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EPIDEMIOLOGY

CYP1A1 and *CYP1B1* genetic polymorphisms, smoking and breast cancer risk in a Finnish Caucasian population

Pia Sillanpää · Liisa Heikinheimo · Vesa Kataja · Matti Eskelinen · Veli-Matti Kosma · Matti Uusitupa · Harri Vainio · Katja Metsola · Ari Hirvonen

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Abstract We investigated the associations between two *CYP1A1* polymorphisms (*Ile462Val* and *Thr461Asn*) and one *CYP1B1* polymorphism (*Leu432Val*) and breast cancer risk. The study population consisted of 483 breast cancer patients and 482 healthy population controls, all of homogenous Finnish origin. No statistically significant overall associations were found between the *CYP1A1* and *CYP1B1* genotypes and breast cancer risk. However, a significant increase in the breast cancer risk was seen for women who had smoked 1–9 cigarettes/day and carried the *CYP1B1 432Val* allele; the OR was 2.6 (95% CI 1.07–6.46) for women carrying the *Leu/Val* genotype and 5.1 (95% CI 1.30–19.89, *P* for trend 0.005) for women with the *Val/Val* genotype compared to similarly smoking women homozygous for the *432Leu* allele. Furthermore, when *CYP1B1* genotypes were combined with the previously analyzed *N*-acetyl transferase (*NAT2)* genotypes, a significant increase in breast cancer risk was found among women who had at least one *CYP1B1 432Val* allele together with the *NAT2* slow acetylator genotype (OR 1.52; 95% CI 1.03–2.24) compared to women carrying a combination of *CYP1B1 Leu/Leu* and *NAT2* rapid acetylator genotypes. This risk was seen to be confined to ever smokers; the OR was 2.46 (95% CI 1.11–5.45) for ever smokers carrying at least one *CYP1B1 432Val* allele together with the *NAT2* slow acetylator genotype compared to ever smokers with the *CYP1B1 Leu/Leu* and *NAT2* rapid acetylator genotype combination. Our results suggest that the *CYP1B1* polymorphism may be an important modifier of breast cancer risk in Finnish Caucasian women who have been exposed to tobacco smoke and/or carry the *NAT2* slow acetylator genotype.

Keywords Breast cancer · CYP1A1 · CYP1B1 · NAT2 · Genotype · SNP · Smoking

Abbreviations

BMI	Body mass index
CI	Confidence interval
CYP	Cytochrome P450
E_2	17-β-estradiol
HRT	Hormone replacement therapy
NAT2	*N*-acetyl transferase

P. Sillanpää · L. Heikinheimo · H. Vainio · K. Metsola · A. Hirvonen (✉)
Finnish Institute of Occupational Health, Topeliuksenkatu 41 a A, Helsinki 00250, Finland
e-mail: ari.hirvonen@ttl.fi

V. Kataja
Department of Oncology, Kuopio University Hospital, Kuopio, Finland

M. Eskelinen
Department of Surgery, Kuopio University Hospital, Kuopio, Finland

V.-M. Kosma
Department of Clinical Pathology, Kuopio University Hospital, Kuopio, Finland

V.-M. Kosma
Department of Pathology and Forensic Medicine, Institute of Clinical Medicine, University of Kuopio, Kuopio, Finland

M. Uusitupa
Department of Clinical Nutrition, School of Public Health and Clinical Nutrition, University of Kuopio, Kuopio, Finland

OC	Oral contraceptive
OR	Odds ratio
PAH	Polycyclic aromatic hydrocarbon
PCB	Polychlorinated biphenyl
PCR	Polymerase chain reaction
RFLP	Restriction fragment length polymorphism

Introduction

Most of the established risk factors for breast cancer are linked to prolonged estrogen exposure, like early age at menarche, late age at menopause, nulliparity and obesity in postmenopausal women [1]. Also some life style factors, such as the use of hormone replacement therapy (HRT) and alcohol are known to contribute to the increased risk, while the studies on the association between smoking and breast cancer risk have given discrepant results [2, 3]. Tobacco smoke contains a wide variety of known carcinogens including polycyclic aromatic hydrocarbons (PAHs) and nitrosoamines. However, in some studies tobacco smoking has been suggested to have protective effect against breast cancer, possibly due to earlier menopause and thus shorter exposure to estrogen [4]. Increased level of 2-hydroxyestradiol induced by smoking has also been suggested as one potential anti-estrogenic mechanism of tobacco smoke [5].

Cytochrome P450 (CYP) 1A1 and 1B1 enzymes are involved in the metabolism of both estradiol and PAHs to mutagenic intermediates capable of causing DNA damage [6–8]. CYP1A1 mediates hydroxylation of 17-β-estradiol (E_2) mainly to 2-hydroxyestradiol and CYP1B1 preferably to 4-hydroxyestradiol [6, 7]; the latter metabolite has been shown to be carcinogenic [9, 10]. The expression of CYP1A1 and CYP1B1 is induced by PAHs and recently CYP1B1 was also demonstrated to be induced by E_2 in estrogen receptor positive cells [11, 12]. Different expression levels of these enzymes as well as inter-individual differences due to genetic variations may thus further contribute to variations in individual susceptibility to breast cancer.

To date, several genetic polymorphisms have been identified in the *CYP1A1* and *CYP1B1* genes [13, 14]. In CYP1A1, in addition to a *T3801C* polymorphism detectable by *Msp*I restriction enzyme [15], two polymorphisms leading to an amino acid change exist in exon 7 (*Thr461Asn* and *Ile462Val*) [16, 17]. A strict linkage disequilibrium have been reported between the *CYP1A1 3801C* and *CYP1A1 462Val* alleles in Caucasian population [16, 18, 19]. Among African–Americans, an additional polymorphic site exists in the 3′ non-coding region of *CYP1A1*; a *T3205C* creating a novel *Msp*I restriction site [20]. Functional significance of the different *CYP1A1* genotypes in human lymphocytes has been studied with somewhat inconclusive results [21–24]. One study showed that subjects with the variant *CYP1A1 462Val* allele had an increased inducibility of CYP1A1 mRNA and threefold increase in enzymatic activity, whereas *T3801C* polymorphism had no effect on the induction of CYP1A1 [22]. Similarly, lymphocyte CYP1A1 enzyme activity was significantly increased among subjects with the variant *CYP1A1 462Val* allele according to two other studies [21, 24]. The variant *CYP1A1 3801C* allele has also been reported to be more readily inducible or having higher activity than the wild type in human lymphocytes [23, 24].

In *CYP1B1*, two polymorphisms, *Leu432Val* and *Asn453Ser* are located in a catalytically important heme binding domain in exon 3 [25, 26]. Two linked amino acid substitutions in exon 2 (*Arg48Gly* and *Ala119Ser*) constitute an additional allele [25, 27]. Functional assays of *CYP1B1 Leu432Val* polymorphisms in bacterial expression systems have shown that the *CYP1B1 432Val* allele encodes an enzyme with higher activity towards E_2 than the *432Leu* variants [28, 29]. However, one study performed in bacterial expression system suggested that all variant enzymes (with amino acid substitutions at codon 48, 119, 432 or 453) were associated with increased catalytic efficiency for the 4-hydroxylation of E_2 [30]. Instead, the *CYP1B1*2* (*48Gly* and *119Ser*) variant has not been shown to alter the catalytic activity towards E_2 [27, 31]. One study using (–)-*trans*-(7R,8R)-benzo[*a*]pyrene 7, 8-dihydrodiol as a substrate in a bacterial system found no major differences in catalytical properties for studied *CYP1B1* variants [28].

In earlier studies a significant overall association has been found between the *CYP1A1* polymorphisms and breast cancer risk [32–39]. However, only three out of eight studies reported positive association among Caucasian population [32, 37, 38]. Moreover, in all other Caucasian studies as well as in two Asian studies no significant overall association was seen [40–49]. When exposure to tobacco smoke or polychlorinated biphenyls (PCBs) has been taken into account, stronger evidence for the association between *CYP1A1* genotypes has been found [38, 44–46, 49–51].

Two earlier studies have found an overall association between *CYP1B1 Leu432Val* polymorphism and breast cancer risk [52, 53]. Moreover, the *432Val* allele has been associated with estrogen and/or progesterone receptor positive tumors [26, 54], breast cancer risk among HRT users [55] and current smokers in a case-only study [56].

In contrast, at least five case-control studies have not found any significant association between the *432Val* allele and breast cancer risk [57–61].

In this study, the potential modifying role of the *CYP1A1* and *CYP1B1* genotypes was studied in a homogenous Finnish study population consisting of 483 incident breast cancer patients and 482 healthy population controls. We also evaluated the potential interaction between the *CYP1B1* genotypes and the previously [62] analyzed *N*-acetyl transferase *(NAT2)* genotypes.

Materials and methods

Subjects

This case-control study is an extension of the Kuopio Breast Cancer Study [63, 64]. This study was approved by the Joint Committee of the University of Kuopio and Kuopio University Hospital. Participation was based on written consent. Women with a suspect breast lump, mammographic abnormality or a breast symptom necessitating further examinations were invited to Kuopio University Hospital (Finland) between 1990 and 1995 for diagnostic procedures. Detailed data were collected from all study subjects by a trained study nurse before any diagnostic procedures were initiated. Among other things, the questionnaires outlined socioeconomic background, family history of breast cancer, history of benign breast disease, reproductive and medical history, smoking habits, current alcohol intake and body size indicators. Smoking inquiries included the data about the amount of daily smoking (cigarettes/day), the duration of smoking (in years), and the time since cessation of smoking (in years). Exposure to passive smoking at work and/or at home was also resolved (in years).

A total of 516 women were eventually diagnosed with histologically confirmed breast cancer. Because all interviewed women agreed to donate a blood sample and only 12 women who were later diagnosed with breast malignancy refused to participate, the co-operation rate for the cases was 98%. The recruitment protocol missed 51 women within the hospital, all being private patients not entering the hospital through the standard referral system. Additional 11 cases were lost due to nurses' one-month strike in spring 1995. Only 26 breast cancer patients from the hospital catchment area were treated elsewhere according to the files of the Finnish Cancer Registry. The contact rate for the cases, calculated as described in Slattery et al. [65], was therefore 86% and the overall response rate 84%.

Healthy population controls with no previous breast problems or symptoms were drawn from the Finnish National Population Register covering the catchment area of cases. They were initially contacted by a letter explaining the study protocol and later called up by a study nurse. The exact contact rate is not available for controls. Overall, 514 controls were interviewed in parallel with the breast cancer cases, all of whom agreed to donate blood. The co-operation rate among controls was 72%. The main reason for non-participation was refusal.

Lymphocyte DNA was available for 483 breast cancer patients and for 488 controls. Six subjects among controls were excluded because they had an earlier breast cancer diagnosis ($n = 4$) or they were of non-Finnish origin ($n = 2$). Consequently, the final case group included 483 patients (44.3–91.6 years, mean 58.9 years) and the final control population consisted of 482 subjects (37.5–77.2 years, mean 53.5 years).

Genotyping analyses

Lymphocyte DNA was extracted by standard techniques. The *CYP1A1* genotypes were determined using 100 ng of DNA as template in a PCR-based restriction fragment length polymorphism (RFLP) method previously described by Cascorbi et al. [16]. Briefly, a 204 bp PCR amplification product was digested with *BseM* I (Fermentas, Amherst, NY, USA) or *Bsa* I (New England Biolabs, Inc., Beverly, MA, USA) restriction enzymes for determination of the polymorphisms *CYP1A1 Ile462Val* and *CYP1A1 Thr461Asn* genotypes, respectively.

For *CYP1B1* genotyping, 50 ng of DNA was amplified by AmpliTaq Gold DNA polymerase which is included in TaqMan Universal Master mix (Applied Biosystems, Foster city, CA, USA). The detection of alleles was based on fluorogenic TaqMan MGB probes using the ABI PRISM 7000 Sequence Detection Systems (Applied Biosystems, Foster City, CA, USA). Primer Express™ Version 2.0 software (Applied Biosystems, Foster city, CA, USA) was used for designing short amplicons for detecting the *CYP1B1 432Val* allele. A 108 bp PCR product was amplified using specific forward (5′-ACC TCT GTC TTG GGC TAC CAC AT-3′) and reverse (5′-TGG ATC AAA GTT CTC CGG GTT A-3′) primers. Allele specific TaqMan MGB-probes (Applied Biosystems, Cheshire, UK) 5′-VIC-AT CAT GAC CCA **C**TG AA-3′ and 5′-FAM-AAT CAT GAC CCA **G**TG AA-3′ were used for detecting the *CYP1B1 432Leu* allele and the *432Val* allele, respectively. The PCR reaction was performed in a total volume of 20 µl containing 1× TaqMan

Universal Master mix, 900 nM of each primer and 200 nM of each probe. Two initial hold steps of 2 min at 50°C and 10 min at 94°C were followed by 40 cycles of two-step PCR with denaturation at 94°C for 15 s and annealing and extension at 60°C for 1 min. For quality control purposes 144 of the TaqMan genotyping results were confirmed by a PCR–RFLP method published earlier [26].

All obscure results as well as a random repertoire of 10% of all samples, were re-examined for the quality assurance of the laboratory work. All the results were interpreted by two independent investigators. No discrepancies were found in the replicate analyses.

The *NAT2* was genotyped by a TaqMan method as previously described in [62].

Statistical methods

The observed *CYP1A1* and *CYP1B1* genotype frequencies were compared to the expected genotype frequencies to determine whether they are in Hardy–Weinberg equilibrium (HWE).

Associations between *CYP1A1* or *CYP1B1* genotypes and breast cancer risk were analysed by unconditional logistic regression to estimate adjusted odds ratios (ORs) and 95% confidence intervals (CIs) using the SPSS 9.0 statistical package (SPSS Inc, Chicago, IL, USA). Known or suspected risk factors for breast cancer were used as adjusting variables in the multivariate logistic models. These covariates included age (<45, 45–53, 53–61, >61 years), age at menarche (≤12, 13–14, ≥ 15 years), age at first full-term pregnancy (nulliparous, <25, 25–30, >30 years), number of full-term pregnancies (continuous), history of benign breast disease (no/yes), first-degree (mother, sister, daughter) family history of breast cancer (no/yes), smoking (never/ever), use of alcohol (never/ever) and body mass index (BMI) (<25.4 kg/m^2, ≥25.4 kg/m^2). Subjects were excluded from the logistic regression if any of the adjusting variables were missing.

Women who had ever smoked daily for at least 3 months were classified as smokers and those who reported smoking at reference date were considered current smokers. Women who reported ever being exposed to passive smoking at work or at home were classified as passive smokers. Adjusted estimates for active and passive smoking and breast cancer risk were calculated in order to evaluate the potential association between smoking habits and breast cancer risk. The factors of interest were smoking status (never active/passive, only passive, former and current smokers), duration of smoking (1–14 and ≥15 years), number of cigarettes smoked per day (1–9 and ≥10) and pack-years of cigarettes (<5 and ≥5). Pack-years were calculated as number of packs (20 cigarettes per pack) smoked per day multiplied by the number of smoking years.

Women were considered postmenopausal if they had reported natural menopause, or had gone through bilateral oophorectomy. Women who were hysterectomized with intact ovaries (ovary) (40 cases and 41 controls) or for whom details of the operations were unknown (6 cases and 2 controls) were classified postmenopausal if they were no longer menstruating and were older than 51 years (median for menopause among Finnish women). All the rest were classified premenopausal.

Possible associations between the *CYP1A1* and *CYP1B1* genotypes and smoking, use of oral contraceptives (OCs) (never/ever) or HRT (never/ever), were examined by stratified analyses. The interactive effects were assessed by the likelihood ratio tests to compare the goodness of fit of the models with and without the interaction term taking into account other adjusting variables. The association between *CYP1A1* or *CYP1B1* genotypes and the expression of estrogen or progesterone receptors in the tumour was also evaluated.

Based on the data from previous studies, homozygous for the *CYP1A1 461Thr* and *462Ile* alleles and *CYP1B1 432Leu* alleles were chosen to serve as the referent category in all separate analyses for these locuses, respectively [35, 41, 51, 55, 57]. In order to increase statistical power the heterozygous and homozygous variant genotypes were combined as the risk genotype group.

For the combined analyses the rapid *NAT2* acetylator genotypes were grouped with the *CYP1B1 Leu/Leu* genotypes to act as a reference group.

Results

Our previous studies have shown a high (>0.91) waist-to-hip ratio, first-degree family history of breast cancer, and history of benign breast disease to be associated with increased risk of breast cancer in this study population, whereas parity and use of OCs were associated with decreased risk [64]. Cases (mean age 58.9 years, SD 14.3 years) were somewhat older compared to controls (mean age 53.5 years, SD 10.9 years) ($P < 0.001$) [63, 64]. In contrast, there was no difference in the daily consumption of cigarettes, duration of smoking, pack-years of smoking, BMI or the use of OCs (duration in months) between cases and controls (data not shown).

Table 1 shows the distribution of *CYP1A1* and *CYP1B1* genotypes among cases and controls. All genotype frequencies were in Hardy–Weinberg equilibrium in the controls. Since the *CYP1A1 T3801C* and *CYP1A1 Ile462Val* polymorphisms are closely linked in Caucasian populations [16, 18, 19], only the *Ile462Val* and *Thr461Asn* polymorphisms were studied for *CYP1A1* gene.

Overall, the *CYP1A1* and *CYP1B1* genotype frequencies did not vary significantly between cases and controls (Table 1). When stratified by menopausal status, a tendency of protective effect was seen among premenopausal women carrying the *CYP1A1 462Val* allele (OR 0.51, 95% CI 0.25–1.05) compared to women without the allele. No effect was seen in this context for the *CYP1A1 Thr461Asn* and *CYP1B1 Leu432Val* polymorphisms (data not shown).

No significant association was found between the *CYP1A1* and *CYP1B1* genotypes and breast cancer risk in subgroups defined by estrogen or progesterone receptor status or use of OCs or HRT (never/ever) (data not shown). However, a tendency of increased risk of breast cancer was seen among ever users of OCs with at least one *CYP1B1 432Val* allele (OR 1.50, 95% CI 0.98–2.30) compared to women without this allele. Smoking (never/ever) did not have interaction with *CYP1A1* genotypes (data not shown). In contrast, the *CYP1B1* genotypes appeared to interact with smoking in modifying the individual breast cancer risk; ever smokers with one copy of the *CYP1B1 432Val* allele were at borderline increased risk of breast cancer (OR 1.86, 95% CI 1.01–3.42) compared to ever smokers with the *Leu/Leu* genotype (Table 2). Moreover, when ever smokers were further stratified by daily consumption of cigarettes a statistically significant increase in breast cancer risk was seen for women who had smoked 1–9 cigarettes daily and carried either one (OR 2.63; 95% CI 1.07–6.46) or two (OR 5.09; 95% CI 1.30–19.9) *CYP1B1 432Val* alleles compared to women who had smoked the same amount but carried the *Leu/Leu* genotype (*P* for interaction 0.009) (Table 2.). Similarly, women with at least one *CYP1B1 432Val* allele were at increased risk if they had smoked less than 15 years (OR 2.62, 95% CI 1.06–6.49) compared to women who had smoked the same time but lacked the allele. Similarly, women with at least one *CYP1B1 432Val* allele were at increased risk if they had smoked less than five pack-years (OR 2.73, 95% CI 1.09–6.83) compared to women who had smoked the same amount but carried the *Leu/Leu* genotype.

Although no significant interaction was found between *CYP1B1* and *NAT2* genes ($P = 0.385$) when *CYP1B1* genotypes were combined with the previously analyzed *NAT2* genotypes, women who had at least one *CYP1B1 432Val* allele together with the *NAT2* slow acetylator genotype were found to be at 1.52-fold (95% CI 1.03–2.24) increased risk of breast cancer compared to women simultaneously carrying the *Leu/Leu* and *NAT2* rapid acetylator genotypes. This risk was confined to premenopausal women (OR 1.90; 95% CI 1.02–3.56). Moreover, the risk increased with the number of at risk genotypes (*P* for trend 0.04) (Table 3.). When stratified by smoking habits, this risk was seen to be confined to ever smokers; a 2.46-fold (95% CI 1.11–5.45) risk for breast cancer was seen among women who carried at least one *CYP1B1 432Val* allele (*P* for trend 0.03) together with the *NAT2* slow acetylator genotype compared to ever smokers who carried the *Leu/Leu* genotype together with the *NAT2* rapid acetylator genotype (Table 4.). A further increase in the risk was seen for women who had smoked daily 1–9 cigarettes and carried the high-risk genotype combination; they were at over fourfold (OR 4.41; 95% CI 1.29–15.1) risk of breast cancer

Table 1 Associations between *CYP1A1* and *CYP1B1* genotypes and breast cancer risk

Genotypes	Cases *n* (%)	Controls *n* (%)	OR (95% CI)[a]
CYP1A1 Ile462Val			
Ile/Ile	426 (88.6)	412 (86.0)	1.00
Ile/Val	53 (11.0)	66 (13.8)	–
Val/Val	2 (0.4)	1 (0.2)	–
Ile/Val+Val/Val	55 (11.4)	67 (14.0)	0.76 (0.50–1.14)
CYP1A1 Thr461Asn			
Thr/Thr	474 (98.5)	474 (99.0)	1.00
Thr/Asn	7 (1.5)	5 (1)	1.67 (0.50–5.48)
Asn/Asn	–	–	–
CYP1B1 Leu432Val			
Leu/Leu	193 (40.1)	214 (44.7)	1.00
Leu/Val	226 (47.0)	205 (42.8)	1.19 (0.89–1.59)
Val/Val	62 (12.9)	60 (12.5)	1.17 (0.77–1.78)
Leu/Val + Val/Val	288 (59.9)	265 (55.3)	1.19 (0.91–1.56)

[a] Adjusted for age, age at menarche, number of pregnancies, age at first full-term pregnancy, history of benign breast disease, first-degree family history of breast cancer, smoking, use of alcohol and BMI

Table 2 Association between *CYP1B1* genotypes and breast cancer risk according to smoking habits

Smoking habits	*CYP1B1* genotypes	Cases[a] (%)	Controls[a] (%)	OR (95% CI)[b]
Never active or passive smoking	*Leu/Leu*	86 (41.1)	77 (42.3)	1.00
	Leu/Val	99 (47.4)	78 (42.9)	1.19 (0.75–1.89)
	Val/Val	24 (11.5)	27 (14.8)	0.79 (0.41–1.54)
	Leu/Val + Val/Val	123 (58.9)	105 (57.7)	1.08 (0.70–1.66)
Passive smoking	*Leu/Leu*	61 (40.1)	70 (41.9)	1.00
	Leu/Val	67 (44.1)	81 (48.5)	0.90 (0.54–1.52)
	Val/Val	24 (15.8)	16 (9.6)	1.86 (0.87–4.01)
	Leu/Val + Val/Val	91 (59.9)	97 (58.1)	1.07 (0.66–1.73)
Ever smoking	*Leu/Leu*	43 (37.1)	67 (51.5)	1.00
	Leu/Val	59 (50.9)	46 (35.4)	1.86 (1.01–3.42)
	Val/Val	14 (12.1)	17 (13.1)	1.25 (0.53–2.98)
	Leu/Val + Val/Val	73 (62.9)	63 (48.5)	1.68 (0.95–2.96)
Daily cigarettes				
1–9	*Leu/Leu*	18 (33.3)	39 (60.0)	1.00
	Leu/Val	27 (50.0)	21 (32.3)	2.63 (1.07–6.46)
	Val/Val	9 (16.7)	5 (7.7)	5.09 (1.30–19.89)[c,d]
	Leu/Val + Val/Val	36 (66.7)	26 (40.0)	3.06 (1.32–7.12)
>10	*Leu/Leu*	25 (41.7)	28 (43.8)	1.00
	Leu/Val	30 (50.0)	24 (37.5)	1.28 (0.46–3.55)
	Val/Val	5 (8.3)	12 (18.8)	0.31 (0.06–1.46)
	Leu/Val + Val/Val	35 (58.3)	36 (56.3)	0.89 (0.35–2.26)
Smoking years				
<15	*Leu/Leu*	15 (28.8)	33 (52.4)	1.00
	Leu/Val	31 (59.6)	20 (31.7)	3.28 (1.23–8.74)
	Val/Val	6 (11.5)	10 (15.9)	1.48 (0.41–5.39)
	Leu/Val + Val/Val	37 (71.2)	30 (47.6)	2.62 (1.06–6.49)
>15	*Leu/Leu*	27 (42.9)	34 (50.7)	1.00
	Leu/Val	28 (44.4)	26 (38.8)	1.39 (0.56–3.45)
	Val/Val	8 (12.7)	7 (10.4)	1.09 (0.29–4.00)
	Leu/Val + Val/Val	36 (57.1)	33 (49.3)	1.30 (0.56–3.01)
Pack-years				
<5	*Leu/Leu*	14 (31.1)	35 (56.5)	1.00
	Leu/Val	26 (57.8)	18 (29.0)	3.63 (1.33–9.89)
	Val/Val	5 (11.1)	9 (14.5)	1.29 (0.33–5.12)
	Leu/Val + Val/Val	31 (68.9)	27 (43.5)	2.73 (1.09–6.83)
>5	*Leu/Leu*	27 (40.3)	32 (47.8)	1.00
	Leu/Val	31 (46.3)	27 (40.3)	1.41 (0.58–3.44)
	Val/Val	9 (13.4)	8 (11.9)	1.17 (0.33–4.20)
	Leu/Val + Val/Val	40 (59.7)	35 (52.2)	1.35 (0.59–3.06)

[a] Number of subjects that have provided information on smoking and for whom genotyping was successful

[b] Adjusted for age, age at menarche, number of pregnancies, age at first full-term pregnancy, history of benign breast disease, first-degree family history of breast cancer, use of alcohol and BMI

[c] Interaction between daily cigarette consumption and *CYP1B1* genotypes ($P = 0.009$)

[d] P for trend 0.005

Table 3 Association between combined *CYP1B1* and *NAT2* genotypes and breast cancer risk

Combined genotype	Cases/controls	OR (95 % CI)[a]
CYP1B1 Leu/Leu and *NAT2* rapid	88/105	1.0
CYP1B1 Leu/Leu and *NAT2* slow	103/108	1.15 (0.76–1.74)
CYP1B1 Leu/Val + Val/Val and *NAT2* rapid	119/139	1.04 (0.70–1.55)
CYP1B1 Leu/Val + Val/Val and *NAT2* slow	168/135	1.52 (1.03–2.24)[b]

[a] Adjusted for age, age at menarche, number of pregnancies, age at first full-term pregnancy, history of benign breast disease, first-degree family history of breast cancer, smoking, use of alcohol and BMI

[b] P for trend 0.04

Table 4 Association between combined *CYP1B1* and *NAT2* genotypes and breast cancer risk stratified by smoking

Smoking variable	Combined genotype	Cases/controls	OR (95% CI)[a]
Never smokers	*CYP1B1 Leu/Leu* and *NAT2* rapid	68/69	1.0
	CYP1B1 Leu/Leu and *NAT2* slow	77/78	1.02 (0.62–1.68)
	CYP1B1 Leu/Val + *Val/Val* and *NAT2* rapid	85/97	0.87 (0.54–1.40)
	CYP1B1 Leu/Val + *Val/Val* and *NAT2* slow	128/105	1.31 (0.84–2.06)
Ever smokers	*CYP1B1 Leu/Leu* and *NAT2* rapid	20/36	1.0
	CYP1B1 Leu/Leu and *NAT2* slow	23/30	1.48 (0.63–3.44)
	CYP1B1 Leu/Val + *Val/Val* and *NAT2* rapid	33/33	1.59 (0.70–3.58)
	CYP1B1 Leu/Val + *Val/Val* and *NAT2* slow	40/30	2.46 (1.11–5.45)[b]

[a] Adjusted for age, age at menarche, age at first full-term pregnancy, number of pregnancies, history of benign breast disease, first-degree family history of breast cancer, use of alcohol and BMI

[b] *P* for trend 0.03

compared to women who smoked the same amount daily and were carriers of the *CYP1B1 Leu/Leu* and *NAT2* rapid acetylator genotype combination. Similar association was found among women who had smoked under 15 years (data not shown).

Discussion

The frequency of the *CYP1A1 462Val* allele found in this study (7.1%) is well in accordance with the frequencies found in other Caucasian populations (4–9%) [19, 41]. Instead, the frequency of the *CYP1A1 461Asn* allele was lower in our study population (0.5%), compared to previous studies among other Caucasian population (2.0–5.7%) [66]. Historical and demographic reasons could partly explain this difference [67, 68]. Consequently, the power of the study was far too low for any reliable interpretations of the *CYP1A1 Thr461Asn* and breast cancer risk.

The lack of significant overall association between the studied *CYP1A1* polymorphisms and breast cancer risk is in good agreement with most of the previous studies among Caucasian population [40–46]. Although contradictory results also exist [32–37], only three of them are from Caucasian studies; an increased breast cancer risk was observed among French–Canadian women with at least one variant *CYP1A1 461Asn* allele (OR 3.3, 95% CI 1.1–9.7) [32] and with Caucasian women (in Connecticut) with the *CYP1A1 462Val* allele (OR 2.1, 95% CI 1.1–3.9) [38] whereas a decreased risk was seen among Caucasian women (central European origin) with at least one *CYP1A1 3801C* allele (OR 0.55, 95%CI 0.36–0.82) [37].

A tendency of decreased breast cancer risk was seen in our study among premenopausal women with at least one *CYP1A1 462Val* allele (OR 0.51, 95% CI 0.25–1.05). Similar, statistically significant decrease in the risk has been seen among Japanese women (OR 0.66, 95% CI 0.45–0.96) [33] in whom the frequency of the *CYP1A1 462Val* allele is almost fourfold compared to our population (25.4% vs. 7.1%). A recent meta-analysis found no significant associations between *CYP1A1 T3801C*, *Ile462Val* or *Thr461Asn* polymorphisms and breast cancer risk [69].

In many of the studies that failed to find any overall associations between the *CYP1A1* genotypes and breast cancer, significant associations between the *CYP1A1* polymorphisms and smoking habits or higher levels of serum PCBs in relation to risk of breast cancer have been observed [38, 44–46, 49–51]. However, in accordance with our studies no association between the smoking habits and the *CYP1A1 Ile462Val* or *CYP1A1 Thr461Asn* polymorphisms and breast cancer risk have been found in white Caucasians [32, 41, 42, 46]. The role of PCBs in relation to breast cancer risk could not be evaluated in our study, as the data on the exposure to PCBs was not available.

The frequency of the *CYP1B1 432Val* allele (34%) in the present study was well in accordance with earlier findings in Caucasians (27–45%) [25, 26, 53, 55]. In agreement with most of the previous studies [26, 54, 55, 57–61, 70], no overall association with breast cancer risk was seen for the *CYP1B1* genotypes. However, ever smokers with one *CYP1B1 432Val* allele were at increased risk of breast cancer compared to ever smokers with the *Leu/Leu* genotype. Furthermore, a significant interaction was seen with daily use of cigarettes; the increase was confined to those women who were smoking less than ten cigarettes a day with a significant trend of increasing risk with increasing number of variant alleles.

In a recent case-only study among French women, a similar borderline significant increase in breast cancer risk was found for current smokers carrying two *CYP1B1 432Val* alleles compared to never smokers with at least one *432Leu* allele (OR 2.32, 95% CI 1.00–5.38) [56]. The finding reached statistical significance among

heavier smokers (i.e. >5 cigarettes/day, >20 years or >10 pack-years). In our study no increase in the risk was seen among women smoking more than ten cigarettes/day. However, since the French study was a case only study, the results are not fully comparable. Furthermore, at least two studies have reported no association between breast cancer risk and the *CYP1B1* genotypes in relation to smoking [53, 55].

As NAT2 is also involved in the metabolism of tobacco carcinogens and as we have earlier found a significant association between the slow acetylator status and breast cancer risk in smoking women [62], we also examined the potential combined effects of the *NAT2* and *CYP1B1* genotypes; the study did not have sufficient power to evaluate the *CYP1A1* and *NAT2* genotype combinations in this context.

The concurrent presence of at least one *CYP1B1 432Val* allele and the *NAT2* slow acetylator genotype appeared to pose a statistically significant increase in the risk of breast cancer compared to women with the *CYP1B1 Leu/Leu* genotype together with the *NAT2* rapid acetylator genotype. However, no interaction was found between these two genes. When stratified by smoking, the increased risk seemed to be confined to ever smokers, especially to light smokers (1–9 cigarettes/day) or to those who had smoked less than 15 years. Our finding agrees with the suggestions that the importance of genetic susceptibility might be more significant at lower levels of exposure to carcinogens [71]; the xenobiotic metabolizing enzymes may saturate at higher concentrations of the substrates. Accordingly, NAT2, which is known to be involved in the metabolism of tobacco carcinogens, has been suggested to be associated with increased risk of cancer especially at low doses of tobacco carcinogens in studies by us [62, 72] and others [73, 74].

One shortage in our study is that even though our study size is relatively large, the number of smokers is fairly low. Moreover, the number of pack-years is fairly low. These results should, therefore, be considered preliminary before confirmed in larger studies with adequate number of smokers. Furthermore, due to the multiple comparisons performed, the possibility of a chance finding should also be considered.

In our study, a tendency of increasing risk was seen among ever users of OCs carrying at least one copy of the *CYP1B1 432Val* allele (OR 1.50, 95% CI 0.98–2.31) compared to those with the *CYP1B1 Leu/Leu* genotype. This issue has previously been studied only in a Turkish population where no association between breast cancer and *CYP1B1* genotypes in relation to use of OCs was found [53]. One possible mechanistic explanation for our finding is that increased CYP1B1 activity may result in producing higher levels of genotoxic 4-OHE_2.

Finally, the *CYP1B1 Val/Val* genotype has been shown to be more prevalent in the tumors expressing the ER and/or PR [26, 54] or among patients who have used HRT for more than 4 years [55]. However, in agreement with two previous studies [55, 75], no association between ER and/or PR status and *CYP1B1 Val* was seen in our study. Neither were no associations found when stratified by the use of postmenopausal hormones.

In summary, we found no significant overall associations between the *CYP1A1* and *CYP1B1* genotypes and breast cancer risk. However, the *CYP1B1 432Val* allele was associated with increased risk of breast cancer among light smokers. Moreover, as a novel finding, a significant trend of increasing risk with increasing number of the putative at-risk genotypes of *CYP1B1* and *NAT2* was seen. This association was confined to light smokers.

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