Short Communication

The Effects of ABCB1 3’-Untranslated Region Variants on mRNA Stability

Received June 7, 2007; accepted October 15, 2007

ABSTRACT:

Genetic variation in ABCB1, encoding P-glycoprotein (P-gp), is a potential cause of interindividual variation in drug response. Numerous studies have focused on the effects of coding region variants on P-gp expression and function, whereas few noncoding region variants have been investigated. The 3’-untranslated region (UTR) regulates mRNA levels or stability via RNA-protein interactions with mRNA degradation machinery. mRNA stability is a key regulatory step controlling ABCB1 mRNA expression that ultimately affects P-gp levels and function. We hypothesized that ABCB1 3’-UTR polymorphisms alter mRNA stability by disrupting RNA-protein interactions. An ethnically diverse panel of DNA samples was sequenced to identify 3’-UTR polymorphisms and determine allele frequencies. The three most common variants, along with reference ABCB1, were stably expressed in cells in order to measure mRNA half-life. The calculated half-life for ABCB1 reference in HEK293 cells was 9.4 ± 1.3 h and was similar to that estimated for the 3’-UTR variants. Endogenous ABCB1 mRNA decay was similar in lymphoblastoid cell lines carrying 3’-UTR variant and reference alleles. Although the examined ABCB1 3’-UTR variants have no effect on ABCB1 mRNA stability, these data represent one of the first attempts to determine the influence of genetic variation in UTRs on ABCB1 mRNA levels.

P-glycoprotein (P-gp) is a member of the ATP-binding cassette (ABC) transporter superfamily and its physiological role is to remove intracellular compounds via energy-dependent efflux. The distribution of P-gp in various barrier and excretory tissues can hinder drug therapy because many different types of drugs are P-gp substrates (Litman et al., 2001; Szakacs et al., 2006). Furthermore, variability in drug pharmacokinetics is widely observed for P-gp substrates, suggesting that there are interindividual differences in P-gp expression and function (Eap et al., 2002; Goh et al., 2002; Urquhart et al., 2007). The kinetics of P-gp function are dependent upon transport activity and the abundance of P-gp molecules. It is generally accepted that the structure of P-gp, encoded by the ABCB1 gene, regulates P-gp transport function. However, the mechanisms that control membrane P-gp expression levels are expected to be distinct from those that control transport activity.

The level of ABCB1 mRNA expression is an important determinant of P-gp expression levels. Interindividual differences in ABCB1 mRNA expression levels observed in the liver (Schuetz et al., 1995), small intestine (Moriya et al., 2002; Nakamura et al., 2002; Lindell et al., 2003), and kidney (Uwai et al., 2004; Owen et al., 2005; Haensch et al., 2007). An in vitro study measured different mRNA stabilities between the C and T alleles of 3435 (Wang et al., 2005), so it is possible that this variant causes changes at the cellular level that may extend to clinical phenotypes.

Mechanisms that alter mRNA levels can change P-gp expression and potentially P-gp transport activity. Steady-state ABCB1 mRNA levels reflect the balance between gene transcription (i.e., synthesis) and mRNA stability (i.e., decay). Recent evidence has demonstrated that the 3’-UTR of mRNA is an important regulatory site controlling interactions with mRNA degradation machinery (Hollams et al., 2002; Tourriere et al., 2002; Mangus et al., 2003; Wilkie et al., 2003). 3’-UTR RNA-binding proteins that recognize specific mRNA sequence elements and secondary structure dictate the fate of mRNA transcripts. Polymorphisms in the 3’-UTR of ABCB1 could disrupt RNA-protein interactions, resulting in altered mRNA stability.

The current studies were designed to test whether polymorphisms in the 3’-UTR of ABCB1 affected mRNA stability. ABCB1 3’-UTR polymorphisms were identified in an ethnically diverse panel of DNA samples. Stable cell lines expressing ABCB1 reference and the three most common 3’-UTR variants were established in order to measure the rate of ABCB1 mRNA decay after cessation of transcription. The estimated mRNA half-life served as a measure of ABCB1 mRNA stability.

Materials and Methods

Materials. TRIzol, Zeocin, hygromycin, the pcDNA5/FRT and pOG44 plasmids, and HEK293 Flp-In (Flp293) cells were obtained from Invitrogen (Carlsbad, CA). Actinomycin D (Sigma-Aldrich, St. Louis, MO) was dissolved with respect to mRNA levels. It was initially linked with increased mRNA expression (Moriya et al., 2002); however, data from subsequent studies are not in agreement (Siegmund et al., 2002; Uwai et al., 2004; Owen et al., 2005; Haensch et al., 2007). An in vitro study measured different mRNA stabilities between the C and T alleles of 3435 (Wang et al., 2005), so it is possible that this variant causes changes at the cellular level that may extend to clinical phenotypes.

Mechanisms that alter mRNA levels can change P-gp expression and potentially P-gp transport activity. Steady-state ABCB1 mRNA levels reflect the balance between gene transcription (i.e., synthesis) and mRNA stability (i.e., decay). Recent evidence has demonstrated that the 3’-UTR of mRNA is an important regulatory site controlling interactions with mRNA degradation machinery (Hollams et al., 2002; Tourriere et al., 2002; Mangus et al., 2003; Wilkie et al., 2003). 3’-UTR RNA-binding proteins that recognize specific mRNA sequence elements and secondary structure dictate the fate of mRNA transcripts. Polymorphisms in the 3’-UTR of ABCB1 could disrupt RNA-protein interactions, resulting in altered mRNA stability.

The current studies were designed to test whether polymorphisms in the 3’-UTR of ABCB1 affected mRNA stability. ABCB1 3’-UTR polymorphisms were identified in an ethnically diverse panel of DNA samples. Stable cell lines expressing ABCB1 reference and the three most common 3’-UTR variants were established in order to measure the rate of ABCB1 mRNA decay after cessation of transcription. The estimated mRNA half-life served as a measure of ABCB1 mRNA stability.

Materials and Methods

Materials. TRIzol, Zeocin, hygromycin, the pcDNA5/FRT and pOG44 plasmids, and HEK293 Flp-In (Flp293) cells were obtained from Invitrogen (Carlsbad, CA). Actinomycin D (Sigma-Aldrich, St. Louis, MO) was dissolved

ABBREVIATIONS: P-gp, P-glycoprotein; UTR, untranslated region; ABC, ATP binding cassette; Flp293, HEK293 Flp-In; SNP, single-nucleotide polymorphism; PCR, polymerase chain reaction; IRES, internal ribosome entry site; AU, adenine-uracil.
in 100% dimethyl sulfoxide and stored at −20°C. Marine IgG2a MRK16 antibody was obtained from Kamiya Biomedical Co. (Seattle, WA) and goat anti-mouse IgG allophycocyanin from Invitrogen. Cell culture media and reagents were purchased from the University of California, San Francisco, Cell Culture Facility.

**Cell Culture.** Growth media for HEK293 Flp-In cells contained Dulbecco’s modified Eagle’s with high glucose (4.5 g/l) supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin, and either 100 µg/ml Zeocin (parental cells) or 75 µg/ml hygromycin (ABCB1 stable cells). Human lymphoblast cell lines (GM12814, GM12851, GM17102, GM12892, GM18506, and GM18562) were obtained from the Coriell Cell Repository and maintained in suspension with RPMI 1640 medium supplemented with 15% fetal bovine serum and 1% penicillin/streptomycin. All cell lines were passaged every 2 to 4 days in T25 (lymphoblasts) or T75 (HEK293) flasks and maintained in 5% CO₂ at 37°C.

**Identification of ABCB1 3’-UTR Variants.** Genomic DNA from 247 human samples from the Coriell Institute was used to identify 3’-UTR polymorphisms in ABCB1. Direct sequencing of the 3’-UTR was performed with forward and reverse primers (5’-GGTTTTCAGAATGGCAGATGCT-3’ and 5’-CTGTTAACCATTCCCTCCACAAA-3’), respectively) using previously reported methods (Kroetz et al., 2003; Lebman et al., 2003).

**ABCB1 Plasmids.** Full-length ABCB1 cDNA was subcloned into pcDNA5/FRT and used as a template to create 3’-UTR variant plasmids. The +193A>G variant was already present and was changed back to the reference nucleotide. Mutagenesis primers were designed for +89A>T (forward 5’-AACATCTAGAATTGTTAGGATCTGTT and reverse 5’-ACAAGATACCTTCTCAATAATTCTG ATGGT) and +146G>A (forward 5’-GTCTTGAGAGCTTCAATAATAGGAACAG and reverse 5’-CTGT TCTTTTAATATTAGGATCTGCTAGAC) and reversion of +193A>G (forward 5’-AAGTGAGGAAATTACATGTTTAAACTGCAT and reverse 5’-ATGCGATTTAATTAGTCTTCTCACC). The QuikChange Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA) with Pfu turbo polymerase was used according to the manufacturer’s protocol to introduce the desired variants. Direct sequencing verified the base changes.

**ABCB1 Flp293 Stable Cell Lines.** HEK293 Flp-In cells were seeded at 5 × 10⁵ cells/well in 6-well plates in medium without antibiotics. One day later, cells were transfected with 3.6 µg of p0G44, 0.4 µg of ABCB1/pcDNA5/FRT, and 6 µl of Lipofectamine 2000 in a total well volume of 2 ml. Fresh medium was added −5 h after transfection. The following day, cells were split into six new wells and, 2 to 3 h later, medium containing 75 µg/ml hygromycin was added. Drug selection lasted 10 to 14 days, and fresh hygromycin medium was added every 2 to 3 days. Surviving cell colonies were propagated and screened for surface P-gp expression by flow cytometry. P-gp positive clones were further screened for ABCB1 mRNA expression using TaqMan quantitative real-time PCR.

**ABCB1 mRNA Half-Life in Flp293 Stable Cells and Lymphoblast Cell Lines.** ABCB1 reference, +89A>T, +146G>A, and +193A>G Flp293 cell lines were seeded in 12-well plates at 6 × 10⁵ cells/well and allowed to grow for 24 h. For each cell line, triplicate wells were seeded for each time point. Medium containing 75 µg/ml actinomycin D was added to the wells, and cells were harvested over a 24-h period. Total RNA was isolated from each sample using TRizol and quantitated using UV spectrophotometry (NanoDrop Technologies, Wilmington, DE). Equal amounts of total RNA were reverse transcribed using Moloney murine leukemia virus reverse transcriptase (Promega, Madison, WI) according to the manufacturer’s instructions. TaqMan primers (forward 5’-TGTATTGCTGAAAAACCCCTTTT and reverse 5’-TCCAGGCTCAGCTCCTGGA) and probe (5’-6-FAM)-ACGGAGGCTCAAATGCGCAACATT(BHQ1) for ABCB1 were designed using Primer Express (Applied Biosystems, Foster City, CA) and were purchased from Integrated DNA Technologies (Coralville, IA). The 25-µl reaction volume contained 10× TaqMan Buffer (UCSF Genome Core, San Francisco, CA), 5.5 mM MgCl₂, 200 µM deoxynucleoside-5'-triphosphates (Promega, Madison, WI), 0.625 U AmpliTaq Gold (Applied Biosystems), 1 µl of cDNA, 500 nM forward and reverse primers, 200 nM probe, and PCR-grade H₂O. Reactions were run on an ABI Prism 7700 and cycling conditions were as follows: 12 min at 95°C followed by 45 cycles of 15 s at 95°C and 1 min at 60°C. The threshold limit was set so that it intersected all samples during the log-linear phase of amplification, and the corresponding CT values for each set of triplicates were normalized to the average t = 0 value using the equation for percentage of mRNA remaining: \[ \frac{\text{CT}_{t}}{\text{CT}_{0}} = 10^{\frac{t}{k}} \] + 100, where x equals time after t = 0 in hours. The decay slope (k) for percentage of mRNA versus time was determined using logarithmic data fitting (Excel; Microsoft, Redmond, WA). The half-life was calculated from the equation \( t_{1/2} = \ln(2)/k \). All experiments were carried out in triplicate.

For each lymphoblastoid cell line, cells were seeded in three wells of a six-well plate at 5 × 10⁵ cells/well in 4 ml of medium. After 24 h, actinomycin D was added to each well at a final concentration of 7.5 µg/ml and 0.5-ml aliquots were removed at each time point (0, 2, 4, 8, 12, and 24 h). Cell aliquots were pelleted, and TRizol was added to isolate total RNA. ABCB1 mRNA expression and ABCB1 mRNA half-life were determined as described above except that double the amount of RNA was reverse-transcribed, and double the volume of cDNA was used in each TaqMan reaction. The experiment was performed twice.

**Statistical Analysis.** Half-life values from three experiments for ABCB1 reference, +89A>T, +146G>A, and +193A>G Flp293 cell lines were averaged, and the standard deviation was calculated. Student’s t test was used to determine whether the mean half-lives for the variants were different from reference with an α value of 0.05.

**Results**

**ABCB1 3’-UTR Variants.** The 3’-UTR of ABCB1 was sequenced in 247 DNA samples from ethnically diverse populations from the Coriell Institute. Nine polymorphic sites were identified in this 380-base pair region with allele frequencies ranging from 0.5 to 14%. ABCB1 3’-UTR variants were chosen for study based on their relatively high frequency in any one ethnic group and occurrence as a single nucleotide substitution. There are three polymorphisms that meet these criteria and for which stable cell lines were generated: +89A>T, +146G>A, and +193A>G. The +89A>T variant is found in African Americans and Caucasians, whereas +146G>A is found only in African Americans (Table 1). ABCB1 +193A>G is a cosmopolitan polymorphism found in all four of the sampled ethnic populations.

**Genetic Analysis of the ABCB1 3’-UTR.** Little research has been done on the 3’-UTR of ABCB1, so predictive measures were used to ascertain the potential impact of ABCB1 +89A>T, +146G>A, and +193A>G on mRNA stability. Sequence alignments with other mammalian species using ClustalW (version 1.83) were used to compare the 3’-UTR of human ABCB1 with corresponding sequences from mouse, rat, and rhesus monkey. The sequences ranged in length from 350 to 401 base pairs, and 175 base positions were 100% identical compared with the human sequence (Fig. 1). Using the human sequence as a reference, the sequence similarity of these four species is 46%, and it increases to 90% if only human and rhesus monkey are compared. There are six 3’-UTR variant sites that are 100% identical in the three other species: +21, +89, +146, +193, +252, and +355. The other three variant sites are only conserved in rhesus monkey. The multiple species alignment also revealed contiguous sections of 100% conservation that could indicate functional importance, so the ABCB1 3’-UTR sequence was analyzed against a database of known 5’- and 3’-UTR sequence elements (Pesole and Liuni, 1999). An internal ribosome entry site (IRES) element, which enables translation initiation independent of the 5’ cap, was found between +282 and +380; however, IRES elements generally are located in the 5’-UTR (Pesole et al., 1999). No other potential RNA binding sites were identified using UTRscan (http://www.ba.ibr.cnrs.fr/UTR).

**mRNA Half-Life of ABCB1 3’-UTR Variants in Flp293 Stable Cells.** Stable cell lines expressing ABCB1 reference, +89A>T, +146G>A, and +193A>G were established using the Flp-In system, which is designed to insert a single copy of a gene at the same genomic location. The host Flp293 cells originate from HEK293 cells and have endogenous ABCB1 mRNA expression. However, the
ABC1 Flp293 reference, +89A>T, +146G>A, and +193A>G stable cell lines demonstrate significant overexpression of ABCB1. TaqMan real-time PCR determined ABCB1 mRNA levels increased ~80-fold, and P-gp surface expression quantitated by flow cytometry showed a ~25-fold increase compared with the host Flp293 cells. Additionally, the steady-state mRNA levels before actinomycin D exposure (t = 0) for the ABCB1 3′-UTR variants differed by no more than 15% of reference (n = 3; data not shown).

The mRNA stability of transcripts is commonly determined by measuring mRNA half-life. ABCB1 Flp293 reference, +89A>T, +146G>A, and +193A>G stable cell lines were exposed to actinomycin D to stop whole-genome transcription. The ABCB1 mRNA levels over a 24-h period were normalized to the t = 0 point to convert the data into percentage of mRNA remaining for each cell line. A plot of percentage of mRNA versus time shows the rate of ABCB1 mRNA decay, and one study hypothesized the AU-rich regions of the c-myc 3′-UTR showed that ABCB1 mRNA degradation is an important cellular mechanism controlling P-gp expression that ultimately affects apparent P-gp activity. The 3′-UTR of mRNA transcripts is considered the key regulatory region that interacts with mRNA degradation machinery. Protein binding sites and secondary structure in the 3′-UTR will either promote or repress mRNA degradation, resulting in changes to steady-state mRNA levels (Hollams et al., 2002; Tourriere et al., 2002; Mangus et al., 2003; Wilkie et al., 2003). Our studies established a stable expression system to test the hypothesis that ABCB1 3′-UTR variants alter mRNA stability. Standard methods for generating stable cell lines are unable to control for multiple plasmid insertion and transcriptional differences due to random genomic integration of the plasmid(s). The Flp-In system eliminated copy number and transcriptional differences due to random genomic integration of the plasmid(s). The numerous efforts to associate ABCB1 coding region SNPs with clinical phenotypes have not produced conclusive results. It is possible that unidentified SNPs in linkage disequilibrium with the commonly studied SNPs may be causative. To our knowledge, the +89A>T, +146G>A, and +193A>G variants have not been investigated and were chosen for study based on a high allele frequency in at least one ethnic group and a significant degree of evolutionary conservation at these sites. It is important for ABCB1 pharmacogenetic research to examine noncoding polymorphisms, especially in relation to their probable mechanistic effects in vitro.

Regulatory proteins bind to specific elements in the 3′-UTR and interact with RNA degradation machinery by promoting or repressing degradation (Tourriere et al., 2002). A common sequence motif containing adenine-uracil (AU) repeats is thought to confer rapid degradation, and one study hypothesized the AU-rich regions of the c-myc and ABCB1 3′-UTRs could destabilize β-globin mRNA. In HepG2 cells, c-myc, ABCB1, and β-globin mRNA have half-lives of 30 min, 8 h, and >24 h, respectively (Prokopcak et al., 1999). Chimeras containing the β-globin coding region with either the ABCB1 3′-UTR or the c-myc 3′-UTR showed that β-globin half-life was only decreased by c-myc (Prokopcak et al., 1999). It should be noted that our measured half-life for reference ABCB1 in HEK293 cells (~9.4 h) is very similar to that seen in HepG2 cells.

The mRNA stability of ABCB1 is a regulated process dependent on multiple factors, such as stress and cell type. The human leukemia cell line K562 is a disease-state model for studying mechanisms controlling P-gp overexpression due to drug exposure. ABCB1 mRNA half-life in K562 cells is 1 h, but short-term incubation with drugs, such as vinblastine and doxorubicin, increased the half-life to 10 h with no changes in transcriptional activity (Yague et al., 2003). Studies in rats have revealed that carcinogenesis in the liver can

### Table 1: Alleles and frequencies of ABCB1 3′-UTR polymorphisms

<table>
<thead>
<tr>
<th>Position</th>
<th>Alleles</th>
<th>NCBI SNP ID (Build 126)</th>
<th>Golden Path Position (3/06 Build)</th>
<th>Allele Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>+21</td>
<td>T&gt;C</td>
<td>rs28364275</td>
<td>chr7:86971474</td>
<td>AA (n = 100)</td>
</tr>
<tr>
<td>+77</td>
<td>ACTT&gt;A</td>
<td>rs2235052</td>
<td>chr7:86971418</td>
<td>CA (n = 100)</td>
</tr>
<tr>
<td>+89</td>
<td>A&gt;T</td>
<td>rs17064</td>
<td>chr7:86971406</td>
<td>AS (n = 30)</td>
</tr>
<tr>
<td>+146</td>
<td>G&gt;A</td>
<td>rs28364277</td>
<td>chr7:86971349</td>
<td>ME (n = 10)</td>
</tr>
<tr>
<td>+169</td>
<td>G&gt;GACAGAGA rs28364278</td>
<td>chr7:86971326</td>
<td>8.5 0 0 0</td>
<td></td>
</tr>
<tr>
<td>+193</td>
<td>A&gt;G</td>
<td>rs3842</td>
<td>chr7:86971302</td>
<td>13 14 15 5</td>
</tr>
<tr>
<td>+232</td>
<td>A&gt;C</td>
<td>rs28364279</td>
<td>chr7:86971243</td>
<td>4.5 0 0 0</td>
</tr>
<tr>
<td>+316</td>
<td>G&gt;A</td>
<td>rs28364280</td>
<td>chr7:86971179</td>
<td>3 0 0 0</td>
</tr>
<tr>
<td>+355</td>
<td>T&gt;C</td>
<td>NA</td>
<td>chr7:86971140</td>
<td>0 0 1.7 0</td>
</tr>
</tbody>
</table>

AA, African American; AS, Asian American; CA, Caucasian American; ID, identification; ME, Mexican American; NCBI, National Center for Biotechnology Information.

SNP location is relative to the TGA stop codon.

GACAAGA rs28364278 chr7:86971326 9.5 0.5 0 0

AA, African American; AS, Asian American; CA, Caucasian American; ID, identification; ME, Mexican American; NCBI, National Center for Biotechnology Information.

SNP location is relative to the TGA stop codon.
modulate the mRNA stability of Pgp1–3 (human P-gp orthologs) by dramatically increasing their normally short half-life (2 versus 12 h) (Lee et al., 1998). Subsequent studies determined that the increased mRNA stability in rat liver tumors was associated with a decrease in P-gp mRNA fragments, indicating there was less mRNA degradation (Lee et al., 2005). Even in healthy organs free of stress, rat P-gp mRNA has tissue-specific half-lives that range from 2 to 12 h (Lee and Ling, 2003).

There are no literature reports discussing possible regulatory sites in the 3′/UTR of ABCB1. ClustalW was used to align the ABCB1 3′-UTRs of human, rhesus monkey, rat, and mouse. Variant positions are designated in the human sequence (underlined), and homology for each reference allele in all (dark highlight) or some (light highlight) species is shown. Complete homology for each base position is marked with an asterisk. UTRscan predicted an IRES element from +282 to +381, and it is shown in the human sequence with a dashed underline.

The stability of mRNA may be altered by 3′-UTR polymorphisms if recognition of specific mRNA sequence and secondary structure by regulatory proteins is disrupted (Shen et al., 1999; Hollams et al., 2002; Tourriere et al., 2002). A polymorphism in the 3′-UTR of

![Fig. 1. ABCB1 3′-UTR sequence alignment with selected mammalian species. ClustalW was used to align the ABCB1 3′-UTRs of human, rhesus monkey, rat, and mouse. Variant positions are designated in the human sequence (underlined), and homology for each reference allele in all (dark highlight) or some (light highlight) species is shown. Complete homology for each base position is marked with an asterisk. UTRscan predicted an IRES element from +282 to +381, and it is shown in the human sequence with a dashed underline.](image-url)
human tumor necrosis factor-α changes binding affinity for a multi-
protein complex that contains the HuR regulatory protein (Di Marco 
et al., 2001). HuR binds AU-rich elements in the 3′-UTR of certain
genes (Peng et al., 1998) and has been shown to stabilize mRNA 
taining tumor necrosis factor-α 3′-UTR sequence motifs (Dean et 
al., 2001). There is one report that the 3435C>T synonymous variant decreases mRNA stability (Wang et al., 2005), but to our knowledge no pharmacogenetic research of this type has been conducted for 
ABCB1 3′-UTR variants. Thus, our mRNA half-life data represent novel findings as to the effects of the 89A>T, 146G>A, and 193A>G polymorphisms have on ABCB1 mRNA stability and demonstrate the utility of using stable cell lines made with Flp-In technology for these measurements. Similarly, studies in lymphoblas-
toid cell lines carrying 3′-UTR variants suggest that decay of endog-
enous ABCB1 mRNA is not affected by these specific polymor-
phisms. Future mRNA stability experiments should investigate other

ABCB1 3′-UTR variants and/or haplotypes. Furthermore, the 5′-UTR may play a role in mRNA stability based on the closed-loop hypoth-
esis (Wilkie et al., 2003). The possible interactions between both UTRs in the cytosol suggest 5′-UTR polymorphisms could modify regulatory protein interactions that propagate to the 3′-UTR and affect mRNA stability.

Acknowledgments. We acknowledge the efforts of the University of California, San Francisco, Genomics Core Facility in the identifi-
cation of the 3′-UTR variants. Conrad C. Huang, Susan J. Johns, Michiko Kawamoto, and Doug Stryke were instrumental in the compi-
lation and display of the resequencing data.

The Department of Biopharmaceutical Sciences, JASON M. GOW University of California,  
San Francisco, California  
LESLIE W. CHENN  
DEANNA L. KROETZ

References
translated region of tumor necrosis factor alpha mRNA is a target of the mRNA-stabilizing factor HuR.


translated region of TNFα mRNA impairs binding of the post-transcriptional regulatory 

Eap CB, Buciuc T, and Baumann P (2002) Interindividual variability of the clinical pharma-
co-kinetics of methadone: implications for the treatment of opioid dependence. Clin Pharma-
cother 41:1153–1193.

al. (2002) Explaining interindividual variability of docetaxel pharmacokinetics and pharma-
3690.

RW, and Cascorbi I (2007) Influence of polymorphisms of ABCB1 and ABCG2 on mRNA and 


ABCB1 (MDR1, multidrug resistance transporter) gene. Pharmacogenetics 13:481– 
494.

Leibman MK, Huang CC, DeYoung J, Carlson EL, Taylor TR, de la Cruz M, Johns SJ, Stryke 
genes reveals evolutionary and functional constraints. Proc Natl Acad Sci U S A 100:5896– 
5901.


Lee CH and Ling V (2003) Superinduction of P-glycoprotein messenger RNA expression in vivo 


Litman T, Dudley TE, Stein WD, and Bates SE (2001) From MDR to MXT: new understanding of 
965.


Matys V, Fricke E, Geffers R, Gossling E, Haubrock M, Hehl R, Hornschild K, Karas D, Kel AE, 
Kel-Margoulis OV, et al. (2003) TRANSFAC: transcriptional regulation, from patterns to 

Moriya Y, Nakamura T, Horinouchi M, Sakaeda T, Tamura T, Aoyama N, Shirakawa T, Gotob 
A, Fujimoto S, Matsuoka M, et al. (2002) Effects of polymorphisms of MDR1, MRPI, and 
MRP2 genes on their mRNA expression levels in duodenal enterocytes of healthy Japanese subjects. 

Nakamura T, Sakaeda T, Horinouchi M, Tamura T, Aoyama N, Shirakawa T, Matsuoka M, Kasuga 
M, and Okumura K (2002) Effect of the mutation (C3435T) at exon 26 of the MDR1 gene on 
expression level of MDR1 message ribonucleic acid in duodenal enterocytes of healthy Japanese 

the C3435T and G2677(T/A) polymorphisms in the ABCB1 gene and P-glycoprotein 

regulatory protein interactions that propagate to the 3′-UTR of certain 

Pesole G and Liuni S (1999) Internet resources for the functional analysis of 5′-

UTrdb: a specialized database of 5′ and 3′ untranslated regions of eukaryotic mRNAs. 

mRNA is an inefficient mRNA destabilizer. Biochem Biophys Res Commun 261:627– 
634.

Schuetz EG, Furuya KN, and Schuetz JD (1995) Interindividual variation in expression of 
P-glycoprotein in normal human liver and secondary hepatic neoplasms. J Pharmacol Exp 
Ther 275:1011–1018.

FIG. 2. mRNA half-life of ABCB1 reference and 3′-UTR variants in Flp293 stable 
cells. The mRNA decay of ABCB1 reference (■) and three 3′-UTR variants 
(+89A>T (▲), +146G>A (●), and +193A>G (●●)) was measured for 24 h in 
the presence of 7.5 μg/ml actinomycin D. The reference cell line without actino-
mycin D served as a negative control (▲▲). The percentage of mRNA remaining was 
calculated as described in Materials and Methods. Each point is the mean ± S.D. 
of three experiments.


Address correspondence to: Deanna L. Kroetz, UCSF Box 2911, 1550 4th St, RH584E, San Francisco, CA 94158-2911. E-mail: deanna.kroetz@ucsf.edu