

# Pharmacogenetics and Regulation of Human Cytochrome P450 1B1: Implications in Hormone-Mediated Tumor Metabolism and a Novel Target for Therapeutic Intervention

Tristan M. Sissung, Douglas K. Price, Alex Sparreboom, and William D. Figg

*Clinical Pharmacology Research Core and Cancer Therapeutics Branch,  
National Cancer Institute, Bethesda, Maryland*

## Abstract

Several of the hormone-mediated cancers (breast, endometrial, ovarian, and prostate) represent major cancers in both incidence and mortality rates. The etiology of these cancers is in large part modulated by the hormones estrogen and testosterone. As advanced disease develops, the common treatment for these cancers is chemotherapy. Thus, genes that can alter tissue response to hormones and alter clinical response to chemotherapy are of major interest. The cytochrome P450 1B1 (CYP1B1) may be involved in disease progression and modulate the treatment in the above hormone-mediated cancers. This review will focus on the pharmacogenetics of CYP1B1 in relation to hormone-mediated cancers and provide an assessment of cancer risk based on CYP1B1 polymorphisms and expression. In addition, it will provide a summary of CYP1B1 gene regulation and expression in normal and neoplastic tissue. (*Mol Cancer Res* 2006;4(3):135–50)

## Introduction

The cytochrome P450 1B1 (CYP1B1) is a heme-thiolate monooxygenase that is involved in the NADPH-dependent phase I monooxygenation of a variety of substrates, including fatty acids, steroids, and xenobiotics. CYP1B1 was discovered when it was found to be transcriptionally induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin within a human keratinocyte cell line (1). Before the identification of CYP1B1 as a novel metabolic enzyme, it has been detected in mouse endometrial stromal cells as a polycyclic aromatic hydrocarbon-inducible CYP (2) and was subsequently characterized (3). Since then, much interest has been placed on the inducibility of CYP1B1, especially given that it is differen-

tially expressed within the tumor microenvironment of several human cancers (4–6). Although CYP1B1 is expressed in normal tissues (6, 7), it is expressed at much higher levels in tumor cells compared with the surrounding normal tissue (4, 5). The overexpression of CYP1B1 has been implicated in premalignant progression (8), and given its differential expression in tumor tissue, it may be considered a drug and vaccine target for the treatment of several types of cancer (9, 10).

CYP1B1 expression is clinically relevant in neoplastic progression, tumor metabolism, and cancer treatment. Although CYP1B1 expression has been observed in multiple cancers examined to date (colon, lung, renal, bladder, and glioma), it shows particularly high expression in many of the hormone-mediated cancers (prostate, breast, endometrial, and ovarian; refs. 5, 11, 12). CYP1B1 is also implicated in the etiology of hormone-mediated tumors, as it is responsible for hormone metabolism and the formation of toxic metabolites from both endogenous and exogenous molecules (13–16). Thus, CYP1B1 induction is an important factor in determining risk associated with hormone-mediated cancers. In addition to its relevance in cancer risk, CYP1B1 is involved in the metabolism of some clinically relevant anticancer agents used in the treatment of hormone-mediated cancers. Polymorphisms within the gene have also been implicated in differential cancer risk. This review will focus on the pharmacogenetics of CYP1B1 in relation to hormone-mediated cancers and provide an assessment of cancer risk based on CYP1B1 polymorphisms and expression. In addition, it will provide a summary of CYP1B1 gene regulation and expression in normal and neoplastic tissue.

## Gene and Protein Structure of CYP1B1

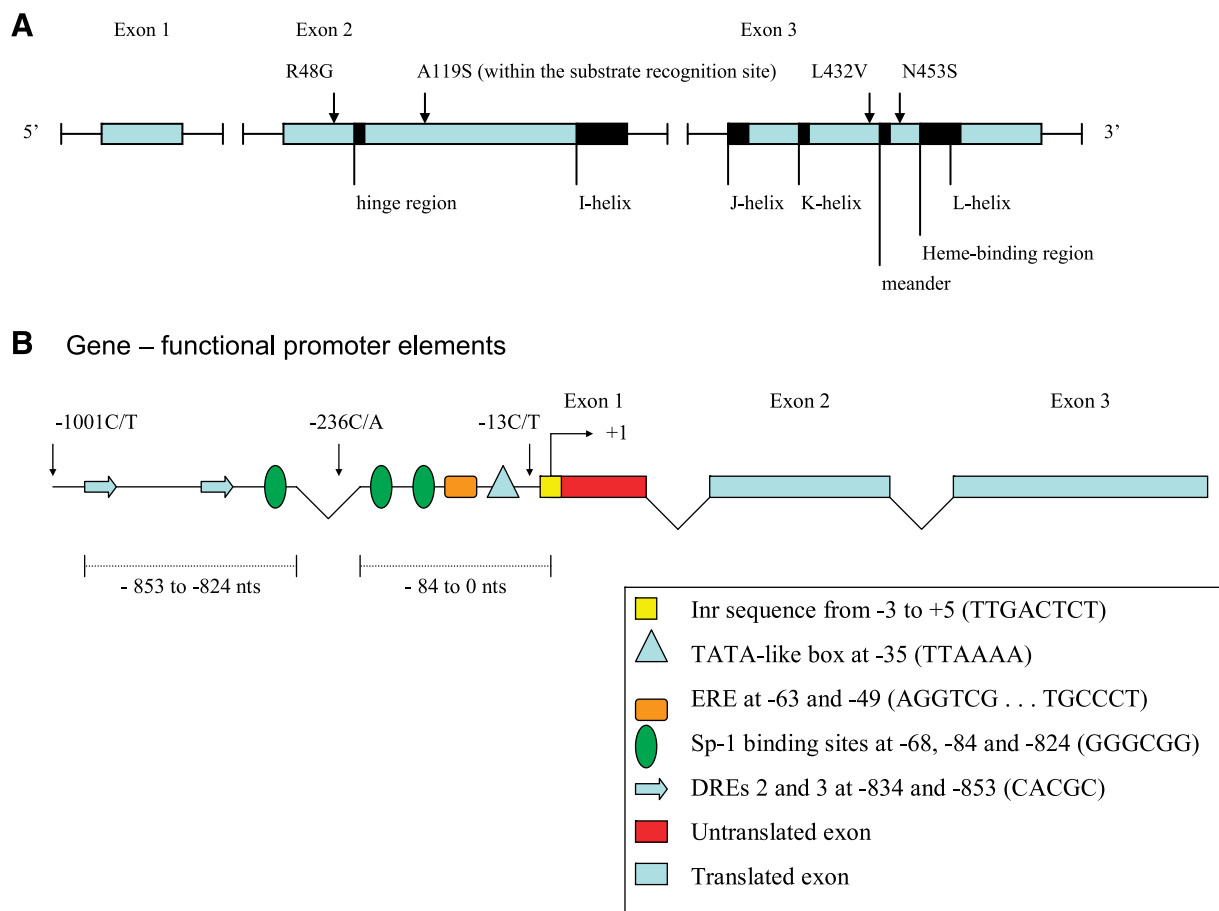
The CYP1B1 gene (Genbank accession no. U03688) is contained within three exons and two introns on chromosome 2p21 and spans ~8.5 kb of genomic DNA (Genbank accession no. U56438; see Fig. 1A). It encodes a 543–amino acid protein product that is found normally expressed in the nucleus of most cell types in which it is expressed and exhibits cytoplasmic and nuclear localization in tubule cells of the kidney and secretory cells of breast tissue (7). Although a crystal structure for CYP1B1 has not been elucidated, its structure can be inferred based on conserved sequences found in many P450s (ref. 17; see Fig. 1A).

Received 7/19/05; revised 1/18/06; accepted 1/23/06.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

**Requests for reprints:** William D. Figg, Medical Oncology Clinical Research Unit, Clinical Pharmacology Research Core, National Cancer Institute, 9000 Rockville Pike, Building 10, Room 5A01, Bethesda, MD 20892. Phone: 301-402-3623; Fax: 301-402-8606. E-mail: wdfigg@helix.nih.gov

Copyright © 2006 American Association for Cancer Research.  
doi:10.1158/1541-7786.MCR-05-0101



**FIGURE 1.** Gene and promoter structure of *CYP1B1*. **A.** Gene structure and location of polymorphisms in *CYP1B1* (adapted from ref. 17). **B.** Functional promoter elements and polymorphisms in *hCYP1B1*.

## Normal Regulation of *CYP1B1*

### Gene Regulation

*CYP1B1* is regulated by several key transcription factors, such as the aryl hydrocarbon receptor (AhR), AhR nuclear translocator (ARNT) complex (AhR/ARNT), the Sp1 transcription factor, a cyclic AMP (cAMP)–response element–binding protein (CREB), and estrogen receptor (ER). Epigenetic factors, post-transcriptional modifications, and degradation pathways have also been recently explored. Given that *CYP1B1* is transcriptionally activated in several human cancers and is being considered as a potential target for anticancer therapy (10), a full understanding of its transcriptional regulation may be important in treating *CYP1B1*-positive tumors.

### AhR/ARNT-Mediated Transcription

In normal tissue, *CYP1B1* is transcriptionally activated when a ligand (i.e., 2,3,7,8-tetrachlorodibenzo-*p*-dioxin) binds the cytoplasmic AhR complex consisting of the AhR, heat shock protein-90, XAP2, and p23 proteins (refs. 18-24; see Fig. 2). Ligand binding exposes a nuclear localization sequence site contained within the AhR that mediates the translocation of the ligand-bound AhR complex to the nucleus where it dissociates, allowing the AhR to form a heterodimer with the nuclear resident protein ARNT (25-29). The AhR/ARNT heterodimer

subsequently binds to dioxin-responsive elements (DRE) in the *CYP1B1* enhancer region using basic helix-loop-helix motifs located within the amino termini of the AhR and ARNT (28, 30, 31).

The first clone of the *CYP1B1* gene was generated by Tang et al. (32), who initially described several putative DRE (GCGTG; refs. 33, 34), Sp1 enhancer elements (35), TATA-like box (TTAAAA; refs. 35, 36), initiator sequence (TTGACTCT; ref. 35), and the transcription start site. The promoter region was also found to contain putative steroidogenic factor-1, AP2, and E-box domains (37, 38). The TATA-like box, initiator sequence, and Sp1 elements were found to be necessary for enhanced activity (39). *CYP1B1* gene expression was then examined in cancer cell lines and found to be differentially expressed (37). The cell-specific gene expression profiles were attributed to a functional DRE located at -833 (DRE2) from the transcriptional start site by luciferase reporter constructs (37). A subsequent experiment found that another functional DRE located at -853 (DRE3) also binds to the AhR/ARNT (38). Both DRE2 and DRE3 may mutually regulate basal transcription and AhR-mediated inducibility of *CYP1B1* under the control of a Sp1 enhancer located at -824 (38). Gel shift assays also showed that the AhR/ARNT also binds to DREs located at -1024

(DRE6) and -1490 (DRE7), although the functional consequences of such an interaction remain unclear (ref. 38). (See Fig. 1B for known functional elements of the *CYP1B1* promoter).

#### cAMP-Mediated Transcription

The far upstream enhancer region (-5298 to -5110) of the *CYP1B1* gene contains several steroidogenic factor-1 elements that interact with two cAMP-responsive elements (CRE1 and CRE2). Steroidogenic factor-1 and cAMP-enhanced activator protein-1 (consisting of Fos-Fos or Fos-Jun) complexes bind steroidogenic factor-1 sites within the promoter and cooperatively participate in transcription along with CREB and CREB-binding protein complexes bound to the CRE element within the far upstream enhancer region (ref. 40; see Fig. 2). Steroidogenic factor-1-mediated transcription of *CYP1B1* through sites within the far upstream enhancer region and activator protein-1 sites may be more important in tissues where *CYP1B1* is not regulated by the AhR, and where cAMP signal transduction pathways are important (i.e., adrenals, testes, and ovary; ref. 40).

#### Epigenetic Regulation

Promoter methylation of *CYP1B1* has been associated with decreased activity of this gene (41). Recently, prostate-specific increases in expression of *CYP1B1* were found to be regulated by promoter hypomethylation, thus confirming the importance of promoter methylation in *CYP1B1* gene expression (42). *CYP1B1* methylation takes place at multiple CpG sites within the *CYP1B1* gene, some of which are contained within key promoter elements, such as DRE1, DRE2, DRE3, and Sp1 binding sites at -72 and -80 (42). Methylation at these sites may decrease the accessibility of DNA-binding sites for

proteins involved in AhR-mediated regulation (42) and may alter estrogen-mediated regulation of *CYP1B1*. DNA methylation of *CYP1B1* has also been associated with survival in breast cancer patients treated with tamoxifen (43). Histone methylation has also been associated with alterations in chromatin structure; therefore, gene expression of *CYP1B1* could also be regulated through chromatin remodeling. Chromatin structure is also altered by histone acetylation, and histone H3 acetylation has been observed in the far upstream enhancer region and other *CYP1B1* promoter elements through the interaction of histone acetyltransferase and CREB-binding protein (40). Additionally, promoter methylation of some *CYP1B1* effectors and associated metabolic enzymes (including several steroid receptor genes and catechol-*O*-methyltransferase) have also been linked to differential gene expression in hormone-dependent cancers compared with normal tissue (44-49). Thus, epigenetic regulation through methylation and acetylation of histones within the *CYP1B1* promoter region is a key determinant of *CYP1B1* transcription, and the degree of epigenetic regulation may be tissue specific, with those tissues relying on cAMP-mediated transcription of *CYP1B1* most likely having a different chromatin structure than other tissues.

#### Post-transcriptional Regulation and Degradation

A post-transcriptional mechanism may also be involved in *CYP1B1* induction wherein multiple polyadenylation signal sequences are contained (1). Shehin et al. (37) propose that these sites may be regulated in a cell-specific manner by either mRNA processing or random use of signals with varying strengths (see Fig. 1C).

The degradation of *CYP1B1* was recently shown to be mediated by the proteases, polyubiquitination, and proteasomal

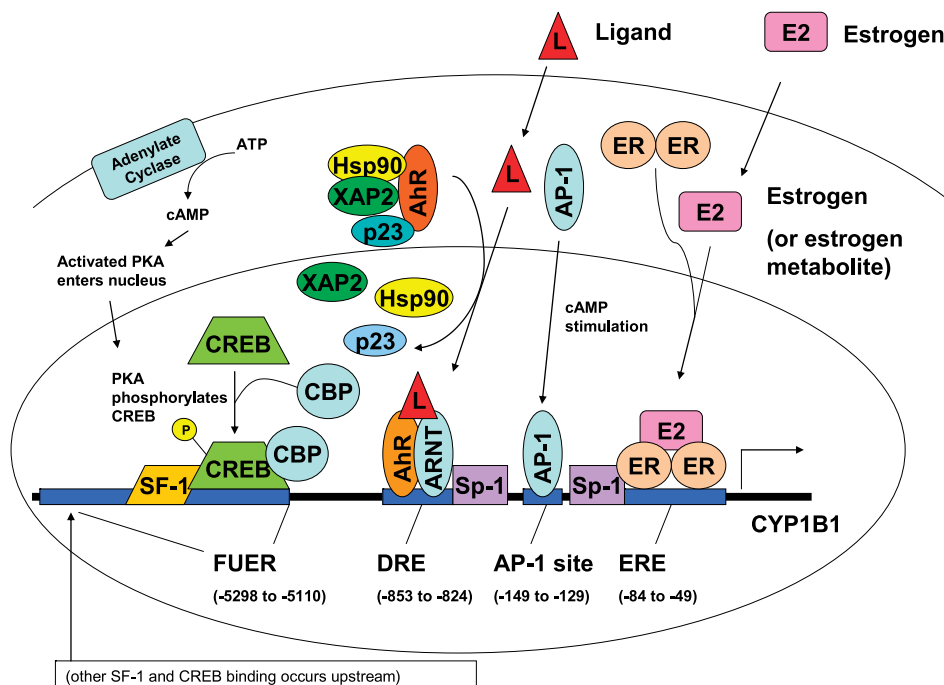


FIGURE 2. AhR-, ER-, and cAMP-mediated *CYP1B1* induction.

degradation but not by phosphorylation in COS-1 cells (50). Although it cannot be excluded that degradation of CYP1B1 in this cell line is different from the tumor degradation pathway, a polymorphism that increases the degradation efficiency of CYP1B1 (N453S) is also correlated with decreased cancer risk, suggesting that an increased rate of CYP1B1 degradation mediates a protective effect against tumorigenesis (51). The amino acid substitution does not cause an increase in ubiquitination rate, and the structural alterations responsible for this phenomenon are currently unknown (see Polymorphisms; ref. 50).

#### Sex Steroid Hormonal Regulation

An estrogen-responsive element was recently shown to be involved in ER $\alpha$  regulation of CYP1B1 (52). This estrogen-responsive element may cooperate with Sp1 sites located nearby (see Fig. 1B). Estrogen is required for maximal AhR expression and constitutive inducibility of CYP1B1 in MCF-7 cells (53), indicating a possible role for estrogen in the constitutive expression of CYP1B1 in breast tumors. Furthermore, the ER $\alpha$  and ER $\beta$  status of hormone-mediated cancers is correlated with CYP1B1 expression (54) through CYP1B1-mediated transformation of estrogen into 4-hydroxyestradiol (4-OHE<sub>2</sub>; refs. 55, 56). However, the effect of estrogen on the expression of CYP1B1 is most likely tissue specific. ER-negative cell lines do not show induction of CYP1B1 in the presence of estrogen, whereas increased ER expression coincides with increased CYP1B1 expression on estrogen stimulation (52). Furthermore, some tissues do not rely on estrogen for CYP1B1 expression and metabolism (57).

The progesterone receptor may also be involved in CYP1B1 pathways. The formation of 4-OHE<sub>2</sub> by CYP1B1 also results in an increased rate of cell proliferation and expression of estrogen-inducible genes, such as the PR (55, 56). Furthermore, CYP1B1 and PR genotypes are associated with increased risk of cancer in the Japanese population (58).

CYP1B1 catalyzes the formation of 6-hydroxytestosterone (59). It is known that androgens cause differential expression of CYP1B1 in mice expressing humanized CYP1B1 by down-regulating CYP1B1 (60), but the molecular mechanisms that mediate this occurrence are currently unknown. Furthermore, there is some evidence that exogenous substrates that induce CYP1B1, such as (benzo[a]pyrene and benzo[a]pyrene diol epoxide), also cause reduced expression of the AR, an effect that is reversed by CYP1B1 antagonists (61). However, the implications of CYP1B1 metabolism on AR signaling are unclear.

#### Tissue-Specific Expression in Normal and Neoplastic Tissues

##### Detection in Normal Tissue

CYP1B1 has been detected in several normal tissues (see Table 1) and is mainly expressed extrahepatically (6). Before the discovery of antibodies specific for CYP1B1, mRNA expression had been detected in numerous tissues and cell types (15, 62-66). This could suggest a functional role for CYP1B1 in the bioactivation of numerous procarcinogens, including endogenous substrates, such as estrogens (7).

##### Problems with mRNA Detection

CYP1B1 mRNA detection does not always correlate with CYP1B1 protein expression (67). RNA-PCR studies have shown that expression of CYP1B1 is equivalent in normal and tumor tissues, whereas immunohistochemical analysis and activity assays indicate that protein expression is increased in tumors (4, 5, 63, 68). This could suggest that (a) a certain threshold of mRNA expression must be present before the protein can be expressed, (b) cell-specific post-transcriptional modifications must be present before protein expression is achieved, and (c) proteolytic degradation can modulate CYP1B1 protein levels (50, 69). Thus, cell-specific CYP1B1

**Table 1. CYP1B1 Tissue-Specific Expression in Hormone-Mediated Cancers and Corresponding Normal Tissue**

Cancer Type	Tumor Tissue*		Ref.	Normal Tissue <sup>†</sup>		Ref.
	No. CYP1B1 Positive/No. Tested	Most Common Intensity of Staining		No. CYP1B1 Positive/No. Tested	Most Common Intensity of Staining	
Breast						
Invasive ductal	52/64	Moderate to strong <sup>‡</sup>	(4, 5)	5/5	Strong	(6, 7)
Invasive lobular	6/8	Moderate to strong <sup>‡</sup>	(4, 5)			
Uterus	7/7	Strong	(5)			
Endometrioid carcinoma	33/35	Moderate to strong	(147)	8/9	Weak	(7)
Ovary	7/7	Strong	(5)	6/8	Moderate	(7)
Serous cystadenocarcinoma	91/99	Moderate to strong	(147)			
Mucinous cystadenocarcinoma	20/23	Strong	(147)			
Clear cell adenocarcinoma	6/7	Weak to strong	(147)			
Malignant mixed Mullerian tumor	3/3	Strong	(147)			
Prostate						
Prostate carcinoma	25/33	Weak to moderate	(8)	6/6	Moderate to strong	(7)
Prostatic intraepithelial neoplasia	2/2	Moderate	(8)			
Metaplastic urothelium	8/8	Moderate to strong	(8)			
Benign prostatic hyperplasia	27/33	Moderate	(8)			
Testis	8/8	Strong	(5)	ND	ND	—

\*Assessed with various antibodies from the following publications: Murray et al. (5) and McFadyen et al. (4), which are not sensitive enough to detect CYP1B1 in normal tissues.

<sup>†</sup>Assessed with a highly sensitive antibody developed by Tang et al. (6).

<sup>‡</sup>Expression was moderate to high in most grade 2 and 3 breast cancers.

transcriptional activity must be assessed using immunologic and activity assays in conjunction with mRNA detection. Polyclonal and monoclonal antibodies have been generated against several peptide sequences within CYP1B1 that are currently used in immunohistochemical analysis and provide excellent protein detection levels (4-6). For the above reasons, this review will not consider experiments that *only* use mRNA detection (i.e., Northern blotting and reverse transcription-PCR) alone without detecting protein expression (i.e., Western blotting and immunohistochemistry) to assess CYP1B1 expression.

#### *Constitutive Expression of CYP1B1 in Several Types of Cancer*

Since the initial development of antibodies specific to CYP1B1 (4, 5), much research has been conducted to investigate the inducibility of CYP1B1. CYP1B1 is overexpressed in several carcinomas and is involved in the premalignant progression of some neoplastic tissue (8). Although CYP1B1 is expressed in several normal tissues (6, 70, 71), it is differentially overexpressed in the tumor microenvironment (4, 5). However, it is difficult to assess differences in tumor expression versus normal tissue expression given that different antibodies with differing sensitivities are used to assess expression via immunohistochemistry. Thus, comparisons between antibodies must be made with caution. A summary of CYP1B1 expression in hormone-mediated cancers and their corresponding normal tissue is provided (see Table 1).

### **Carcinogenic Metabolism**

#### *Metabolism of Procarcinogens*

The procarcinogen metabolism of CYP1B1 is very important to understanding the role of CYP1B1 in cancer initiation and progression. There are numerous procarcinogens that CYP1B1 activates, such as polycyclic aromatic hydrocarbons, aromatic and heterocyclic amines, and aflatoxin B1. The role of CYP1B1 in exogenous procarcinogen metabolism has been extensively reviewed (see refs. 15, 59, 72, and 73 for a more complete coverage of procarcinogen metabolism).

#### *Estrogen Metabolism*

The concentration of circulating estrogens is much to low (nanomolar range) for CYP1B1 to play a major role in estrogen metabolism as a whole given that CYP1B1 has rather low affinity for estrogens ( $\sim 10 \mu\text{mol/L}$ ). At low levels, estrogen is primarily metabolized by CYP3A4 (74). However, CYP3A4 is often not found expressed in estrogen-responsive tissues, such as breast, depending on the ethnicity of the population under investigation (75-78). Furthermore, plasma and tissue levels of estrogen levels are not always concordant. Tissue estrogen levels in some patients are often found to be 10- to 50-fold higher than would be predicted from plasma levels due to tissue-specific synthesis of estrogens, particularly in breast tissue where CYP1B1 is colocalized with aromatase (reviewed in ref. 79) and in the ovarian surface epithelium where estrogen levels are  $\sim 100$ -fold greater than circulating levels and follicular levels are higher still (80). Therefore, CYP1B1 metabolism may play a key role in estrogen metabolism in

some estrogen-responsive tissues, especially those that express aromatase (CYP2C19) or show increased uptake of estrogens. Thus, CYP1B1 metabolites may also be more concentrated in these tissues as is apparent from circulating levels. CYP2C19 and CYP1B1 are up-regulated by cAMP and could be up-regulated together in some tissues where AhR levels are low (i.e., ovary and testes; refs. 40, 81). Furthermore, CYP1B1 may have a clinically relevant role in intratumoral metabolism of estrogens where it is often up-regulated and may be involved in tumor formation and progression through the formation of toxic metabolites, especially when colocalized with overexpressed aromatase. Aromatase up-regulation and subsequent increases in tumor-specific estrogen levels within the tumor tissue has been observed in cancers of the breast (82) and endometrium (83, 84), and drastic increases in tissue estrogen levels have been observed in ovarian cancer (80, 85).

CYP1B1 is currently thought to be the most efficient estrogen hydroxylase (86, 87) and was the first estrogen metabolizing enzyme identified that is also transcriptionally activated by estrogen (52). CYP1B1 catalyzes the extrahepatic 4-hydroxylation of  $17\beta$ -estradiol into the less active metabolites, 4-OHE<sub>2</sub> (major product) and 2-hydroxyestradiol (2-OHE<sub>2</sub>; minor product; refs. 11, 88-90). 4-OHE<sub>2</sub> can either be converted into 4-methoxyestradiol by catechol-*O*-methyltransferase or undergo redox cycling resulting in reactive quinones and semiquinones that covalently bind tubulin and DNA resulting in carcinogenesis (refs. 87, 91, 92; see Fig. 2). However, 2-OHE<sub>2</sub> treatment does not induce tumors and is much less carcinogenic than 4-OHE<sub>2</sub> (93).

The estrogen-induced toxicity model has been consistently validated in studies aimed at determining the molecular mechanisms of estrogen toxicity. Cavalieri et al. showed that 4-OHE<sub>2</sub>, when converted to its corresponding quinone and semiquinone metabolites, covalently binds purines in DNA, and this reaction results in the formation of abasic sites (91). Several studies have subsequently arisen that have shown that these abasic sites are present *in vivo* and contribute to carcinogenesis (94-99). These studies have also shown that catechol estrogen quinones bind proteins within the cell, further contributing to carcinogenesis (94). The toxicity of 4-OHE<sub>2</sub> can be abrogated via at least two known mechanisms. Methoxyestrogens can exert feedback inhibition on CYP1B1-mediated estrogen metabolism (100), and glutathione conjugation by glutathione *S*-transferase P1 can deactivate the quinone derivative of 4-OHE<sub>2</sub> (ref. 101). (For a more complete description of estrogen metabolism and estrogen-induced toxicity, see refs. 79, 102-105; see Fig. 2).

In summary, 4-OHE<sub>2</sub> and its metabolites contribute to carcinogenesis by the formation of DNA adducts and protein binding. Such reactions have been observed *in vivo* and are known to cause carcinogenesis and increase cancer incidence through the increase in the 4-OHE<sub>2</sub>:2-OHE<sub>2</sub> brought about by CYP1B1 metabolism. Therefore, the efficiency of CYP1B1 metabolism of estrogen, those enzymes that regulate estrogen biosynthesis, and those enzymes that regulate the clearance of catechol estrogens are important in the determination of cancer risk.

## Polymorphisms

A summary of all known single nucleotide polymorphisms (SNP), including five different missense mutations and seven common different common haplotypes, is provided on the Human Cytochrome P450 Allele Nomenclature Committee home page (<http://www.imm.ki.se/CYPalleles/cyp1b1.htm>). Of these, five SNPs [C142G (R48G), G355T (A119S), C4326G (L432V), C4360G (A443G), and A4390G (N453S)] are known to result in amino acid substitutions.

The C142G and G355T polymorphisms (*CYP1B1\*2*) are tightly linked (106) and result in amino acid substitutions (CYP1B1.2) that have not been found involved in the catalytic properties of CYP1B1 when not considered in combination with other functional alleles (107, 108) and are located near the hinge region of CYP1B1 (see Fig. 1A; ref. 17). However, increased basal mRNA levels of *CYP1B1* have been observed in cell culture (109), suggesting a possible role for these polymorphisms in CYP1B1-mediated carcinogenesis. Similarly, the A443G polymorphism has not been associated with the functional properties of CYP1B1 when considered outside the context of a haplotype (107, 108).

The C4326G transition (*CYP1B1\*3*) leading to the corresponding amino acid transition [L432V (CYP1B1.3)] is associated with increased catalytic activity of the CYP1B1 enzyme in several studies (90, 110, 111). A possible cause of this increase in catalytic activity is changes in the tertiary (or quaternary) structure of the CYP1B1 protein, as the CYP1B1.3 polymorphism is located near a catalytically important heme-binding domain in CYP1B1 (refs. 17, 106; see Fig. 1A). Furthermore, the *CYP1B1\*3* transition is also responsible for significant increases in AhR-mediated *CYP1B1* gene expression during AhR-mediated signaling events (109).

The A4390G polymorphism (*CYP1B1\*4*) leading to the corresponding amino acid transition (CYP1B1.4) is not associated with catalytic changes in the protein product but has been associated with increases in the CYP1B1 degradation rate. The levels of immunologically active CYP1B1.4 are a factor of 2 lower than other alleles because the 453S allele results in rapid proteolytic degradation of the CYP1B1.4 protein isoform. The increase in degradation rate can provide a

rationale for the observed decreased activity of CYP1B1 in ethoxyresorufin de-ethylase assays and decreased genotoxicity from CYP1B1 metabolism (50).

Other polymorphisms in the promoter region of *CYP1B1* have been identified that could be involved in altering *CYP1B1* gene expression and are attributed to increased cancer risk (ref. 112; see Table 2 for a summary of polymorphisms and their functions).

Many studies have considered *CYP1B1* in the context of haplotype, and three common haplotypes have been found in several different populations. The haplotypes are described by the aforementioned Allele Nomenclature Committee and are labeled as *CYP1B1\*5*, *CYP1B1\*6*, and *CYP1B1\*7*. The corresponding protein variants are labeled as CYP1B1.5, CYP1B1.6, and CYP1B1.7.

### *Expression and Function of CYP1B1 Polymorphisms in Bacterial, Insect, and Mammalian Expression Systems*

CYP1B1 polymorphisms have been evaluated in a variety of expression systems, including bacteria, yeast, Sf9 insect cells, and mammalian COS-1 cells. It is not surprising that the effects of *CYP1B1* polymorphisms on catalytic activity and protein processing vary between each expression system. McLellan et al. were the first to express two *CYP1B1* polymorphisms in both yeast and COS-1 cells (108). This group found that CYP1B1.1 and CYP1B1.2 variants did not vary functionally either by protein processing or by the catalytic efficiency of estrogen hydroxylation (108). However, other functionally important CYP1B1 variants, including CYP1B1.3 and CYP1B1.4, were not evaluated, and other studies have shown that both CYP1B1.3 and CYP1B1.4 are important in determining catalytic efficiency and the protein processing of CYP1B1 in both COS-1 and other cellular expression systems. Recently, others have shown that the CYP1B1.4 variant increases the degradation efficiency of CYP1B1 by proteases and the proteasome in COS-1 cells, although the precise mechanism to explain these results remains to be elucidated (50, 113). When expressed by bacteria in an *ex vivo* assay, the CYP1B1.3 variant alone was found have the lowest catalytically efficiency toward estrogen 4-hydroxylation reactions and the lowest ratio of 4-OHE<sub>2</sub>:2-OHE<sub>2</sub> formation. However, when the *CYP1B1\*2* and

**Table 2. Polymorphisms and Their Consequences**

Nucleotide Transition*	Amino Acid Transition†	Functional Consequence	Ref.
-1001C/T	—	Possible promoter alteration	(112)
-263G/A	—	Possible promoter alteration	(112)
13C/T	—	Possible promoter alteration	(112)
142C/G	R48G	Increased basal <i>CYP1B1</i> gene expression	(109)
		No alterations in <i>CYP1B1</i> catalytic properties	(108)
355G/T	A119S	Increased basal <i>CYP1B1</i> gene expression	(109)
		No alterations in <i>CYP1B1</i> catalytic properties	(108)
4326C/G	L432V	Significant increase in <i>CYP1B1</i> gene expression during AhR signaling events.	(109)
		Alter heme binding domain and increased catalytic activity of enzyme with the V substitution	(90, 110, 111)
4390A/G	N453S	Significant decrease in protein expression due to an increase in <i>CYP1B1</i> degradation efficiency by proteolysis.	(50)

NOTE: Summary of selected polymorphisms within CYP1B1 and their functional consequences.

\*Numbering corresponds to transcription start site.

†Numbering corresponds to the amino acid in the expressed protein.

**Table 3. Summary of Case-Control Studies Assessing CYP1B1 Polymorphisms in Relation to Cancer Risk Assessments**

Cancer	Ethnicity	Case/Control	Association of Cancer Risk with CYP1B1 Genotype	Genotype	OR (95% CI)	Ref.	
Breast	<i>L432V*</i>	ND	173/154	Yes (increases risk)	V/V and V/L	3.30 (1.76-6.19)	(14)
		Mixed ancestry	1,339/1,370	No		0.9 (0.7-1.1)	(132)
		Caucasian	164/164	No		0.7 (0.4-1.5)	(106)
		African American	59/59	No		1.6 (0.4-2.9)	(106)
		Chinese	186/200	Yes (increases risk)	L/L	2.3 (1.2-4.3)	(136)
		ND	453/453	No		1.0 (0.72-1.45)	(124)
		Turkish	84/103	Yes (increases risk)	V/V and V/L	2.32 (1.26-4.25)	(129)
		Korean	241/290	No		1.0 (0.7-1.6)	(133)
Endometrial	<i>A119S†</i>	Caucasian	689/1,549	No		1.2 (0.9-1.4)	(146)
		Japanese	113/202	Yes (increases risk)	S/S	3.32 (1.38-8.01)	(54)
	<i>L432V‡</i>	Caucasian/African American	371/420	Yes (moderate increase)	V/V	1.34 (0.90-1.98)	(145)
		ND	222/666	No		1.1 (0.75-1.59)	(51)
	<i>N453S§</i>	Caucasian	689/1,549	No		0.8 (0.7-1.0)	(146)
		Japanese	113/202	Yes (increases risk)	V/V	2.49 (1.10-5.66)	(54)
		ND	222/666	Decreased risk	N/S and S/S	0.62 (0.42-0.91)	(51)
		Caucasian	689/1,549	No		1.0 (0.8-1.3)	(136)
Ovarian	<i>L432V  </i>	Mixed ancestry	129/144	Yes (increases risk)	V/V	3.8 (1.2-11.4)	(148)
		Caucasian	223/280	No		???	(149)
Prostate	<i>A119S  </i>	Japanese	117/200	Yes (increases risk)	S/S	4.02 (1.73-9.38)	(16)
		<i>L432V¶</i>	Japanese	136/255	Yes (increases risk)	V/V	4.80 (1.21-19.05)
	<i>L432V¶</i>	Japanese	117/200	No	—	1.34 (0.53-3.35)	(16)
		Caucasian American	50/50	Yes (increases risk)	V/V	3.3 (1.9-9.0)	(154)

\*Test for homogeneity of ORs: estimated exact *Ps* (Monte Carlo); *P* = 0.014. Data are too heterogeneous to pool. Pooled OR (95% CI), 1.08 (0.94-1.25).

† Test for homogeneity of ORs: exact *Ps* (Zelen); *P* = 0.055. Data are marginally too heterogeneous to pool. Pooled OR (95% CI), 0.99 (0.73-1.34).

‡ Test for homogeneity of ORs: exact *Ps* (Zelen); *P* = 0.18. Data are sufficiently homogeneous to pool. Pooled OR (95% CI), 0.94 (0.80-1.11); *P* = 0.48.

§ Test for homogeneity of ORs: exact *Ps* (Zelen); *P* = 0.43. Data are homogeneous. Pooled OR (95% CI), 1.08 (0.72-1.63); *P* = 0.81.

|| Testing for homogeneity of ORs was not warranted for these data.

¶ Test for homogeneity of ORs: exact *Ps* (Zelen); *P* = 0.13. Data are sufficiently homogeneous to pool. Pooled OR (95% CI), 0.43 (0.24-0.77); *P* = 0.0059.

*CYP1B1*\*3 alleles were expressed together, the highest catalytic efficiency toward estrogen hydroxylation was observed, and when the *CYP1B1*\*3 and *CYP1B1*\*4 alleles were expressed in the same construct, the highest ratio of 2:4 hydroxylation of estrogen was observed (110). Interestingly, Shimada et al. showed that CYP1B1.1 had a higher catalytic efficiency toward estrogen than did CYP1B1.2, whereas the ratio of 4-hydroxylated and 2-hydroxylated estrogens was greater in CYP1B1.3 using *Escherichia coli* together with human NADPH-P450 reductase (114). CYP1B1.3 and CYP1B1.4 were also found to have similar catalytic activities toward estrogen hydroxylation when expressed in insect cells (115). Finally, when CYP1B1 was expressed in yeast cells, all of the CYP1B1 variants had similar reaction kinetics toward estrogen metabolism. However, the CYP1B1.6 and CYP1B1.7 variants (both of which contain the R48G, A119S, and L432V amino acid substitutions) had the highest  $K_m$  and the lowest  $V_{max}$ , indicating that haplotype has an important effect on protein folding. Taken together, these results are unclear and difficult to interpret. We suggest that if eukaryotic cells are taken to better represent the mRNA and protein processing capabilities of human cells, (a) the CYP1B1.4 variant is likely associated with increased CYP1B1 degradation efficiency corresponding to lower protein levels, whereas the reaction kinetics toward estrogen are unlikely to be different from any other variant; (b) *CYP1B1* haplotype may be more relevant in risk assessments as the protein folding is most likely different

between the various *CYP1B1* haplotypes causing alterations in ligand binding and catalysis in the expressed protein; and (c) it is still unclear which polymorphisms and haplotypes are likely to be important in assessing reaction kinetics as no study has evaluated CYP1B1 estrogen metabolism in human cells.

### Contribution of Polymorphisms to Increased/Decreased Risk

#### *Risk Associated with Specific Cancers*

**Estrogen-Mediated Cancers.** Prolonged exposure to estrogens is a major etiologic factor in the causation of estrogen-mediated cancers (116, 117). Two prevailing hypotheses regarding the etiology of estrogen-mediated cancers prevail in the literature. The first argues that estrogen-mediated cancers occur due to spontaneous mutations brought about by increases in cell proliferation from increased estrogen levels (102). The second hypothesis argues that estrogen-mediated cancers arise due to the genotoxic effects of estrogen (79, 80, 85, 102, 118) particularly through the genotoxic effects of the CYP1B1 metabolites, such as 4-OHE<sub>2</sub> (91, 92). Both hypotheses are not mutually exclusive, and both may contribute significantly to the etiology of estrogen-mediated cancers.

Polymorphisms in the *CYP1B1* gene (especially the *CYP1B1*\*3 allele) may be important determinants of estrogen-mediated cancer risk in part because of the role of CYP1B1 in genotoxic metabolism of estrogen and ER status (11, 14, 15, 54,

107, 110, 119). Estrogen hydroxylase activity and the formation of toxic 4-OHE<sub>2</sub> is increased in the metabolically hyperactive CYP1B1.3 variants resulting in the increased carcinogenicity of estrogen (11, 86, 90, 91, 107, 110, 111, 120). Furthermore, haplotype analysis indicates that certain combinations of alleles can drastically alter the kinetic properties of CYP1B1 in the formation of 4-OHE<sub>2</sub>. Whereas most haplotypes do not affect the catalytic properties of CYP1B1 toward E<sub>2</sub>, the CYP1B1\*6 haplotype was associated with drastic increases in K<sub>m</sub> for the conversion of E<sub>2</sub> into 4-OHE<sub>2</sub> (107). Thus, differential estrogen metabolism due to polymorphic variants of CYP1B1 may influence estrogen metabolism and estrogen-mediated cancer risk. However, a distinction must be made between the activity of CYP1B1 in premenopausal and postmenopausal women as the levels of circulating and tissue estrogens differ widely in these populations. In premenopausal women, estrogens are primarily secreted into the circulation from the adrenal cortex and the ovaries where they act on target tissues. In postmenopausal women, estrogen synthesis occurs in the peripheral tissues from circulating estrogen precursors (reviewed in ref. 121). A large multiethnic study has indicated that CYP1B1 genotype is not associated with serum estrogen levels in premenopausal women (122). Another study investigating circulating estrogens in postmenopausal women has indicated that CYP1B1 genotype is not correlated with

circulating estrogen levels (123), whereas another large study indicated that serum levels of estrogen are increased with the CYP1B1\*1 genotype, whereas the CYP1B1\*3 genotype correlates with an increased percentage of ER-positive tumors (124), thus confirming a previous observation (106). The major weakness of the current studies evaluating CYP1B1 polymorphisms and circulating estrogen levels is that only the L432V polymorphism (the N453S polymorphism was evaluated in the latter study) was investigated, whereas the L432V polymorphism may only be relevant in combination with other CYP1B1 alleles in both exons 2 and 3 (107). The association between CYP1B1 genotype and estrogen metabolism is unclear at present due to the presence of several conflicting studies in the literature that will be explained below.

**Breast Cancer.** Breast tumors frequently overexpress CYP1B1 resulting in increased levels of 4-OHE<sub>2</sub> and an increased ratio of 4-OHE<sub>2</sub>:2-OHE<sub>2</sub> concentrations in the tumor microenvironment (4, 68, 125). The formation of 4-OHE<sub>2</sub> was shown to result in breast carcinogenesis in rats (126). Perhaps some of the more interesting data implicating the role of estrogens in breast cancer come from a study examining the effects of aromatase inhibitors versus antiestrogens. This study found that patients treated with tamoxifen develop breast tumors at a greater rate than patients treated with aromatase inhibitors, suggesting that estradiol-mediated toxicity and other

**Table 4. Polymorphisms in Controls**

Polymorphism	Ethnicity	Controls	Variants, n (%)			p	q	Ref.	
R48G	Japanese	200	R/R	R/G	G/G			(16)	
		202	99 (49.5)	72 (36.0)	29 (14.5)			(54)	
A119S	Caucasian	1,382	A/A	A/S	S/S			(134)	
		200	699 (50.6)	556 (40.2)	127 (9.2)			(16)	
		202	151 (75.5)	38 (19.0)	11 (5.5)			(54)	
L432V	Caucasian	202	L/L	V/L	V/V			(134)	
		164	153 (75.7)	38 (18.8)	11 (5.5)			(123)	
		148	49 (30)	96 (59)	19 (12)			(134)	
	Caucasian American	1,380	425 (30.1)	676 (49.0)	279 (20.2)			(130)	
		200	99 (49.5)	72 (36.0)	29 (14.5)			(154)	
		189	61 (32.3)	93 (49.2)	35 (18.5)			(106)	
	African American	59	26 (44)	30 (51)	3 (5)			(154)	
		52	28 (54)	21 (40)	3 (6)			(130)	
	German	112	59 (52.7)	39 (34.8)	14 (12.5)			(136)	
	Chinese	200	29 (15)	127 (64)	44 (22)			(154)	
	Japanese	109	73 (67.0)	35 (32.0)	1 (1.0)			(130)	
		200	141 (70.5)	46 (23.0)	13 (6.5)			(135)	
	N453S	Several Kadlubar	324	230 (71)	88 (27)	6 (2)	0.160	0.84	(156)
			337	ND	ND	ND			(16)
			200	141 (70.5)	46 (23.0)	13 (6.5)			(54)
202			143 (70.8)	46 (22.8)	13 (6.4)			(129)	
103			59 (57)	32 (31)	12 (12)			(133)	
290			229 (79)	56 (19)	5 (2)			(124)	
453			162 (36)	200 (44)	91 (20)			(51)	
666			193 (29.5)	316 (48.2)	146 (22.3)				
137			36 (26.27)	64 (46.7)	37 (27.0)				
N453S			Caucasian	164	N/N	N/S	S/S		
	1,379	111 (68)		49 (30)	4 (2)			(134)	
	59	926 (67.2)		412 (30.0)	40 (2.9)			(106)	
	200	55 (93)		4 (7)	0 (0)			(16)	
	202	200 (100)		0 (0)	0 (0)			(54)	
	453	202 (100)		0 (0)	0 (0)			(124)	
	666	299 (66)		137 (30)	17 (4)			(51)	
	666	424 (64.4)		206 (31.3)	28 (4.3)				

NOTE: Summary of selected polymorphisms in control populations.



toxic pathways likely play a role in tumor formation in patients treated with antiestrogens alone (127). Furthermore, CYP1B1 has also been shown to be up-regulated in a breast cancer cell line treated with tamoxifen, suggesting that toxic CYP1B1 estrogen metabolites may contribute to carcinogenesis and progression in patients treated with antiestrogens (128). However, others have found that exogenous carcinogen metabolism by CYP1B1 in breast tissue may be a more important determinant in the assessment of breast cancer susceptibility than 4-OHE<sub>2</sub> formation (129). The localization of CYP1B1 is both nuclear and cytoplasmic (this localization is only seen in mammary tumors and secretory cells of the kidneys) possibly modulating the effects of CYP1B1 in breast tissue (7, 78).

The L432V polymorphism is one of three key discriminators of breast cancer status in a medium-sized and well-controlled case-control study of 98 SNPs in 45 genes (14). However, the same study showed that no individual SNP examined had >60% predictive power when assessing cancer risk. Several case-control and family-based studies have shown that individuals with two hyperactive 432V alleles are at increased risk for developing breast cancer (14, 43, 129, 130), whereas many have indicated that this allele is not associated with an individual's breast cancer risk (refs. 124, 131-135; see Table 3). Two of the above studies have also shown that breast cancer risk increases in the daughters of mothers with the 432V/V genotype independent of the daughter's own genotype perhaps through prenatal exposure to estrogen and its metabolites (43, 131). The 432L/L genotype has also been associated with increased incidence of breast cancer (106, 136), especially in postmenopausal Chinese women (136). The inconsistency between these studies may show the variability of other genetic and environmental factors linked to CYP1B1 expression and metabolism during breast carcinogenesis in different populations. These could include but are certainly not limited to body mass index (129), catechol-*O*-methyltransferase status (131), CYP1A1 status (43), ER status (124), having undergone menopause, and exposure to environmental carcinogens (137).

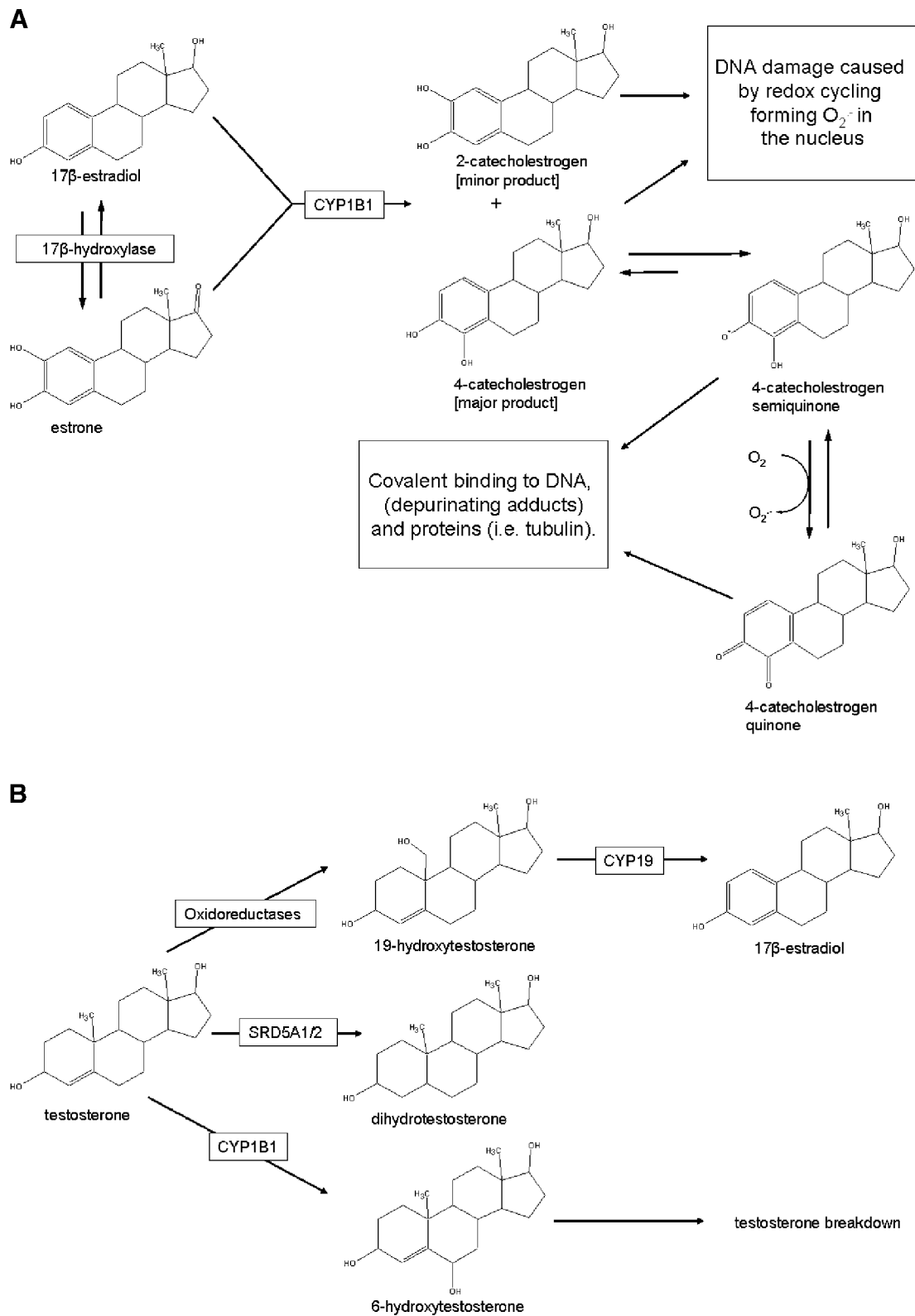
Case-only and case-control studies (see Table 4 for controls) have also been published that have examined the gene-environment interaction and the interaction of CYP1B1 polymorphisms with other genetic and cellular factors. Such studies have found that smoking confers a further increase in breast cancer risk in individuals with the CYP1B1\*3/\*3 genotype (137). A prevailing hypothesis is that CYP1B1 is transcriptionally induced by polyaromatic hydrocarbons and other aromatic hydrocarbons (major constituents of cigarette smoke) resulting in increased levels of CYP1B1. Those individuals that carry the hyperactive CYP1B1\*3/\*3 genotype are more likely to metabolize a polycyclic aromatic hydrocarbons and endogenous procarcinogens into toxic metabolites resulting in a further increase in exposure to carcinogens compared with nonsmokers (137). As is the case for prostate cancer, DNA methylation may be an important determinant of CYP1B1 expression in breast and has been shown to predict the response to tamoxifen therapy (41). A body mass index > 24 kg/m<sup>2</sup> has also been associated with an increased incidence of breast cancer in those women carrying the CYP1B1\*3

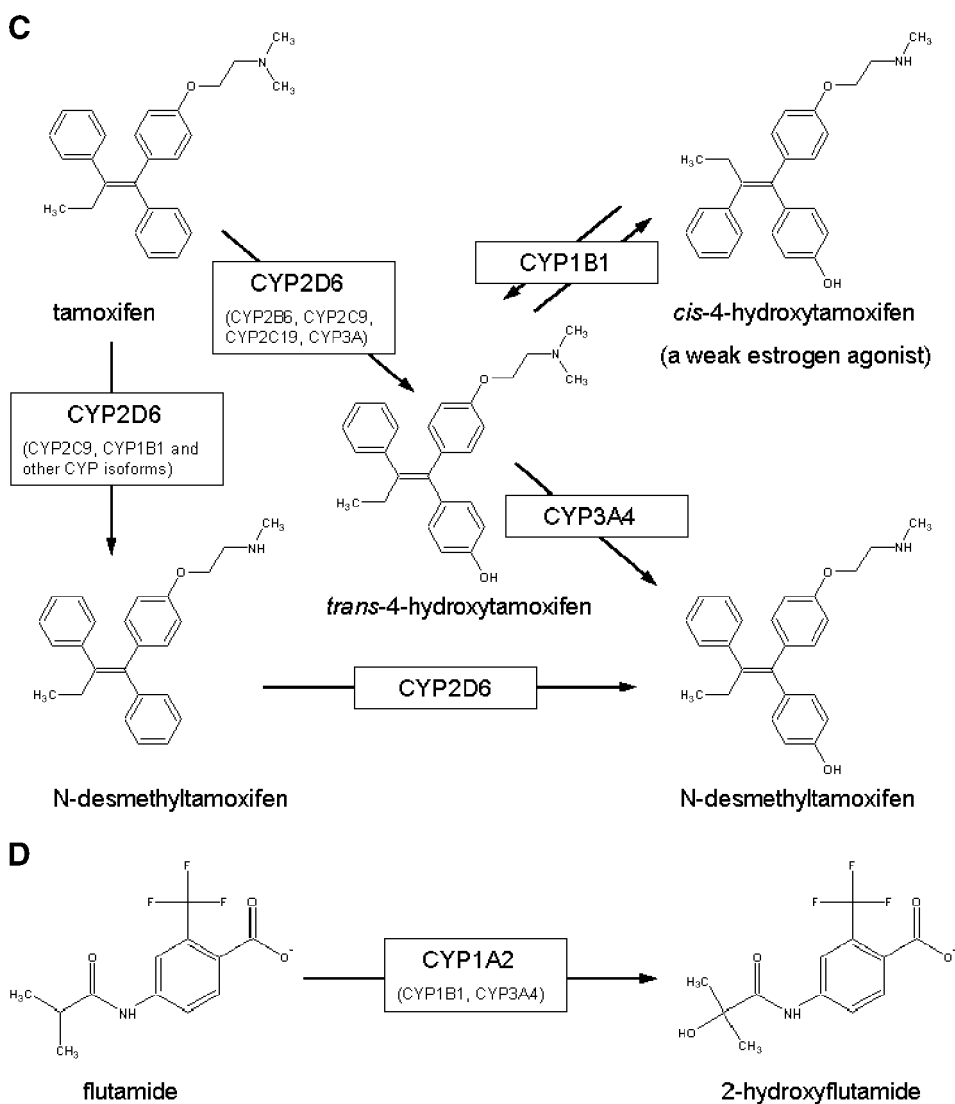
allele (129). Interestingly, increased body mass index results in higher levels of circulating endogenous estrogens and this may be a contributing factor to breast cancer risk in obese women (138).

The preceding case-control studies are often conflicting as is often observed with case-control studies into genetic factors with low penetrance. Indeed, a test of homogeneity of odds ratios (OR) indicates that there is strong evidence that the ORs are too heterogeneous to be pooled [test of homogeneity of OR: asymptotic *P*s (Breslow and Day); *P* = 0.017; see Table 3]. The conflicting results may be due to several factors, including population selection bias, small sample sizes, interpopulation variability of CYP1B1 genotype frequencies, and other factors. The largest studies investigating the role of CYP1B1 in breast cancer risk have found no correlation with any CYP1B1 genotype. Perhaps the most pertinent factor contributing to the interstudy variability is that although CYP1B1 has been most extensively examined in the context of its contribution to breast cancer risk, no study to date has considered CYP1B1 polymorphisms in exon 2 of the gene, nor has any study made risk assessments in the context of haplotypes. Both of these factors may be very important in determining CYP1B1 catalytic efficiency and the influence of CYP1B1 on risk assessments. Nevertheless, the common OR [95% confidence interval (95% CI)] is estimated to be 1.12 (0.97-1.29). Thus, across the six studies, on average, there is no association between the genotype as classified and patients with breast cancer and controls. However, the homogeneity test would suggest that there are very large differences between studies. Therefore, the association between CYP1B1\*3 genotype and breast cancer remains unclear.

**Endometrial Cancer.** The highest levels of CYP1B1 are found in the endometrium (11). Endometrial myoma tissue has significantly elevated 4-OHE<sub>2</sub> levels compared with the surrounding normal myometrium, an effect that is abrogated by inhibition of CYP1B1 (139). Furthermore, 4-OHE<sub>2</sub> production was shown to be responsible for endometrial carcinoma in mice (87). These data suggest an important role for CYP1B1 in the induction of cancers of the uterus.

CYP1B1 may also be involved in the causation of endometrial cancers brought about by tamoxifen therapy (140-142). Tamoxifen is a potent antiestrogen used for the treatment of several estrogen-mediated cancers. CYP1B1 is the primary catalyst of *trans-cis* isomerization of *trans*-4-hydroxytamoxifen to 4'-hydroxytamoxifen (a weak estrogen agonist; see Fig. 3; ref. 143). The net result of such a conversion is the inactivation of *trans*-4-hydroxytamoxifen and the formation of a weak promoter of estrogen signaling. Indeed, clinical resistance to tamoxifen therapy has been associated with the increased formation of *cis*-hydroxytamoxifen (144), and CYP1B1-mediated metabolism may be directly responsible. Cell lines treated with tamoxifen in the absence of estrogen were shown to up-regulate CYP1B1 possibly through the estrogen-responsive element on the CYP1B1 promoter (52). CYP1B1 promoter methylation has also been associated with increases in overall survival after tamoxifen therapy, and this relationship is likely due to decreased CYP1B1 expression resulting in decreased tamoxifen metabolism (41). Thus,





**FIGURE 3** Continued. **C.** Phase I metabolism of tamoxifen by the cytochrome *P*450. CYP1B1 catalyzes the *trans-cis* isomerization of *trans*-4-hydroxytamoxifen to the weak estrogen agonist *cis*-4-hydroxytamoxifen. This isomerization occurs in tumors in which CYP1B1 has been up-regulated and is associated with a drug-resistant phenotype. **D.** Phase I metabolism of flutamide by the cytochrome *P*450. Although CYP1A2 and CYP3A4 are thought to be the main phase I metabolizing enzymes that interact with flutamide, CYP1B1 metabolism of flutamide may also occur within tissues that express CYP1B1 at high levels.

CYP1B1 may be involved in the disposition of tumors toward tamoxifen and its expression may be responsible for clinical resistance to tamoxifen therapy within the tumor tissue as well as endometrial toxicity. Further research is necessary to define the role of CYP1B1 in tamoxifen metabolism, especially in breast cancer where the role of tamoxifen metabolism is unclear.

Sharma et al. show that tissue-specific CYP1B1 expression in uterine tissue is partially responsible for the conversion of tamoxifen into the genotoxic metabolite,  $\alpha$ -hydroxytamoxifen, which forms DNA adducts resulting in subsequent carcinogenesis (142). Furthermore, 4-hydroxytamoxifen has been shown to induce the expression of CYP1B1 through a promoter-mediated pathway possibly involving the CYP1B1 estrogen-responsive element (52). It is hypothesized that as tamoxifen

increases CYP1B1 expression, toxicity may result through the conversion of estrogen into 4-OHE<sub>2</sub> (52). These data could possibly explain the molecular mechanisms governing the increased incidence of endometrial cancers during prolonged tamoxifen therapy. However, little has been done to verify these data.

The 432V/V and 119S/S genotypes have been associated with increased cancer risk (54). Other studies have indicated that the 432V/V may be more relevant in assessing endometrial cancer risk in premenopausal women (51), whereas the 453S/S genotype has been correlated with decreased cancer risk (51). The 119S/S allele was strongly correlated with positive ER $\alpha$  and ER $\beta$  status, whereas the 432V/V allele was weakly correlated (54). Another recent study found an association between CYP1B1\*3 and CYP1A1/2 genotypes (145). Although

the risk was low to moderate when *CYP1B1\*1* was assessed alone (OR, 1.34; 95% CI, 0.9-1.98), when risk of endometrial cancer was assessed in combination with *CYP1A1/2* low-risk alleles, the risk was significantly reduced (OR, 0.29; 95% CI, 0.15-0.56; ref. 145). However, a large study was recently published in which no association was found between polymorphisms in the *CYP1B1* gene and cancer risk (146). Although the sample size was larger, the discrepancy between the findings of these studies could again be attributed to genetic, environmental, and possible dietary differences between the two sample populations. Thus, *CYP1B1*-induced carcinogenesis could contribute to the disease etiology of endometrial cancer, wherein the formation of genotoxic catechol estrogens, positive ER status, and prolonged tamoxifen therapy cause tumor formation. However, this has not been shown in endometrial cancer.

Our meta-analysis reveals that the current case-control studies that have evaluated the A119S polymorphism in relation to endometrial cancers are marginally too heterogeneous to be pooled [test of homogeneity of ORs: exact *P*s (Zelen); *P* = 0.055]. The common OR (95% CI) is 0.99 (0.73-1.34) for the A119S SNP, although due to the heterogeneity of ORs the comparison between studies must be interpreted with caution. However, the published ORs for the *CYP1B1\*3* and *CYP1B1\*4* are sufficiently homogeneous to be pooled [test of homogeneity of OR: exact *P*s (Zelen); *P* = 0.18 and 0.67, respectively]. The common ORs [95% CI; 0.94 (0.80-1.11); *P* = 0.48 and 1.08 (0.72-1.63); *P* = 0.81, respectively] indicate that there is no statistically significant association between *CYP1B1\*3* or *CYP1B1\*4* genotype and patients with endometrial cancer versus controls (see Table 3).

**Ovarian Cancer.** Moderate to strong expression of *CYP1B1* is observed in ovarian carcinomas, with metastatic tumors expressing higher levels of *CYP1B1* than nonmetastatic tumors (147). Furthermore, the expression of *CYP1B1* was weakly correlated with survival on docetaxel treatment in a pilot study involving 20 patients with ovarian cancer (147). Thus, *CYP1B1* is likely an important metabolic enzyme that modulates the effectiveness of drug treatment and aggressiveness of ovarian tumors.

The *CYP1B1* genotypes associated with ovarian cancer risk are controversial. The 432V/V and 432V/L alleles have been associated with increased cancer risk in the Hawaiian population (consisting of Asians and Caucasians; ref. 148). However, a recent negative study found no difference between 432V/V cases and controls in Caucasians (149). In the first (positive) study, a significant increase in risk was seen in smokers who carried at least one *CYP1B1* 432V allele, *CYP1A1* (*MspI*) *m2* allele, one *COMT Met* allele, or two *CYP1A2 A* alleles compared with never-smokers who carry *CYP1A1* (*MspI*) *m1/m1*, *CYP1B1 L/L*, *COMT V/V*, or *CYP1A2 A/A* alleles (148). Thus, *CYP1B1* status alone or in combination with other factors may modulate the risk of ovarian cancer development. However, both studies that have assessed *CYP1B1* alleles versus the risk of developing ovarian cancer suffer from small sample sizes, and no definitive conclusions can be made from them at present. We did not conduct a meta-analysis of the current literature pertaining to ovarian cancer, as there are not enough data to warrant such an analysis.

### Androgen-Mediated Cancers

*CYP1B1* catalyzes the 6-hydroxylation of testosterone (see Fig. 3; ref. 59). The sex steroid hormones have been shown to be involved in the neoplastic progression of prostate and testicular cancer, and *CYP1B1* metabolizes several of these (for a more complete review of sex steroid hormone-induced prostate cancer, see ref. 150). Mouse models expressing h*CYP1B1* increased expression of *CYP1B1* on the removal of androgen signaling via chemical or surgical castration (60). *CYP1B1* is also known to activate several carcinogens that are suspected to be involved in prostate cancer development (151). Thus, *CYP1B1* is relevant to androgen-mediated cancers, such as those of the prostate and testis.

**Prostate Cancer.** Estrogen exposure has been implicated in the disease etiology of prostate cancer (152), and *CYP1B1* has been implicated as an important gene up-regulated in prostate cancer (153). Those alleles associated with alterations in promoter or protein function may be responsible for modulating the metabolic effects of *CYP1B1* in tumor tissue (16, 58, 112, 154). *CYP1B1* is frequently found in prostate carcinomas but usually at either weak or moderate staining intensity (see Table 1; ref. 8). Furthermore, differential expression of *CYP1B1* within the tumor is found in the neoplastic progression of prostate cancer and has been observed in prostatic intraepithelial neoplasia (8). *CYP1B1* is also being considered as a target for prostate-specific anticancer therapy, and it may metabolize flutamide (see Fig. 3), resulting in differential tumor response to flutamide therapy (10).

Flutamide is a nonsteroidal antiandrogen used for the treatment of prostate cancer. The formation of 2-hydroxyflutamide by *CYP1B1* was observed using the ethoxyresorufin de-ethylase assay (10). Interestingly, the antiandrogenic properties of flutamide may result in increased expression of *CYP1B1*, as androgens reduce induction of *CYP1B1* in humanized mouse studies (60). This suggests that *CYP1B1*-mediated metabolism could provide a protective effect against flutamide treatment in those tumors that express *CYP1B1*, and flutamide can cause the induction of *CYP1B1* through indirect means. Furthermore, androgen-mediated tissues show an increased reliance on estrogen signaling during flutamide treatment (49, 155). *CYP1B1* induction could modulate this signaling and cause toxicity in flutamide-treated patients through the metabolism of estrogens and the formation of 4-OHE<sub>2</sub>. Although flutamide may be metabolized by *CYP1B1*, little, if any, research has been published confirming or disputing these data. Thus, flutamide metabolism by *CYP1B1* is still controversial but could be significant in prostate cancer treatment.

Polymorphically expressed *CYP1B1* may also be involved in cancer risk assessments. The 48G/G, 432V/V, and A119S/S *CYP1B1* polymorphisms were found to be associated with increased cancer risk alone or in combination with other factors (16, 58, 112, 154). Whereas the 432V/V allele was responsible for increased prostate cancer risk, the 432V/V and 432V/L polymorphisms further increased risk associated with an *Alu* repeat in exon 7 of the progesterone receptor, suggesting a possible role of *CYP1B1* in progesterone receptor pathways (58). The *CYP1B1\*3* allele was also associated with increased cancer incidence in a Caucasian population; however, this study

suffered from a very low sample size (154). Another report found that the A119S was associated with increased cancer risk in Japanese prostate cancer cases, whereas other alleles (*CYP1B1\*1*, *CYP1B1\*3*, and *CYP1B1\*4*) were not associated (16). This study concluded that differences in risk assessments between other studies were due to population type-dependent differences. However, a subsequent study in a larger population of Japanese showed an increased risk for developing prostate cancer associated with the *CYP1B1\*3* allele (58). The first *CYP1B1* haplotype study was also conducted in the context of prostate cancer by Chang et al. (112). In this experiment, the haplotype CGCCG [for consecutive SNPs -1001C/T, -263G/A, -13C/T, +142C/G (R48G), and +355G/T (L432V)] was associated with an increase in prostate cancer incidence. Interestingly, the TATGT allele for the same series of SNPs was associated with decreased prostate cancer incidence. Chang et al. attribute the results of this experiment either to a founder effect or to the CGCCG series of SNPs causing unfavorable promoter and catalytic differences in the *CYP1B1* gene (112).

The case-control studies that have investigated the *CYP1B1\*3* allele are sufficiently homogeneous to be pooled [test of homogeneity of ORs: exact *Ps* (Zelen);  $P = 0.13$ ]. Interestingly, the common OR (95% CI) is estimated to be 0.43 (0.24-0.77;  $P = 0.0059$ ) for these studies (see Table 3). Thus, there is a strong association between the L432V polymorphism genotypes and patients with cancer and controls. However, further investigation into the effects of *CYP1B1\*3* on prostate cancer risk is needed to clarify this relationship.

## Conclusion

*CYP1B1* is emerging as an important biomarker and metabolic intermediary in cancers that are modulated by sex hormones. Many histologic samples taken from neoplastic tissues show increased levels of immunoreactive *CYP1B1* protein compared with the surrounding normal tissue, suggesting a possible role for *CYP1B1* in neoplastic progression and tumor metabolism. The up-regulation of *CYP1B1* is mediated by certain sex hormones and their metabolites in addition to environmental carcinogens, such as polyaromatic hydrocarbons. Once up-regulated, *CYP1B1* catalyzes the conversion of steroid hormones and exogenous substrates into toxic metabolites that increase the genotoxic and oxidative load on the cell and modulate cell signaling. This could further explain the role of *CYP1B1* in neoplastic progression.

At present, there are large inconsistencies in studies that have examined the contribution of different *CYP1B1* alleles to cancer risk, and the reason for between-study differences is unclear. Most of the risk assessments currently available in the literature are based on small sample sizes. Given that the contribution of any *CYP1B1* allele to increased cancer risk would likely be small, variability in population sampling and low study power is the most plausible explanation for such inconsistencies. Other possible explanations for between-study variability could be (a) that *CYP1B1* contributes to increased risk in combination with other factors (i.e., exposure to carcinogens, increased body mass index, ER status, tissue steroid hormone levels, estrogen use, other genes that participate in *CYP1B1*-mediated pathways, etc.), (b) that other endogenous and environmental factors are not controlled for in

many of the above studies, (c) between-population variability in *CYP1B1* allele frequencies, and (d) selection bias. However, many of the aforementioned studies do not report consideration of such factors. Indeed, those studies that have found positive correlations between *CYP1B1* genotype and cancer incidence generally have assessed *CYP1B1* alleles in combination with other factors. This suggests that polymorphisms within *CYP1B1* depend on life-style and environmental factors that act in concert to increase (or decrease) an individual's susceptibility to developing certain cancers. Further investigation into the role of *CYP1B1* in cancer risk is needed to fully ascertain this gene-environment interaction along with further assessment of other genes that may contribute to *CYP1B1*-mediated carcinogenesis and disease progression, and careful controls should be set in case-control studies that investigate polymorphic variants of *CYP1B1* and associated cancer risk.

## Acknowledgments

We thank Dr. Seth Steinberg for his assistance in the statistics of the meta-analysis.

## References

- Sutter TR, Tang YM, Hayes CL, et al. Complete cDNA sequence of a human dioxin-inducible mRNA identifies a new gene subfamily of cytochrome *P450* that maps to chromosome 2. *J Biol Chem* 1994;269:13092-9.
- Savas U, Christou M, Jefcoate CR. Mouse endometrium stromal cells express a polycyclic aromatic hydrocarbon-inducible cytochrome *P450* that closely resembles the novel *P450* in mouse embryo fibroblasts (*P450EF*). *Carcinogenesis* 1993;14:2013-8.
- Savas U, Bhattacharyya KK, Christou M, Alexander DL, Jefcoate CR. Mouse cytochrome *P-450EF*, representative of a new 1B subfamily of cytochrome *P-450s*. Cloning, sequence determination, and tissue expression. *J Biol Chem* 1994;269:14905-11.
- McFadyen MC, Breeman S, Payne S, et al. Immunohistochemical localization of cytochrome *P450 CYP1B1* in breast cancer with monoclonal antibodies specific for *CYP1B1*. *J Histochem Cytochem* 1999;47:1457-64.
- Murray GI, Taylor MC, McFadyen MC, et al. Tumor-specific expression of cytochrome *P450 CYP1B1*. *Cancer Res* 1997;57:3026-31.
- Tang YM, Chen GF, Thompson PA, Lin DX, Lang NP, Kadlubar FF. Development of an antipeptide antibody that binds to the C-terminal region of human *CYP1B1*. *Drug Metab Dispos* 1999;27:274-80.
- Muskhelishvili L, Thompson PA, Kusewitt DF, Wang C, Kadlubar FF. *In situ* hybridization and immunohistochemical analysis of cytochrome *P450 1B1* expression in human normal tissues. *J Histochem Cytochem* 2001;49:229-36.
- Carnell DM, Smith RE, Daley FM, et al. Target validation of cytochrome *P450 CYP1B1* in prostate carcinoma with protein expression in associated hyperplastic and premalignant tissue. *Int J Radiat Oncol Biol Phys* 2004; 58:500-9.
- Luby TM, Cole G, Baker L, Komher JS, Ramstedt U, Hedley ML. Repeated immunization with plasmid DNA formulated in poly(lactide-co-glycolide) microparticles is well tolerated and stimulates durable T cell responses to the tumor-associated antigen cytochrome *P450 1B1*. *Clin Immunol* 2004;112:45-53.
- Rochat B, Morsman JM, Murray GI, Figg WD, McLeod HL. Human *CYP1B1* and anticancer agent metabolism: mechanism for tumor-specific drug inactivation? *J Pharmacol Exp Ther* 2001;296:537-41.
- Hayes CL, Spink DC, Spink BC, Cao JQ, Walker NJ, Sutter TR. 17 $\beta$ -Estradiol hydroxylation catalyzed by human cytochrome *P450 1B1*. *Proc Natl Acad Sci U S A* 1996;93:9776-81.
- McKay JA, Melvin WT, Ah-See AK, et al. Expression of cytochrome *P450 CYP1B1* in breast cancer. *FEBS Lett* 1995;374:270-2.
- Hatanaka N, Yamazaki H, Oda Y, Guengerich FP, Nakajima M, Yokoi T. Metabolic activation of carcinogenic 1-nitropyrene by human cytochrome *P450 1B1* in *Salmonella typhimurium* strain expressing an *O*-acetyltransferase in SOS/umu assay. *Mutat Res* 2001;497:223-33.
- Listgarten J, Damaraju S, Poulin B, et al. Predictive models for breast cancer susceptibility from multiple single nucleotide polymorphisms. *Clin Cancer Res* 2004;10:2725-37.

15. Shimada T, Hayes CL, Yamazaki H, et al. Activation of chemically diverse procarcinogens by human cytochrome *P*-450 1B1. *Cancer Res* 1996; 56:2979–84.
16. Tanaka Y, Sasaki M, Kaneuchi M, Shiina H, Igawa M, Dahiya R. Polymorphisms of the CYP1B1 gene have higher risk for prostate cancer. *Biochem Biophys Res Commun* 2002;296:820–6.
17. Stoilov I, Akarsu AN, Alozie I, et al. Sequence analysis and homology modeling suggest that primary congenital glaucoma on 2p21 results from mutations disrupting either the hinge region or the conserved core structures of cytochrome P4501B1. *Am J Hum Genet* 1998;62:573–84.
18. Cuthill S, Poellinger L, Gustafsson JA. The receptor for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the mouse hepatoma cell line Hepa 1c1c7. A comparison with the glucocorticoid receptor and the mouse and rat hepatic dioxin receptors. *J Biol Chem* 1987;262:3477–81.
19. Denis M, Cuthill S, Wikstrom AC, Poellinger L, Gustafsson JA. Association of the dioxin receptor with the *M*<sub>1</sub> 90,000 heat shock protein: a structural kinship with the glucocorticoid receptor. *Biochem Biophys Res Commun* 1988;155:801–7.
20. Denison MS, Harper PA, Okey AB. Ah receptor for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Codistribution of unoccupied receptor with cytosolic marker enzymes during fractionation of mouse liver, rat liver and cultured Hepa-1c1 cells. *Eur J Biochem* 1986;155:223–9.
21. Okey AB, Bondy GP, Mason ME, et al. Temperature-dependent cytosol-to-nucleus translocation of the Ah receptor for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in continuous cell culture lines. *J Biol Chem* 1980;255:11415.
22. Perdev EH. Association of the Ah receptor with the 90-kDa heat shock protein. *J Biol Chem* 1987;262:13802.
23. Perdev GH, Poland A. Purification of the Ah receptor from C57BL/6J mouse liver. *J Biol Chem* 1988;263:9848.
24. Poland A, Glover E, Bradfield CA. Characterization of polyclonal antibodies to the Ah receptor prepared by immunization with a synthetic peptide hapten. *Mol Pharmacol* 1991;39:20–6.
25. Henry EC, Rucci G, Gasiewicz TA. Characterization of multiple forms of the Ah receptor: comparison of species and tissues. *Biochemistry* 1989;28:6430–40.
26. Ikuta T, Eguchi H, Tachibana T, Yoneda Y, Kawajiri K. Nuclear localization and export signals of the human aryl hydrocarbon receptor. *J Biol Chem* 1998; 273:2895–904.
27. Prokipek RD, Okey AB. Physicochemical characterization of the nuclear form of Ah receptor from mouse hepatoma cells exposed in culture to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Arch Biochem Biophys* 1988;267:811–28.
28. Reyes H, Reisz-Porszasz S, Hankinson O. Identification of the Ah receptor nuclear translocator protein (Arnt) as a component of the DNA binding form of the Ah receptor. *Science* 1992;256:1193–5.
29. Wilhelmsson A, Cuthill S, Denis M, Wikstrom AC, Gustafsson JA, Poellinger L. The specific DNA binding activity of the dioxin receptor is modulated by the 90 kD heat shock protein. *EMBO J* 1990;9:69–76.
30. Denison MS, Fisher JM, Whitlock JP, Jr. Protein-DNA interactions at recognition sites for the dioxin-Ah receptor complex. *J Biol Chem* 1989;264: 16478–82.
31. Dolwick KM, Swanson HI, Bradfield CA. *In vitro* analysis of Ah receptor domains involved in ligand-activated DNA recognition. *Proc Natl Acad Sci U S A* 1993;90:8566–70.
32. Tang YM, Wo YY, Stewart J, et al. Isolation and characterization of the human cytochrome *P*450 CYP1B1 gene. *J Biol Chem* 1996;271:28324–30.
33. Fisher JM, Wu L, Denison MS, Whitlock JP, Jr. Organization and function of a dioxin-responsive enhancer. *J Biol Chem* 1990;265:9676–81.
34. Whitlock JP, Jr. Genetic and molecular aspects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin action. *Annu Rev Pharmacol Toxicol* 1990;30:251–77.
35. Smale ST, Schmidt MC, Berk AJ, Baltimore D. Transcriptional activation by Sp1 as directed through TATA or initiator: specific requirement for mammalian transcription factor IID. *Proc Natl Acad Sci U S A* 1990;87:4509–13.
36. Reznikoff WS, Siegele DA, Cowing DW, Gross CA. The regulation of transcription initiation in bacteria. *Annu Rev Genet* 1985;19:355–87.
37. Shehin SE, Stephenson RO, Greenlee WF. Transcriptional regulation of the human CYP1B1 gene. Evidence for involvement of an aryl hydrocarbon receptor response element in constitutive expression. *J Biol Chem* 2000; 275:6770–6.
38. Tsuchiya Y, Nakajima M, Yokoi T. Critical enhancer region to which AhR/ARNT and Sp1 bind in the human CYP1B1 gene. *J Biochem (Tokyo)* 2003;133: 583–92.
39. Wo YY, Stewart J, Greenlee WF. Functional analysis of the promoter for the human CYP1B1 gene. *J Biol Chem* 1997;272:26702–7.
40. Zheng W, Jefcoate CR. Steroidogenic factor-1 interacts with cAMP response element-binding protein to mediate cAMP stimulation of CYP1B1 via a far upstream enhancer. *Mol Pharmacol* 2005;67:499–512.
41. Widschwendter M, Siegmund KD, Muller HM, et al. Association of breast cancer DNA methylation profiles with hormone receptor status and response to tamoxifen. *Cancer Res* 2004;64:3807–13.
42. Tokizane T, Shiina H, Igawa M, et al. Cytochrome *P*450 1B1 is over-expressed and regulated by hypomethylation in prostate cancer. *Clin Cancer Res* 2005;11:5793–801.
43. Han W, Kang D, Park IA, et al. Associations between breast cancer susceptibility gene polymorphisms and clinicopathological features. *Clin Cancer Res* 2004;10:124–30.
44. Sasaki M, Dharia A, Oh BR, Tanaka Y, Fujimoto S, Dahiya R. Progesterone receptor B gene inactivation and CpG hypermethylation in human uterine endometrial cancer. *Cancer Res* 2001;61:97–102.
45. Sasaki M, Kaneuchi M, Sakuragi N, Dahiya R. Multiple promoters of catechol-*O*-methyltransferase gene are selectively inactivated by CpG hypermethylation in endometrial cancer. *Cancer Res* 2003;63:3101–6.
46. Sasaki M, Kotcherquina L, Dharia A, Fujimoto S, Dahiya R. Cytosine-phosphoguanine methylation of estrogen receptors in endometrial cancer. *Cancer Res* 2001;61:3262–6.
47. Sasaki M, Nakajima K, Perincher G, et al. Frequent genotype changes at –308 of the human tumor necrosis factor- $\alpha$  promoter region in human uterine endometrial cancer. *Oncol Rep* 2000;7:369–73.
48. Sasaki M, Oh BR, Dharia A, Fujimoto S, Dahiya R. Inactivation of the human androgen receptor gene is associated with CpG hypermethylation in uterine endometrial cancer. *Mol Carcinog* 2000;29:59–66.
49. Sasaki M, Tanaka Y, Perincher G, et al. Methylation and inactivation of estrogen, progesterone, and androgen receptors in prostate cancer. *J Natl Cancer Inst* 2002;94:384–90.
50. Bandiera S, Weidlich S, Harth V, Broede P, Ko J, Friedberg T. Proteasomal degradation of human cytochrome *P*450 1B1 (CYP1B1): effect of the Asn<sup>453</sup>Ser polymorphism on the post-translational regulation of CYP1B1 expression. *Mol Pharmacol* 2004.
51. McGrath M, Hankinson SE, Arbeitman L, Colditz GA, Hunter DJ, De Vivo I. Cytochrome *P*450 1B1 and catechol-*O*-methyltransferase polymorphisms and endometrial cancer susceptibility. *Carcinogenesis* 2004;25:559–65.
52. Tsuchiya Y, Nakajima M, Kyo S, Kanaya T, Inoue M, Yokoi T. Human CYP1B1 is regulated by estradiol via estrogen receptor. *Cancer Res* 2004;64: 3119–25.
53. Spink DC, Katz BH, Hussain MM, Pentecost BT, Cao Z, Spink BC. Estrogen regulates Ah responsiveness in MCF-7 breast cancer cells. *Carcinogenesis* 2003; 24:1941–50.
54. Sasaki M, Tanaka Y, Kaneuchi M, Sakuragi N, Dahiya R. CYP1B1 gene polymorphisms have higher risk for endometrial cancer, and positive correlations with estrogen receptor  $\alpha$  and estrogen receptor  $\beta$  expressions. *Cancer Res* 2003; 63:3913–8.
55. Schutze N, Vollmer G, Knuppen R. Catecholestrogens are agonists of estrogen receptor dependent gene expression in MCF-7 cells. *J Steroid Biochem Mol Biol* 1994;48:453–61.
56. Schutze N, Vollmer G, Tiemann I, Geiger M, Knuppen R. Catecholestrogens are MCF-7 cell estrogen receptor agonists. *J Steroid Biochem Mol Biol* 1993; 46:781–9.
57. Berge G, Mollerup S, OVrebo S, et al. Role of estrogen receptor in regulation of polycyclic aromatic hydrocarbon metabolic activation in lung. *Lung Cancer* 2004;45:289–97.
58. Fukatsu T, Hirokawa Y, Araki T, et al. Genetic polymorphisms of hormone-related genes and prostate cancer risk in the Japanese population. *Anticancer Res* 2004;24:2431–7.
59. Crespi CL, Penman BW, Steimel DT, Smith T, Yang CS, Sutter TR. Development of a human lymphoblastoid cell line constitutively expressing human CYP1B1 cDNA: substrate specificity with model substrates and promutagens. *Mutagenesis* 1997;12:83–9.
60. Hwang DY, Cho JS, Chae KR, et al. Differential expression of the tetracycline-controlled transactivator-driven human CYP1B1 gene in double-transgenic mice is due to androgens: application for detecting androgens and antiandrogens. *Arch Biochem Biophys* 2003;415:137–45.
61. Lin P, Chang JT, Ko JL, Liao SH, Lo WS. Reduction of androgen receptor expression by benzo[*a*]pyrene and 7,8-dihydro-9,10-epoxy-7,8,9,10-tetrahydro-benzo[*a*]pyrene in human lung cells. *Biochem Pharmacol* 2004;67:1523–30.
62. Hakkola J, Raunio H, Purkunen R, et al. Detection of cytochrome *P*450 gene expression in human placenta in first trimester of pregnancy. *Biochem Pharmacol* 1996;52:379–83.

63. Huang Z, Fasco MJ, Figge HL, Keyomarsi K, Kaminsky LS. Expression of cytochromes *P450* in human breast tissue and tumors. *Drug Metab Dispos* 1996; 24:899–905.
64. Kivisto KT, Griese EU, Fritz P, et al. Expression of cytochrome *P450* 3A enzymes in human lung: a combined RT-PCR and immunohistochemical analysis of normal tissue and lung tumours. *Naunyn Schmiedebergs Arch Pharmacol* 1996;353:207–12.
65. Rieder CR, Ramsden DB, Williams AC. Cytochrome *P450* 1B1 mRNA in the human central nervous system. *Mol Pathol* 1998;51:138–42.
66. Vadlamuri SV, Glover DD, Turner T, Sarkar MA. Regiospecific expression of cytochrome *P450*1A1 and 1B1 in human uterine tissue. *Cancer Lett* 1998; 122:143–50.
67. Spink DC, Spink BC, Cao JQ, et al. Differential expression of CYP1A1 and CYP1B1 in human breast epithelial cells and breast tumor cells. *Carcinogenesis* 1998;19:291–8.
68. Liehr JG, Ricci MJ. 4-Hydroxylation of estrogens as marker of human mammary tumors. *Proc Natl Acad Sci U S A* 1996;93:3294–6.
69. McFadyen MC, Rooney PH, Melvin WT, Murray GI. Quantitative analysis of the Ah receptor/cytochrome *P450* CYP1B1/CYP1A1 signalling pathway. *Biochem Pharmacol* 2003;65:1663–74.
70. Gibson P, Gill JH, Khan PA, et al. Cytochrome *P450* 1B1 (CYP1B1) is overexpressed in human colon adenocarcinomas relative to normal colon: implications for drug development. *Mol Cancer Ther* 2003;2:527–34.
71. Lin P, Chang H, Ho WL, Wu MH, Su JM. Association of aryl hydrocarbon receptor and cytochrome *P450*1B1 expressions in human non-small cell lung cancers. *Lung Cancer* 2003;42:255–61.
72. Shimada T, Oda Y, Gillam EM, Guengerich FP, Inoue K. Metabolic activation of polycyclic aromatic hydrocarbons and other procarcinogens by cytochromes *P450* 1A1 and *P450* 1B1 allelic variants and other human cytochromes *P450* in *Salmonella typhimurium* NM2009. *Drug Metab Dispos* 2001;29:1176–82.
73. Thier R, Bruning T, Roos PH, Bolt HM. Cytochrome *P450* 1B1, a new keystone in gene-environment interactions related to human head and neck cancer? *Arch Toxicol* 2002;76:249–56.
74. Lee AJ, Kosh JW, Conney AH, Zhu BT. Characterization of the NADPH-dependent metabolism of 17 $\beta$ -estradiol to multiple metabolites by human liver microsomes and selectively expressed human cytochrome *P450* 3A4 and 3A5. *J Pharmacol Exp Ther* 2001;298:420–32.
75. Miyoshi Y, Taguchi T, Kim SJ, Tamaki Y, Noguchi S. Prediction of response to docetaxel by immunohistochemical analysis of CYP3A4 expression in human breast cancers. *Breast Cancer* 2005;12:11–5.
76. Murray GI, Foster CO, Barnes TS, et al. Expression of cytochrome *P450*1A in breast cancer. *Br J Cancer* 1991;63:1021–3.
77. Murray GI, Weaver RJ, Paterson PJ, Ewen SW, Melvin WT, Burke MD. Expression of xenobiotic metabolizing enzymes in breast cancer. *J Pathol* 1993; 169:347–53.
78. Oyama T, Morita M, Isse T, et al. Immunohistochemical evaluation of cytochrome *P450* (CYP) and p53 in breast cancer. *Front Biosci* 2005;10: 1156–61.
79. Jefcoate CR, Liehr JG, Santen RJ, et al. Tissue-specific synthesis and oxidative metabolism of estrogens. *J Natl Cancer Inst Monogr* 2000;27:95–112.
80. Lindgren PR, Backstrom T, Cajander S, et al. The pattern of estradiol and progesterone differs in serum and tissue of benign and malignant ovarian tumors. *Int J Oncol* 2002;21:583–9.
81. Zhao Y, Agarwal VR, Mendelson CR, Simpson ER. Estrogen biosynthesis proximal to a breast tumor is stimulated by PGE<sub>2</sub> via cyclic AMP, leading to activation of promoter II of the CYP19 (aromatase) gene. *Endocrinology* 1996; 137:5739–42.
82. Geisler J. Breast cancer tissue estrogens and their manipulation with aromatase inhibitors and inactivators. *J Steroid Biochem Mol Biol* 2003;86: 245–53.
83. Berstein L, Kovalevskij A, Zimarina T, et al. Aromatase and comparative response to its inhibitors in two types of endometrial cancer. *J Steroid Biochem Mol Biol* 2005;95:71–4.
84. Berstein L, Zimarina T, Kovalevskij A, Maximov S, Volkov O, Thijssen JH. CYP19 gene expression and aromatase activity in endometrial cancer tissue: importance of the type of the disease. *Neoplasma* 2005;52:115–8.
85. Ho SM. Estrogen, progesterone and epithelial ovarian cancer. *Reprod Biol Endocrinol* 2003;1:73.
86. Han X, Liehr JG. DNA single-strand breaks in kidneys of Syrian hamsters treated with steroidal estrogens: hormone-induced free radical damage preceding renal malignancy. *Carcinogenesis* 1994;15:997–1000.
87. Newbold RR, Liehr JG. Induction of uterine adenocarcinoma in CD-1 mice by catechol estrogens. *Cancer Res* 2000;60:235–7.
88. Badawi AF, Cavalieri EL, Rogan EG. Role of human cytochrome *P450* 1A1, 1A2, 1B1, and 3A4 in the 2-, 4-, and 16 $\alpha$ -hydroxylation of 17 $\beta$ -estradiol. *Metabolism* 2001;50:1001–3.
89. Lee AJ, Cai MX, Thomas PE, Conney AH, Zhu BT. Characterization of the oxidative metabolites of 17 $\beta$ -estradiol and estrone formed by 15 selectively expressed human cytochrome *P450* isoforms. *Endocrinology* 2003;144:3382–98.
90. Shimada T, Watanabe J, Kawajiri K, et al. Catalytic properties of polymorphic human cytochrome *P450* 1B1 variants. *Carcinogenesis* 1999;20: 1607–13.
91. Cavalieri E, Frenkel K, Liehr JG, Rogan E, Roy D. Estrogens as endogenous genotoxic agents-DNA adducts and mutations. *J Natl Cancer Inst Monogr* 2000;27:75–93.
92. Cavalieri EL, Rogan EG. A unifying mechanism in the initiation of cancer and other diseases by catechol quinones. *Ann N Y Acad Sci* 2004;1028:247–57.
93. Li JJ, Li SA, Oberley TD, Parsons JA. Carcinogenic activities of various steroidal and nonsteroidal estrogens in the hamster kidney: relation to hormonal activity and cell proliferation. *Cancer Res* 1995;55:4347–51.
94. Cavalieri EL, Devanesan P, Bosland MC, Badawi AF, Rogan EG. Catechol estrogen metabolites and conjugates in different regions of the prostate of Noble rats treated with 4-hydroxyestradiol: implications for estrogen-induced initiation of prostate cancer. *Carcinogenesis* 2002;23:329–33.
95. Cavalieri EL, Li KM, Balu N, et al. Catechol ortho-quinones: the electrophilic compounds that form depurinating DNA adducts and could initiate cancer and other diseases. *Carcinogenesis* 2002;23:1071–7.
96. Cavalieri EL, Rogan EG. A unified mechanism in the initiation of cancer. *Ann N Y Acad Sci* 2002;959:341–54.
97. Cavalieri EL, Rogan EG, Chakravarti D. Initiation of cancer and other diseases by catechol ortho-quinones: a unifying mechanism. *Cell Mol Life Sci* 2002;59:665–81.
98. Chakravarti D, Mailander PC, Li KM, et al. Evidence that a burst of DNA depurination in SENCAR mouse skin induces error-prone repair and forms mutations in the H-ras gene. *Oncogene* 2001;20:7945–53.
99. Devanesan P, Todorovic R, Zhao J, Gross ML, Rogan EG, Cavalieri EL. Catechol estrogen conjugates and DNA adducts in the kidney of male Syrian golden hamsters treated with 4-hydroxyestradiol: potential biomarkers for estrogen-initiated cancer. *Carcinogenesis* 2001;22:489–97.
100. Dawling S, Roodi N, Parl FF. Methoxyestrogens exert feedback inhibition on cytochrome *P450* 1A1 and 1B1. *Cancer Res* 2003;63:3127–32.
101. Hachey DL, Dawling S, Roodi N, Parl FF. Sequential action of phase I and II enzymes cytochrome *P450* 1B1 and glutathione S-transferase P1 in mammary estrogen metabolism. *Cancer Res* 2003;63:8492–9.
102. Dickson RB, Stancel GM. Estrogen receptor-mediated processes in normal and cancer cells. *J Natl Cancer Inst Monogr* 2000;27:135–45.
103. Raftogianis R, Creveling C, Weinshilboum R, Weisz J. Estrogen metabolism by conjugation. *J Natl Cancer Inst Monogr* 2000;27:113–24.
104. Thompson PA, Ambrosone C. Molecular epidemiology of genetic polymorphisms in estrogen metabolizing enzymes in human breast cancer. *J Natl Cancer Inst Monogr* 2000;27:125–34.
105. Yager JD, Liehr JG. Molecular mechanisms of estrogen carcinogenesis. *Annu Rev Pharmacol Toxicol* 1996;36:203–32.
106. 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.
107. Aklillu E, Oscarson M, Hidestrand M, Leidvik B, Otter C, Ingelman-Sundberg M. Functional analysis of six different polymorphic CYP1B1 enzyme variants found in an Ethiopian population. *Mol Pharmacol* 2002;61:586–94.
108. McLellan RA, Oscarson M, Hidestrand M, et al. Characterization and functional analysis of two common human cytochrome *P450* 1B1 variants. *Arch Biochem Biophys* 2000;378:175–81.
109. Landi MT, Bergen AW, Baccarelli A, et al. CYP1A1 and CYP1B1 genotypes, haplotypes, and TCDD-induced gene expression in subjects from Seveso, Italy. *Toxicology* 2005;207:191–202.
110. 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;60:3440–4.
111. Li DN, Seidel A, Pritchard MP, Wolf CR, Friedberg T. Polymorphisms in *P450* CYP1B1 affect the conversion of estradiol to the potentially carcinogenic metabolite 4-hydroxyestradiol. *Pharmacogenetics* 2000;10:343–53.
112. Chang BL, Zheng SL, Isaacs SD, et al. Polymorphisms in the CYP1B1 gene are associated with increased risk of prostate cancer. *Br J Cancer* 2003;89:1524–9.

113. Bandiera S, Weidlich S, Harth V, Broede P, Ko Y, Friedberg T. Proteasomal degradation of human CYP1B1: effect of the Asn<sup>453</sup>Ser polymorphism on the post-translational regulation of CYP1B1 expression. *Mol Pharmacol* 2005; 67:435–43.
114. Shimada T, Watanabe J, Inoue K, Guengerich FP, Gillam EM. Specificity of 17 $\beta$ -oestradiol and benzo[a]pyrene oxidation by polymorphic human cytochrome P4501B1 variants substituted at residues 48, 119 and 432. *Xenobiotica* 2001; 31:163–76.
115. Spink DC, Spink BC, Zhuo X, Hussain MM, Gierthy JF, Ding X. NADPH- and hydroperoxide-supported 17 $\beta$ -estradiol hydroxylation catalyzed by a variant form (432L, 453S) of human cytochrome P450 1B1. *J Steroid Biochem Mol Biol* 2000;74:11–8.
116. Jordan VC. Antiestrogens: clinical applications of pharmacology. *J Soc Gynecol Investig* 2000;7:S47–8.
117. Nandi S, Guzman RC, Yang J. Hormones and mammary carcinogenesis in mice, rats, and humans: a unifying hypothesis. *Proc Natl Acad Sci U S A* 1995; 92:3650–7.
118. Yager JD. Endogenous estrogens as carcinogens through metabolic activation. *J Natl Cancer Inst Monogr* 2000;27:67–73.
119. Ingelman-Sundberg M, Oscarson M, McLellan RA. Polymorphic human cytochrome P450 enzymes: an opportunity for individualized drug treatment. *Trends Pharmacol Sci* 1999;20:342–9.
120. Liehr JG. Catecholestrogens in the induction of tumors in the kidney of the Syrian hamster. *Adv Pharmacol* 1998;42:824–8.
121. Simpson ER. Aromatization of androgens in women: current concepts and findings. *Fertil Steril* 2002;77 Suppl 4:S6–10.
122. Lurie G, Maskarinec G, Kaaks R, Stanczyk FZ, Le Marchand L. Association of genetic polymorphisms with serum estrogens measured multiple times during a 2-year period in premenopausal women. *Cancer Epidemiol Biomarkers Prev* 2005;14:1521–7.
123. Tworoger SS, Chubak J, Aiello EJ, et al. Association of CYP17, CYP19, CYP1B1, and COMT polymorphisms with serum and urinary sex hormone concentrations in postmenopausal women. *Cancer Epidemiol Biomarkers Prev* 2004;13:94–101.
124. De Vivo I, Hankinson SE, Li L, Colditz GA, Hunter DJ. Association of CYP1B1 polymorphisms and breast cancer risk. *Cancer Epidemiol Biomarkers Prev* 2002;11:489–92.
125. Larsen MC, Angus WG, Brake PB, Eltom SE, Sukow KA, Jefcoate CR. Characterization of CYP1B1 and CYP1A1 expression in human mammary epithelial cells: role of the aryl hydrocarbon receptor in polycyclic aromatic hydrocarbon metabolism. *Cancer Res* 1998;58:2366–74.
126. Li KM, Todorovic R, Devanesan P, et al. Metabolism and DNA binding studies of 4-hydroxyestradiol and estradiol-3,4-quinone *in vitro* and in female ACI rat mammary gland *in vivo*. *Carcinogenesis* 2004;25:289–97.
127. Yue W, Wang JP, Li Y, et al. Tamoxifen versus aromatase inhibitors for breast cancer prevention. *Clin Cancer Res* 2005;11:925–30s.
128. Brockdorff BL, Skouv J, Reiter BE, Lykkesfeldt AE. Increased expression of cytochrome P450 1A1 and 1B1 genes in anti-estrogen-resistant human breast cancer cell lines. *Int J Cancer* 2000;88:902–6.
129. Kocabas NA, Sardas S, Cholerton S, Daly AK, Karakaya AE. Cytochrome P450 CYP1B1 and catechol O-methyltransferase (COMT) genetic polymorphisms and breast cancer susceptibility in a Turkish population. *Arch Toxicol* 2002;76:643–9.
130. Sasaki M, Tanaka Y, Kaneuchi M, Sakuragi N, Dahiya R. Alleles of polymorphic sites that correspond to hyperactive variants of CYP1B1 protein are significantly less frequent in Japanese as compared to American and German populations. *Hum Mutat* 2003;21:652.
131. Ahsan H, Chen Y, Whitemore AS, et al. A family-based genetic association study of variants in estrogen-metabolism genes COMT and CYP1B1 and breast cancer risk. *Breast Cancer Res Treat* 2004;85:121–31.
132. Le Marchand L, Donlon T, Kolonel LN, Henderson BE, Wilkens LR. Estrogen metabolism-related genes and breast cancer risk: the multiethnic cohort study. *Cancer Epidemiol Biomarkers Prev* 2005;14:1998–2003.
133. Lee KM, Abel J, Ko Y, et al. Genetic polymorphisms of cytochrome P450 19 and 1B1, alcohol use, and breast cancer risk in Korean women. *Br J Cancer* 2003;88:675–8.
134. Rylander-Rudqvist T, Wedren S, Granath F, et al. Cytochrome P450 1B1 gene polymorphisms and postmenopausal breast cancer risk. *Carcinogenesis* 2003; 24:1533–9.
135. Watanabe J, Shimada T, Gillam EM, et al. Association of CYP1B1 genetic polymorphism with incidence to breast and lung cancer. *Pharmacogenetics* 2000; 10:25–33.
136. Zheng W, Xie DW, Jin F, et al. Genetic polymorphism of cytochrome P450-1B1 and risk of breast cancer. *Cancer Epidemiol Biomarkers Prev* 2000; 9:147–50.
137. Saintot M, Malaveille C, Hautefeuille A, Gerber M. Interactions between genetic polymorphism of cytochrome P450-1B1, sulfotransferase 1A1, catechol-o-methyltransferase and tobacco exposure in breast cancer risk. *Int J Cancer* 2003; 107:652–7.
138. Cook L. Hormones, genes, and cancer. New York (NY): Oxford University Press; 2003. p. 371–9.
139. Liehr JG, Ricci MJ, Jefcoate CR, Hannigan EV, Hokanson JA, Zhu BT. 4-Hydroxylation of estradiol by human uterine myometrium and myoma microsomes: implications for the mechanism of uterine tumorigenesis. *Proc Natl Acad Sci U S A* 1995;92:9220–4.
140. Colacurci N, De Seta L, De Francis P, Mele D, Fortunato N, Cassese S. Tamoxifen effects on endometrium. *Panminerva Med* 2000;42:45–7.
141. Czernobilsky B, Lifschitz-Mercer B. Endometrial pathology. *Curr Opin Obstet Gynecol* 1997;9:52–6.
142. Sharma M, Shubert DE, Sharma M, et al. Biotransformation of tamoxifen in a human endometrial explant culture model. *Chem Biol Interact* 2003;146:237–49.
143. Crewe HK, Notley LM, Wunsch RM, Lennard MS, Gillam EM. Metabolism of tamoxifen by recombinant human cytochrome P450 enzymes: formation of the 4-hydroxy, 4'-hydroxy and N-desmethyl metabolites and isomerization of *trans*-4-hydroxytamoxifen. *Drug Metab Dispos* 2002;30: 869–74.
144. Osborne CK, Wiebe VJ, McGuire WL, Ciocca DR, DeGregorio MW. Tamoxifen and the isomers of 4-hydroxytamoxifen in tamoxifen-resistant tumors from breast cancer patients. *J Clin Oncol* 1992;10:304–10.
145. Doherty JA, Weiss NS, Freeman RJ, et al. Genetic factors in catechol estrogen metabolism in relation to the risk of endometrial cancer. *Cancer Epidemiol Biomarkers Prev* 2005;14:357–66.
146. Rylander-Rudqvist T, Wedren S, Jonasdottir G, et al. Cytochrome P450 1B1 gene polymorphisms and postmenopausal endometrial cancer risk. *Cancer Epidemiol Biomarkers Prev* 2004;13:1515–20.
147. McFadyen MC, Cruickshank ME, Miller ID, et al. Cytochrome P450 CYP1B1 over-expression in primary and metastatic ovarian cancer. *Br J Cancer* 2001;85:242–6.
148. Goodman MT, McDuffie K, Kolonel LN, et al. Case-control study of ovarian cancer and polymorphisms in genes involved in catecholestrogen formation and metabolism. *Cancer Epidemiol Biomarkers Prev* 2001;10: 209–16.
149. Cecchin E, Russo A, Campagnutta E, Martella L, Toffoli G. Lack of association of CYP1 B1\*3 polymorphism and ovarian cancer in a Caucasian population. *Int J Biol Markers* 2004;19:160–3.
150. Henderson BE, Ross RK, Pike MC, Casagrande JT. Endogenous hormones as a major factor in human cancer. *Cancer Res* 1982;42:3232–9.
151. Williams JA, Martin FL, Muir GH, Hewer A, Grover PL, Phillips DH. Metabolic activation of carcinogens and expression of various cytochromes P450 in human prostate tissue. *Carcinogenesis* 2000;21: 1683–9.
152. Bosland MC. The role of steroid hormones in prostate carcinogenesis. *J Natl Cancer Inst Monogr* 2000;27:39–66.
153. Chaib H, Cockrell EK, Rubin MA, Macoska JA. Profiling and verification of gene expression patterns in normal and malignant human prostate tissues by cDNA microarray analysis. *Neoplasia* 2001;3:43–52.
154. Tang YM, Green BL, Chen GF, et al. Human CYP1B1 Leu<sup>432</sup>Val gene polymorphism: ethnic distribution in African-Americans, Caucasians and Chinese; oestradiol hydroxylase activity; and distribution in prostate cancer cases and controls. *Pharmacogenetics* 2000;10:761–6.
155. Burns KH, Agno JE, Chen L, et al. Sexually dimorphic roles of steroid hormone receptor signaling in gonadal tumorigenesis. *Mol Endocrinol* 2003; 17:2039–52.
156. Yoshimura K, Hanaoka T, Ohnami S, et al. Allele frequencies of single nucleotide polymorphisms (SNPs) in 40 candidate genes for gene-environment studies on cancer: data from population-based Japanese random samples. *J Hum Genet* 2003;48:654–8.



# Molecular Cancer Research

## Pharmacogenetics and Regulation of Human Cytochrome *P* 450 1B1: Implications in Hormone-Mediated Tumor Metabolism and a Novel Target for Therapeutic Intervention

Tristan M. Sissung, Douglas K. Price, Alex Sparreboom, et al.

*Mol Cancer Res* 2006;4:135-150.

**Updated version** Access the most recent version of this article at:  
<http://mcr.aacrjournals.org/content/4/3/135>

**Cited articles** This article cites 154 articles, 58 of which you can access for free at:  
<http://mcr.aacrjournals.org/content/4/3/135.full#ref-list-1>

**Citing articles** This article has been cited by 17 HighWire-hosted articles. Access the articles at:  
<http://mcr.aacrjournals.org/content/4/3/135.full#related-urls>

**E-mail alerts** [Sign up to receive free email-alerts](#) related to this article or journal.

**Reprints and Subscriptions** To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at [pubs@aacr.org](mailto:pubs@aacr.org).

**Permissions** To request permission to re-use all or part of this article, use this link  
<http://mcr.aacrjournals.org/content/4/3/135>.  
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.