



Human Genome Epidemiology (HuGE) Review

Meta- and Pooled Analyses of the Cytochrome P-450 1B1 Val432Leu Polymorphism and Breast Cancer: A HuGE–GSEC Review

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The association between the cytochrome P-450 1B1 (*CYP1B1*) Val432Leu polymorphism and breast cancer was assessed through a meta-analysis of all published case-control studies and a pooled analysis of both published and unpublished case-control studies from the Genetic Susceptibility to Environmental Carcinogens (GSEC) database (http://www.upci.upmc.edu/research/ccps/ccontrol/g_intro.html). GSEC is a collaborative project that gathers information on studies of metabolic gene polymorphisms and cancer. Thirteen articles were included in the meta-analysis (14,331 subjects; 7,514 cases, 6,817 controls); nine data sets were included in the pooled analysis (6,842 subjects; 3,391 cases, 3,451 controls). A summary meta- or pooled estimate of the association between the *CYP1B1* Val432Leu polymorphism and breast cancer could not be calculated because of statistically significant heterogeneity in the point estimates among studies. No association between the *CYP1B1* Val432Leu polymorphism and breast cancer was observed in Asians (for *Val/Val* and *Val/Leu* combined, odds ratio (OR) = 1.0, 95% confidence interval (CI): 0.8, 1.2). An inverse association was observed in populations of mixed/African origin (OR = 0.8, 95% CI: 0.7, 0.9). The pooled analysis suggested a possible association in Caucasians (for *Val/Val* and *Val/Leu* combined, OR = 1.5, 95% CI: 1.1, 2.1), with effect modification across age categories. The observed effect of age on the association in Caucasians indicates that further studies are needed on the role of *CYP1B1* Val432Leu in estrogen metabolism according to age, ethnicity, and menopausal status.

breast neoplasms; *CYP1B1*; cytochrome P-450 enzyme system; genetics; hormones; meta-analysis; polymorphism, genetic; review [publication type]

Abbreviations: CI, confidence interval; CYP, cytochrome P-450; GSEC, Genetic Susceptibility to Environmental Carcinogens; OR, odds ratio.

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BACKGROUND

Gene

The cytochrome P-450 1B1 (*CYP1B1*) gene maps to chromosome 2p22–p21 (1) and contains three exons and two introns (2). The entire coding sequence of the gene is contained in exons 2 and 3. The gene is expressed in monocytes and macrophages, as shown by the measurement of both mRNA and proteins (3, 4), and in several other extrahepatic tissues and organs, such as the kidney, prostate, breast, uterus, ovary, and placenta (5–9).

CYP1B1 is an inducible enzyme regulated through the aryl hydrocarbon receptor (10) and is activated through this receptor by polycyclic aromatic hydrocarbons and dioxin-like compounds (11). It is capable of activating a variety of carcinogens, such as arylamines, and is involved in the metabolism of estradiol, like several genes of the cytochrome P-450 (CYP) family; *CYP1B1* encodes for an enzyme that catalyzes the formation of both 2- and 4-hydroxyestrone, with higher activity on the 4- compound (12, 13).

Levels of *CYP1B1* gene expression in white blood cells have been investigated according to gender; results showed that females have higher expression than males (14), although these data were not confirmed in other studies (15, 16). *CYP1B1* gene expression during pregnancy has also been examined; it was found to be up-regulated in one study (17) but not in another (18).

Gene variants

There are several variants of *CYP1B1* (<http://www.cypalleles.ki.se/>). There is a common C→G transversion at position 1666 in exon 3, resulting in an amino acid substitution of leucine (Leu) with valine (Val) at codon 432 (13, 19). Exon 3 encodes the heme-binding domain, a region that is critical to the catalytic function of the gene (19). Several other polymorphisms in the *CYP1B1* gene have been described as well (<http://www.cypalleles.ki.se/>); four of them result in amino acid substitutions (20). They are: Arg→Gly at codon 48, Ala→Ser at codon 119, Leu→Val at codon 432, and Asn→Ser at codon 453.

Population frequency

The valine substitution is reported to be present at an allele frequency of 0.43 in Caucasians, while in African Americans the allele frequency is 0.75 (21) and in Asians it is 0.23 (22).

We calculated the pooled frequencies of the *CYP1B1* Val432Leu polymorphism from published studies on healthy subjects. A MEDLINE search of articles published through August 2005 produced 29 papers, with a total of 10,252 subjects belonging to different ethnic groups. The frequency of the *Leu/Leu* genotype is 0.70 (95 percent confidence interval (CI): 0.69, 0.72) in Asians and 0.31 (95 percent CI: 0.30,

0.32) in Caucasians, while it is lower in African-Americans: 0.08 (95 percent CI: 0.05, 0.11) (table 1).

Gene function

The Leu432Val allele of the *CYP1B1* gene is the most common allele in populations of European and African descent (23). The single nucleotide polymorphism that causes this amino acid change could affect mRNA stability or be in linkage disequilibrium with other variants in *CYP1B1* transcriptional regulatory sequences, such as response elements or regulating elements of RNA degradation (24). The Val432Leu polymorphism seems to have the largest impact on the catalytic properties of the enzyme; the *Val432* allele displays threefold higher 4-hydroxylase activity than the *Leu432* allele (25).

In vitro studies indicate that the valine allele has higher 4-hydroxyestradiol:2-hydroxyestradiol and 4-hydroxyestrone:2-hydroxyestrone ratios than the leucine allele (12). Several other in vitro studies have also indicated that the leucine substitution may influence estrogen metabolism (13, 25–27).

Higher catalytic activities towards estrogens have also been suggested for the *Gly48*, *Ser119*, and *Ser453* alleles (26). One study showed that among postmenopausal controls, plasma estradiol levels of *Ser453* allele carriers were higher than those of noncarriers, but no association with breast cancer was found (28).

Disease

Breast cancer is the most common cancer in women (29), and more than 1,000,000 new cases are diagnosed every year worldwide (30). The most established risk factors for breast cancer relate to cumulative exposure of the breast tissue to endogenous hormones, particularly estrogen (31). Factors that have been strongly implicated and are more or less directly related to estrogen exposure are early menarche, late age at first birth, nulliparity, oral contraceptive use, hormone replacement therapy (32, 33), low physical exercise levels, obesity (34), and alcohol intake (35); however, each of them accounts for only a small proportion of the risk (36).

Family history of breast cancer is an important predictor of breast cancer. Genetic factors, such as breast cancer genes *BRCA1* and *BRCA2*, the autosomal dominant susceptibility genes, account for less than 5 percent of cases (37–39). Allelic variations in genes involved in carcinogen metabolism (*CYP1A1*, *CYP2E1*, *GSTM1/T1P1*, *NAT1/2*, etc.), DNA repair (*XRCC1/3*, *ERCC2/4*, *ATM*, *AGT*, etc.), steroid hormone metabolism (*CYP19*, *CYP17*, *CYP1B1*, *COMT*, *ER-α*, etc.), and signal transduction and cell cycle control (*TGF-β*, *IGF-1*, *TNF-β*, *IL-1B*, *IL-1RN*, etc.) have been considered as possible cofactors in breast cancer risk.

METHODS

Selection criteria

We assessed the association between the *CYP1B1* Val432Leu polymorphism and breast cancer through a meta-analysis of all published papers and a pooled analysis

TABLE 1. Frequencies of the cytochrome P-450 1B1 (*CYP1B1*) Val432Leu polymorphism in healthy populations included in a meta-analysis

Ethnic group	Reference nos. of published articles	No. of subjects	<i>Leu/Leu</i>		<i>Val/Leu</i>		<i>Val/Val</i>	
			%	95% CI*	%	95% CI	%	95% CI
African Americans	19, 21, 27, 60	343	8.5	5.5, 11.4	46.4	41.1, 51.7	45.2	39.9, 50.5
Asians	21, 40, 49, 60–63	2,691	70.5	68.7, 72.2	23.6	22.0, 25.2	5.5	4.6, 6.4
Caucasians	19, 21, 28, 43, 46, 47, 55, 60, 62, 64–69	4,928	31.0	29.7, 32.3	46.9	45.5, 48.2	22.1	20.9, 23.2
Other or mixed	44, 45, 56, 57, 60, 66, 70, 71	2,290	34.5	32.6, 36.5	41.0	39.0, 43.0	24.5	22.7, 26.3

* CI, confidence interval.

of both published and unpublished studies. We searched MEDLINE and EMBASE up to September 2005, using different combinations of the keywords “cytochrome P-450 1B1,” “*CYP1B1*,” and “breast,” without any restriction on language. We supplemented the computer search by consulting bibliographies from the articles found through the MEDLINE search and by looking at a review paper (40).

An initial screening of all abstracts produced 22 articles containing information on both *CYP1B1* Val432Leu polymorphisms and breast cancer. Eligible studies were case-control, genotype-based studies that reported the frequency of the *CYP1B1* Val432Leu polymorphism or the odds ratio for the relation between *CYP1B1* Val432Leu and breast cancer. Both hospital-based and population-based case-control studies were included in the analysis. Of the selected papers, four were excluded because they were case-only studies, one was excluded because it was a family-based study, one was excluded because it reported data only on *CYP1B1* Val432Leu gene expression, and one was excluded because it was a review of published data (41). The subjects included in the paper by Kocabas et al. (42) completely overlapped with those in a previous article (43); therefore, only the study that included detailed analyses of the *CYP1B1* Val432Leu polymorphism (42) was included. The subjects in the papers by Li et al. (44) and Zhu et al. (45) partially overlapped; therefore, we included the study with the largest numbers of cases and controls (45). The final number of articles considered for the present meta-analysis was 13, comprising a total of 14,331 subjects (7,514 cases and 6,817 controls). A description of these studies is given in table 2.

All of the selected papers assessed the association between *CYP1B1* Val432Leu polymorphisms and breast cancer. Of these papers, eight also evaluated the association between other polymorphisms in the *CYP1B1* gene and breast cancer risk: Four analyzed the Asn453Ser polymorphism (19, 28, 44, 46), three the Arg48Gly polymorphism (40, 47, 48), and five the Ala119Ser polymorphism (46–49).

Data collection

The pooled analysis was performed using the Genetic Susceptibility to Environmental Carcinogens (GSEC) database. The International Collaborative Study on Genetic Susceptibility to Environmental Carcinogens (http://www.upci.upmc.edu/research/ccps/ccontrol/g_intro.html) is a collaborative project that gathers information from both published and unpublished case-control studies on metabolic gene

polymorphisms and cancer. The design of this study is explained in detail elsewhere (50). A questionnaire was provided to each investigator at the time of enrollment in the study, collecting information on the study design, the selection and source of controls, the laboratory methods used for genotyping, the source of DNA for genotype analysis, and the response rates for both cases and controls. Some of this information has been previously published (51). Studies that had information on *CYP1B1* Val432Leu and breast cancer were selected from the GSEC database. The investigators of the published studies for which data were not available through the GSEC project were then contacted and asked to provide their data for this specific pooled analysis.

Of the 13 studies included in the meta-analysis, we were able to obtain data from seven (see table 2 for details). The GSEC database contained two additional studies with unpublished data on the *CYP1B1* Val432Leu polymorphism and breast cancer; therefore, the final number of data sets included in the pooled analysis was nine, with a total of 6,842 subjects (3,391 cases and 3,451 controls).

Statistical analysis

For the meta-analysis, the reported frequencies of *CYP1B1* *Val/Leu* and *Val/Val* in cases and controls were extracted from the published studies, and the corresponding study-specific crude odds ratios and 95 percent confidence intervals for breast cancer risk were calculated. The departure of *CYP1B1* Val432Leu frequencies from expectation under Hardy-Weinberg equilibrium was tested (52). Egger's test for assessment of publication bias (53) was performed on the overall data set and according to ethnicity (Caucasian, Asian, or other) and source of controls (healthy controls or hospitalized controls). Funnel plots were used for a graphical representation of publication bias.

The *Q* statistic was used to test the hypothesis of homogeneity among all of the studies and according to ethnicity and type of controls, with *p* values less than 0.05 indicating the presence of heterogeneity among studies. A fixed-effects model was used when the test for heterogeneity was not statistically significant, while a random-effects model was employed when heterogeneity across studies was statistically observed (54).

Summary odds ratios were calculated for all of the studies combined. Since two of the most important causes of heterogeneity among studies were ethnicity and source of controls, we calculated separate odds ratios to assess the association

TABLE 2. Characteristics of case-control studies included in a meta-analysis of the cytochrome P-450 1B1 (*CYP1B1*) Val432Leu polymorphism and breast cancer

Author (ref. no.) and year of publication	No. of cases	No. of controls	Location	Source of controls	Menopausal status	Matching criteria	Val/Val		Val/Leu		Val/Val + Val/Leu		
							OR*,†	95% CI*	OR†	95% CI	OR†	95% CI	
Studies of Caucasians‡													
Listgarten et al. (55), 2004	173	154	Canada	Hospital	Both	Age	3.3	1.8, 6.2	2.2	1.3, 3.5	2.5	1.6, 3.9	
Rylander-Rudqvist et al. (46), 2003§	1,484	1,336	Sweden	Healthy	Postmenopausal	Age	1.0	0.8, 1.3	1.0	0.9, 1.2	1.0	0.9, 1.2	
De Vivo et al. (28), 2002	453	453	United States	Healthy	Both	Year of birth, menopausal status, estrogen replacement therapy	1.3	0.9, 1.8	1.5	1.1, 2.0	1.4	1.1, 1.9	
Kocabas et al. (43), 2002§	84	103	Turkey	Healthy	Both	Age, menopausal status	1.3	0.5, 3.4	2.7	1.4, 5.0	2.3	1.3, 4.1	
Bailey et al. (19), 1998§,¶	164	164	United States	Hospital	Postmenopausal	Age, race	1.3	0.7, 2.7	0.8	0.5, 1.4	0.9	0.6, 1.5	
Dunning et al. (47), 2004	1,617	845	United Kingdom	Healthy		Age, ethnicity, residence	0.9	0.7, 1.1	0.8	0.6, 1.0	0.8	0.7, 1.0	
Zimarina et al. (48), 2004§	192	132	Russia and Norway	Healthy			1.6	0.8, 3.0	0.7	0.4, 1.2	0.9	0.6, 1.5	
Studies of Asians													
Wen et al. (40), 2005§	1,110	1,195	Shanghai, China	Healthy	Both	Age	0.9	0.5, 1.6	1.0	0.8, 1.2	1.0	0.8, 1.2	
Lee et al. (61), 2003§	241	290	South Korea	Hospital	Both	Age	1.5	0.4, 4.9	1.0	0.7, 1.6	1.1	0.7, 1.6	
Watanabe et al. (49), 2000	336	324	Japan	Healthy			1.1	0.4, 3.3	0.9	0.6, 1.3	0.9	0.7, 1.3	
Meta-analysis of Asians	1,687	1,809					1.0	0.6, 1.6	1.0	0.8, 1.2	1.0	0.8, 1.2	
<i>p</i> value													
<i>Q</i> test							0.77		0.86		0.85		
Egger's test							0.25		0.76		1.00		
Studies of other ethnic groups													
Thyagarajan et al. (56), 2004	164	338	United States	Healthy	Both	Study center, age at visit	1.1	0.7, 1.8	1.3	0.8, 2.0	1.2	0.8, 1.8	
Li/Zhu (44, 45), 2002/2003§	98	54	United States	Hospital	Both		0.4	0.1, 1.0	0.4	0.1, 1.1	0.4	0.1, 1.0	
Le Marchand et al. (57), 2005	1,339	1,370	United States	Healthy	Both	Ethnicity	0.7	0.6, 0.9	0.8	0.7, 1.0	0.8	0.7, 1.0	
Bailey et al. (19), 1998§,¶	59	59	United States	Hospital		Age, race	0.6	0.1, 2.9	0.5	0.1, 2.5	0.6	0.1, 2.5	
Meta-analysis of others	1,660	1,821					0.7	0.6, 0.9	0.9	0.7, 1.0	0.8	0.7, 0.9	
<i>p</i> value													
<i>Q</i> test							0.23		0.14		0.10		
Egger's test							0.85		0.76		1.00		

* OR, odds ratio; CI, confidence interval.

† Reference category: *Leu/Leu*.‡ Meta-estimates are not reported for Caucasians because of the statistically significant test for heterogeneity (*Q* test: $p < 0.001$).

§ Study was included in the pooled analysis.

¶ Bailey et al. (19) presented genotype frequencies according to ethnicity (Caucasian or African-American); therefore, this study was included in both the analysis on Caucasians and the analysis on persons of other ethnicity).

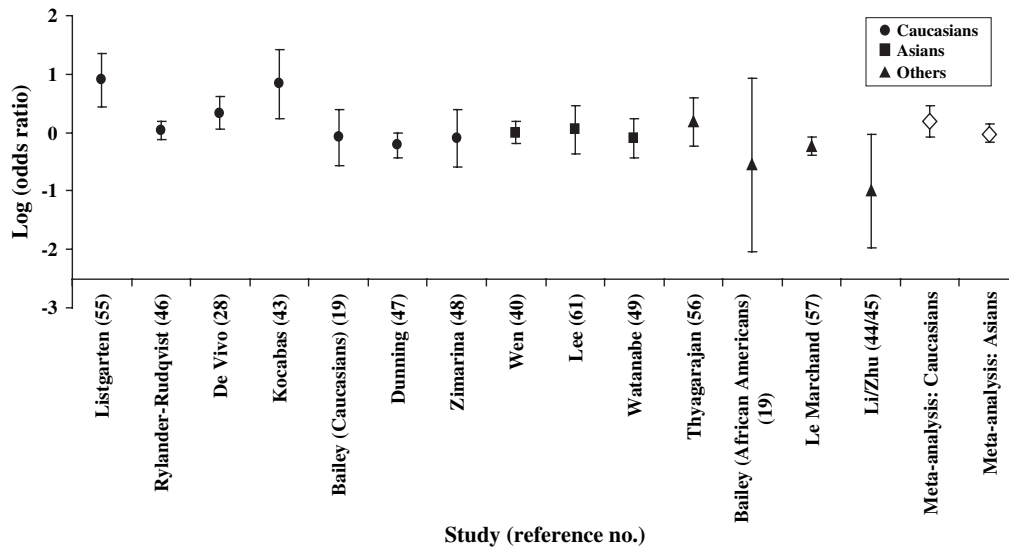


FIGURE 1. Study-specific and meta-analysis odds ratios for the relation between cytochrome P-450 1B1 (*CYP1B1*) Val432Leu polymorphisms (Val/Leu + Val/Val) and breast cancer, according to ethnicity. Bars, 95% confidence interval.

between breast cancer and the *CYP1B1* Val432Leu polymorphism in subgroups of studies performed in different ethnic groups (Caucasians, Asians, others) and in subgroups of studies including hospitalized or healthy controls.

Individual data on breast cancer and *CYP1B1* Val432Leu were extracted from the GSEC data set and were used for the pooled analysis. We assessed associations between several variables and breast cancer risk by using general linear models for continuous variables and by calculating crude odds ratios for binary variables. Crude odds ratios for the association between the *CYP1B1* Val432Leu polymorphism and breast cancer were calculated, and the chi-squared test for trend was performed. We then calculated adjusted odds ratios using multivariate logistic regression models. The pooled data were subsequently stratified according to ethnicity. The large number of subjects included in the pooled analysis allowed us to perform further stratified analyses according to age and histologic type.

RESULTS

Meta-analysis

The study-specific odds ratios and the meta-odds ratios for all of the studies are presented in table 2. Figure 1 contains a graphical representation of the study-specific odds ratios. Bailey et al. (19) reported the results of separate analyses for two different ethnic groups (Caucasians and African Americans); therefore, this study was included both in the analysis of Caucasians (by calculating the odds ratios for Caucasians only) and in the analysis of persons of other ethnic groups (by calculating the odds ratios for African Americans only). Among the seven studies on Caucasians, three reported no association between the *CYP1B1* Val432Leu polymorphism and breast cancer (19, 46, 48), while three

reported that the valine allele had a risk effect (28, 42, 55); one study reported an inverse association between the valine allele and breast cancer (47). Statistical significance for the association between breast cancer and the *CYP1B1* Val/Val polymorphism was reached in only one study (55) (odds ratio (OR) = 3.3, 95 percent CI: 1.8, 6.2).

Among Asians, the three included studies did not find any significant association between breast cancer risk and the *CYP1B1* Val432Leu polymorphism.

Among the other studies conducted in African Americans (19) or in mixed populations (45, 56, 57), two (19, 56) presented odds ratios spread around the null effect, while two (45, 57) reported an inverse association between the Val/Val genotype and breast cancer (OR = 0.4 (95 percent CI: 0.1, 1.0) and OR = 0.7 (95 percent CI: 0.6, 0.9), respectively) and for the Val/Val and Val/Leu genotypes combined (OR = 0.4 (95 percent CI: 0.1, 1.0) and OR = 0.8 (95 percent CI: 0.7, 0.9), respectively).

The overall meta-odds ratio for the combination of the *CYP1B1* Val/Val and Val/Leu genotypes was null; it is not reported because of the large heterogeneity (Q test: $p < 0.001$), although there was no evidence of publication bias (Egger's test: $p = 0.26$). When a stratified analysis according to ethnicity was performed, no association was observed between the *CYP1B1* Val432Leu polymorphism and breast cancer among Asians (for the Val/Val and Val/Leu genotypes combined, OR = 1.0, 95 percent CI: 0.8, 1.2), while a significant inverse association between the valine allele and breast cancer was observed for the four studies including African Americans or mixed populations (for the Val/Val and Val/Leu genotypes combined, OR = 0.8, 95 percent CI: 0.7, 0.9). The result of the test for heterogeneity was strongly statistically significant for the Caucasian studies (Q test: $p < 0.001$); therefore, a combined estimate was not calculated for this ethnic group. Among the 13 studies,

TABLE 3. Association between the cytochrome P-450 1B1 (*CYP1B1*) Val432Leu polymorphism and breast cancer in a pooled analysis of studies from the Genetic Susceptibility to Environmental Carcinogens (GSEC) project

Ethnic group and <i>CYP1B1</i> polymorphism	Crude OR*	95% CI*	Adjusted OR†	95% CI	<i>p</i> value	
					<i>Q</i> test	Egger's test‡
Asians (<i>n</i> = 2,937)						
<i>Leu/Leu</i>	1.0§		1.0§			
<i>Val/Leu</i>	1.0	0.8, 1.2	1.0	0.9, 1.2	0.73	
<i>Val/Val</i>	1.0	0.6, 1.7	1.0	0.8, 1.3	0.58	
<i>Val/Leu</i> + <i>Val/Val</i>	1.0	0.9, 1.2	1.0	0.9, 1.2	0.69	
Caucasians (<i>n</i> = 2,176)						
<i>Leu/Leu</i>	1.0§		1.0§			
<i>Val/Leu</i>	1.3	1.1, 1.6	1.6	1.1, 2.3	0.08	0.77
<i>Val/Val</i>	1.8	1.4, 2.3¶	1.1	0.8, 1.4	0.09	0.23
<i>Val/Leu</i> + <i>Val/Val</i>	1.4	1.2, 1.7	1.5	1.1, 2.1	0.05	0.93
African Americans (<i>n</i> = 1,615)						
<i>Leu/Leu</i>	1.0§		1.0§			
<i>Val/Leu</i>	0.9	0.7, 1.1	0.9	0.7, 1.2	0.49	
<i>Val/Val</i>	0.9	0.7, 1.1	0.9	0.8, 1.1	0.65	
<i>Val/Leu</i> + <i>Val/Val</i>	0.9	0.7, 1.1	0.9	0.7, 1.1	0.56	

* OR, odds ratio; CI, confidence interval.

† Adjusted for study, smoking (ever/never), age (<45, 45–50, 51–54, 55–59, or ≥60 years), and body mass index (weight (kg)/height (m)²; continuous variable).

‡ Egger's test was not performed in Asians and African Americans because only two studies were available for each ethnic group.

§ Reference category.

¶ *p*-trend < 0.0001.

nine included healthy controls and four included hospitalized controls; the odds ratios were similar in the two groups of studies, but with statistically significant heterogeneity.

Data on the relation between breast cancer and three other *CYP1B1* polymorphisms (Asn453Ser, Ala119Ser, and Arg48Gly) were reported in the studies selected for the *CYP1B1* Val432Leu meta-analysis. Of the four studies including information on Asn453Ser, none reported a significant association with breast cancer. Of the five studies on the Ala119Ser polymorphism, one (49) found a higher risk of breast cancer in subjects carrying at least one serine allele (OR = 1.57, 95 percent CI: 1.12, 2.20), while another study (48) reported an inverse association between the serine allele and breast cancer (for the *Ala/Ser* and *Ser/Ser* genotypes combined, OR = 0.32, 95 percent CI: 0.18, 0.60). The same study found an inverse association between the Arg48Gly polymorphism and breast cancer (for the *Arg/Gly* and *Gly/Gly* genotypes combined, OR = 0.13, 95 percent CI: 0.07, 0.23), while the other two studies found no association between the Arg48Gly polymorphism and breast cancer.

Pooled analysis

The pooled analysis included nine studies, for a total of 3,391 cases and 3,451 controls. Among the personal and reproductive variables for which data were available in the

GSEC pooled analysis, family history of cancer, late age at first birth, late age at menopause, and low number of live-births were found to be associated with breast cancer. No association was observed for active smoking, reported exposure to environmental tobacco smoke, or alcohol consumption (data not shown).

The crude and adjusted odds ratios for the association between the *CYP1B1* Val432Leu polymorphism and breast cancer are presented in table 3. An overall estimate was not calculated because of statistically significant heterogeneity, as previously observed in the meta-analysis. Crude odds ratios were found to be significantly higher than 1.00 in Caucasians for both the *Val/Leu* and *Val/Val* genotypes, with a significant trend, but after adjustment for study, age, and smoking, the association was only present for *Val/Leu* alone and the *Val/Leu* + *Val/Val* genotypes combined. No significant effect of the *CYP1B1* Val432Leu polymorphism on breast cancer risk was observed in Asian or African-American subjects.

Nonsignificant borderline heterogeneity was found among the six studies including Caucasian subjects. No evidence of publication bias was observed in the pooled analysis.

The availability of individual data allowed us to analyze the data according to age groups. A significant effect of age on the association between *CYP1B1* Val432Leu and breast cancer was found in Caucasians, while the same effect was

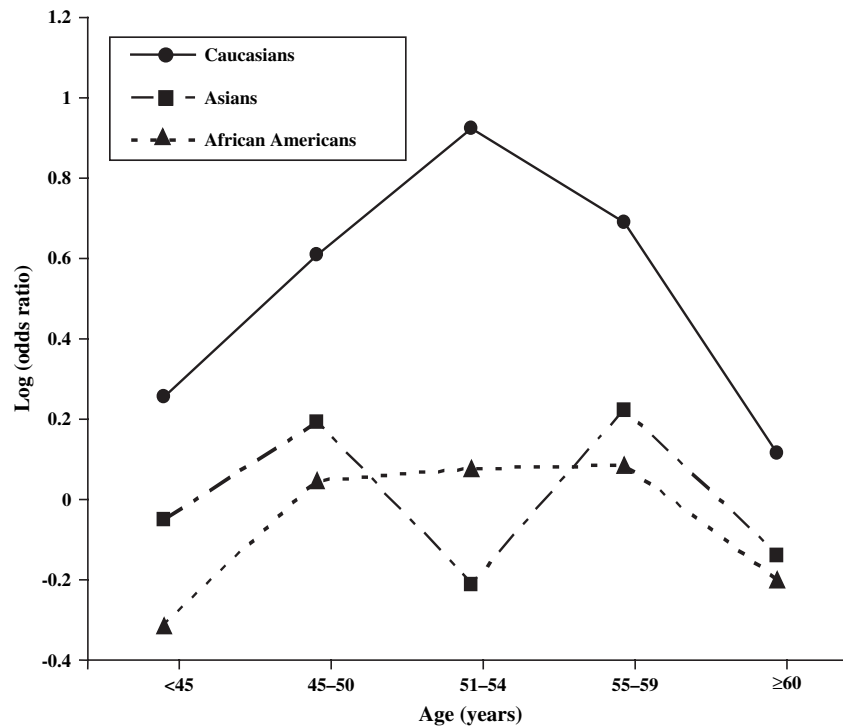


FIGURE 2. Association between cytochrome P-450 1B1 (*CYP1B1*) Val432Leu polymorphisms (*Val/Leu* + *Val/Val*) and breast cancer, according to age and ethnicity, in a pooled analysis of studies from the Genetic Susceptibility to Environmental Carcinogens (GSEC) project. The numbers of Asian, Caucasian, and African-American subjects with *CYP1B1* Val432Leu polymorphisms in each age class were, respectively: <45 years: 316, 277, and 64; 45–50 years: 174, 186, and 24; 51–54 years: 67, 94, and 94; 55–59 years: 68, 226, and 257; ≥60 years: 78, 651, and 698.

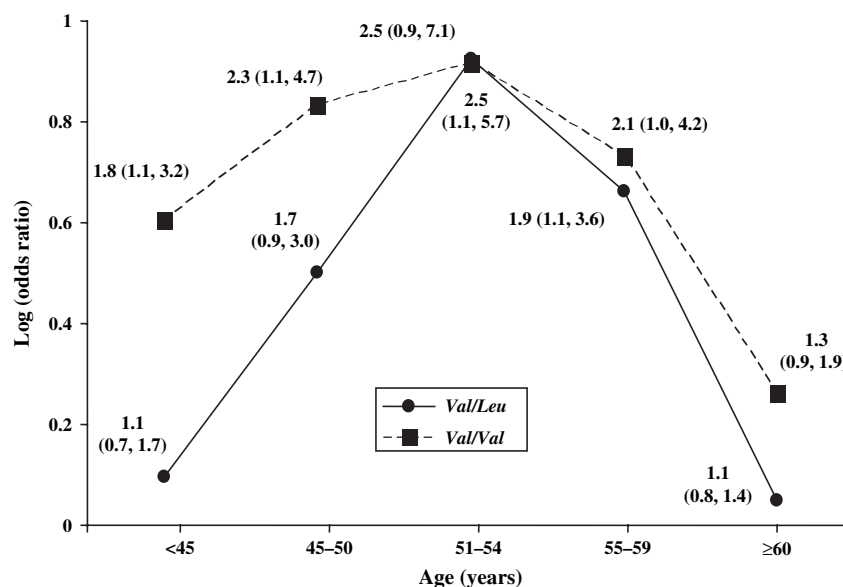


FIGURE 3. Association between cytochrome P-450 1B1 (*CYP1B1*) Val432Leu polymorphisms (*Val/Leu* + *Val/Val*) and breast cancer, according to age, among Caucasians in a pooled analysis of studies from the Genetic Susceptibility to Environmental Carcinogens (GSEC) project. Corresponding odds ratios (with 95% confidence intervals in parentheses) are presented near each data point. The numbers of subjects with *CYP1B1* Val432Leu polymorphisms in each age class were: <45 years: 191 (*Val/Leu*) and 86 (*Val/Val*); 45–50 years: 123 (*Val/Leu*) and 63 (*Val/Val*); 51–54 years: 70 (*Val/Leu*) and 24 (*Val/Val*); 55–59 years: 154 (*Val/Leu*) and 72 (*Val/Val*); ≥60 years: 458 (*Val/Leu*) and 193 (*Val/Val*).

TABLE 4. Association between the cytochrome P-450 1B1 (*CYP1B1*) Val432Leu polymorphism and breast cancer in a pooled analysis of studies from the Genetic Susceptibility to Environmental Carcinogens (GSEC) project, according to histologic type

Histologic type and <i>CYP1B1</i> polymorphism	Crude OR*	95% CI*	Adjusted OR†	95% CI
Ductal (n = 1,549)				
Leu/Leu	1.0‡		1.0‡	
Val/Leu	1.1	1.0, 1.3	1.0	0.8, 1.1
Val/Val	1.2	1.0, 1.5§	0.9	0.8, 1.1
Val/Leu + Val/Val	1.1	1.0, 1.3	0.9	0.8, 1.1
Lobular (n = 176)				
Leu/Leu	1.0‡		1.0‡	
Val/Leu	1.5	1.1, 2.1	0.9	0.6, 1.4
Val/Val	1.8	1.2, 2.9¶	1.0	0.8, 1.3
Val/Leu + Val/Val	1.6	1.2, 2.2	1.0	0.7, 1.4

* OR, odds ratio; CI, confidence interval.

† Adjusted for study, race, smoking (ever/never), age (<45, 45–50, 51–54, 55–59, or ≥60 years), and body mass index (weight (kg)/height (m)²; continuous variable).

‡ Reference category.

§ *p*-trend = 0.04.

¶ *p*-trend = 0.002.

not observed in the other ethnic groups (figures 2 and 3). For Caucasians, the risk of breast cancer increased between the ages of 45 years and 59 years, while it was lower among older and younger women.

We performed a further stratified analysis in order to assess the effect of histologic type on the association between *CYP1B1* Val432Leu and breast cancer (table 4). Only ductal and lobular breast cancers were considered, since they represented the most common histologic types in the data set. The adjusted odds ratios did not indicate any association between the *CYP1B1* Val432Leu polymorphism and either of the two histologic types.

DISCUSSION

This meta-analysis confirmed the lack of association between the *CYP1B1* Val432Leu polymorphism and breast cancer risk among Caucasian and Asian subjects, as reported in a previous meta-analysis with smaller numbers (40). A significant inverse association between the valine allele and breast cancer risk was observed in the studies including African Americans or mixed populations.

The pooled analysis suggests a possible association of both the Val/Leu and Val/Val genotypes with breast cancer in Caucasians; however, no significant effect was observed in Asian or African-American subjects. To our knowledge, this was the first comprehensive pooled analysis assessing the role of the *CYP1B1* Val432Leu polymorphism in breast cancer. Since the pooled data set included additional information on several cofactors, it was possible to adjust the

point estimates for potentially confounding factors and to perform stratified analyses.

Among the genes involved in hormone production and metabolism, *CYP1B1* has been shown to have an important role in estrogen metabolism, catalyzing principally the formation of 4-hydroxyestradiol, a carcinogenic metabolite that retains estrogenic activity (16). This enzyme also hydroxylates the estradiol in the C-2 position, although to a lower extent. *CYP1B1* appears to be implicated in the metabolic activation of a number of environmental carcinogens, such as arylamines, heterocyclic amines, benzo(*a*)pyrene, and polycyclic aromatic hydrocarbons (11, 58). The change in amino acid from valine to leucine has been shown to increase the activity of the *CYP1B1* enzyme on a variety of substrates, including procarcinogens and gonadal steroid hormones (12). The increased formation of 4-hydroxyestrone induced by the *CYP1B1* enzyme could be a possible risk factor for breast cancer (56). We reported previously (59) that women with the leucine substitution had higher 2-/16- hydroxyestrone metabolites than women carrying the other *CYP1B1* genotypes. Despite the premises, most of the individual studies included in the present analysis showed no association between the *CYP1B1* Val432Leu polymorphism and breast cancer, and there was a large amount of heterogeneity. One possible explanation may be the relatively small sample size in some studies (40); another could be the observed differences in reported genotype frequencies across studies, as well as some differences in the original study designs. While most of the articles were case-control studies, the study by Dunning et al. (47) was a case-cohort study, and the studies by Le Marchand et al. (57) and De Vivo et al. (28) were case-control studies nested within a cohort. However, when these three studies were excluded from the meta-analysis, the test of heterogeneity still remained significant (results not shown). The studies included in the pooled analysis were all case-control studies by definition; therefore, the heterogeneity observed in the pooled analysis is unlikely to be explained by differences in study design.

Since breast cancer is a hormone-related disease, we considered age as a possible reason for heterogeneity. In fact, we observed that the three Caucasian studies responsible for the statistical heterogeneity in the meta-analysis (28, 42, 55) all included younger women; therefore, we hypothesized that age could modify the effect of *CYP1B1* Val432Leu on cancer risk. We tested this hypothesis through the pooled analysis, where we found a significant effect of age on the association between *CYP1B1* Val432Leu and breast cancer in Caucasians. The risk of breast cancer seemed to be higher for the central age classes (45–59 years), while it was lower in either older or younger women. Aside from chance, a possible explanation for this could involve the change in estrogen levels, and therefore in estrogen metabolism, that occurs around menopause. Changes in hormonal patterns, in conjunction with weight changes, could make polymorphisms in genes involved in estrogen metabolism key elements during the menopausal transition, while they could be less important in younger or older women. This hypothesis, deriving from the large number of subjects included in our pooled analysis, has never been put forward, but it needs to be tested in a large study of women of different age groups.

Potential public health impact

At the moment, the potential public health impact of these results is minimal, since there is not sufficient evidence for a role of the *CYP1B1* Val432Leu polymorphism in breast cancer etiology. Therefore, population testing for the *CYP1B1* Val432Leu polymorphism is not indicated.

Laboratory testing

The detailed methods used for determining the presence of the *CYP1B1* Val432Leu polymorphism were described in each article. Most of the studies included in the present analyses used genomic DNA extracted from blood. Two studies utilized breast tissue (46, 55) in addition to blood; one study utilized only breast tissue (19). Most investigators reported the use of polymerase chain reaction, with different reaction conditions and control samples. Rylander-Rudqvist et al. (46) reported the use of two different techniques, multiplex fluorescent solid-phase minisequencing and dynamic allele-specific hybridization; Dunning et al. (47) used TaqMan assays (Applied Biosystems, Warrington, United Kingdom) and sequencing; De Vivo et al. (28) used automated DNA sequencing; Watanabe et al. (49) used single-strand conformation polymorphism analysis; and Listgarten et al. (55) reported the use of an external genomic service.

Conclusions and implications for research

Overall, a possible association between the *CYP1B1* Val432Leu polymorphism and breast cancer could be detected in Caucasians, while a negative association was suggested in populations of mixed/African-American origin. Among Caucasians, a significant effect of age on the association was found in the pooled analysis. The risk of breast cancer seemed to be higher for the central age classes (45–59 years), while it decreased for older and younger women. Further studies are needed in order to investigate the effect of *CYP1B1* Val432Leu on estrogen metabolism in different age groups and ethnic groups and in premenopausal women versus postmenopausal women.

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