Cytochrome P450 IB1 (CYP1B1) Pharmacogenetics: Association of Polymorphisms with Functional Differences in Estrogen Hydroxylation Activity¹

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ABSTRACT

Activation of 17β-estradiol (E2) through the formation of catechol estrogen metabolites, 2-OH-E2 and 4-OH-E2, and the C-16α hydroxylation product, 16α-OH-E2, has been postulated to be a factor in mammmary carcinogenesis. Cytochrome P450 IB1 (CYP1B1) exceeds other P450 enzymes in both estrogen hydroxylation activity and expression level in breast tissue. To determine whether inherited variants of CYP1B1 differ from wild-type CYP1B1 in estrogen hydroxylase activity, we expressed recombinant wild-type and five polymorphic variants of CYP1B1: variant 1 (codon 48Arg→Gly), variant 2 (codon 119Ala→Ser), variant 3 (codon 432Val→Leu), variant 4 (codon 453Asn→Ser), and variant 5 (48Arg, 119Ser, 432Leu, 453Ser). The His-tagged proteins were purified by nickel-nitriol-triacetic acid (Ni-NTA) chromatography and analyzed by electrophoresis and spectrophotometry. We performed assays of E2 hydroxylation activity and quantitated production of 2-OH-E2, 4-OH-E2, and 16α-OH-E2 by gas chromatography/mass spectrometry. Wild-type CYP1B1 formed 4-OH-E2 as main product (Km = 40 ± 8 μM; kcat = 4.4 ± 0.4 min−1; kcat/Km = 110 mM−1 min−1), followed by 2-OH-E2 (Km = 34 ± 4 μM; kcat = 1.9 ± 0.1 min−1; kcat/Km = 55 mM−1 min−1) and 16α-OH-E2 (Km = 39 ± 5.7 mM; kcat = 0.3 ± 0.02 min−1; kcat/Km = 7.6 mM−1 min−1). The CYP1B1 variants also formed 4-OH-E2 as the main product but displayed 2.4- to 3.4-fold higher catalytic efficiencies kcat/Km than the wild-type enzyme, ranging from 270 mM−1 min−1 for variant 4, to 370 mM−1 min−1 for variant 2. The variant enzymes also exceeded wild-type CYP1B1 with respect to 2- and 16α-hydroxylation activity. Thus, inherited alterations in CYP1B1 estrogen hydroxylation activity may be associated with significant changes in estrogen metabolism and, thereby, may possibly explain interindividual differences in breast cancer risk associated with estrogen-mediated carcinogenicity.

INTRODUCTION

Metabolic activation of E2³ has been postulated to be a factor in mammmary carcinogenesis. E2 is metabolized via two major pathways: formation of catechol estrogens, the 2-OH and 4-OH derivatives; and C-16α hydroxylation. Two enzymes, CYP1A1 and CYP1B1, are responsible for the hydroxylation to the 2-OH and 4-OH catechol estrogens (i.e., 2-OH-E2 and 4-OH-E2). The 2-OH and 4-OH catechol estrogens are oxidized to semiquinones and quinones. The latter are reactive electrophilic metabolites and are capable of forming DNA adducts (1, 2). Further DNA damage results from quinone-semiquinone redox cycling, generated by enzymatic reduction of catechol estrogen quinones to semiquinones and subsequent auto-oxidation back to quinones (3–6). C-16α hydroxylation has also been suggested to be involved in breast carcinogenesis (7, 8).

Although other cytochrome P450 enzymes, such as CYP1A2 and CYP3A4, are involved in hepatic and extrahepatic estrogen hydroxylation, CYP1A1 and CYP1B1 display the highest levels of expression in breast tissue (9, 10). In turn, CYP1B1 exceeds CYP1A1 in its catalytic efficiency as an E2 hydroxylase and differs from CYP1A1 in its principal site of catalysis (11–13). CYP1B1 has its primary activity at the C-4 position of E2, whereas CYP1A1 has its primary activity at the C-2 position in preference to 4-hydroxylation. The 4-hydroxylation activity of CYP1B1 has received particular attention because of the fact that the 2-OH and 4-OH catechol estrogens differ in carcinogenicity. Treatment with 4-OH-E2, but not 2-OH-E2, induced renal cancer in Syrian hamster (14, 15). Analysis of renal DNA demonstrated that 4-OH-E2 significantly increased 8-hydroxydeoxyguanosine levels, whereas 2-OH-E2 did not cause oxidative DNA damage (16). Similarly, 4-OH-E2 induced DNA single-strand breaks whereas 2-OH-E2 had a negligible effect (17). Comparison of the corresponding catechol estrogen quinones showed that E2→3,4-quinone produced two to three orders of magnitude higher levels of depurinating adducts than E2→2,3-quinone (18). In addition to the induction of renal cancer in the hamster model, 4-OH-E2 is capable of inducing uterine adenocarcinoma, a hormonally related cancer, in mice. Administration of E2, 2-OH-E2, and 4-OH-E2 induced endometrial carcinomas in 7, 12, and 66%, respectively, of treated CD-1 mice (19). Finally, examination of microsomal E2 hydroxylation in human breast cancer showed significantly higher 4-OH-E2/2,3-OH-E2 ratios in tumor tissue than in adjacent normal breast tissue (20). All of these findings support a causative role of 4-OH catechol estrogens in carcinogenesis and implicate CYP1B1 as a key player in the process.

Mutations and polymorphisms have both been identified in the CYP1B1 gene. Primary congenital glaucoma, a rare autosomal recessive eye disorder, has been linked to homozygous frameshift and missense mutations in affected Turkish and Saudi Arabian families (21–23). Six polymorphisms of the CYP1B1 gene have been described in the Anglo-American population, of which four result in amino acid substitutions (Table 1; Refs. 23, 24). We described two of these amino acid substitutions in exon 3, which encodes the heme-binding domain: codon 432Val→Leu and codon 453Asn→Ser (24). Stoilov et al. (23) described the other two amino acid substitutions in codons 48Arg→Gly and 119Ala→Ser in exon 2. Polymorphisms are inherited and, therefore, dictate exposure levels to metabolites for life. Thus, inherited alterations in the activity of CYP1B1 hold the potential to define differences in estrogen metabolism and, thereby, possibly explain interindividual differences in breast cancer risk associated with estrogen-mediated carcinogenesis. However, to support this hypothesis, formal proof is needed that these inherited enzyme variants are indeed associated with significant changes in estrogen metabolism. In the present study, we determined whether the polymorphic variants of CYP1B1 differ from wild-type CYP1B1 in 2-, 3-, and 16α-estradiol hydroxylation activities.

MATERIALS AND METHODS

Construction of CYP1B1 Bacterial Expression Plasmid. To facilitate expression and purification of CYP1B1, the hydrophobic NH2-terminal 25 amino acids were replaced by six histidine residues. This was accomplished by designing primers to contain BamHI and KpnI sites, respectively, at their 5’ ends to allow amplification of wild-type and polymorphic CYP1B1 cDNA. The amplification reaction was carried out with 1 μg of cDNA in a 100-μl volume containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 5 μl DMSO, 200 μM each of the four deoxyribonucleotides, native Pfu DNA

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³ The abbreviations used are: E2, 17β-estradiol; CYP1B1, cytochrome P450 401B1; CYP1A1, cytochrome P450 1A1; TMS, trimethylsilyl; Ni-NTA, nickel-nitriol-triacetic acid; DMSO, dimethylsulfoxide.
polymerase (2.5 units; Stratagene, La Jolla, CA) and each oligonucleotide at 150 ng/mL. Amplification conditions consisted of a denaturing step at 95°C, annealing at 62°C, and extension at 72°C for a total of 24 cycles. Each amplified cDNA was purified using the QIAquick PCR purification kit (Qiagen; Valencia, CA), digested with BamHI and KpnI, and purified by centrifugation through a Chromaspin-100 column (Clontech, Palo Alto, CA). Each 1.6-kb PCR fragment was then ligated into the similarly digested vector pQE-30 (Qiagen) that encodes the NH2-terminal hexahistidine tag. Each ligated vector/insert was transformed into XL1-Blue cells for amplification. The amplified plasmid DNA was then transformed into DH5αF’Iq using the methods described by the manufacturer. Colonies harboring the correct sequence (as judged by restriction digest and DNA sequencing) were picked and used to express the respective CYP1B1 protein.

Expression and Purification of Recombinant CYP1B1. Recombinant wild-type and variant CYP1B1 proteins were expressed in Escherichia coli. Strain DH5αF’Iq cells were grown for 12 h at 37°C in 50 ml of modified terrific broth medium containing 100 μg of ampicillin/ml, 25 μg of kanamycin/ml, 1 mM thiamine, and 10 mM glucose. The cells were then grown at 33°C in the same medium with added trace elements as described previously (25) until the A600 nm was between 0.6 and 0.9. Mild induction with 8 mM lactose yielded optimal enzyme production if 0.5 mM β-aminolevulinic acid was added and cells were grown at 23°C for 40 h while shaking at 150 rpm. After 40 h, cells were harvested by centrifugation at 6,500 × g for 10 min, and the P450 content in the bacterial cell lysate was determined by Fe3+↔Fe2+ difference spectra. Spheroplasts were prepared with the use of lysozyme and disrupted by sonication. The pellet obtained after centrifugation at 10,000 × g for 20 min was discarded, and the microsomal membranes in the supernatant was used as a source for purification. The membranes were pelleted by overnight centrifugation at 110,000 × g, and the resultant supernatant was discarded because it generally contained <3% of the P450 content. The red 110 K pellet was resuspended in 200 ml of solubilization buffer [100 mM NaPO4 (pH 8.0), 0.4 mM NaCl, 40% glycerol (v/v), 10 mM β-mercaptoethanol, 10 mM aprotinin, 0.5% sodium cholate (w/v), and 1.0% Triton N-101 (w/v)], and the suspension was stirred overnight. Centrifugation at 110,000 × g for 90 min yielded a clear pellet, which was discarded, and a supernatant that contained most of the P450. The supernatant was applied to a preequilibrated Ni-NTA column (1 ml of resin per 50 nmol of enzyme). The column was washed with at least 50 column volumes of buffer [100 mM NaPO4 (pH 8.0), 0.4 mM NaCl, 40% glycerol (v/v), 10 mM β-mercaptoethanol, 0.25% sodium cholate (w/v), and 10 mM imidazole], followed by a second wash with the same buffer containing 40 mM imidazole to remove unbound proteins and Triton N-101. The His-tagged protein was eluted with two column volumes of buffer [100 mM NaPO4 (pH 8.0), 0.4 mM NaCl, 40% glycerol (v/v), 10 mM β-mercaptoethanol, 0.25% sodium cholate (w/v), and 400 mM imidazole], and the eluate dialyzed against buffer containing 0.1 M NaPO4 (pH 8.0), 0.4 M NaCl, 40% glycerol (v/v), and 0.1 mM DTT. The purity of the protein was assessed by SDS-PAGE and silver staining and by Western immunblots using both anti-(His) and anti-CYP1B1 antibodies.

Site-Directed Mutagenesis. Part of our initial studies of the CYP1B1 gene, including DNA sequence analysis, was carried out with human breast cancer cell lines. In analyzing the CYP1B1 gene in cell lines, we determined that BT-20 cells contain the CYP1B1 sequence designated as wild type. Accordingly, CYP1B1 cDNA from BT-20 cells served as the source for site-directed mutagenesis and the corresponding pQE-30 wild-type CYP1B1 plasmid was used as template to generate variant CYP1B1 cDNA encoding the substitutions in codon 48, 119, 432, and 453 (Table 1). Complementary 25 base oligonucleotide primers were synthesized to contain the selected mutated nucleotides in the center and were purified by PAGE. We used the primers in the QuiChange site-directed mutagenesis method as specified by the manufacturer (Stratagene). After 12 PCR cycles with TurboPhu DNA polymerase, the reaction was digested with DpnI and transformed into XL1-Blue cells. Successful mutagenesis was verified by nucleotide sequence analysis. Transformation into DH5αF’Iq cells, expression, and purification of variant CYP1B1 were performed as described above.

Spectrophotometric Analysis. All of the spectra were recorded using an Aninco DW2a/601is instrument (On-Line Instrument Systems, Bogart, GA). Wavelength maxima were determined using the peak finder or second derivative software. The high-spin content was estimated from the second derivative spectrum of the ferric enzyme as described previously (26). P450 and cytochrome P420 concentrations were determined as described previously (27).

Assay of CYP1B1 E2 Hydroxylation Activity. Purified CYP1B1 (200 pmol) was reconstituted with a 2-fold molar amount of recombinant rat NADPH-P450 reductase (400 pmol), purified as described previously (28), and with 60 μg of 1α,25-dihydroxy-vitamin D3-phosphocholine in the presence of sodium cholate (0.005%, w/v; Ref. 29) in 0.4 ml of 100 mM potassium phosphate buffer (pH 7.4) containing varying concentrations of E2 (2, 3, 6, 9, 12, 15, 20, 40, 60, 80, and 100 μM) and 1 mM ascorbate. A NADPH-generating system consisting of 5 mM glucose 6-phosphate and 0.5 units of glucose-6-phosphate dehydrogenase/ml was added and reactions were initiated by adding NADP+ to a final concentration of 0.5 mM. Reactions proceeded for 10 min at 37°C with gentle shaking and then were terminated by the addition of 2 ml of CH2Cl2.

Extraction and Gas Chromatography/Mass Spectrometry Analysis of E2 and Metabolites. A deuterated internal standard (100 μl of 8 ng/tube E2-d2, 4, 16, 16-d4, in methanol; CDN Isotopes, Pointe-Claire, Quebec, Canada) was added, and all of the steroids were extracted into CH2Cl2 by vortex mixing for 30 s. The CH2Cl2 fraction (1.5 ml) was evaporated to dryness under air and volatile TMS derivatives were prepared by heating the residue with 100 μl of 50% N-O-bistrimethylsilyl)trifluoroacetamide/1% trimethyl chlorosilane in acetonitrile at 56°C for 30 min. The TMS derivatives of E2 and its metabolites were separated by gas chromatography (H-P 5890, Hewlett-Packard, Wilmington, DE) on a 5% phenyl methyl silicone stationary-phase fused silica capillary column (30 m × 0.2 mm × 0.5 μm film, HP-5; Hewlett-Packard). Helium carrier gas was used at a flow of 1 ml/min. The injector was operated at 250°C, with 2 μl injected in the splitless mode, with a purge (60 ml/min helium) time of 0.6 min. The oven temperature was held at 180°C for 0.5 min, then raised to 6°C/min to 250°C, at which it was held for 17 min, then raised to 300°C at 8°C/min to give a total run time of 35.42 min. This program permitted adequate separation of a wide range of estrogen metabolites. Retention times for the TMS derivatives were: E2 and E2-d2, 20.6 min; 2-OH-E2, 26.6 min; 4-OH-E2, 28.7 min; and 16α-OH-E2, 30.3 min. The mass spectrometer (H-P 5970) was operated in the electron impact selected ion monitoring mode from 18 to 34 min. Ions monitored were TMS-E2-d4, 420, 288, 330; TMS-E2, 416, 285, 326; TMS-2-OH-E2, 504, 373; TMS-4-OH-E2, 504, 373, 325; TMS-16α-OH-E2, 345, 311, 504. The instrument was calibrated by simultaneous preparation of an 11-point calibration over the range 0.05–10.5 nmol/tube of each compound. Sensitivity was determined to be between 0.02 and 0.04 nmol/tube (400–800 fmol on column) for the various compounds. Preparation of the TMS derivatives improved chromatography and sensitivity significantly. Derivatization was performed at 56°C because the use of a higher temperature resulted in the loss of some estrogen derivatives (particularly the 2-OH metabolite of estrone). Derivatization was demonstrated to be complete at 20 min as evidenced by the absence of detectable amounts of underivatized estrogens in the highest calibrator when the detector was operated in full scan mode. Absolute extraction efficiency for E2, 2-OH-E2, and 4-OH-E2 at 3.5 nmol/tube was 119, 96, and 107%, respectively, assessed by comparison with injections of spiked solvent samples onto the gas chromatograph. The internal standard added before extraction compensated for deviation from 100% recovery.

Statistical Analysis. Kinetic parameters (Km and kcat) were determined by nonlinear regression analysis using the computer program GraphPad PRISM (San Diego, CA).

RESULTS

Initial attempts to express CYP1B1 in E. coli using the pQE-30 vector yielded very low expression levels. Accordingly, we modified...
the expression conditions to achieve higher levels of recombinant protein (400-800 nmol/liter). The modifications included the use of DH5α F’Iq instead of strains recommended by the manufacturer (Qiagen) and the induction of protein expression with lactose instead of isopropyl-β-D-thiogalactopyranoside. The protein modification strategy (i.e., replacement of the NH₂-terminal hydrophobic segment) did not affect the intracellular localization of the recombinant protein in bacterial membranes. However, a much longer centrifugation period was required in the 110,000 × g sedimentation step to pellet the majority of the expressed protein. The presence of the NH₂-terminal hexahistidine allowed purification of the recombinant proteins with relatively high yields (results not presented). Purified wild-type and variant CYP1B1 were electrophoretically homogeneous as judged by SDS-PAGE and silver staining, which revealed a single band at Mᵣ 55,000 for all of the proteins (Fig. 1). Western immunoblots using both anti-(oligo)His and anti-CYP1B1 antibodies also yielded one major band at Mᵣ 55,000 (results not shown).

The reduced-CO difference spectrum of purified recombinant CYP1B1 had a λₘₐₓ at 450 nm and negligible amounts of cytochrome P420, the denatured form of the enzyme (Fig. 2). Examination of the absolute spectra of CYP1B1 revealed that the ferric protein was nearly all in the low-spin state. The low-spin character was further verified by examination of the second derivative spectrum (Fig. 2).

Wild-type and variant CYP1B1 catalyzed E2 hydroxylation at C-2, C-4, and C-16α. Sodium cholate (0.005% w/v) was included in the reconstitution mixtures as suggested by Shimada et al. (29). However, the exclusion of sodium cholate in separate experiments did not significantly affect the observed catalytic properties. The reaction kinetics were determined for each enzyme in duplicate at ten different concentrations of E2 (Fig. 3), and the resulting Kₘ and kₗₐₜ values are presented in Table 2. Wild-type CYP1B1 formed 4-OH-E2 as main product (Kₘ 40 ± 8 μM; kₗₐₜ 4.4 ± 0.4 min⁻¹; kₗₐₜ/Kₘ 110 mM⁻¹min⁻¹), followed by 2-OH-E2 (Kₘ 34 ± 4 μM; kₗₐₜ 1.9 ± 0.1 min⁻¹; kₗₐₜ/Kₘ 55 mM⁻¹min⁻¹) and 16α-OH-E2 (Kₘ 39.4 ± 5.7 μM; kₗₐₜ 0.30 ± 0.02 min⁻¹; kₗₐₜ/Kₘ 7.6 mM⁻¹min⁻¹). The CYP1B1 variants also formed 4-OH-E2 as main product but displayed 2.4- to 3.4-fold higher catalytic efficiencies kₗₐₜ/Kₘ than the wild-type enzyme, ranging from 270 mM⁻¹min⁻¹ for variant 4, to 370 mM⁻¹min⁻¹ for variant 2 (Table 2). The variant enzymes also exceeded wild-type CYP1B1 with respect to 2- and 16α-hydroxylation activity, although the differences were smaller (Table 2). Overall, the 4-hydroxylation activity of the various enzymes was 2- to 4-fold higher than the 2-hydroxylation activity and 15- to 45-fold higher than the 16α-hydroxylation activity.

**DISCUSSION**

Several points are noteworthy about the human CYP1B1 gene: (a) exon 1 is noncoding and no polymorphism has been identified in it; (b) the polymorphism in intron 1 is located 13 nucleotides upstream of the 5’-end of exon 2 and, therefore, unlikely to affect splicing of this exon; (c) the T→A polymorphism at nucleotide 1347 is silent, i.e., the amino acid sequence is not affected; (d) on the basis of the extensive DNA sequence analysis of the CYP1B1 gene in 100 individuals by
Stoilov et al. (23) and our own studies of the CYP1B1 gene (24), it seems unlikely that polymorphisms other than those in codons 48, 119, 432, and 453 are present in the coding region, at least in Caucasians; and (e) multiple sequence alignment for 22 different members of the cytochrome P450 superfamily has shown that the four CYP1B1 polymorphisms associated with amino acid substitutions, i.e., 48Arg→Gly, 119Ala→Ser, 432Val→Leu, and 453Asn→Ser, are near conserved regions (23). In consideration of these data, we focused our analysis on these four polymorphisms.

We modified the recombinant CYP1B1 proteins to allow efficient expression and purification from the bacterial host. The replacement of the NH₂ terminus, which serves to anchor the proteins in the microsomal membrane, by an oligo-His region has been used with several P450s and shown not to affect enzyme activity (29–31). The spectral properties of the expressed proteins were indicative of P450s with low-spin characteristics. Wild-type CYP1B1 has been expressed in yeast and bacteria. Microsomes from the transformed yeast catalyzed the 2- and 4-hydroxylation of E2 with kcat values of 0.27 and 1.4 min⁻¹, respectively (13). The corresponding turnover numbers for the bacterially expressed enzyme were 0.13 and 1.4 min⁻¹, respectively (29). The 2- and 4-hydroxylation activities observed for wild-type CYP1B1 in the present study were higher, possibly attributable to the use of purified enzymes in an optimized reconstitution system. However, all of the studies are in agreement that CYP1B1 preferentially catalyzes E2 4-hydroxylation, although small modifications have been made to facilitate expression. Comparison with other P450 enzymes has shown that the catalytic efficiency of CYP1B1 for 4-hydroxylation was greater than the catalytic efficiencies of CYP1A2 and CYP3A4 for 2-hydroxylation (20- and 18-fold higher, respectively; Refs. 32, 33), which indicates that the E2 4-hydroxylation activity of CYP1B1 has the highest catalytic efficiency of all of the reported E2 hydroxylases. Thus, CYP1B1 seems to be the main cytochrome P450 responsible for the 4-hydroxylation of E2. Previous studies of CYP1B1 have noted trace 16α-hydroxylations (29). Quantification of the 16α-hydroxylation activity of wild-type CYP1B1 revealed a 15-fold lower level than the 4-hydroxylation activity (Table 2).

The variant enzymes exceeded wild-type CYP1B1 with respect to 2-, 4-, and 16α-hydroxylation activity. The largest difference was obtained for variant 2, which displayed 2.3- and 3.4-fold higher 2- and 4-hydroxylation activities, respectively, than the wild-type enzyme. The 4-OH-E2:2-OH-E2 rate ratios of the variant enzymes ranged from 3.0 to 3.8 compared with 2.0 for wild-type CYP1B1. Thus, the variant forms of CYP1B1 may contribute to higher tissue levels of 4-OH-E2. Confirmation of our findings is provided by a recent publication by Shimada et al. (34), who examined the functional effect of two of the four CYP1B1 polymorphisms (codons 119 and 432) on estrogen metabolism. Shimada et al. expressed the recombinant proteins in a bicistronic system linking the CYP1B1 and NADPH-P450 reductase cDNAs and used bacterial membranes rather than purified proteins for analysis. The kcat and Km values were at least 3- to 4-fold lower than those presented in this study. The low kcat values may be attributable to poor coupling of CYP1B1 to NADPH-P450 reductase resulting either from the presence of bacterially derived membrane lipids or insufficient saturation with expressed reductase. The underestimation of the Km values is apparent from the data presented in Shimada et al. The reported Km values ranged between 2.5 and 5.3 μM, whereas the lowest concentration used in calculating these values was 20 μM. The high sensitivity of our gas chromatography/mass spectrometry detection methods allowed the acquisition of data points surrounding the Km values and, therefore, should be a more accurate representation of such catalytic parameters. Nevertheless, qualitatively, both of the studies are in agreement that the variants differ in activity and that Arg48, Ser119, Val432, and Asn453 (our variant 2) seems to have the highest catalytic efficiency for the 4-hydroxylation of estradiol.

Table 2. Estradiol hydroxylation activities of CYP1B1 wild type and variants

<table>
<thead>
<tr>
<th>CYP1B1</th>
<th>Km (μM)</th>
<th>kcat (min⁻¹)</th>
<th>Kcat/Km (min⁻¹)</th>
<th>Kcat/Km (min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>40 ± 8</td>
<td>4.4 ± 0.4</td>
<td>110 ± 24</td>
<td>2.0 ± 0.5</td>
</tr>
<tr>
<td>Variant 1</td>
<td>15 ± 1</td>
<td>3.0 ± 0.6</td>
<td>320 ± 35</td>
<td>4.0 ± 0.7</td>
</tr>
<tr>
<td>Variant 2</td>
<td>10 ± 1</td>
<td>3.0 ± 0.5</td>
<td>370 ± 38</td>
<td>3.7 ± 0.4</td>
</tr>
<tr>
<td>Variant 3</td>
<td>6 ± 1</td>
<td>0.34 ± 0.2</td>
<td>46 ± 10</td>
<td>0.40 ± 0.3</td>
</tr>
<tr>
<td>Variant 4</td>
<td>5 ± 1</td>
<td>0.10 ± 0.2</td>
<td>3.8 ± 0.8</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>Variant 5</td>
<td>2 ± 1</td>
<td>0.02 ± 0.02</td>
<td>7.6 ± 1.3</td>
<td>15 ± 2</td>
</tr>
</tbody>
</table>

* Data represent means ± SEs of duplicate assays. Hydroxylation reactions were conducted as described in “Materials and Methods.”
oxidative damage that may arise on redox cycling of catechol estrogens. (40). The ubiquitous catechol-O-methyltransferase (COMT) inactivates 2-OH and 4-OH catechol estrogens by O-methylation (41). Glutathione S-transferases (GSTs) inactivate catechol estrogen quinones by conjugation with glutathione (42). Genetic variants of each of these enzymes involved in catechol estrogen metabolism have been identified, some with proven or suspected change in function. Inherited alterations in the activity of any of these enzymes hold the potential to define differences in catechol estrogen metabolism and, thereby, explain differences in breast cancer risk associated with estrogen carcinogenesis. However, it is evident that no single genotype can be linked to all breast cancers. This is not surprising given the fact that the enzymes involved in catechol estrogen metabolism also participate in environmental carcinoma metabolism (43).

In conclusion, wild-type and variant CYP1B1 show significant differences in estrogen hydroxylation activities, which may result in different concentrations of 2-OH-E2, 4-OH-E2, and 16α-OH-E2. Because the CYP1B1 polymorphisms are inherited, they will dictate exposure levels to these E2 metabolites for life. Given the carcinogenic and estrogenic potential of 4-OH-E2 and possibly 16α-OH-E2, one may speculate that inheritance of certain CYP1B1 variants may contribute to interindividual differences in breast cancer risk associated with estrogen-mediated carcinogenesis.

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