Subject Review
Pharmacogenetics and Regulation of Human Cytochrome \( P450 \) 1B1: Implications in Hormone-Mediated Tumor Metabolism and a Novel Target for Therapeutic Intervention

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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 differentially 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 glaucoma), 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 \( P450 \)s (ref. 17; see Fig. 1A).
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...
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. DNAmethylation 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
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 ERα 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 ERα and ERβ status of hormone-mediated cancers is correlated with CYP1B1 expression (54) through CYP1B1-mediated transformation of estrogen into 4-hydroxyestadiol (4-OHE2; 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-OHE2 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).

**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

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

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

*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.

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

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 (~ 10 μmol/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 ~ 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 17β-estradiol into the less active metabolites, 4-OHE2 (major product) and 2-hydroxyestradiol (2-OHE2; minor product; refs. 11, 88-90). 4-OHE2 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-OHE2 treatment does not induce tumors and is much less carcinogenic than 4-OHE2 (93).

The estrogen-induced toxicity model has been consistently validated in studies aimed at determining the molecular mechanisms of estrogen toxicity. Cavaliere et al. showed that 4-OHE2, 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-OHE2 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-OHE2 (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-OHE2 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-OHE2;2-OHE2 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, S9 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 to have the lowest catalytically efficiency toward estrogen 4-hydroxylation reactions and the lowest ratio of 4-OHE2:2-OHE2 formation. However, when the CYP1B1*2 and

Table 2. Polymorphisms and Their Consequences

<table>
<thead>
<tr>
<th>Nucleotide Transition*</th>
<th>Amino Acid Transition†</th>
<th>Functional Consequence</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>−1001C/T</td>
<td>—</td>
<td>Possible promoter alteration</td>
<td>(112)</td>
</tr>
<tr>
<td>−263G/A</td>
<td>—</td>
<td>Possible promoter alteration</td>
<td>(112)</td>
</tr>
<tr>
<td>13C/T</td>
<td>—</td>
<td>Possible promoter alteration</td>
<td>(112)</td>
</tr>
<tr>
<td>142G/C</td>
<td>R48G</td>
<td>Increased basal CYP1B1 gene expression</td>
<td>(109)</td>
</tr>
<tr>
<td>355G/T</td>
<td>A119S</td>
<td>No alterations in CYP1B1 catalytic properties</td>
<td>(109)</td>
</tr>
<tr>
<td>4326G/C</td>
<td>L432V</td>
<td>Significant decrease in CYP1B1 expression due to increased CYP1B1 degradation efficiency by proteolysis.</td>
<td>(50)</td>
</tr>
<tr>
<td>4390G/A</td>
<td>N453S</td>
<td>Significant increase in CYP1B1 gene expression during AhR signaling events.</td>
<td>(109)</td>
</tr>
</tbody>
</table>

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.
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 alleles) have 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_2 (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, 61) the CYP1B1.4 variant is likely important in assessing reaction kinetics as no study has evaluated CYP1B1 estrogen metabolism in human cells.

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

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Ethnicity</th>
<th>Case/Control</th>
<th>Association of Cancer Risk with CYP1B1 Genotype</th>
<th>Genotype</th>
<th>OR (95% CI)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>L432V*</td>
<td>ND</td>
<td>Yes (increases risk)</td>
<td>V/V and V/L</td>
<td>3.30 (1.76-6.19)</td>
<td>(14)</td>
</tr>
<tr>
<td></td>
<td>Mixed ancestry</td>
<td>1.339/1,370</td>
<td>No</td>
<td></td>
<td>0.9 (0.7-1.1)</td>
<td>(132)</td>
</tr>
<tr>
<td></td>
<td>Caucasian</td>
<td>164/164</td>
<td>No</td>
<td></td>
<td>0.7 (0.4-1.5)</td>
<td>(106)</td>
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<tr>
<td></td>
<td>African American</td>
<td>59/99</td>
<td>No</td>
<td></td>
<td>1.6 (0.4-2.9)</td>
<td>(106)</td>
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<tr>
<td></td>
<td>Chinese</td>
<td>186/200</td>
<td>Yes (increases risk)</td>
<td>L/L</td>
<td>2.3 (1.2-4.3)</td>
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<tr>
<td></td>
<td>ND</td>
<td>453/453</td>
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<td></td>
<td>Turkish</td>
<td>84/103</td>
<td>Yes (increases risk)</td>
<td>V/V and V/L</td>
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<td>(129)</td>
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<tr>
<td></td>
<td>Korean</td>
<td>241/290</td>
<td>No</td>
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<td>1.0 (0.71-1.6)</td>
<td>(133)</td>
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<tr>
<td>Endometrial</td>
<td>A119S*</td>
<td>Caucasian</td>
<td>689/1,549</td>
<td>No</td>
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<tr>
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<td>113/202</td>
<td>Yes (increases risk)</td>
<td>S/S</td>
<td>3.32 (1.38-8.01)</td>
<td>(54)</td>
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<tr>
<td></td>
<td>L432V*</td>
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<td>Yes (moderate increase)</td>
<td>V/V</td>
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<td>ND</td>
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<tr>
<td></td>
<td>Caucasian</td>
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<td></td>
<td>0.8 (0.7-1.0)</td>
<td>(146)</td>
</tr>
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<td>113/202</td>
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<td>V/V</td>
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<tr>
<td></td>
<td>N453S*</td>
<td>ND</td>
<td>Decreased risk</td>
<td>N/S and S/S</td>
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<td>Japanese</td>
<td>117/200</td>
<td>No</td>
<td></td>
<td>1.0 (0.8-1.3)</td>
<td>(136)</td>
</tr>
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<td></td>
<td></td>
<td></td>
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<td>(16)</td>
<td></td>
</tr>
<tr>
<td>Ovarian</td>
<td>L432V*</td>
<td>Mixed ancestry</td>
<td>129/144</td>
<td>Yes (increases risk)</td>
<td>V/V</td>
<td>3.8 (1.2-11.4)</td>
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<td>223/280</td>
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<td>???</td>
<td>(149)</td>
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<tr>
<td>Prostate</td>
<td>A119S*</td>
<td>Japanese</td>
<td>117/200</td>
<td>Yes (increases risk)</td>
<td>S/S</td>
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<tr>
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<td>L432V*</td>
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<td>V/V</td>
<td>4.80 (1.21-19.05)</td>
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<tr>
<td></td>
<td>Japanese</td>
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<td></td>
<td>1.34 (0.53-3.35)</td>
<td>(16)</td>
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<tr>
<td></td>
<td>Caucasian American</td>
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<td>Yes (increases risk)</td>
<td>V/V</td>
<td>3.3 (1.9-9.0)</td>
<td>(154)</td>
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</table>

*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.
Estrogen hydroxylase activity and the formation of toxic 4-OHE2 is increased in the metabolically hyperactive CYP1B1.3 variants resulting in the increased carcinogenicity of estrogen (11, 86, 90, 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-OHE2. Whereas most haplotypes do not affect the catalytic properties of CYP1B1 toward E2, the CYP1B1*6 haplotype was associated with drastic increases in $K_m$ for the conversion of E2 into 4-OHE2 (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-OHE2 and an increased ratio of 4-OHE2:2-OHE2 concentrations in the tumor microenvironment (4, 68, 125). The formation of 4-OHE2 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

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Ethnicity</th>
<th>Controls</th>
<th>Variants, n (%)</th>
<th>p</th>
<th>q</th>
<th>Ref.</th>
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<tbody>
<tr>
<td>R48G</td>
<td>Japanese</td>
<td>200</td>
<td>99 (49.5)</td>
<td>72  (36.0)</td>
<td>29 (14.5)</td>
<td>(16)</td>
</tr>
<tr>
<td></td>
<td>202</td>
<td>100 (49.5)</td>
<td>73 (36.1)</td>
<td>29  (14.4)</td>
<td>(54)</td>
<td></td>
</tr>
<tr>
<td>A119S</td>
<td>Caucasian</td>
<td>1,382</td>
<td>699 (50.6)</td>
<td>556 (40.2)</td>
<td>127 (9.2)</td>
<td>(134)</td>
</tr>
<tr>
<td></td>
<td>Japanese</td>
<td>200</td>
<td>151 (75.5)</td>
<td>38  (19.0)</td>
<td>11  (5.5)</td>
<td>(16)</td>
</tr>
<tr>
<td>L432V</td>
<td>Caucasian</td>
<td>164</td>
<td>49 (30)</td>
<td>96  (59)</td>
<td>19  (12)</td>
<td>(106)</td>
</tr>
<tr>
<td></td>
<td>148</td>
<td>46 (31.1)</td>
<td>76  (51.3)</td>
<td>26  (17.6)</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>1,380</td>
<td>425 (30.1)</td>
<td>676 (49.0)</td>
<td>279 (20.2)</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Caucasian American</td>
<td>200</td>
<td>99 (49.5)</td>
<td>72  (36.0)</td>
<td>29  (14.5)</td>
<td>(130)</td>
</tr>
<tr>
<td></td>
<td>189</td>
<td>61 (32.3)</td>
<td>93  (49.2)</td>
<td>35  (18.5)</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>African American</td>
<td>59</td>
<td>26 (44)</td>
<td>30  (51)</td>
<td>3   (5)</td>
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<td></td>
<td>52</td>
<td>28 (54)</td>
<td>21  (40)</td>
<td>3   (6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>German</td>
<td>112</td>
<td>59 (52.7)</td>
<td>39  (34.8)</td>
<td>14  (12.5)</td>
<td>(130)</td>
</tr>
<tr>
<td></td>
<td>Chinese</td>
<td>200</td>
<td>29 (15)</td>
<td>127 (64)</td>
<td>44 (22)</td>
<td>(136)</td>
</tr>
<tr>
<td></td>
<td>201</td>
<td>29 (15)</td>
<td>127 (64)</td>
<td>44 (22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>109</td>
<td>73 (67.0)</td>
<td>35  (52.0)</td>
<td>1 (1.0)</td>
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<td></td>
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<td>141 (70.5)</td>
<td>46  (23.0)</td>
<td>13 (6.5)</td>
<td>(130)</td>
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<tr>
<td></td>
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<td>230 (71)</td>
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<td>6   (2)</td>
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</tr>
<tr>
<td></td>
<td>337</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>A119S</td>
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<td>200</td>
<td>141 (70.5)</td>
<td>46  (23.0)</td>
<td>13 (6.5)</td>
<td>(130)</td>
</tr>
<tr>
<td></td>
<td>202</td>
<td>143 (70.8)</td>
<td>46  (22.8)</td>
<td>13 (6.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Turkish</td>
<td>103</td>
<td>59 (57)</td>
<td>32  (31)</td>
<td>12 (12)</td>
<td>(129)</td>
</tr>
<tr>
<td></td>
<td>Korean</td>
<td>290</td>
<td>229 (79)</td>
<td>56  (19)</td>
<td>5   (2)</td>
<td>(133)</td>
</tr>
<tr>
<td></td>
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<td>162 (36)</td>
<td>200 (44)</td>
<td>91 (20)</td>
<td>(124)</td>
</tr>
<tr>
<td></td>
<td>ND</td>
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<td>193 (29.5)</td>
<td>316 (48.2)</td>
<td>146 (22.3)</td>
<td>(51)</td>
</tr>
<tr>
<td></td>
<td>Several Kadlubar</td>
<td>137</td>
<td>36 (26.27)</td>
<td>64  (46.7)</td>
<td>37 (27.0)</td>
<td></td>
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<tr>
<td>N453S</td>
<td>Caucasian</td>
<td>164</td>
<td>111 (68)</td>
<td>49  (30)</td>
<td>4   (2)</td>
<td>(106)</td>
</tr>
<tr>
<td></td>
<td>1,379</td>
<td>926 (67.2)</td>
<td>412 (30.0)</td>
<td>40 (2.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>African American</td>
<td>59</td>
<td>55 (93)</td>
<td>4   (7)</td>
<td>0   (0)</td>
<td>(106)</td>
</tr>
<tr>
<td></td>
<td>Japanese</td>
<td>200</td>
<td>200 (100)</td>
<td>0   (0)</td>
<td>0   (0)</td>
<td>(16)</td>
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<td></td>
<td>202</td>
<td>202 (100)</td>
<td>0   (0)</td>
<td>0   (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>666</td>
<td>424 (64.4)</td>
<td>206 (31.3)</td>
<td>28 (4.3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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-OHE2 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 432V/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/m2 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 (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-OHE2 levels compared with the surrounding normal myometrium, an effect that is abrogated by inhibition of CYP1B1 (139). Furthermore, 4-OHE2 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. A. Metabolic pathway of estrogen. CYP1B1 catalyzes the conversion of estradiol into 2-OHE$_2$ and 4-OHE$_2$. The metabolites of this reaction are both genotoxic and bind proteins within the cell. B. Metabolic pathway of testosterone. CYP1B1 catalyzes the conversion of testosterone into 6-hydroxytestosterone that undergoes subsequent breakdown. Given that a large proportion of testosterone is converted to estradiol, CYP1B1 may also be important in the toxic metabolism of testosterone metabolites in the tissues. CYP3A4/5, CYP2C9, and CYP2C19 are also involved in the breakdown of testosterone in the liver by the conversion of testosterone to form 2-oxo, 6-oxo, 11-oxo, 15-oxo, and 16-oxo-hydroxytestosterone (not included).
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, α-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-OHE2 (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 ERα and ERβ 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

**FIGURE 3** Continued. C. Phase I metabolism of tamoxifen by the cytochrome P450. 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 P450. 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.
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 Ps (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 Ps (Zelen); P = 0.18 and 0.67, respectively]. The common ORs [95% CI; 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 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 hCYP1B1 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 anticanter 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-hydroxy-flutamide by CYP1B1 was observed using the ethoxyresorufin de-ethyrase 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-OHE2. 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
further investigation into the effects of there is a strong association between the L432V polymorphism Interestingly, the common OR (95% CI) is estimated to be 0.43 the haplotype CGCCG [for consecutive SNPs CYP1B1 haplotype study was also conducted in the context of CYP1B1*3 allele 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 \(-1001\text{C/T}, -263\text{G/A}, -13\text{C/T}, +142\text{C/G (R48G)}, \) 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 reasons 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.

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**References**


