Molecular Mechanisms of Polymorphic CYP3A7 Expression in Adult Human Liver and Intestine*

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Human CYP3A enzymes play a pivotal role in the metabolism of many drugs, and the variability of their expression among individuals may have a strong impact on the efficacy of drug treatment. However, the individual contributions of the four CYP3A genes to total CYP3A activity remain unclear. To elucidate the role of CYP3A7, we have studied its expression in human liver and intestine. In both organs, expression of CYP3A7 mRNA was polymorphic. The recently identified CYP3A7*1C allele was a consistent marker of increased CYP3A7 expression both in liver and intestine, whereas the CYP3A7*1B allele was associated with increased CYP3A7 expression only in liver. Because of the replacement of part of the CYP3A7 promoter by the corresponding region of CYP3A4, the CYP3A7*1C allele contains the proximal ER6 motif of CYP3A4. The pregnane X and constitutively activated receptors were shown to bind with higher affinity to CYP3A4-ER6 than to CYP3A7-ER6 motifs and transactivated only promoter constructs containing CYP3A4-ER6. Furthermore, we identified mutations in CYP3A7*1C in addition to the ER6 motif that were necessary only for activation by the constitutively activated receptor. We conclude that the presence of the ER6 motif of CYP3A4 mediates the high expression of CYP3A7 in subjects carrying CYP3A7*1C.

Cytochrome P450 enzymes play a pivotal role in the oxidative, peroxidative, and reductive metabolism of many endogenous compounds, procarcinogens, and drugs. The CYP3A subfamily composed of CYP3A4, CYP3A5, CYP3A7, and CYP3A43 in humans is of special importance because it accounts for as much as 30–50% of total CYP3A content (1). At least 50% of all medicines are metabolized by enzymes of the CYP3A subfamily (2). The most abundant CYP3A isoform in liver and intestine is CYP3A4. Its interindividually hepatic expression varies 60-fold (3), and the in vivo function as assessed by clearance displays at least a 20-fold difference (4). Induction by xenobiotics (e.g. rifampin) and endogenous compounds (e.g. steroid hormones) further modulates the variability of CYP3A4 expression among individuals. The induction of CYP3A4 and most likely that of other CYP3A genes is mediated by the nuclear receptor NR1I2 (pregnane X receptor (PXR) (5)) (reviewed in Ref. 5). CYP3A4-inducing compounds bind to PXR and stimulate the transcriptional activity of the receptor. Additional nuclear receptors such as NR1I3 (constitutively activated receptor (CAR)) and NR1I1 (vitamin D receptor) have also been implicated in the transcriptional regulation of CYP3A4 (6, 7). Although the substrate specificity of CYP3A5 is similar to that of CYP3A4, CYP3A5 has been regarded to be less important for drug elimination because it is expressed at much lower levels than CYP3A4 in most livers of Caucasian origin (8). CYP3A4 is expressed at very low levels in adult human livers, accounting for only 0.1–0.2% of CYP3A4 transcripts (9, 10). Therefore, its contribution to the elimination of CYP3A substrates is regarded to be negligible (10). This variability in CYP3A expression and function explains why the intensity and duration of drug action and the occurrence of side effects show large patient-to-patient variability. Although a recent analysis suggests that, depending on the drug, 60–90% of patient-to-patient variability in CYP3A function is caused by genetic factors (3), the sources for variability in constitutive CYP3A expression remain largely unknown. However, a common genetic polymorphism in intron 3 of CYP3A5 was recently identified. It results in high expression genotypes and explains the >10-fold increase in CYP3A5 protein expression observed in 10–30% of livers of Caucasian origin (8, 11). In some persons, CYP3A5 can contribute to >50% of total CYP3A content, thus exceeding CYP3A4 levels. Correspondingly, CYP3A5 has been proposed to contribute substantially to the elimination of CYP3A substrates (11). These findings could explain why there is less variability in the in vivo clearance than one would predict based on the >60-fold variability in CYP3A4 expression.

The role of CYP3A7 in the biotransformation of CYP3A substrate in adult liver and intestine is not known. CYP3A7 accounts for 30–50% of total cytochrome P450 in fetal liver (13) and was at first regarded to be exclusively expressed there (13). Since then, CYP3A7 expression was detected in 54–88% of adult livers (14, 15). However, quantitative data on CYP3A7 expression in adult livers are missing. Moreover, the mechanisms responsible for expression of CYP3A7 in adult livers remain unknown.

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1 The abbreviations used are: PXR, pregnane X receptor; CAR, constitutively activated receptor; RXR, retinoic X receptor; HNF, hepatocyte nuclear factor.
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Using a large collection of human livers, we report here that CYP3A7 mRNA is polymorphically expressed in both liver and intestine, with ~11% of subjects belonging to a distinct subgroup of high expression phenotype. Two-thirds of the subjects in this group carry the CYP3A7*1C or (less frequently) the CYP3A7*1B promoter allele. The CYP3A7*1C allele is the exclusive marker of high CYP3A7 expression in the intestine. Functional differences between CYP3A7 and CYP3A7*1C proximal promoter ER6 (gapped repeat separated by 6 base pairs) motifs in binding and activation by the nuclear receptors PXR and CAR were identified as the mechanisms responsible for the high CYP3A7 expression in CYP3A7*1C carriers.

EXPERIMENTAL PROCEDURES

Human Tissue Samples—Normal human liver tissue samples were obtained from patients of Caucasian origin undergoing liver resection at the Department of Surgery, Campus Virchow-Clinic, Humboldt University (Berlin, Germany) (16). Liver pieces were immediately placed into RNAlater (Ambion Inc.), incubated for 24 h at 4 °C, and then stored frozen at ~80 °C until RNA isolation. Human intestine samples were obtained from patients of Caucasian origin undergoing either gastrectomy or pancreaticoduodenectomy at the Department of Surgery, Robert Bosch Hospital, Stuttgart, Germany. In addition, a liver biopsy was obtained from each of these groups. Normal intestine tissue (duodenum or proximal jejunum) was dissected and either immediately frozen in liquid nitrogen or incubated for 24 h at 4 °C in RNAlater and then stored at ~80 °C until RNA preparation. The matching liver biopsies were processed as the intestine samples.

Genotyping by Sequencing—Genomic DNA was isolated from blood samples of the patients using the QIAamp DNA blood kit (QIAGEN Inc.). To determine the CYP3A7 genotypes, a 614-bp fragment of the CYP3A7 proximal promoter and exon 1 (~426 + 188 with respect to the transcriptional start site) was amplified by PCR using oligonucleotide primers 5'-GGC TCT TGG TGG GAG ATG A3' (bases 110480–110517 of GenBankTM/EBI accession number AF281017) and 5'-GAA CAG TTA CTC ACA GAT AGA GGA GTA TC-3' (bases 111093 to 111106) (12). The standard PCR consisted of 1× PCR buffer with 1.5 mM MgCl2, 200 μM each primer, and 1 unit Taq polymerase (QIAGEN Inc.), and 20 ng of genomic DNA in a total volume of 50 μl. Cycling conditions were as follows: one cycle at 94 °C for 2 min and 34 cycles at 94 °C for 45 s, 62 °C for 45 s, and 72 °C for 1 min, followed by a final cycle at 72 °C for 7 min, respectively. The PCR products were purified using the QIAQuick gel extraction kit (QIAGEN Inc.), directly sequenced using the ABI BigDye terminator cycle sequencing kit (Applied Biosystems) and an ABI 3700 DNA analyzer (Applied Biosystems), and analyzed using the PHRED/PHRAP/POLYPHRED/CONSED software package (University of Washington, Seattle, WA).

Restriction Fragment Length Polymorphism Assay for CYP3A7*1C—A restriction fragment length polymorphism assay was developed for the CYP3A7*1C allele. Oligonucleotide primers 5'-ATG ACC TAA GAA GAT GGA GATA CTG ATG A3' (bases 110513–110517 of GenBankTM/EBI accession number AF281017) and 5'-GAA CAG TTA CTC ACA GAT AGA GGA GTA TC-3' (bases 111093 to 111106) (12). The standard PCR consisted of 1× PCR buffer with 1.5 mM MgCl2 (QIAGEN Inc.), 200 μM dATP, 200 μM dCTP, 200 μM dGTP, 200 μM dTTP, 0.5 μM each primer, 1.25 units Taq polymerase (QIAGEN Inc.), and 20 ng of genomic DNA in a total volume of 50 μl. Cycling conditions were as follows: one cycle at 94 °C for 1 min and 34 cycles at 94 °C for 45 s, 62 °C for 45 s, and 72 °C for 1 min, followed by a final cycle at 72 °C for 7 min, respectively. The PCR products were purified using the QIAQuick gel extraction kit (QIAGEN Inc.), directly sequenced using the ABI BigDye terminator cycle sequencing kit (Applied Biosystems) and an ABI 3700 DNA analyzer (Applied Biosystems), and analyzed using the PHRED/PHRAP/POLYPHRED/CONSED software package (University of Washington, Seattle, WA).

Plasmid Constructs—The eukaryotic expression plasmid for human PXR has been described previously (17). To construct a eukaryotic expression plasmid for human CAR, the open reading frame of human CAR was amplified by PCR from human liver cDNA using primers 5'-ATG AAT TCC ACC ATG GCC AGT AGG GAA GAT GAG CTG-3' (bases 110153–110173 of GenBankTM/EBI accession number AF281017), which introduced an EcoRI site and an optimized Kozak consensus sequence, and 5'-GTT AGC ATT GGA CAC GTC CAC TCT TTT-3' and probe 5'-CCA GAT CAT TGC TGA GGT CAG CAG C-3'. Amplification and cloning of the appropriate CYP3A7 promoter region were verified by sequencing of the resulting plasmid, pGL3-CYP3A7*-869, and a HindIII restriction site on the 5'-3' ends of the amplified fragment, respectively. The PCR product was digested with KpnI and HindIII and cloned into appropriately digested vector pCDNA3 (Invitrogen), creating pCDhCAR1, and verified by sequencing.

The CYP3A7 proximal promoter (~869 to +50) was amplified from human genomic DNA by PCR with oligonucleotides 5'-GGG TTA CAT CCT CAT GGC TGT CCT TT3' and 5'-ACA AGG TTC CCT TCT GGT GCG TCT TG3', which were designed with the PrimerExpress software (Applied Biosystems). The PCR products were purified using the QIAQuick gel extraction kit (QIAGEN Inc.) and digested with the restriction endonuclease SspI. The wild-type allele fragment has two SspI sites (positions 110734/110739 and 112353/112358), resulting in three fragments of 1617, 585, and 266 bp. In contrast, in the CYP3A7*1C allele, the site at position 110734/110739 is destroyed through mutation of the T at position 110739 to G corresponding to position –167 of the CYP3A7 promoter sequence. The two fragments have sizes of 2202 and 266 bp, respectively (see also Fig. 2).

RNA Analysis and Real-time PCR—Total RNA was prepared from human liver and intestine samples using the RNaseasy kit (QIAGEN Inc.) according to the recommendations of the manufacturer. RNA was treated with DNase I to remove contaminating genomic DNA. First-strand cDNA was generated from 0.5 to 1 μg of total RNA using random hexamer primers and TaqMan reverse transcription reagents (Applied Biosystems) according to the standard protocol of the manufacturer. PCRs were set up with cDNA corresponding to 35 or 40 ng of total RNA for liver or intestine samples, respectively, and the TaqMan Universal PCR Mastermix (Applied Biosystems). Primers were used at a final concentration of 300 nM. Oligonucleotides for CYP3A7, CYP3A4, and CYP3A5, or of a plasmid containing CYP3A7 cDNA were used to create the calibration curve. Oligonucleotides used for CYP3A4 were as follows: primers 5'-ATG AGG AAC TAT TGG AGG TTT GAC A-3' and 5'-ATC ATG AGA GGC CCA CAG AAG-3', and probe 5'-TAT TTC TGA TGG TCA ACA GAC TAT GCT GCC TAT C-3'. The specificity of the assay was determined using in vitro transcribed CYP3A4, CYP3A5, and CYP3A34 RNAs. Serial dilutions of in vitro transcribed CYP3A7 or of a plasmid containing CYP3A7 cDNA were used to create the calibration curve. Oligonucleotides used for CYP3A4 were as follows: primers 5'-CTC ACG TGG GCT TCC TCT ATC TAT-3' and 5'-AAG CCT TTA TGG TAG GAC AAA ATA TTT-3', and probe 5'-TCT AGG GCC CAC ACC TCT GCC T-3'. The specificity of the assay was determined using in vitro transcribed CYP3A5, CYP3A7, and CYP3A34. Serial dilutions of in vitro transcribed CYP3A4 were used to create the calibration curve. Oligonucleotides used for CYP3A7 were as follows: primers 5'-CTG CCA GCC ATC TTA GGG ACT GG-3', and 5'-GTT AGC ATT GGA CAC GTC CAC TCT TTT-3', and probe 5'-CCA GAT CAT TGC TGA GGT CAG CAC-3'. To create the calibration curve for the villin assay, serial dilutions of Caco-2 TCT cDNA were used. The expression levels of CYP3A7 and CYP3A4 in liver samples were normalized with respect to the expression of 18 S rRNA, as determined using the TaqMan ribosomal RNA control reagents (Applied Biosystems) and serial dilutions of HepG2 cDNA for the calibration curve. CYP3A7 and CYP3A4 expression levels in intestine samples were normalized with respect to the expression levels of villin.

Statistical Analysis—CYP3A7 expression data were tested for gaussian distribution by calculating a probability plot with Statgraphics Plus Version 2.0 for Windows (Statistical Graphics Corp.). The two-tailed Mann-Whitney U test was used to analyze for statistically significant associations between CYP3A7 expression and CYP3A7 genotype. These analyses were performed using GraphPAD InStat Version 3.05 for Windows 95.

Plasmid constructs—The GenBank accessions of the constructs were 16884400 for full length constructs of the CYP3A7 promoter and 16884401 for the CYP3A7 proximal promoter.

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...translated system (Promega). Oligonucleotides for the Freiburg, Germany) and the TNT T7 Quick Coupled transcription/...cultur...es of the ER6 motif of CYP3A7 into the corresponding ER6 motif of CYP3A4 (TGAACCTCAAAGGAGGCTCA, mutated bases in boldface italics), were introduced into the CYP3A7 construct, creating CYP3A7(ER6A4). This construct was further mutated. First, the mutation at position −188 of CYP3A7*1C was introduced, creating CYP3A7*1A4: −188,ER6. Second, the mutation at position −129 of CYP3A7*1C was introduced, creating CYP3A7(A4: −129). Third, the mutations at positions −181, −179, and −178 of CYP3A7*1C were introduced, creating CYP3A7(A4: −3B,ER6). The CYP3A7(A4: −185,ER6) construct was further mutated by introducing the mutations at positions −181, −179, and −178 of CYP3A7*1C, thus creating CYP3A7*(1A7: −129). The introduction of all the mutations and the absence of other, undesired mutations were verified by sequencing.

To create a CYP3A4 promoter reporter gene comparable in size to the CYP3A7 constructs, unidirectional deletion of pGL3-CYP3A4(-1105) (18) was performed with the double-stranded nested deletion kit (Amersham Biosciences). Sequencing identified a clone encompassing the CYP3A4 promoter region from positions −574 to +51, pGL3-CYP3A4(−574/ +51), further referred to as the CYP3A4 construct. Mutations of the ER6 element of the CYP3A4 promoter was done by sequential PCR steps according to standard procedures and converting the ER6 motif into the corresponding ER6 motif of CYP3A7 (TTAATCAATG-GAGGTCA, mutated bases in boldface italics), thus creating CYP3A4(ER6A7). The CYP3A4 construct was further mutated using the QuikChange site-directed mutagenesis kit (Stratagene) according to the recommendations of the manufacturer. The single base pair mutations constituting the CYP3A7*1C allele were introduced either alone or in combination. Thus, the mutations −181, −179, and −178, which convert the ER6 motif of CYP3A7 into the corresponding ER6 motif of CYP3A4 (TGAACCTCAAAGGAGGCTCA, mutated bases in boldface italics), were introduced into the CYP3A7 construct, creating CYP3A7(ER6A4). This construct was further mutated. First, the mutation at position −188 of CYP3A7*1C was introduced, creating CYP3A7*1A4: −188,ER6. Second, the mutation at position −129 of CYP3A7*1C was introduced, creating CYP3A7(A4: −129). Third, the mutations at positions −181, −179, and −178 of CYP3A7*1C were introduced, creating CYP3A7(A4: −3B,ER6). The CYP3A7(A4: −185,ER6) construct was further mutated by introducing the mutations at positions −181, −179, and −178 of CYP3A7*1C, thus creating CYP3A7*(1A7: −129). The introduction of all the mutations and the absence of other undesired mutations were verified by sequencing.

Electrophoretic Mobility Shift Assays—Electrophoretic mobility shift assays were performed as previously described (17). The human PXR, CAR, and RXRα proteins were synthesized using the using expression plasmids pCBhPXR (17), pCBhCAR1, and pCMX-hRXRα (kindly provided by R. Schuler, Klinik fuer Tumorbiologie, University of Freiburg, Germany) and the TNT T7 Quick Coupled transcription/translation system (Promega). Oligonucleotides for the MDR1 −7.8-kb enhancer nuclear receptor response element DR1(−4) (wild-type) were as described (17). Oligonucleotides for the CYP3A4 and CYP3A7 proximal ER6 PXR-binding sites were as follows: ER6-A4 sense, 5′−GAT CCA ATA TTA ACT CAA TGG AGG TCA GTG A−3′; ER6-A4 antisense, 5′−GAT CTC ACT GAC CTC CTT TGA GGT CAT GAT A−3′; ER6−3A7 sense, 5′−GAT CCA ATA TTA ACT CAA TGG AGG TCA GTG A−3′; and ER6−3A7 antisense, 5′−GAT CTC ACT GAC CTC CTT TGA GGT CAT GAT A−3′. Retarded complexes were quantified with the BAS1800 II phosphor-storage scanner (Fuji) and AIDA software (Raytest, Straubenhardt, Germany).

CYP3A7 is polymorphically expressed in adult livers and significantly contributes to hepatic CYP3A expression—CYP3A7 mRNA expression was analyzed in a total of 127 liver samples obtained from adult individuals. CYP3A7 was expressed in every liver. However, interindividual variability of CYP3A7 expression was very pronounced, showing a >700-fold difference (Table I). Statistical analysis demonstrated a clear deviation from gaussian distribution, with a distinct subgroup of 11% of livers that expressed >25,000 (log10 4.4) transcripts/ng of total RNA (Fig. 1, A and B). To compare the expression of CYP3A7 with that of CYP3A4, we analyzed CYP3A4 expression in the same samples (Table I). On average, CYP3A7 transcripts accounted for only 1.5% of combined CYP3A4 and CYP3A7 transcripts. However, in the 11% of livers expressing >25,000 CYP3A7 transcripts/ng of total RNA, its contribution was 7.7 ± 5.1% (median of 6.4% and range of 2.4−20.7%). In contrast, the corresponding value was 1.2 ± 2.0% (median of 0.46% and range of 0.01−14.5%) in the 89% of livers expressing lower levels of CYP3A7. The difference between the two groups was statistically significant (Mann-Whitney U test, p < 0.001). CYP3A7*1C and CYP3A7*1B are associated with high hepatic CYP3A7 expression—Recently, several single nucleotide polymorphisms have been described in the CYP3A7 proximal promoter region, and three alleles (CYP3A7*1B, CYP3A7*1C, and CYP3A7*1D) were defined in Caucasians (11). To investigate the role of these alleles in CYP3A7 expression, we sequenced the proximal promoter region of CYP3A7, encompassing these alleles, in the subjects of the liver bank. The results, presented in Table II, were in accordance with the previously published allele frequencies (11). A restriction fragment length polymorphism assay (Fig. 2) was used to confirm the sequencing data regarding the CYP3A7*1C allele. Subsequently, this assay was used to screen for the presence of this allele. CYP3A7 expression was significantly increased in the livers of all subjects heterozygous or homozygous for the CYP3A7*1C allele (Mann-Whitney U test, p < 0.001) (Fig. 3). The CYP3A7*1B allele was detected in two subjects, and it was also significantly associated with high CYP3A7 expression (Mann-Whitney U test, p < 0.05) (Fig. 3). A third CYP3A7*1B heterozygote was present in the study group from which we obtained intestine and liver samples. This subject also showed strongly increased CYP3A7 expression in the liver (Fig. 4B). In contrast, CYP3A7*1D was not associated with high CYP3A7 expression (data not shown). CYP3A7*1B, CYP3A7*1C, and CYP3A7*1D were never found together in any subject investigated. With the exception of one CYP3A7*1C heterozygote, all subjects with the CYP3A7*1C or CYP3A7*1B allele belonged to the group expressing CYP3A7*1C or CYP3A7*1B allele belonged to the group expressing...
CYP3A7*1C Is a Predictor of High CYP3A7 Expression in Adult Intestine—The expression of CYP3A7 was studied in 23 human intestine samples. Because the samples were derived from surgical specimens containing variable amounts of epithelial enterocytes, CYP3A7 expression was normalized using the expression of the epithelium-specific gene villin (21). Most intestine samples expressed very low amounts of CYP3A7 (71 ± 178, range of 3–679 copies/ng of total RNA). However, two intestine specimens showed 34- and 38-fold more CYP3A7 transcripts, respectively, than the mean of all the other samples (p < 0.01) (Fig. 4A). They were obtained from the two subjects heterozygous for the CYP3A7*1C allele in this study group. To elucidate whether CYP3A7 was co-regulated in liver and intestine, we analyzed CYP3A7 expression in the liver samples matching 15 of these intestine samples. The expression of hepatic CYP3A7 was increased in the two subjects who were heterozygous for either CYP3A7*1B or CYP3A7*1C as well as in one additional subject (Fig. 4B). No matching liver sample was available from the second CYP3A7*1C heterozygote. Thus, in contrast to liver, high intestinal CYP3A7 expression was exclusively associated with the presence of the CYP3A7*1C allele.

TABLE II

| Genotype | Individuals | Heterozygous | Homozygous | Allele Frequency |
|----------|-------------|--------------|------------|-----------------|
| CYP3A7*1B | 104         | 2            | 0          | 0.96            |
| CYP3A7*1C | 127         | 7            | 1          | 3.54            |
| CYP3A7*1D | 104         | 2            | 0          | 0.96            |

FIG. 1. A distinct subset of adult human livers exhibit high CYP2A7 expression. CYP2A7 expression was analyzed in adult human livers (n = 127). The CYP2A7 expression data were normalized with respect to the expression of 18 S rRNA and log 10-transformed. A, probability plot for CYP2A7 expression data. The frequencies of log 10-transformed CYP2A7 expression data were transformed to probits. The plot includes a least square regression line for comparison. B, frequency distribution of log 10-transformed CYP2A7 expression. Closed arrows indicate subjects homozygous or heterozygous for CYP3A7*1C; open arrows denote subjects heterozygous for CYP3A7*1B.

FIG. 2. Restriction fragment length polymorphism assay for detection of the CYP3A7*1C allele. The scheme shows the genomic region of CYP3A7 amplified by PCR. The sizes (in bp) of fragments derived by digestion with SspI (S) are shown for the CYP3A7 wild-type allele (wt) and the CYP3A7*1C allele (*1C). The gel shows an electrophoretic separation of SspI digests of DNA samples from subjects homozygous for the CYP3A7 wild-type allele (wt/wt), heterozygous for the CYP3A7*1C allele (wt/*1C), and homozygous for the CYP3A7*1C allele (*1C/*1C). The flanking lanes show size markers; the numbers indicated are sizes in bp.

FIG. 3. CYP3A7*1C and CYP3A7*1B are associated with high CYP3A7 expression in adult human livers. Genotypes are indicated as described in the legend to Fig. 2. wt/*1B indicates subjects heterozygous for CYP3A7*1B. CYP3A7 expression is presented in box and whisker diagrams. Minimum, maximum, and median values (in the box) are shown as lines; the box shows the 25–75th percentile. “Non-CYP3A7*1B” and “non-CYP3A7*1C” subjects with increased CYP3A7 expression (>25,000 transcripts/ng of total RNA) are depicted individually as open circles. Differences were tested for statistical significance using the Mann-Whitney U test (*, p < 0.05; ***, p < 0.001). Non-CYP3A7*1B and non-CYP3A7*1C subjects with increased CYP3A7 expression (>25,000 transcripts/ng of total RNA) were included in the Mann-Whitney U test.
**Fig. 4. Impact of CYP3A7 promoter alleles on intestinal versus hepatic CYP3A7 expression.** A, the distribution of intestinal CYP3A7 expression is presented in box and whisker diagrams as described in the legend to Fig. 3. CYP3A7 expression values were normalized with respect to the expression of villin. The statistical significance of the association between CYP3A7 expression and the presence of the CYP3A7*1C allele was analyzed by the Mann-Whitney U test (**, p < 0.01). wt/wt, homozygous for the CYP2A7 wild-type allele; wt/*1C, heterozygous for the CYP3A7*1C allele. B, shown is CYP3A7 expression in matched liver and intestine samples. CYP3A7 expression values are given as copies of CYP3A7 mRNA/ng of total RNA. Liver expression data (gray bars, left y axis) were normalized with respect to the expression of 18 S rRNA; expression values given for intestines (black bars, right y axis) were normalized with respect to the expression of villin. Letters indicate the donors of the sample pairs. CYP3A7*1B and CYP3A7*1C heterozygotes are marked with *1B and *1C, respectively.

**Mutation of the ER6 Motif Is Responsible for PXR-dependent Transcriptional Activation of CYP3A7*1C—**Because CYP3A7*1C was detected in half of the subjects highly expressing CYP3A7 and was the only allele associated with high CYP3A7 expression in both liver and intestine, we attempted to elucidate the mechanism underlying its effect. In the CYP3A7*1C allele, 60 bp of the CYP3A7 promoter (~188 to ~129) are replaced by the corresponding region of CYP3A4 (11). This replacement results in a difference of 7 bases between the CYP3A7 wild-type and CYP3A7*1C alleles. Two bases alter the proximal ER6 element found in all CYP3A4 gene promoters (Fig. 5). The nuclear receptor PXR binds to the proximal ER6 motif (22). Consequently, in CYP3A7*1C, the proximal ER6 PXR-binding site of CYP3A7 is replaced by the corresponding ER6 motif of CYP3A4 (Fig. 5). CYP3A4 is highly expressed in adult liver and small intestine, and PXR has been described to be a key regulator for CYP3A4 expression. Therefore, we hypothesized that the presence of CYP3A4-ER6 in CYP3A7*1C could be responsible for the increased expression of CYP3A7 in individuals carrying the CYP3A7*1C allele. Fig. 6A demonstrates a markedly stronger binding of PXR/RXR heterodimers to the ER6 motif of CYP3A4 than to the ER6 motif of CYP3A7. This result suggested that the high CYP3A7 expression, associated with the CYP3A7*1C allele, may indeed be caused by the higher affinity of binding of PXR for CYP3A4-ER6 compared with CYP3A7-ER6 and consequently by a higher transactivation potential of PXR at the CYP3A7*1C promoter.

**Fig. 5. Proximal promoter region mutated in the CYP3A7*1C allele and hypothesized consequences for gene expression.** In the CYP3A7*1C promoter allele, the region between positions ~188 and ~129 is replaced by the corresponding region of CYP3A4. Bases different between CYP3A7 and CYP3A4 in the region replaced are depicted as open circles. Numbering is with respect to the transcriptional start site. Also indicated is the localization of the ER6 element. The size of the arrows indicates the hypothesized strength of gene expression in adults, depending on the presence of CYP3A4-ER6. Open ellipse, the ER6 motif of CYP3A7; closed ellipses, the ER6 motif of CYP3A4.
CYP3A7 promoter and their identity in CYP3A7 versus CYP3A7*1C and CYP3A4. The G > T mutation at position –188 significantly contributed to CAR-dependent activation of CYP3A7*1C. Combining this mutation with CYP3A4-ER6 resulted in a significant increase in CAR-dependent transactivation compared with the construct containing only CYP3A4-ER6 (Fig. 8B, constructs 1 and 2). However, the full extent of CAR-mediated activation of a CYP3A7*1C reporter (Fig. 8B, construct 6) was not yet achieved. Mutation of –129A > C or –181T > A/–179T > C/–178A > T, each combined with CYP3A4-ER6, did not show any significant increase in activation by CAR compared with the CYP3A7 reporter gene containing only CYP3A4-ER6. In contrast, combining mutation –188G > T, mutations –181T > A/–179T > C/–178A > T, and CYP3A4-ER6 conferred a CAR-dependent activation on the CYP3A7 reporter, which was almost identical to that observed with the CYP3A7*1C reporter (Fig. 8B, constructs 5 and 6). Consequently, activation by CAR depended not only on the ER6 CAR-binding site, but also on neighboring nucleotides. Nucleotide T at position –188 seemed to be of special importance, as it had the strongest effect on CAR-mediated activation of all mutations tested together with CYP3A4-ER6. At least one of the mutations, –181T > A/–179T > C/–178A > T, also contributed to CAR-mediated activation, but only in the presence of –188G > T and CYP3A4-ER6. The importance of nucleotide T at position –188 is further highlighted by the results presented in the lower part of Fig. 8B. Mutation of –188T > G in the CYP3A4
promoter reduced CAR-dependent activation by ~80%. In conclusion, we have identified specific sequence features responsible for differences in the activation potential by PXR and CAR. In contrast to PXR, several additional sites in the promoter sequence, besides the ER6 motif, are required for CAR-mediated activation of CYP3A4 and CYP3A7*1C.

**DISCUSSION**

In this study, we have described the polymorphic expression of CYP3A7 in adult human liver and intestine and analyzed the functional consequences of two promoter alleles for the expression of the gene. CYP3A7 mRNA was found in all livers, but showed pronounced interindividual variability. There was a non-gaussian distribution, with 11% of livers expressing >25,000 transcripts/ng of total RNA. Because at the present time no CYP3A7-specific antibody is available, expression at the protein level could not be studied. Nearly two-thirds of the subjects in the subgroup with hepatic expression of >25,000 transcripts of CYP3A7 mRNA/ng of total RNA carried the CYP3A7*1C allele or, less frequently, the CYP3A7*1B allele. The mechanism of the increased CYP3A7 expression in the subjects without these alleles remains to be elucidated, but it could involve additional genetic markers or induction by xenobiotics. Only one subject in this group had been treated with nifedipine, which is a known inducer of CYP3A4 expression in vitro (24). However, the expression of CYP3A4 in the corresponding liver was low (data not shown). Thus, it is very likely that the high expression of CYP3A7 in subjects without the CYP3A7*1C or CYP3A7*1B allele is caused by yet unknown genetic variants rather than by induction, but this remains to be verified experimentally. Induction can be ruled out as the mechanism of high CYP3A7 expression in the subjects carrying CYP3A7*1C or CYP3A7*1B because none of these patients was treated with known CYP3A inducers.

The increased CYP3A7 expression observed in ~11% of adult human livers could have consequences for drug biotransformation. For a number of drugs, data on substrate affinity and specificity using cDNA-expressed CYP3A4, CYP3A5, and CYP3A7 demonstrate similarity (25). On the other hand, there are also examples of pronounced differences in substrate affinity among CYP3A isoforms, including CYP3A7 (26, 27). For example, CYP3A7 metabolizes retinoic acid 25 times more efficiently than CYP3A4 (26). Based on the expression levels of CYP3A7 in the subjects of the high expression subgroup, which come up to 20% of the combined CYP3A4 and CYP3A7 pool, CYP3A7 could contribute to up to 80% of total biotransformation of retinoic acid. Thus, the polymorphic expression of CYP3A7 in adult human livers could be responsible for part of the variability of CYP3A activity among individuals.

In contrast to previous studies that did not detect CYP3A7 expression in fetal and adult human intestine (28–30), we demonstrated CYP3A7 expression in every intestine sample investigated. This is in all probability due to the more sensitive method used. However, high amounts of CYP3A7 transcripts were present only in intestine samples of subjects with the CYP3A7*1C allele. This observation demonstrates differences in the regulation of CYP3A7 between liver and intestine. In addition to the CYP3A7*1C-dependent regulation, further mechanisms responsible for high CYP3A7 expression (such as CYP3A7*1B-dependent regulation) obviously exist in liver. However, half of the subjects from whom we obtained intestine samples, including the CYP3A7*1C and CYP3A7*1B heterozygotes, were treated with known inducers of CYP3A4 (omeprazole, nifedipine, reserpine, or St. John’s wort). Because the CYP3A7*1C heterozygotes showed only low intestinal CYP3A4 expression, it is unlikely that their high intestinal CYP3A7 expression was caused by induction. This assumption is further supported by a lack of association between treatment with
inducers and CYP3A7 expression (data not shown) and the tissue-specific effects of the two alleles determining CYP3A7 expression. In addition, reserpine, which was administered to one CYP3A7*1C heterozygote, did not induce CYP3A7 expression in an intestinal cell line, whereas CYP3A4 was induced by reserpine in the same cell line (31).

Furthermore, we have provided a mechanistic explanation for the increased expression of CYP3A7 in the individuals carrying the more frequent marker of the CYP3A7 polymorphism, the CYP3A7*1C allele. The CYP3A7*1C mutation has arisen through replacement of part of the CYP3A7 promoter by the corresponding region of CYP3A4. This led to the substitution of CYP3A7-ER6 for CYP3A4-ER6. The ER6 motif in the proximal promoter of the CYP3A4 gene is one of the two elements mediating PXR-dependent activation. The second element is the distal xenobiotic-responsive enhancer module (32). Two bases are different between the proximal ER6 motifs of CYP3A4 and CYP3A7 (33). We showed that the presence of CYP3A4-ER6 was responsible for the high expression of CYP3A7 in carriers of the CYP3A7*1C allele. PXR exhibited stronger binding to CYP3A4-ER6 than to CYP3A7-ER6. However, in contrast to the results presented by Pascussi et al. (33), this differential binding also resulted in a dramatic difference in the PXR-mediated transactivation of CYP3A4 and CYP3A7 promoters. The discrepancy is most likely due to differences between the natural CYP3A7 promoter used in our study and the reporter gene containing multimerized CYP3A7-ER6 motifs used by Pascussi et al. (33). Our data clearly demonstrate that the proximal ER6 motifs of CYP3A4 and CYP3A7 are functionally different with respect to binding and activation by PXR. This difference appears to be the mechanism of the CYP3A7*1C effect on CYP3A7 expression.

PXR is not the only transcription factor binding to the proximal ER6 motif of CYP3A4 and activating its transcription. Recently, it has been shown that CAR and vitamin D receptor, which belong to the same subfamily of nuclear receptors (NR11) as PXR, also regulate CYP3A4 expression (6, 7, 23). Similarly to PXR, CAR showed stronger binding to CYP3A4-ER6 than to CYP3A7-ER6 and activated CYP3A4 and CYP3A7*1C promoters. But in contrast to PXR, the presence of CYP3A4-ER6, although necessary, was not sufficient for CAR activation. Additional single base mutations of the CYP3A7*1C allele proved to be necessary. We identified position –188 as the most crucial one, but also positions –181, –179, and –178 were involved in the activation by CAR. The –188G>T mutation in CYP3A7*1C creates a putative HNF-3-binding site, whereas mutations of positions –181, –179, and –178 are not located within any of the known transcription factor-binding sites (34). The –129A>C mutation in CYP3A7*1C destroys a functional HNF-3-binding site of the CYP3A7 promoter (35) and creates a putative octamer motif (34). The strong dependence of CAR-mediated activation on position –188 and, to a more limited extent, on at least one of positions –181, –179, and –178 probably reflects functional interactions between CAR and other transcription factors binding to these sequences. The nature of these interactions and the role of HNF-3 have to be elucidated in future studies.

The involvement of PXR in the induction of CYP3A4 genes is established, but data on its role in the regulation of constitutive CYP3A4 expression are less convincing. A targeted deletion of PXR did not alter the constitutive expression of CYP3A4 in one strain of mice (36), whereas it resulted in a 3-fold reduction of CYP3A4 expression in another mouse strain (37). The data presented in our study support the hypothesis that nuclear receptors binding to the proximal ER6 motif may play a role in the constitutive expression of CYP3A. The described CYP3A7*1C-dependent mechanism of high CYP3A7 expression is based on functional differences between the proximal ER6 motifs of CYP3A4 and CYP3A7. The proximal ER6 motif of CYP3A4, but not that of CYP3A7, mediates activation by the nuclear receptors PXR and CAR. Therefore, the induction of CYP3A7 observed in most, although not all, primary adult hepatocyte cultures and hepatoma cell lines after treatment with the PXR ligands rifampin, clotrimazole, and RU486 (15, 33) could be mediated by the recently described xenobiotic-responsive enhancer module in the CYP3A7 far upstream regulatory region that is functionally conserved between CYP3A4 and CYP3A7 (38).

No clues regarding the mechanism of action of the CYP3A7*1B allele can be derived from the sequence context of the single nucleotide polymorphism that constitutes the allele. The mechanism has to be clarified in future functional studies. In conclusion, this study provides clear evidence for an association of the CYP3A7*1B and/or CYP3A7*1C allele with high expression of CYP3A7 in adult liver and intestine. These alleles could serve as markers for the variable CYP3A activity. Furthermore, we identified functional differences between the ER6 motifs of CYP3A4 and CYP3A7 proximal promoters in binding and activation by the nuclear receptors PXR and CAR. Taken together, these results provide a mechanistic explanation for the increased CYP3A7 expression in CYP3A7*1C carriers. Whether the proximal ER6 motif and these nuclear receptors participate also in the developmental switch from CYP3A7 to CYP3A4 expression in liver, occurring immediately after birth (39), remains to be elucidated in future studies.

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Note Added in Proof—The high prevalence of CYP3A7*1C alleles in subjects expressing high levels of CYP3A7 transcripts is in agreement with expression data presented together with the original description of this allele (11).

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