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Cloning of *CYP2J2* Gene and Identification of Functional Polymorphisms

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ABSTRACT

CYP2J2 is abundant in cardiovascular tissue and active in the metabolism of arachidonic acid to eicosanoids that possess potent anti-inflammatory, vasodilatory, and fibrinolytic properties. We cloned and sequenced the entire *CYP2J2* gene (~40.3 kb), which contains nine exons and eight introns. We then sequenced the *CYP2J2* exons and intron-exon boundaries in 72 healthy persons representing African, Asian, and European/white populations as part of the National Institutes of Health/National Institute of Environmental Health Sciences Environmental Genome Single Nucleotide Polymorphism Program. A variety of polymorphisms were found, four of which resulted in coding changes (Arg158Cys, Ile192Asn, Asp342Asn, and Asn404Tyr). A fifth variant (Thr143Ala) was identified by screening a human heart cDNA library. All five variant cDNAs of CYP2J2 were generated by site-directed mutagenesis and expressed in Sf9 insect cells by using a baculovirus system. The recombinant wild-type and variant CYP2J2 proteins immunoreacted with peptide-based antibodies to CYP2J2 and displayed typical cytochrome P450 (P450) CO-difference spectra; however, the Asn404Tyr and Ile192Asn variants also had prominent spectral peaks at 420 nm. The ability of these variants to metabolize arachidonic acid and linoleic acid was compared with that of wild-type CYP2J2. Three variants (Asn404Tyr, Arg158Cys, and Thr143Ala) showed significantly reduced metabolism of both arachidonic acid and linoleic acid. The Ile192Asn variant showed significantly reduced activity toward arachidonic acid only. The Asp342Asn variant showed similar metabolism to wild-type CYP2J2 for both endogenous substrates. Based on these data, we conclude that allelic variants of the human *CYP2J2* gene exist and that some of these variants result in a P450 protein that has reduced catalytic function. Insofar as CYP2J2 products have effects in the cardiovascular system, we speculate that these variants may be functionally relevant.

Human cytochromes P450 participate in the metabolism of endogenous lipids and in the detoxification of a variety of xenobiotics (Nelson et al., 1996). There are 17 recognized P450 gene families in humans and 42 subfamilies with more than 55 unique P450 genes already identified in the human genome (see http://drnelson.utmem.edu/CytochromeP450.html). The CYP2 family contains about one-third of all human P450s, making it the largest human P450 family. The CYP2J subfamily, which was originally described by Kikuta and coworkers in 1991 (Kikuta et al., 1991), contains a single human P450 gene, *CYP2J2*, which has been mapped to the short arm of chromosome 1 (Wu et al., 1996; Ma et al., 1998). Human CYP2J2 is expressed at high levels in the heart, predominantly in cardiac myocytes and endothelial cells lining small and large coronary arteries (Wu et al., 1996, 1997; Node et al., 1999). CYP2J2 is also expressed in other tissues, including the liver, kidney, lung, pancreas, and gastrointestinal tract (Wu et al., 1996; Zeldin et al., 1996a, 1997a,b). CYP2J2 has been shown to metabolize both AA and LA, as well as several xenobiotics, including diclofenac and bufuralol (Wu et al., 1996; Scarborough et al., 1999; Moran et al., 2000).

[1] The new sequence reported in this article was submitted to GenBank and assigned the accession number AF272142. The allelic variants identified in this article have been submitted to the Human Cytochrome P450 Allele Nomenclature Committee and given the following names in accordance with their published recommendations: CYP2J2*1 (wild type), CYP2J2*2 (Thr143Ala), CYP2J2*3 (Arg158Cys), CYP2J2*4 (Ile192Asn), CYP2J2*5 (Asp342Asn), CYP2J2*6 (Asn404Tyr), and CYP2J2*7 (G-50T).

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ABBREVIATIONS: P450, cytochrome P450; AA, arachidonic acid; LA, linoleic acid; EET, epoxyeicosatrienoic acid; HETE, hydroxyeicosatetraenoic acid; EOA, epoxyoctadecenoic acid; SNP, single nucleotide polymorphism; BAC, bacterial artificial chromosome; RACE, rapid amplification of cDNA ends; PCR, polymerase chain reaction; UTR, untranslated region; Sf, *Spodoptera frugiperda*; HPLC, high-performance liquid chromatography; bp, base pair(s); SRS, substrate recognition site; CYPOR, NADPH-cytochrome P450 oxidoreductase.

The major products generated by CYP2J2-catalyzed AA metabolism are *cis*-epoxyeicosatrienoic acids (5,6-, 8,9-, 11,12-, and 14,15-EETs) and 19-HETE (Wu et al., 1996). The EETs are further metabolized by soluble epoxide hydrolase to the corresponding *vic*-dihydroxyeicosatrienoic acids (Chacos et al., 1983; Zeldin et al., 1993). EETs have been shown to have numerous biological functions. In the heart, EETs dilate coronary arteries by activating Ca^{2+}-sensitive K^+ channels (Oltman et al., 1998; Campbell and Harder, 1999), improve recovery of heart contractile function after prolonged global ischemia (Wu et al., 1997), and affect the activity of cardiac L-type Ca^{2+} channels (Xiao et al., 1998; Chen et al., 1999). EETs are leading candidates for endothelium-derived hyperpolarizing factor, the nitric oxide/prostacyclin-independent component of endothelium-dependent vasorelaxation (Campbell et al., 1996; Fisslthaler et al., 1999). In endothelial cells, CYP2J2-derived EETs have been shown to inhibit cell adhesion molecule expression and leukocyte adhesion to vascular wall, protect against hypoxia-reoxygenation injury, and induce tissue-plasminogen activator gene transcription (Node et al., 1999, 2001; Yang et al., 2001). EETs have also been extensively studied in the kidney where they have been shown to affect renal vascular tone; inhibit Na^+ reabsorption and K^+ secretion; affect Na^+-K^+-ATPase activity; and modulate the actions of several renal hormones, including angiotensin II, arginine vasopressin, and renin (McGiff, 1991; Capdevila et al., 2000). The potent effects of EETs on renal vascular tone and fluid/electrolyte transport suggest that they may play a role in the pathogenesis of human hypertension (McGiff, 1991; Capdevila et al., 2000).

CYP2J2 is also active in the metabolism of LA to *cis*-epoxyoctadecenoic acids (9,10- and 12,13-EOAs), which affect cardiac function. EOAs have been shown to be increased in burn patients and persons with adult respiratory distress syndrome (Ozawa et al., 1988; Kosaka et al., 1994). Patients with extensive burns have 100 times higher levels of these compounds in their plasma than normal patients (Kosaka et al., 1994). These epoxides are known to cause severe lung edema and cardiac arrest within 1 h of intravenous injection in dogs (Fukushima et al., 1988). Moreover, it is now known that the EOAs are bioactivated by soluble epoxide hydrolase to their corresponding diols, which are cytotoxic (Moghaddam et al., 1997).

An SNP is a site of a single base-pair variation in DNA. SNPs are the most abundant form of human genetic variation and their detection promises to provide insights into common disease susceptibility and pharmacogenetic traits. There are currently more than 1.6 million SNPs deposited into public databases, and it is predicted that there are as many as 10 million SNPs in the human population (Lai, 2001). Genetic factors are important in nearly every human disease, resulting in susceptibility or resistance, and can influence a person's response to environmental factors. We hypothesize that variations in the coding and/or regulatory regions of the human *CYP2J2* gene will lead to changes in CYP2J2 expression and/or enzymatic activity and result in altered CYP2J2-dependent metabolism of AA and LA. This in turn might lead to effects on heart and kidney function and ultimately to altered susceptibility to human disease. To begin to address these hypotheses, we first cloned and sequenced the entire *CYP2J2* gene and characterized its intron/exon organization. We then sequenced *CYP2J2* exons and intron-exon boundaries in genomic DNA prepared from cells isolated from a group of 72 healthy persons to identify variations in the *CYP2J2* gene sequence. Finally, we used site-directed mutagenesis to generate the variant cDNAs, expressed the variant recombinant proteins by using baculovirus, and examined their functional relevance with respect to the metabolism of the endogenous substrates AA and LA.

Experimental Procedures

Materials. Restriction enzymes and T4 polynucleotide kinase were purchased from New England Biolabs (Beverly, MA). [α-^{32}P]dCTP, [1-^{14}C]LA, and [1-^{14}C]AA were purchased from PerkinElmer Life Sciences (Boston, MA). Polymerase chain reaction reagents, including AmpliTaq DNA polymerase were purchased from Applied Biosystems (Foster City, CA). All other chemicals and reagents were purchased from Sigma-Aldrich Chemical (St. Louis, MO) unless otherwise stated.

CYP2J2 Gene Cloning and Sequence Analysis. A human lymphocyte genomic bacterial artificial chromosome (BAC) library (Genome Systems, St. Louis, MO) was screened with the full-length CYP2J2 cDNA (1.85 kb). Three positive clones were identified (clones 16121, 16122, and 16123), propagated in DH19B *Escherichia coli* host by using the pBeloBAC11 single copy cloning vector, and their BAC DNA purified using KB-100 Magnum columns (Genome Systems) according to the manufacturer's instructions. Each clone was partially sequenced using CYP2J2-specific primers. One of these clones (clone 16122), which contained the entire *CYP2J2* gene, was digested with either *Hin*dIII or *Pst*I, shotgun cloned into pBluescript SK(+), and overlapping *CYP2J2* gene fragments were fully sequenced using a total of 136 oligonucleotide primers that spanned the length of the entire gene. Sequencing was performed using the dRhodamine Terminator Cycle Sequencing kit (Applied Biosystems) on an ABI model 377 Stretch automated DNA sequencer. Fragments were assembled using GCG Fragment Assembly System software (Genetics Computer Group, Inc., Madison, WI).

Southern Blots. Southern blot analysis of human genomic DNA was performed using a modification of the procedure described by Sambrook et al. (1989). Briefly, genomic DNAs were digested with either *Eco*RI, *Pst*I, *Hin*dIII, or *Bam*HI; electrophoresed on 0.8% agarose gels; transferred to HybondN nylon membranes (Amersham Biosciences, Inc., Piscataway, NJ); and hybridized with the radiolabeled 1.85-kb CYP2J2 cDNA probe at 68°C in QuickHyb solution (Stratagene, La Jolla, CA). The CYP2J2 cDNA was radiolabeled with [α-^{32}P]dCTP by using the Megaprime DNA labeling system (Amersham Biosciences, Inc.) and purified using G-50 Sephadex columns.

5′ Rapid Amplification of cDNA Ends (5′ RACE). The transcriptional start site of the *CYP2J2* gene was determined using the 5′ RACE kit from Invitrogen (Carlsbad, CA). Total RNA was isolated from human heart tissue by using an RNeasy kit (QIAGEN, Valencia, CA) and first strand cDNA synthesis was specifically primed by the oligonucleotide CypRaceP1 (5′-GCTCCTTCCATGCCTGGCCACTTGAC-3′) complementary to nucleotides 389 to 414 of the CYP2J2 cDNA (GenBank U37143). The following conditions were used for first strand synthesis: 2.5 pmol of primer CypRaceP1; 2 μg of total RNA; 20 mM Tris-HCl, pH 8.4, 50 mM KCl, 2.5 mM $MgCl_2$, 400 μM concentrations of each dNTP, and 200 units of SuperScript II reverse transcriptase in a final volume of 15.5 μl of diethyl pyrocarbonate-water. After denaturing at 70°C for 10 min, reverse transcription was performed for 30 min at 42°C. The reaction was terminated by incubation at 70°C for 15 min and the RNA template was removed by incubation with RNase at 37°C for 30 min. The first strand cDNA was purified using a GLASSMAX DNA isolation spin cartridge according to the manufacturer's protocol (Invitrogen). A dC-tail was added to the 3′ end of the first strand cDNA by incubating it at 37°C for 10 min in the following solution: 10 mM Tris-HCl, pH 8.4, 25 mM KCl, 1.5 mM $MgCl_2$, 200 μM dCTP, and 1 μl of TdT. PCR was done using the dG-anchor primer from the 5′ RACE kit and

the following internal CYP2J2-specific primer CypRaceP2 (5′-GCAAGCCAGTAATAAGAAGTGCAG-3′) complementary to nucleotides 265 to 288 of the CYP2J2 cDNA. The final composition of the PCR reaction buffer was as follows: 20 mM Tris-HCl, pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$, 400 nM each primer, 200 μM each dNTP, tailed cDNA, and 2.5 units of AmpliTaq gold DNA polymerase (Applied Biosystems). The cycling protocol was as follows: 94°C for 1 min (1 cycle); 94°C for 1 min, 55°C for 45 s, and 72°C for 45 s (42 cycles); and 72°C for 5 min (1 cycle). PCR products were separated by agarose gel electrophoresis. DNA bands were excised from the gel, purified using a QIAQuick PCR purification kit (QIAGEN), and sequenced directly using the internal primer CypRaceP3 (5′-GTGCGACTGCTCGAAGTCCACA-3′).

DNA Samples. Genomic DNA was prepared from lymphoblast cells isolated from 72 different humans. These samples were carefully selected from the Human Genetic Cell Repository sponsored by the National Institutes of Health housed at the Coriell Institute (Camden, NJ) to represent all three major racial/ethnic groups (Africans, Asians, and Europeans/whites). Allele frequency data derived from these 72 samples for *GSTM1*, *GSTP1*, *NAT2*, and *CYP2E1* genes have been shown to be consistent with published data from other large sample sets (Fritsche et al., 2000). The Human Genetic Cell Repository maintains and distributes cells and DNA derived from those cells to research scientists around the world for the purpose of gene identification and SNP discovery. These highly characterized, viable, and contaminant-free cell cultures and the high-quality, well characterized DNA samples derived from these cultures have been subjected to rigorous quality control (for details, see http://locus.umdnj.edu/nigms). The same 72 samples used in our analysis are also being used in the National Institutes of Health/National Institute of Environmental Health Sciences Environmental Genome SNP Program, a large effort that is examining polymorphisms in selected environmental response genes (for details and a complete listing of National Institutes of Health/National Institute of Environmental Health Sciences Environmental Genome SNP Program genes, see http://manuel.niehs.nih.gov/egsnp/home.htm). Moreover, other large international SNP discovery efforts (e.g., the National Center for Biotechnology Information SNP Consortium) are also using lymphoblast cells from the Coriell Human Genetic Cell Repository for their work. The healthy persons represented in our sample were of the following ancestries: 24 Africans (16 African-Americans, eight African pygmies), 24 Asians (five Indo-Pakistani, five native Taiwanese, five mainland Chinese, five Cambodian, three Japanese, three Melanesian), and 24 European/white (nine from Utah, five Druze-Lebanon, five Adygei-Eastern Europe, five Russian). Because the samples were preexisting and anonymous, the protocol received an exemption from the National Institutes of Health/National Institute of Environmental Health Sciences Institutional Review Board.

Sequencing to Identify CYP2J2 Polymorphisms. Sequence analysis was performed at the Lawrence Livermore National Laboratory (Livermore, CA). Separate PCR reactions were performed on each DNA sample to amplify the nine exons of the *CYP2J2* gene, the proximal promoter region, and the 3′-UTR. Specific primer sets were designed based on the nucleotide sequence of the *CYP2J2* gene (GenBank AF272142) by using Oligo Primer Analysis software (National Biosciences, Inc., Plymouth, MN). These intron-based primers correspond to regions located approximately 75 nucleotides from the CYP2J2 intron-exon boundaries and also contain 5′ sequences that correspond to universal M13 forward and M13 reverse primers (Table 1). Each amplification reaction was carried out in a reaction volume of 50 μl in the presence of 50 ng of genomic DNA, 0.5 μM each primer, 0.2 μM dNTPs, 10× *Taq* polymerase buffer, 0.5 μl of *Taq* polymerase antibody, and 2.5 units of AmpliTaq DNA polymerase. Reactions to amplify exons 1, 5, and 6 also included 5% dimethyl sulfoxide. The following PCR conditions were used to amplify the *CYP2J2* gene fragments: 9 min at 94°C (1 cycle); 30 s at 94°C, 45 s at 63°C, and 1 min at 72°C (35 cycles); and 7 min at 72°C (1 cycle).

Dye primer cycle sequencing reactions were performed according to the manufacturer's instructions for the DYEnamic direct cycle sequencing kit and loaded on an ABI model 377 Stretch DNA sequencer. The initial data analysis was performed with the ABI Prism DNA sequence analysis software, version 2.1.2. Chromatograms were imported into a SUN Microsystems UNIX workstation (Sun Microsystems Inc., Mountain View, CA) and reanalyzed with Phred, version 0.961028, assembled with Phrap, version 0.960213, and the resultant data viewed with Consed, version 4.1. Information on Phred, Phrap, and Consed may be obtained at http://www.genome.washington.edu. "PolyPhred", version 2.1, a software package that uses the output from Phred, Phrap, and Consed, was used to identify SNPs in heterozygote subjects (Nickerson et al., 1997).

Screening of a Human Heart cDNA Library. A human heart λgt10 cDNA library (Stratagene) was screened with the radiolabeled 1.85-kb CYP2J2 cDNA probe by using methods described previously (Wu et al., 1996). Seven duplicate positive clones were identified, plaque-purified, rescued into pBluescript SK(+), replicated in DH5α-competent *E. coli*, and their inserts fully sequenced.

Site-Directed Mutagenesis. The CYP2J2 variants were generated by in vitro mutagenesis with the Chameleon double-stranded site-directed mutagenesis kit (Stratagene) and the plasmid CYP2J2/pBluescript (clone SW2-14) containing the full-length CYP2J2 cDNA (Wu et al., 1996). The primers used to introduce amino acid substitutions are listed in Table 2. The entire coding region, including the mutated sites, was verified by complete sequencing with the Dye Terminator Cycle Sequencing kit (PerkinElmer) by using GCG SeqWeb software to align sequences.

Expression of Recombinant Wild-Type and Variant CYP2J2 Proteins. Coexpression of CYP2J2 (wild type and variants) and CYPOR in Sf9 insect cells was accomplished with the pAcUw51-CYPOR shuttle vector (provided by Dr. Cosette Serabjit-Singh, GlaxoSmithKline, Research Triangle Park, NC) and the BaculoGold baculovirus expression system (BD PharMingen, San Diego, CA) by using methods similar to those described previously (Lee et al., 1995; Wu et al., 1997; Ma et al., 1999; Qu et al., 2001). Briefly, the CYP2J2 wild-type and variant cDNAs were directionally subcloned into the

TABLE 1
Primers used for amplification of CYP2J2 exons
Sequencing primers are shown in uppercase.

Exon	Size	Forward Primer Sequence	Reverse Primer Sequence
	bp		
1	637	5′-GTTTTCCCAGTCACGACGcaggcacagttccaatcatag-3′	5′-AGGAAACAGCTATGACCATcacctcttcctgcccttcatc-3′
2	464	5′-GTTTTCCCAGTCACGACGacacgcactcctctcaatatgat-3′	5′-AGGAAACAGCTATGACCATcttctcagggagtgacttcaatc-3′
3	552	5′-GTTTTCCCAGTCACGACGtcaagtgccttaatgatcttc-3′	5′-AGGAAACAGCTATGACCATccttacactgcatctgacata-3′
4	335	5′-GTTTTCCCAGTCACGACGtggccagctcctcatatatgg-3′	5′-AGGAAACAGCTATGACCATccatctgtggctgacatcggc-3′
5	430	5′-GTTTTCCCAGTCACGACGgggtcaaggataaccaaggtt-3′	5′-AGGAAACAGCTATGACCATttaggcaccaagtttgtgatc-3′
6	389	5′-GTTTTCCCAGTCACGACGaagagtctgccttctgaagag-3′	5′-AGGAAACAGCTATGACCATcccgacttcaggcagcacatc-3′
7	581	5′-GTTTTCCCAGTCACGACGcctaaccaggctcaaggagat-3′	5′-AGGAAACAGCTATGACCATctagttgaagactacacatggag-3′
8	431	5′-GTTTTCCCAGTCACGACGctctagcagaggcaatgaatg-3′	5′-AGGAAACAGCTATGACCATgaagccggaggatgaccaccc-3′
9A	517	5′-GTTTTCCCAGTCACGACGctgcgctgttcctcaggtgta-3′	5′-AGGAAACAGCTATGACCATacgtgccatgtcttcttactt-3′
9B	277	5′-GTTTTCCCAGTCACGACGgaggatagcaatctctgctagg-3′	5′-AGGAAACAGCTATGACCATgataacgcatgcacagtgctacc-3′

*Bam*HI/*Xho*I sites of the pAcUw51-CYPOR vector and the identity of the resulting constructs confirmed by sequence analysis. The plasmids contain two independent promoters: the p10 promoter to control expression of the CYPOR and the polyhedrin promoter to control expression of the CYP2J2. Recombinant wild-type and variant CYP2J2 proteins were obtained by cotransfection of Sf9 cells with linear wild-type BaculoGold DNA and either wild-type or variant CYP2J2 DNA in pAcUw51-CYPOR. Recombinant viruses were selected by visualizing plaques on agarose overlays (Copeland and Wang, 1993). Clear plaques were picked without further plaque purification, amplified, and used to infect Sf9 cells at a multiplicity of infection of 5 to 10 in the presence of δ-aminolevulinic acid and iron citrate (100 μM each). Cells coexpressing recombinant wild-type or variant CYP2J2 and CYPOR were harvested 72 h after infection and were used to prepare microsomal fractions as described previously (Zeldin et al., 1995). P450 content was determined spectrally according to the method described by Omura and Sato (1964) by using a DW-2000 spectrophotometer (Aminco, Urbana, IL).

Protein Immunoblotting. Polyclonal antibodies against the CYP2J2-specific peptides HMDQNFGNRPVTPMR (amino acids 103–117; anti-CYP2J2pep1) and FNPDHFLENGQFKKRE (amino acids 421–436; anti-CYP2J2pep4) were raised in New Zealand White rabbits as described previously (Ma et al., 1999). Antibodies to rat CYPOR were purchased from Gentest (Woburn, MA). Microsomal proteins from Sf9 cells coexpressing recombinant wild-type or variant CYP2J2 and CYPOR were separated on 12% Tris-glycine gels (80 × 80 × 1 mm) purchased from Novex (San Diego, CA), and the resolved proteins were transferred electrophoretically onto nitrocellulose membranes. Membranes were immunoblotted with either anti-CYP2J2pep1 serum, anti-CYP2J2pep4 serum, or anti-CYPOR IgG, goat anti-rabbit IgG conjugated to horseradish peroxidase (Bio-Rad, Hercules, CA), and the ECL Western blotting detection system (Amersham Biosciences, Inc.) as described previously (Wu et al., 1996, 1997; Ma et al., 1999). Preimmune serum, collected from the rabbits before immunization, did not cross-react with recombinant CYP2J2 or with microsomal fractions prepared from uninfected Sf9 cells.

Enzymatic Characterization. The ability of recombinant wild-type and variant CYP2J2 proteins to metabolize [1-^{14}C]AA and [1-^{14}C]LA was evaluated in HPLC assays. Briefly, microsomal proteins from transfected Sf9 cells (final concentration 0.1–0.2 nmol of spectral P450/ml) were incubated with either freshly purified [1-^{14}C]AA or freshly purified [1-^{14}C]LA (final concentration 100 μM) at 37°C in a buffer containing 0.05 M Tris-Cl, pH 7.5, 0.15 M KCl, 0.01 M $MgCl_2$, 8 mM sodium isocitrate, 0.5 IU/ml isocitrate dehydrogenase, and 1 mM NADPH. After 30 min, aliquots were withdrawn and the reaction products were extracted with diethyl ether, dried under a nitrogen stream, analyzed by reverse phase HPLC, and quantified by on-line liquid scintillation by using a model 150TR flow scintillation analyzer (Packard Instrument Co., Meriden, CT) as described previously (Wu et al., 1997; Moran et al., 2000). All products were identified based on coelution with authentic standards. Control studies demonstrated that uninfected Sf9 cell microsomes and baculovirus-infected Sf9 cell microsomes expressing recombinant CYPOR but containing no spectrally evident P450 did not significantly metabolize AA or LA; that no products were formed when NADPH was omitted from the reactions; and that the quantitative assessment of the rates of product formation reflected initial rates.

TABLE 2
Primers used for site-directed mutagenesis of CYP2J2
The underlined bases indicate single nucleotides changes.

Mutation	Sequences of Primers
Thr143Ala	5′-AGGTTCACTCTGGCAGCACTAAGG-3′
Arg158Cys	5′-GCTTAGAGGAATGCATTCAGGAGG-3′
Ile192Asn	5′-CCAATATCAATTGCTCCATCACC-3′
Asp342Asn	5′-AGCTGAGATTAACAGAGTGATTGG-3′
Asn404Tyr	5′-GATCCTGACCTATTTGACGGCGC-3′

Statistical Analysis. Approximate allele frequencies for *CYP2J2* coding and promoter SNPs were calculated by dividing the number of mutations identified by the total number of chromosomes sampled ($N = 144$) as described previously (Fritsche et al., 2000; Dai et al., 2001). Statistical power to detect frequency differences between racial/ethnic groups was low due to the small sample size and so p values are not reported. Metabolism data were analyzed by the Student's t test with a two-tailed distribution assuming equal variance. Differences were considered significant if $p < 0.05$.

Results

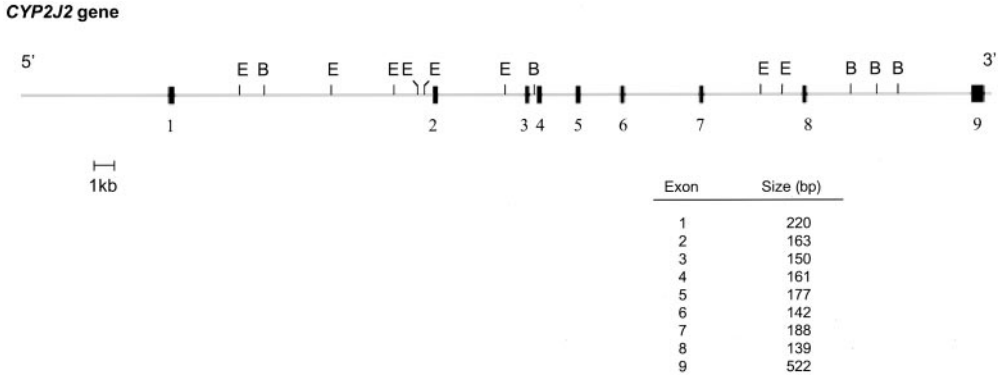
CYP2J2 Gene Cloning and Sequence Analysis. Screening of a human BAC library with the CYP2J2 cDNA probe yielded three genomic clones, one of which (clone 16122) was selected for further study. Sequence analysis of clone 16122 revealed it contained the entire *CYP2J2* gene (~40.3 kb), including ~6.0 kb of 5′-flanking region and ~1.0 kb of 3′-UTR (GenBank AF272142).[1] There are multiple putative Sp1 binding sites in the proximal CYP2J2 5′-flanking region located at positions −45, −50, −64, and −84 relative to the transcriptional start site (see below). CYP2J2 also has a TATA-less promoter. The multiple putative Sp1 binding sites and the TATA-less promoter are consistent with the housekeeping nature of this P450 gene (Kadonaga et al., 1986). The CYP2J2 gene, like all previously described CYP2 family genes, contains nine exons and eight introns (Fig. 1). The exons vary in length from 139 to 522 nucleotides, with the eighth and ninth exons containing nucleotides that encode the putative heme-binding peptide. The introns vary in length from 393 nucleotides (intron 3) to 10.4 kb (intron 1) (Fig. 1; Table 3). Splice sites are highly conserved among all CYP2 family members.

To determine whether the *CYP2J2* gene has closely related subfamily members, Southern blot analysis was performed. Human genomic DNAs were digested with the restriction enzymes *Eco*RI, *Pst*I, *Hin*dIII, or *Bam*HI, subjected to gel electrophoresis, transferred to nylon membranes, and hybridized with the 1.85-kb CYP2J2 cDNA probe. As shown in Fig. 2, only a few fragments were detected with each restriction enzyme. This simple pattern of hybridization is consistent with the existence of a single copy gene without closely related subfamily members. In fact, all of the bands detected in Fig. 2 are predictable based on the known locations of the *Eco*RI, *Pst*I, *Hin*dIII, and *Bam*HI restriction sites in the *CYP2J2* gene. The single copy nature of the human *CYP2J* gene subfamily was confirmed by searching existing databases.

Identification of Major CYP2J2 Transcriptional Start Site by 5′ RACE. 5′ RACE was used to identify the transcriptional start site of the *CYP2J2* gene. Gel electrophoretic separation of PCR products generated using two nested CYP2J2-specific primers yielded a prominent band of ~340 bp in three independent experiments (Fig. 3). An additional band of minor intensity (~230 bp) was also observed in one of the three RACE experiments. The prominent 340-bp product and the much weaker 230-bp band were both excised from the gel and sequenced. Sequence analysis showed that the minor RACE product (230 bp) started within the already known CYP2J2 mRNA sequence and therefore probably represented an amplificate derived from a shortened, partially

degraded mRNA molecule. In contrast, sequence analysis of the 340-bp RACE product elongated the known mRNA sequence to the 5′ end by 21 bp. An identical 5′ end sequence was found in each of three independent RACE experiments. Based on these data, we conclude that the major transcriptional starting point of the *CYP2J2* gene is located 26 bp upstream of the translational start site (corresponding to position 6005 of the sequence submitted to GenBank, hereafter designated +1 by convention).

Identification of CYP2J2 Variants by Sequencing. We sequenced all nine *CYP2J2* exons, intron-exon boundaries, and ~250 bp of the proximal *CYP2J2* promoter in cells isolated from 72 racially and ethnically diverse humans and identified 19 SNPs. One of the SNPs was located in the proximal *CYP2J2* promoter, eight were in exonic regions, five were in intronic regions, and four were in the 3′-UTR (Table 4). The G-50T SNP was located within one of the four putative Sp1 binding sites in the proximal *CYP2J2* promoter. Of the nine exonic SNPs identified, four resulted in amino acid substitutions and five were silent. Of the four SNPs that resulted in amino acid substitutions, one (Asp342Asn) was a conservative change (i.e., replacement with an amino acid of overall similar chemical properties) and three (Arg158Cys, Ile192Asn, and Asn404Tyr) were nonconservative. The approximate allelic frequencies of the promoter and four exonic SNPs that resulted in amino acid substitutions among three racial/ethnic groups are shown in Table 5. All the subjects were heterozygous for the coding polymorphisms and their estimated allele frequency was relatively low (2.1–4.2%). The Arg158Cys, Ile192Asn, and Asp342Asn variants were observed only among persons of African descent, whereas the Asn404Tyr variant was observed only among white persons. Neither allele was detected in the 24 Asians. In contrast, the G-50T SNP was much more common and present in approximately 17% of Africans, 13% of Asians, and 8% of white in our sample (Table 5). The observed allelic frequency for each coding variant was greater than 1%, and therefore these represent true human genetic polymorphisms; however, due to the large degree of genetic diversity and admixture in human populations, and the difficulty in defining appropriate populations for sampling, the frequency data reported for any individual group must be considered an approximation. One additional nonconservative variation (Thr143Ala) was identified in several clones obtained from screening a human heart cDNA library prepared from pooled RNA from several individual subjects (Table 5). This variant was not identified in any of the 72 individual subjects whose DNA was sequenced and so it is likely that the allelic frequency of this variant is very low. None of the variants are located in the six putative SRSs proposed by Gotoh (1992). All variants except Thr143 are in conserved residues among all CYP2J subfamily members. In addition, Arg158 and Ile192 are also conserved in other CYP2 family P450s, including members of the



Exon	Size (bp)
1	220
2	163
3	150
4	161
5	177
6	142
7	188
8	139
9	522

Fig. 1. Structure and intron/exon organization of the *CYP2J2* gene. The *CYP2J2* gene was isolated from a BAC library as described under *Experimental Procedures*. The gene is >40 kb and contains nine exons/eight introns. Exons 8 and 9 contain nucleotides that encode the putative heme-binding peptide. The locations of *Eco*RI (E) and *Bam*HI (B) restriction enzyme sites are shown.

TABLE 3

Location of Intron-Exon Boundaries in *CYP2J2*

Exons are shown in uppercase and introns in lowercase. Codons at junctions are underlined and the corresponding amino acid is displayed above the sequence.

Intron	Donor	Intron Size	Acceptor
	L70		F71
1	GGTTCAGCTG**gt**aggagtgg	10,436	tatttttc**ag**TTTGTGAAGA
	G125		G125
2	AAGAAAAATG**gt**aagtttct	3,625	ttttcccc**ag**GATTGATTAT
	G175		G175
3	GAGGAGAACG**gt**gagcattg	393	ccaccttc**ag**GACAGCCTTT
	Q228		L229
4	GACATGCCAG**gt**aaggcagc	1,647	tctcaatt**ag**CTCTACAATG
	L287		H288
5	AATGTCAAAG**gt**gagaaaac	1,857	tgttttct**ag**CACACAGGCA
	E335		E335
6	GAAATCCAAG**gt**gagcatgt	2,727	tctttacc**ag**AAAAAGTACA
	L397		G398
7	CCTGCCCAAG**gt**aattaagc	3,767	ctttctct**ag**GGTACCATGA
	G444		G444
8	TTCTCAATAG**gt**aagttgta	7,138	tgtcttac**ag**GAAAGCGGGC

CYP2A, CYP2B, CYP2C, CYP2E, and CYP2J subfamilies. No haplotypes were identified in the study.

Expression of CYP2J2 Variants. Wild-type CYP2J2 and each of the five polymorphic variants were coexpressed with CYPOR in Sf9 insect cells by using the baculovirus expression system. P450 expression levels ranged from 3 to 10 nmol of spectral P450 per liter of infected cells, depending on the variant and the preparation. The expression levels and preparation-to-preparation variability were comparable with those obtained for other P450s by using a similar heterolo-

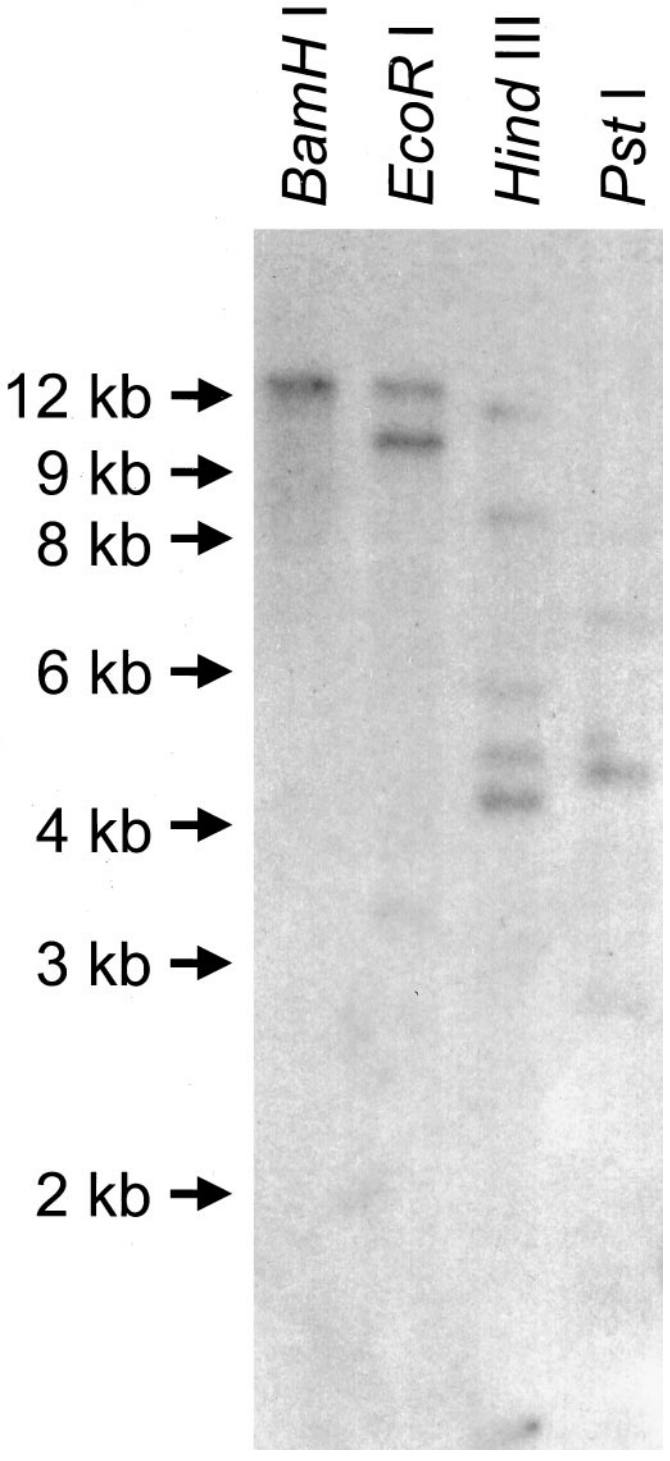


Fig. 2. Southern blot of human genomic DNA with CYP2J2 probe. Human genomic DNA (10 μg) was digested with either *Bam*HI, *Eco*RI, *Hin*dIII, or *Pst*I; electrophoresed; transferred to nylon membranes; and hybridized with the radiolabeled CYP2J2 cDNA probe. The autoradiograph was exposed for 72 h. The positions of molecular weight markers are shown on the left. Results are representative of five independent experiments.

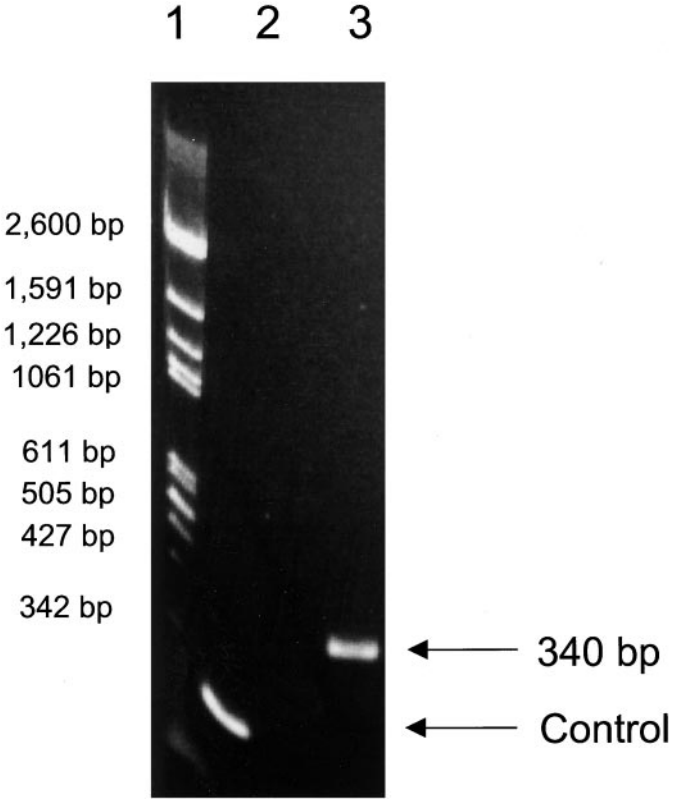


Fig. 3. Identification of CYP2J2 transcriptional start site by 5′ RACE. Human heart total RNA was reverse transcribed and 5′ RACE was performed using *CYP2J2* gene-specific primers or positive control primers as described under *Experimental Procedures*. The resulting products were separated by agarose gel electrophoresis and stained with ethidium bromide. Molecular weight markers are shown in lane 1. The control DNA reaction gives rise to a 180-bp band (lane 2), whereas the *CYP2J2* 5′ RACE reaction produces a single 340-bp band (lane 3), which was sequenced. Results are representative of three independent experiments.

TABLE 4

Polymorphisms in CYP2J2 found in 72 human samples

Nucleotide polymorphism numbering is based on the transcriptional start site.

Nucleotide Polymorphism	Location	Amino Acid Polymorphism
G-50T	Promoter	
C173T	Exon 1	L50L
C208T	Exon 1	F61F
C10770T	Exon 2	H103H
C14558T	Exon 3	R158C
T15054A	Exon 4	I192N
C18944A	Exon 6	R321R
G21734A	Exon 7	D342N
A25687T	Exon 8	N404Y
T33112A	Exon 9	V499V
G10860A	Intron 2	
T10902G	Intron 2	
C14392T	Intron 2	
G14912A	Intron 3	
T18778G	Intron 5	
C33291T	3′-UTR	
T33370A	3′-UTR	
A33465G	3′-UTR	
A33497G	3′-UTR	

gous expression system (Wu et al., 1997; Ma et al., 1999; Oleksiak et al., 2000; Qu et al., 2001). Wild-type CYP2J2 and the variants Thr143Ala, Arg158Cys, and Asp342Asn produced typical cytochrome P450 CO-difference spectra with Soret maxima at 450 nm (Fig. 4A). In contrast, CO-difference spectra for the Ile192Asn and Asn404Tyr variants had prominent 420-nm peaks (Fig. 4A). Although these peaks could be due to the presence of other hemoproteins, they suggest the possibility of improper folding of the protein and/or misincorporation of the heme as reported for other P450s (Imaoka et al., 1993; Iwasaki et al., 1993). Western blot analysis of microsomes prepared from Sf9 cells infected with either wild-type CYP2J2 or variant CYP2J2 baculovirus stocks by using two different peptide-based CYP2J2-specific antibodies revealed primary ~57-kDa bands, indicating that the amino acid substitutions did not significantly affect immunoreactivity or electrophoretic mobility of the recombinant proteins (Fig. 4B). Immunoblots of these same microsomes with the anti-CYPOR IgG revealed a single ~72-kDa band and confirmed that P450 apoprotein/CYPOR protein ratios were comparable in the wild-type and variant CYP2J2 preparations.

Metabolism of AA by Recombinant Wild-Type and Variant CYP2J2 Proteins. To examine whether the amino acid substitutions were associated with alterations in P450 enzyme function, we incubated recombinant wild-type and variant CYP2J2 proteins with radiolabeled AA in the presence of NADPH and an NADPH-regenerating system. As shown in Fig. 5, wild-type CYP2J2 actively metabolized AA to several more polar products. The principal metabolite formed was 14,15-EET followed by lower amounts of 11,12-EET, 8,9-EET, and 19-HETE. The reverse phase HPLC profiles produced by the variant CYP2J2 proteins were qualitatively similar to that of wild-type CYP2J2 in terms of the relative amounts of epoxidation versus hydroxylation and the regiochemistry of olefin epoxidation (Fig. 5). Results from at least eight independent experiments by using a minimum of three different enzyme preparations for each of the recombinant proteins are shown in Fig. 6A. Four of the CYP2J2 variants (Thr143Ala, Arg158Cys, Ile192Asn, and Asn404Tyr) showed statistically significant decreases in enzyme activity, which were 59, 41, 30, and 5% of the wild-type CYP2J2 activity, respectively. In contrast, the Asp342Asn variant metabolized AA at comparable rates to that of wild-type CYP2J2 (Fig. 6A).

Metabolism of LA by Recombinant Wild-Type and Variant CYP2J2 Proteins. As shown in Fig. 7, wild-type CYP2J2 metabolized LA primarily to EOAs. The reverse phase HPLC profiles produced by the variant CYP2J2 proteins were qualitatively similar to that of wild-type CYP2J2 (Fig. 7). Results from at least six independent experiments with a minimum of three different enzyme preparations for each of the recombinant proteins are shown in Fig. 6B. Three of the CYP2J2 variants (Thr143Ala, Arg158Cys, and Asn404Tyr) showed statistically significant decreases in enzyme activity, which were 58, 50, and 10% of the wild-type CYP2J2 activity, respectively. The Ile192Asn variant also had reduced enzyme activity (71% of wild-type CYP2J2); however, this did not reach statistical significance ($p = 0.11$). As in the case of AA, the Asp342Asn variant metabolized LA at comparable rates to that of wild-type CYP2J2 (Fig. 6B).

Discussion

Cytochromes P450 have been the focus of study by toxicologists and pharmacologists in the past because they catalyze the metabolism of a wide range of xenobiotics, including drugs, environmental pollutants, industrial chemicals, and carcinogens. The majority of P450 enzymes involved in xenobiotic metabolism are known to be polymorphic, and this genetic variation has been proposed to influence individual susceptibility to adverse drug reactions and/or toxicity to environmental agents (Nebert et al., 1996; Ingelman-Sundberg, 2001; Pirmohamed and Park, 2001). For example, polymorphisms in the *CYP1A2*, *CYP2C9*, *CYP2C19*, *CYP2D6*, and *CYP3A4* genes have been implicated in the pathogenesis of adverse reactions to antipsychotic, anticoagulant, antiseizure, antidepressant, and anticancer drugs, respectively (Pirmohamed and Park, 2001). Similarly, polymorphisms in the *CYP1A2*, *CYP2A6*, and *CYP2E1* genes may be important in the metabolism of aromatic amine procarcinogens (Nebert et al., 1996; Ingelman-Sundberg, 2001). In contrast, very little is known regarding the presence and/or functional relevance of polymorphisms in P450 genes primarily involved in the metabolism of endogenous substrates such as fatty acids.

CYP2J2 is a human P450 that is constitutively expressed in a number of organs but is most abundant in the cardiovascular system (Wu et al., 1996). CYP2J2 products, which include the EETs (metabolites of AA) and EOAs (metabolites of LA), possess a number of potent biological effects in the heart and vasculature. For example, EETs are vasodilators, improve postischemic cardiac contractile function, reduce vascular inflammation, and increase intravascular fibrinolysis (Wu et al., 1997; Campbell and Harder, 1999; Node et al., 1999, 2001). EETs and/or their hydration metabolites have also been hypothesized to play a role in the pathogenesis of human hypertension (Catella et al., 1990; McGiff, 1991; Cap-

TABLE 5

Allelic frequencies of the *CYP2J2* coding and promoter SNPs

Estimated allele frequencies were calculated by dividing the number of mutations identified by the total number of chromosomes sampled ($N = 144$).

Variant	Nucleotide Polymorphism [a]	Location	Estimated Allele Frequency		
			African ($n = 48$)	Asian ($n = 48$)	White ($n = 48$)
	G-50T	Promoter	0.17	0.13	0.08
R158C	C14558T	Exon 3	0.02	0	0
I192N	T15054A	Exon 4	0.04	0	0
D342N	G21734A	Exon 7	0.02	0	0
N404Y	A25687T	Exon 8	0	0	0.02
T143A	A14513G	Exon 3	N.D.	N.D.	N.D.

N.D., not determined.

[a] Nucleotide number relative to the transcriptional start site.

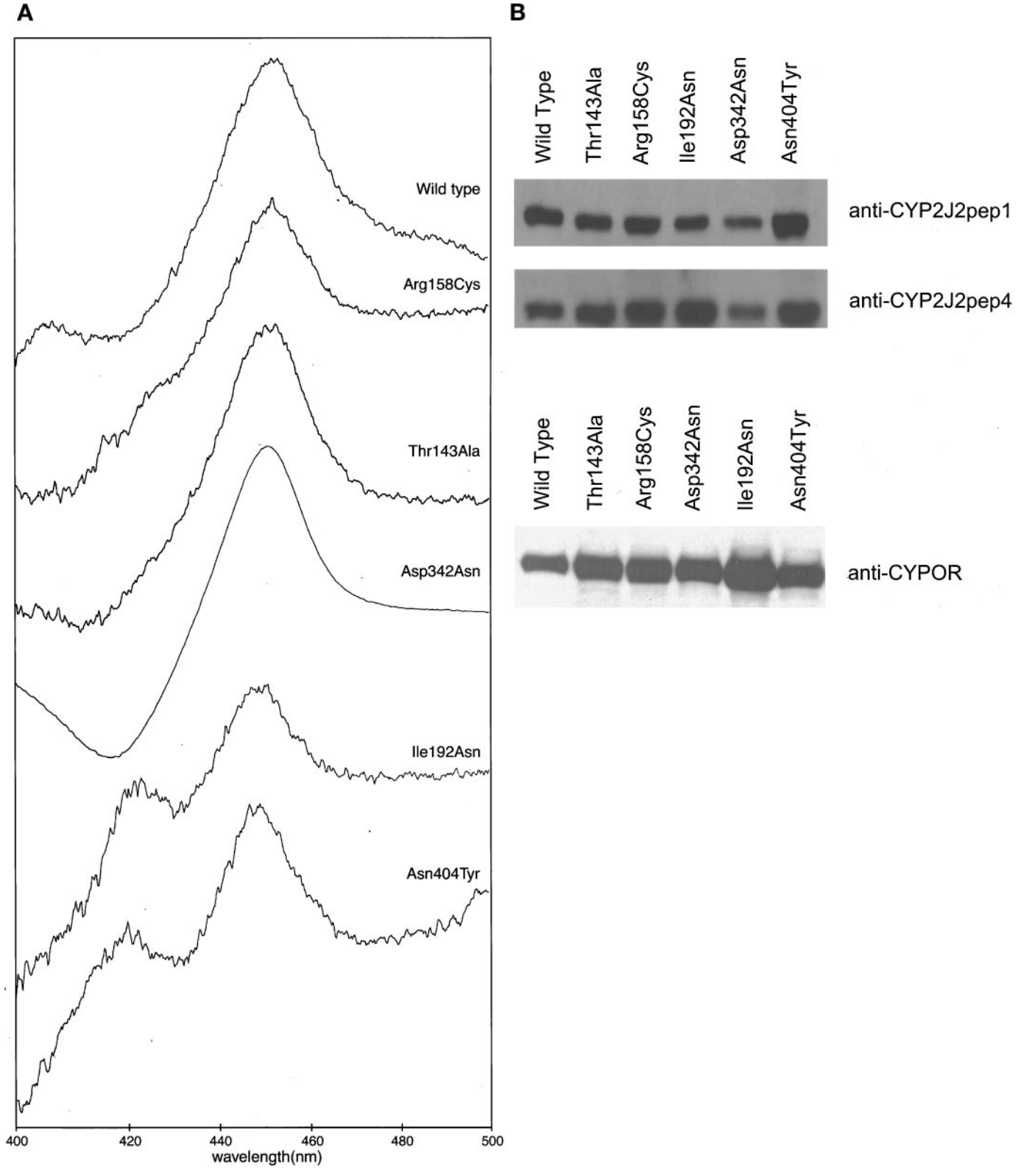


Fig. 4. Reduced CO-difference spectra and immunoblot blot analysis of recombinant wild-type and variant CYP2J2 proteins. A, CO-difference spectra of microsomal fractions prepared from Sf9 cells expressing recombinant CYP2J2 or its variants. All spectra were recorded at room temperature in 0.1 M sodium phosphate buffer. Ordinate, absorbance; abscissa, wavelength (nm). B, microsomal fractions (1 pmol/lane) prepared from Sf9 cells infected with recombinant wild-type or variant CYP2J2 baculovirus were electrophoresed on Tris-glycine gels and the resolved proteins transferred to nitrocellulose membranes. Membranes were immunoblotted with either anti-human CYP2J2pep1 IgG, anti-human CYP2J2pep4 IgG, or anti-rat CYPOR IgG as described under *Experimental Procedures*. The anti-human CYP2J2pep1 and anti-human CYP2J2pep4 recognize different CYP2J2 epitopes.

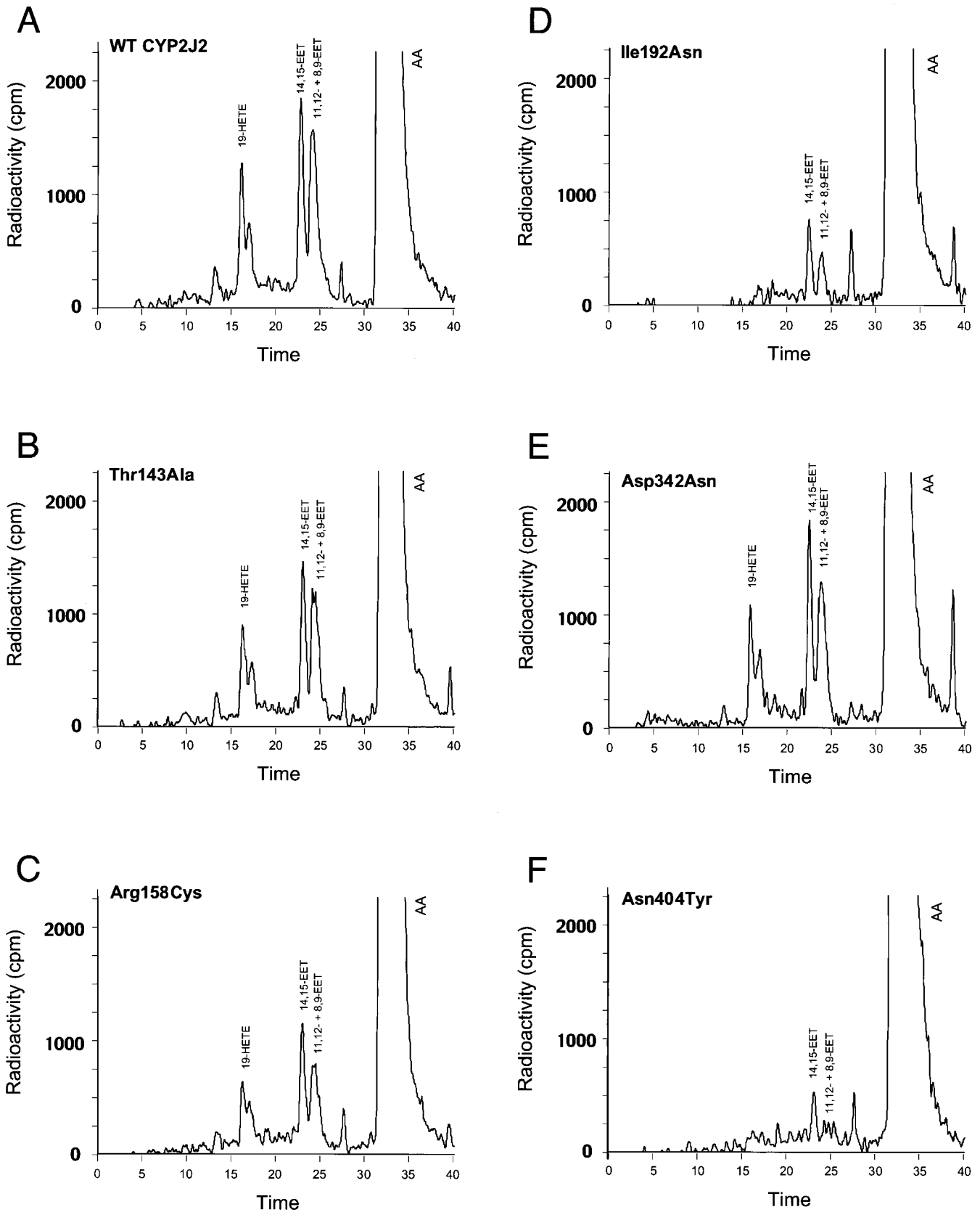


Fig. 5. Metabolism of AA by wild-type CYP2J2 and its variants. Reverse phase HPLC chromatograms of organic soluble metabolites generated during incubations of Sf9 insect cell microsomes coexpressing CYP2J2 (wild-type or variants) and CYPOR with [1-^{14}C]AA. CYP2J2 (wild-type) (A), Thr143Ala (B), Arg158Cys (C), Ile192Asn (D), Asp342Asn (E), and Asn404Tyr (F). Results are representative of at least eight independent experiments for each protein. Products were identified by comparing their reverse and normal phase HPLC properties with those of authentic standards. Ordinate, radioactivity in cpm; abscissa, time in minutes.

devila et al., 2000). EOAs, on the other hand, increase vascular permeability and depress cardiac function (Fukushima et al., 1988; Ozawa et al., 1988). It follows that the biosynthesis of EET and/or EOAs by cardiovascular P450s, including CYP2J2, may have important functional implications. In the present study, we describe the cloning of the *CYP2J2* gene and the discovery of a number of *CYP2J2* polymorphisms that result in amino acid substitutions. We also demonstrate that some of these variants have significantly reduced capacity to metabolize AA and LA to compounds that are known to be biologically active in the cardiovascular system.

The *CYP2J2* gene spans ~40 kb and contains nine exons and eight introns. This latter feature has been shown to be a characteristic of all of *CYP2* family genes. Southern analysis of human genomic DNA suggests the existence of a single copy gene without closely related subfamily members. In this respect, the human *CYP2J* subfamily differs from that in mouse and rat, which are known to contain multiple *CYP2J* genes, probably as a result of gene duplication events in these rodent species (Wu et al., 1997; Zhang et al., 1997; Ma et al., 1999; Scarborough et al., 1999; Qu et al., 2001). The major CYP2J2 transcriptional starting point is located 26 bp upstream from the translational start site. The proximal *CYP2J2* promoter does not contain a TATA box, but does contain multiple putative Sp1 binding sites consistent with the housekeeping nature of this gene. Interestingly, one of the SNPs we identified (G-50T) is located within one of these putative Sp1 binding sites; however, the functional relevance of this noncoding SNP, which is present in approximately 8 to 16% of the population depending on racial/ethnic background, remains to be determined.

We identified five SNPs that resulted in amino acid changes in the CYP2J2 polypeptide sequence. Four of these variants (Thr143Ala, Arg158Cys, Ile192Asn, and Asn404Tyr), each representing a nonconservative amino acid change, showed reduced enzyme activity toward one or more endogenous substrates. The Asn404Tyr variant, which was only detected in whites, metabolized both AA and LA at dramatically reduced rates (5 and 10% of wild-type CYP2J2, respectively). This residue is conserved among all CYP2Js but is not conserved among other CYP2 family P450s except CYP2D6. The Ile192Asn variant, which was detected only in persons of African descent, also exhibited reduced AA metabolism and tended to have reduced LA metabolism compared with wild-type CYP2J2. Interestingly, ClustalW alignments

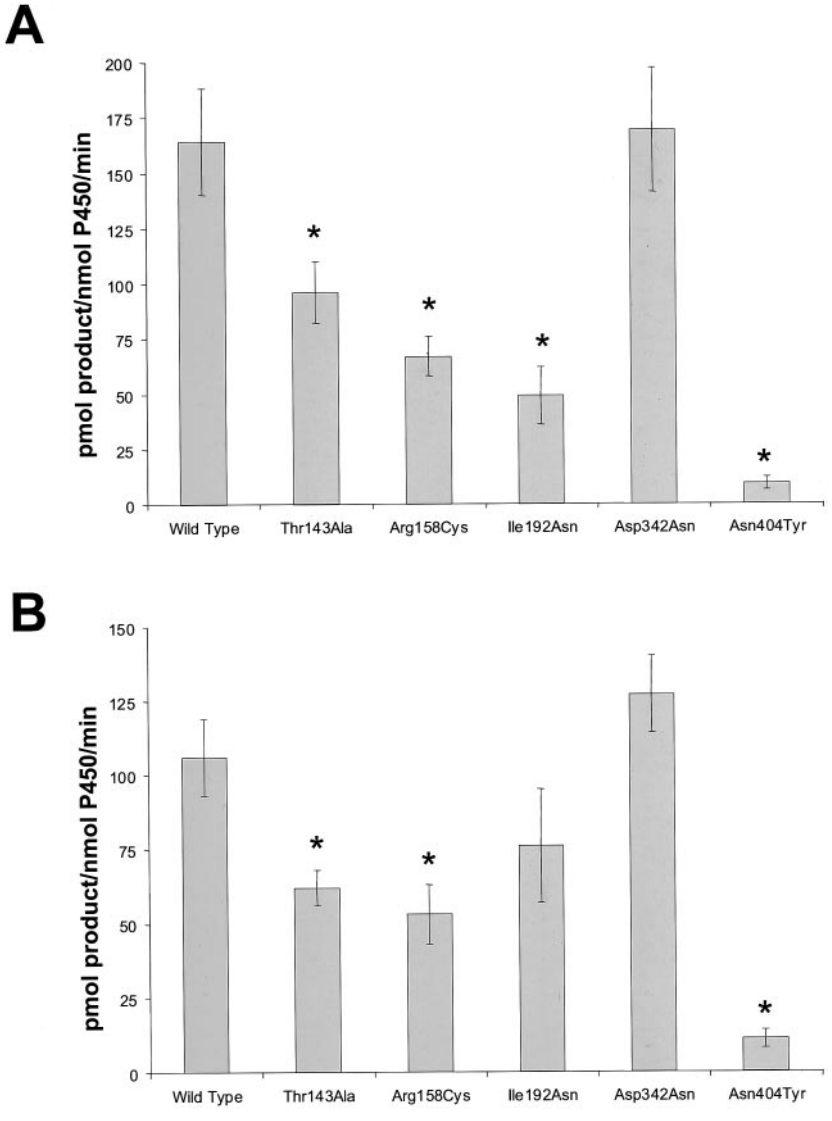


Fig. 6. Rates of AA and LA metabolism by wild-type and variant CYP2J2 proteins. A, quantitative assessment of the rates of product formation (expressed as pmol of product/nmol P450/min) during incubations of radiolabeled AA with wild-type or variant CYP2J2 proteins. Columns show means ± S.E. (n = 8–13/group). B, quantitative assessment of the rates of product formation (expressed as pmol of product/nmol P450/min) during incubations of radiolabeled LA with wild-type or variant CYP2J2 proteins. Columns show means ± S.E. (n = 6–15/group).

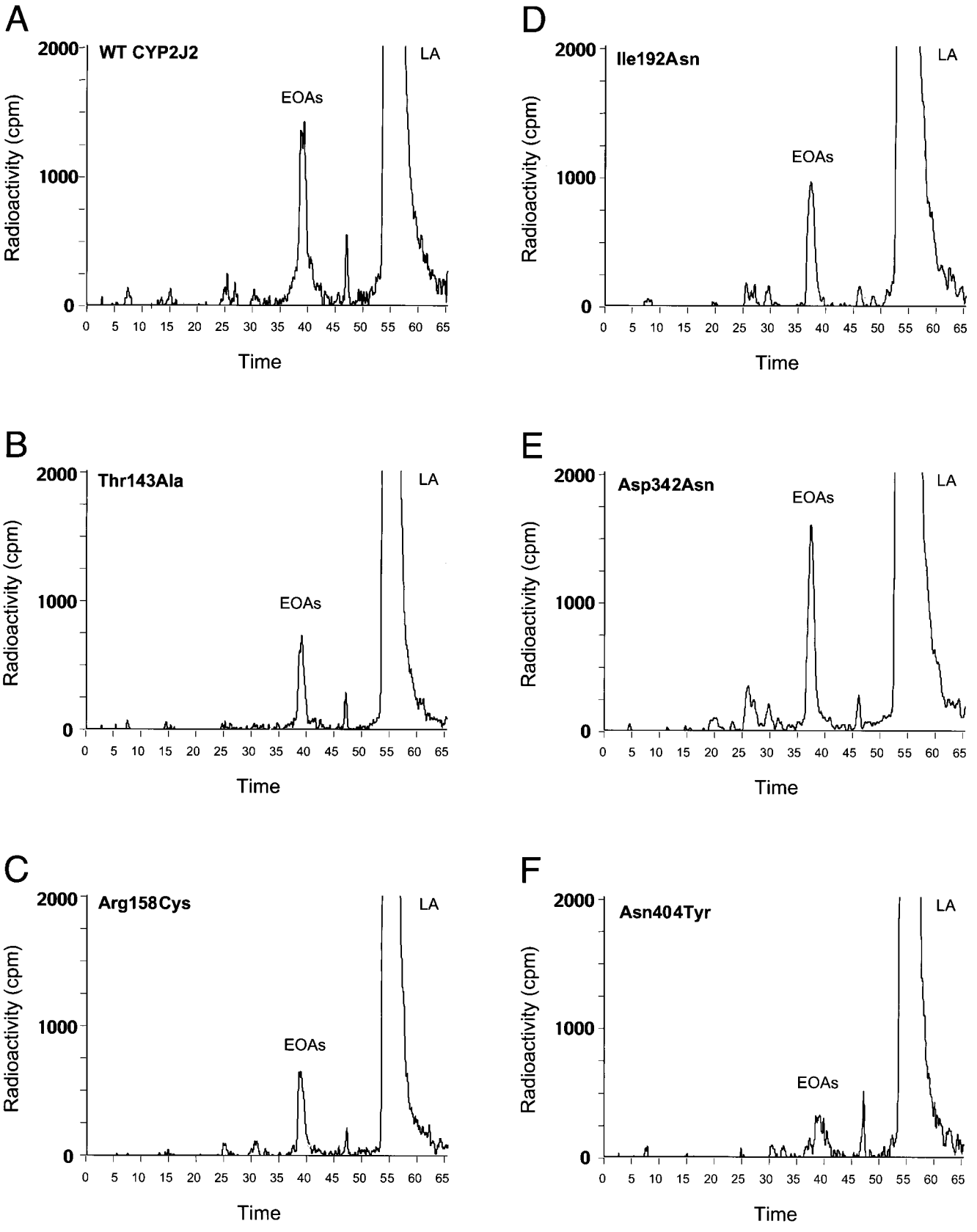


Fig. 7. Metabolism of LA by wild-type CYP2J2 and its variants. Reverse phase HPLC chromatograms of organic soluble metabolites generated during incubations of Sf9 insect cell microsomes coexpressing CYP2J2 (wild-type or variants) and CYPOR with [1-^{14}C]LA. CYP2J2 (wild type) (A), Thr143Ala (B), Arg158Cys (C), Ile192Asn (D), Asp342Asn (E), and Asn404Tyr (F). Results are representative of at least six independent experiments for each protein. Products were identified by comparing their reverse and normal phase HPLC properties with those of authentic standards. Ordinate, radioactivity in cpm; abscissa, time in minutes.

reveal that Ile192 is highly conserved among several different CYP2 family P450s, including members of the CYP2A, CYP2B, CYP2C, CYP2D, CYP2E, and CYP2J subfamilies. The other two variants (Thr143Ala and Arg158Cys) showed statistically significant reduction in the metabolism of both AA and LA compared with wild-type CYP2J2. Although Arg158 is a conserved amino acid residue among members of the CYP2A, CYP2B, CYP2C, CYP2E, and CYP2J subfamilies, Thr143 is not conserved, even among the CYP2Js. Interestingly, none of these variants are located in the six putative SRSs (Gotoh, 1992). Non-SRS mutations have been previously shown to affect P450 enzymatic function (He et al., 1998; Joo et al., 1999).

Remarkably little is known regarding polymorphisms in human P450s involved in AA metabolism. CYP2C8 is one of the primary hepatic enzymes involved in the metabolism of AA to EETs (Zeldin et al., 1995, 1996b) and it has also been suggested that this P450 also contributes to EET biosynthesis in the endothelium (Fisslthaler et al., 1999). We recently reported that the *CYP2C8* gene is polymorphic and that one of the variants, which includes two different amino acid substitutions (Arg139Lys and Lys399Arg), exhibits reduced capacity to metabolize AA (Dai et al., 2001). The frequency of this allele was 13% among whites and 2% among African-Americans. Coding polymorphisms are also known to exist for CYP2C9, CYP2C19, and CYP2E1; however, the effects of these variations on AA metabolism remain unknown. We are unaware of previously published data on *CYP2J2* polymorphisms. At the time of this writing, the National Center on Biotechnology Information dbSNP database (http://www.ncbi.nlm.nih.gov/SNP) listed several CYP2J2 polymorphisms, including the G-50T promoter SNP that we identified herein; however, none of these are associated with amino acid substitutions. Indeed, the frequency of coding SNPs in the *CYP2J2* gene is much lower than that reported for other P450s genes (Ingelman-Sundberg, 2001). We speculate that this may be due to a selection bias against genetic variation in CYP2J2, perhaps because of one or more critical housekeeping functions that it may possess.

We sequenced the *CYP2J2* gene in DNA samples prepared from cells isolated from 72 subjects. Analysis of a sample of this size allows for the identification of relatively abundant SNPs (estimated allele frequency >1%) and would be expected to miss relatively rare mutations. This is an important limitation of our study; however, even a functional polymorphism at the 1% level would potentially affect millions of people in the United States alone. Because of the relatively small sample size, our allele frequency data cannot be extrapolated to the larger population (i.e., detection of a single SNP in a population of 72 subjects may either overestimate or underestimate the true allelic frequency of the SNP). Well designed, population-based studies need to be done in the future to determine the exact allelic frequency of the functionally relevant SNPs discovered herein and to examine whether racial/ethnic differences in the frequency of these SNPs occur in humans. Development of rapid SNP identification methods (e.g., restriction fragment length polymorphism analysis) will greatly facilitate these important studies.

Mutations in P450 genes have been shown to be associated with human disease. For example, mutations in the coding region of the *CYP1B1* gene have been shown to be associated with primary congenital glaucoma (Sarfarazi, 1997; Bejjani et al., 2000; Kakiuchi-Matsumoto et al., 2001). Several studies have reported an association between *CYP2D6* polymorphism and increased risk of Parkinson's disease (Smith et al., 1992; Kurth and Kurth, 1993; McCann et al., 1997). Polymorphisms in the *CYP1A1* gene may be associated with a higher incidence of lung cancer (Houlston, 2000). In contrast, *CYP2E1* gene polymorphisms are associated with reduced incidence of lung cancer in certain populations (Uematsu et al., 1991; Persson et al., 1993). *CYP2D6* polymorphisms have been associated with increased risk of liver, gastrointestinal, and lung cancer (Nebert, 1991). It is not currently known whether any of the *CYP2J2* polymorphisms reported in the present manuscript are associated with human disease; however, we are in the process of assembling cohorts of hypertensive and cardiovascular disease patients (and corresponding age- and sex-matched controls) to determine whether the frequencies of the functionally relevant *CYP2J2* SNPs are altered in these populations. These and other large, well designed case-control studies will be necessary to shed light on the relevance of *CYP2J2* gene polymorphisms to human health and disease.

In summary, we report the cloning of the *CYP2J2* gene and identification of several *CYP2J2* polymorphisms. Of the 20 SNPs identified, five result in amino acid substitutions. Four of the *CYP2J2* variants (Thr143Ala, Arg158Cys, Ile192Asn, and Asn404Tyr) exhibit reduced metabolism of one or more endogenous substrates. One of these variants (Asn404Tyr) is essentially inactive in the metabolism of both AA and LA to fatty acid epoxides, which have effects in the cardiovascular system. We speculate that alterations in the level of production of these physiologically relevant lipid mediators as a result of genetic polymorphism could lead to altered susceptibility to cardiovascular disease and hypertension.

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