianis RB, Wood TC, Jin F, Zheng W, Rebbeck TR. Sulfation pharmacogenetics: SULT1A1 and SULT1A2 allele frequencies in Caucasian, Chinese and African-American subjects. Pharmacogenetics 2001;11:57–68.
49. Williams JA. Single nucleotide polymorphisms, metabolic activation and environmental carcinogenesis: why molecular epidemiologists should think about enzyme expression. Carcinogenesis 2001;22:209–14.
50. Adjei AA, Weinshilboum RM. Catecholestrogen sulfation: possible role in carcinogenesis. Biochem Biophys Res Commun 2002;29: 402–8.
51. Bamber DE, Fryer AA, Strange RC, Elder JB, Deakin M, Rajagopal R, Fawole A, Gilissen RA, Campbell FC, Coughtrie MW. Phenol sulphotransferase SULT1A1*1 genotype is associated with reduced risk of colorectal cancer. Pharmacogenetics 2001;11:679–85.