



Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com



ORIGINAL ARTICLE

17 β -Estradiol up-regulates UDP-glucuronosyltransferase 1A9 expression *via* estrogen receptor α



Sung-joon Cho^b, Miaoran Ning^b, Yanyan Zhang^a, Leah H. Rubin^c,
Hyunyoung Jeong^{a,b,*}

^aDepartment of Pharmacy Practice, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60607, USA

^bDepartment of Biopharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60607, USA

^cDepartment of Psychiatry, University of Illinois at Chicago, Chicago, IL 60607, USA

Received 14 January 2016; received in revised form 16 March 2016; accepted 7 April 2016

KEY WORDS

UGT1A9;
17 β -Estradiol;
Estrogen receptor;
Drug metabolism;
Sex difference

Abstract UDP-glucuronosyltransferase 1A9 (UGT1A9) is a major phase II enzyme responsible for elimination of drugs and endogenous molecules. Clinical data have shown increased elimination of UGT1A9 substrates in pregnant women or oral contraceptive users, but the role of estrogen in the regulation of UGT1A9 expression remains unknown. In this study, we investigated the effect of 17 β -estradiol (E2) on UGT1A9 expression and the role of ER α in the transcriptional regulation of UGT1A9. E2 significantly increased UGT1A9 promoter activity in HepG2 cells in the presence of ER α . UGT1A9 induction by E2 was abrogated by antiestrogen ICI182,780 in HepG2 cells that constitutively express ER α . Results from transient transfection of ER α mutants into HepG2 cells demonstrated that mutation at DNA-binding domain of ER α abrogates increased UGT1A9 promoter activity by E2. Deletion and mutation assays of UGT1A9 promoter revealed a putative ERE located within –2262/–1987 region. Examination of healthy human liver tissues revealed significantly higher UGT1A9 expression in women as compared to men. Together, these findings provide a mechanistic basis for the previous clinical reports and may shed a light on identifying sources for inter-individual variability in UGT1A9-mediated drug metabolism.

© 2016 Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

*Corresponding author at: Department of Pharmacy Practice, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60607, USA. Tel.: +1 312 996 7820.

E-mail address: yjeong@uic.edu (Hyunyoung Jeong).

Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

<http://dx.doi.org/10.1016/j.apsb.2016.04.005>

2211-3835 © 2016 Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

UDP-glucuronosyltransferases (UGTs) are major phase II enzymes and catalyze conjugation of glucuronic acid to substrates, which is an important metabolic and detoxification pathway for drugs and endogenous compounds¹. Drug-metabolizing UGTs in humans are largely divided into two families, *i.e.*, UGT1A (UGT1A1, 1A3, 1A4, 1A5, 1A6, 1A7, 1A8, 1A9, and 1A10) and UGT2B (UGT2B4, 2B7, 2B10, 2B11, 2B15, 2B17, and 2B28)². Among these, UGT1A9 is one of major UGT isoforms expressed in the liver (~6% of hepatic UGT expression)³. UGT1A9 is responsible for the glucuronidation of multiple endogenous molecules (*e.g.*, thyroid hormones) and drugs (*e.g.*, propofol and acetaminophen)⁴⁻⁶.

Clinical data suggest that estrogen may enhance UGT1A9 expression and/or activity. For example, the area under the curve (AUC) ratios of the glucuronide metabolites to propofol were higher in women (1.3- to 2.9-fold) than in men⁷. Users of oral contraceptives exhibited significantly higher systemic clearances of acetaminophen than non-users⁸. Also, pregnant women showed 1.5-fold higher systemic clearances of acetaminophen as compared to nonpregnant controls⁸, and this has been attributed to increased glucuronidation of acetaminophen in pregnant women⁹. These results suggest potential upregulation of UGT1A9 by estrogen; however, whether and how estrogen modulates UGT1A9 expression remains unknown.

17 β -Estradiol (E2) is a primary female sex hormone and a key regulator of growth and differentiation in many tissues including female reproductive tissues, mammary tissues and cardiovascular systems¹⁰. The biological action of E2 is mediated by estrogen receptors (ER), and ER α is the major subtype expressed in the liver^{10,11}. Upon activation by E2, ER binds to estrogen response element (ERE) in the promoter region of target genes for transcriptional regulation of the genes. E2 has been reported to regulate expression of multiple drug-metabolizing enzyme genes including cytochrome P450 (CYP) 2B6, sulfotransferase 2A1, and UGT1A4^{10,12,13}. In this study, we investigated the effect of E2 on UGT1A9 expression and the role of ER α in the transcriptional regulation of UGT1A9. Our results show that estrogen upregulates UGT1A9 expression, and this potentially underlies the reported pharmacokinetic sex differences in UGT1A9-mediated drug metabolism.

2. Materials and methods

2.1. Chemicals and reagents

E2 and ICI182,780 were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Cell cultures

HepG2, HEK293T (American Type Culture Collection, Manassas, VA, USA), and HepG2-ER cells (HepG2 cells stably expressing ER α kindly provided by Dr. David Shapiro, University of Illinois at Urbana-Champaign, USA) were cultured in complete Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 2 mmol/L L-glutamine, 100 U penicillin/mL, 100 μ g streptomycin/mL, and 1% minimum Eagle's medium nonessential amino acids.

2.3. Plasmids

Upstream regulatory region (-2262/+24) of UGT1A9 was subcloned from pLightSwitch_Prom_UGT1A9 (SwitchGear Genomics, Menlo

Park, CA, USA) into pGL3-basic (Promega, Wisconsin, MI, USA) at *Kpn*I and *Nco*I restriction enzyme sites using the following primers: 5'-GGATTGAGGTACCAACCCC-3' (forward) and 5'-AGAGAACTG-CAGCTGAGAGC-3' (reverse). The resulting plasmid was named as pGL3-UGT1A9. For deletion assays, 5'-deletion constructs were prepared using pGL3-UGT1A9 (as a template) and a primer set of the above-mentioned reverse primer and one of the following forward primers: 5'-CGATGGTACCTGGCCTCCCGTGATAC-3' (-1987/+24), 5'-GTGCTGGGACTACAGGTGT-3' (-1950/+24), 5'-CGATGGTACCAGCCTACTGTGCTACTAGAAG-3' (-1873/+24), 5'-CGATGGTACCAGAGATGTTGGGTTTGCAGG-3' (-1182/+24), and 5'-CGATGGTACCTGAACTCATGGGTTCTGGGT-3' (-1133/+24). Expression vectors for wild-type or mutant ER α were previously described¹². Mutation constructs of pGL3-UGT1A9 *cis*-element were prepared using a QuikChange XL Site-Directed Mutagenesis Kit (Agilent Technologies, La Jolla, CA, USA) following the manufacturer's protocol. The following primers were used to make a mutation construct of pGL3-UGT1A9: 5'-GTATGGCGGAAGCG-GAGGTTGCATT-3' (forward) and 5'-AGTGACCCTCCTGCCT-CAGCCTCCAGA-3' (reverse). The following primers were used to make a deletion construct of pGL3-UGT1A9: 5'-CGGGAAGCG-GAGGTTGCATT-3' (forward) and 5'-CTCCTGCCTCAGCCTC-CAGA-3' (reverse).

2.4. Human liver tissues

A total of 115 human liver transplant donor tissues without any gross pathological features were obtained from Corning (Corning, NY, USA) ($n=50$) and Liver Tissues Cell Distribution Systems (LTCDS; Minneapolis, MN; funded by NIH Contract HHSN276201200017C) ($n=65$). The human tissues from Corning were obtained with informed consent in accordance with applicable laws and directly from non-profit organ procurement operations that have provided assurance as to legal compliance. Ethical approval for the LTCDS contract was granted by the University of Minnesota Institutional Review Board (project 1008M87433). Informed written consent was obtained from all participants involved with LTCDS. Liver tissues were stored at -80 °C until analysis. The tissues were from both males ($n=70$, age = 48 \pm 16) and females ($n=45$, age = 51 \pm 14); but ethnicity was unknown.

2.5. RNA isolation and quantitative real-time PCR

Total RNA was isolated from human liver tissues using Trizol (ThermoFisher Scientific, Waltham, MA, USA) and converted to cDNA using High Capacity cDNA Archive Kit (ThermoFisher Scientific). Using the cDNA as template, qRT-PCR was performed using StepOnePlus Real-Time PCR System. PrimeTime[®] Std qPCR Assays (Integrated DNA Technologies, Coralville, IA, USA) were used for CYP3A4 (Hs.PT.58.27726673), UGT1A9 (Hs.PT.58.40314971.g), and HPRT1 (Hs.PT.58.45621572). Relative mRNA levels of genes were determined after normalizing the gene expression levels by those of HPRT1 (2^{- $\Delta\Delta$ Ct} method).

2.6. Luciferase assay

HepG2 or HepG2-ER cells were transfected with 0.7 μ g plasmid (*i.e.*, 0.3 μ g luciferase construct, 0.03 μ g pcDNA3-ER α , 0.369 μ g pcDNA3, and 0.001 μ g pCMV-*Renilla* for HepG2 cells; 0.3 μ g luciferase construct, 0.399 μ g pcDNA3, and 0.001 μ g pCMV-

Renilla for HepG2 cells) using FuGENE[®] HD (Promega, Madison, WI, USA). pCMV-*Renilla* was included to normalize for differences in transfection efficiency. Luciferase activity was measured by using Dual-Luciferase Reporter Assay System (Promega). Firefly luciferase activity was normalized by *Renilla* luciferase activity. The experiments were performed in triplicate and repeated at least twice.

2.7. Electrophoretic mobility shift assay (EMSA)

HEK293T cells were transfected with pcDNA3-ER α , and nuclear extracts were prepared by using CellLytic[™] NuCLEAR[™] Extraction Kit (Sigma, St. Louis, MO, USA). EMSA was performed as previous described¹⁰.

2.8. Statistical analysis

All data were presented as mean \pm standard deviation unless indicated otherwise. For statistical testing among more than two groups, one-way analysis of variance test was performed for multiple comparisons followed by posthoc Dunnett's test using Prism (Graphpad, San Diego, CA, USA). For testing between two groups, Student's *t*-test was performed. Significance was set at $P < 0.05$.

3. Results

3.1. E2 induces UGT1A9 expression via ER α

Considering that estrogen exerts its physiological action through ER, the involvement of ER α (the major subtype in the liver) in UGT1A9 induction by E2 was investigated by using promoter reporter assays. HepG2 cells were co-transfected with pGL3-UGT1A9 (or promoterless pGL3-basic), and ER α expression vector (or empty vector). The transfected cells were treated with vehicle or E2 for 24 h, and luciferase activity was measured. The results showed that E2 significantly increased UGT1A9 promoter activity only in the cells transfected with ER α (Fig. 1A). Such increase in luciferase activity upon E2 treatment was not observed for pGL3-basic vector (data not shown). The increase in UGT1A9 promoter activity by E2 in HepG2 cells was dependent on E2

concentration, and EC₅₀ was estimated as 12.4 nmol/L (Fig. 1B). To verify the involvement of ER α in cells that constitutively express ER α , HepG2-ER cells were used to examine E2 effects on UGT1A9 promoter activity. HepG2-ER cells were transfected with pGL3-UGT1A9, and then treated with E2 (or vehicle) in the presence or absence of ICI182,780, an ER α antagonist. E2 treatment led to a significant increase in UGT1A9 promoter activity, and this was abolished upon co-treatment with ICI182,780 (Fig. 1C). Together, these data indicate that E2 up-regulates UGT1A9 promoter activity via ER α .

3.2. Putative ERE is located in -2262/-1987 of UGT1A9

To define the mode of ER α action in enhancing UGT1A9 promoter activity, the effects of ER α mutants on E2 responsiveness of UGT1A9 promoter were examined. To this end, HepG2 cells were co-transfected with pGL3-UGT1A9, along with one of ER α mutants. ER α mutants included in this study have (1) a deletion of activation function (AF) 1, (2) point mutations in DNA-binding domain (DBD), (3) point mutations in AF2, or (4) a combination of the mutations. The transfected HepG2 cells were treated with E2, and luciferase activity was measured. The results showed that all of the ER α mutants decreased E2 response of pGL3-UGT1A9 (Fig. 2A), a phenomenon previously observed for pGL3-ERE3 where *luc* expression is driven by 3 copies of consensus ERE¹². Importantly, E2 response was abrogated upon mutation at DBD, indicating that direct binding of ER α to its target gene promoter is critical in UGT1A9 induction by E2.

To identify *cis*-element(s) responsible for the enhanced UGT1A9 promoter activity by E2, deletion assays were performed. To this end, a set of luciferase vector containing different 5'-upstream regulatory region of UGT1A9 (-2262/+24, -1987/+24, -1950/+24, -1873/+24, -1182/+24 and -1133/+24) was constructed. HepG2 cells were transfected with one of the luciferase vectors, along with ER α expression vector, followed by E2 (or vehicle) treatment. The result showed that E2 response of UGT1A9 promoter was abolished when -2262/-1987 region was deleted (Fig. 2B). The -2262/-1987 of UGT1A9 was found to harbor a putative ERE (Fig. 2C). To further examine the role of putative ERE, luciferase vectors harboring deleted or mutated ERE of UGT1A9 were constructed. The luciferase vector was co-transfected into HepG2 cells along with ER α , followed by E2 treatment. Dramatic decreases

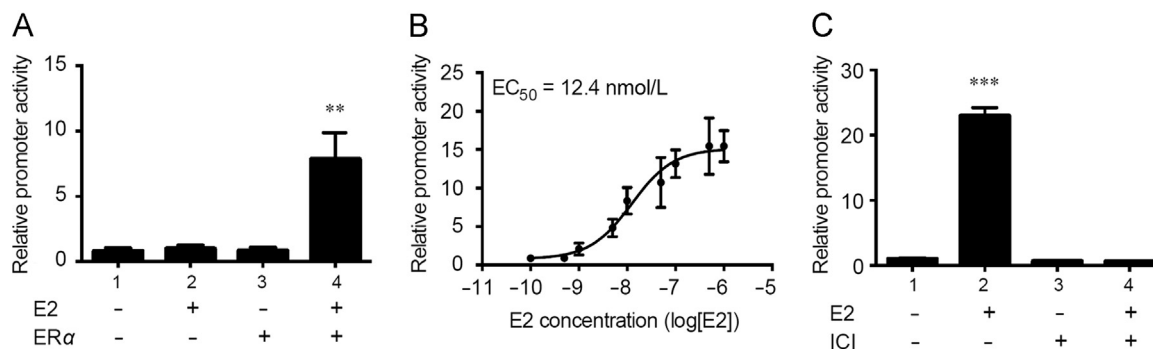


Figure 1 E2 induces UGT1A9 expression via ER α . (A) HepG2 cells were co-transfected with pGL3-UGT1A9, ER α expression vector (or empty vector) and pCMV-*Renilla*. The transfected cells were incubated with vehicle or E2 (100 nmol/L) for 24 h, and luciferase assay was performed. ** $P < 0.01$ vs. lane 1. (B) HepG2 cells were co-transfected with pGL3-UGT1A9, ER α expression vector and pCMV-*Renilla*, followed by treatment with E2 at different concentrations (0.1, 0.5, 1, 5, 10, 50, 100, 500, or 1000 nmol/L). After 24 h, luciferase assay was performed. (C) HepG2-ER cells were transfected with pGL3-UGT1A9 and pCMV-*Renilla*. The transfected cells were treated with E2 (100 nmol/L) and/or ICI182,780 (10 μ mol/L) for 24 h. Then, luciferase assay was performed. *** $P < 0.001$ vs. lane 1.

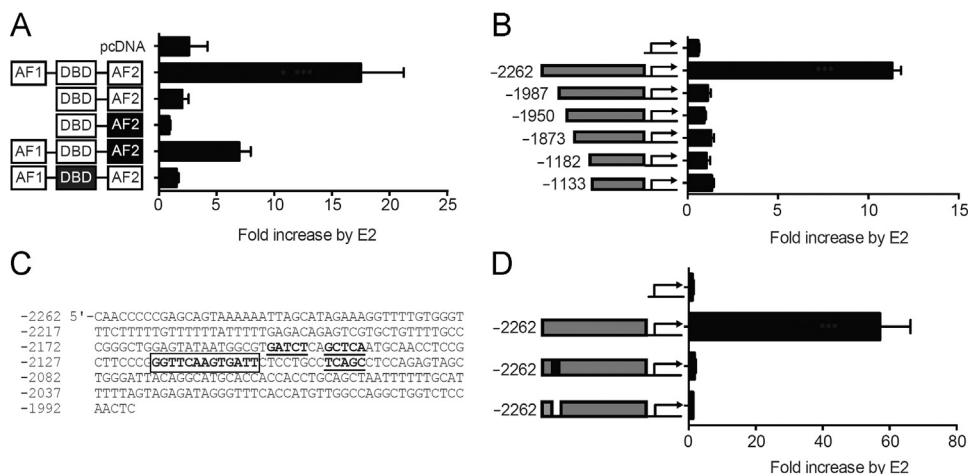


Figure 2 Putative ERE is located in -2262/-1987 of UGT1A9. (A) HepG2 cells were co-transfected with pGL3-UGT1A9, an ER α mutant vector and pCMV-*Renilla*. The cells were treated with vehicle or E2 (100 nmol/L) for 24 h, and luciferase assay was performed. Black box denotes the presence of multiple point mutations in ER α domain. (B) HepG2 cells were transfected with a 5'-deletion construct of pGL3-UGT1A9, ER α expression vector, and pCMV-*Renilla*. The cells were treated with vehicle or E2 (100 nmol/L) for 24 h, and luciferase assay was performed. (C) Putative ERE (boxed) and half ERE (underlined) within -2262/-1987 of UGT1A9 (identified by using ER α matrix²²) are shown. (D) HepG2 cells were co-transfected with UGT1A9 promoter vector harboring wild-type, mutated (black box), or deleted putative ERE, along with ER α expression vector and pCMV-*Renilla*. In the mutation or deletion construct, the putative ERE sequence was mutated to 5'-CCATACAGTGACC-3' or deleted, respectively. The transfected cells were incubated with vehicle or E2 (100 nmol/L) for 24 h, and luciferase assay was performed.

in E2 response were observed when the putative ERE was mutated or deleted (Fig. 2D). These results suggest a key role of the putative ERE in UGT1A9 upregulation by E2.

To determine whether ER α directly binds to the putative ERE of UGT1A9, electrophoretic mobility assays (EMSA) was performed. Short double-stranded DNA probe harboring UGT1A9 putative ERE (the sequence of which is shown in Fig. 3) was incubated with nuclear extracts prepared from HEK293T cells that overexpress ER α . Protein-bound probes were separated from free probes using gel electrophoresis. A DNA probe harboring the previously known consensus ERE sequence was included as a positive control. The consensus ERE probe exhibited a shifted band (Fig. 3, lane 2) that was super-shifted by ER α antibody (Fig. 3, lane 3); however, the UGT1A9 probe did not show any prominent shifted or super-shifted bands (Fig. 3, lane 5 and 6), indicating that ER α did not bind to the UGT1A9 probe.

3.3. mRNA expression of UGT1A9 was higher in women than men in human liver tissues

To verify the role of estrogen in the regulation of UGT1A9 expression, sex difference in UGT1A9 expression was examined in human liver tissues. In healthy human liver tissues ($n=115$), mRNA expression levels of UGT1A9 expression were compared between males ($n=70$, age= 48 ± 16) and females ($n=45$, age= 51 ± 14). *CYP3A4*, a gene known to exhibit sex difference in its expression¹³, was included as a positive control. Statistically significant sex difference was detected in both UGT1A9 and *CYP3A4* expression (Fig. 4).

4. Discussion

In this study, using transient transfection and promoter reporter assays, we showed that E2 upregulates UGT1A9 expression via ER α in HepG2 and HepG2-ER cells. Our results are in agreement

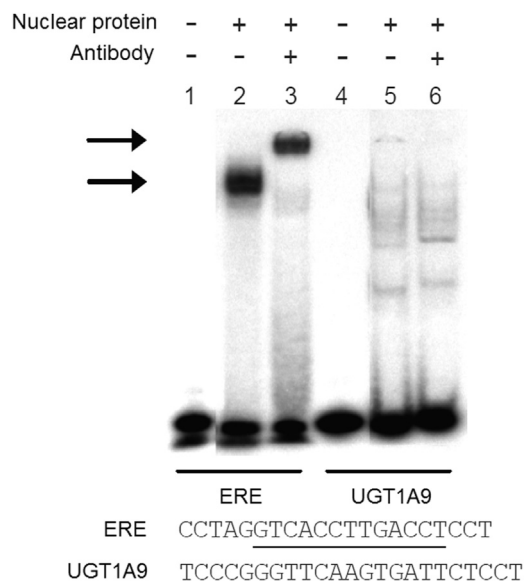


Figure 3 ER α does not bind to UGT1A9 probe. Nuclear proteins were prepared from HEK293T cells transfected with ER α expression vector. The proteins were incubated with ³²P-labeled DNA probe harboring either the consensus ERE or the putative ERE of UGT1A9, in the presence or absence of ER α antibody. The mixture was resolved on non-denaturing gel. The lower arrow indicates the location of shifted bands by ER α binding to ERE. The upper arrow indicates a super-shift complex.

with previous clinical data that showed increased glucuronidation of UGT1A9 substrates (*i.e.*, propofol and acetaminophen) in women (than in men) or oral contraceptive users (than in non-users)⁷⁻⁹. To further verify our findings in HepG2 cells in a more physiologically relevant system, we treated primary human hepatocytes with E2 and examined UGT1A9 expression. The results

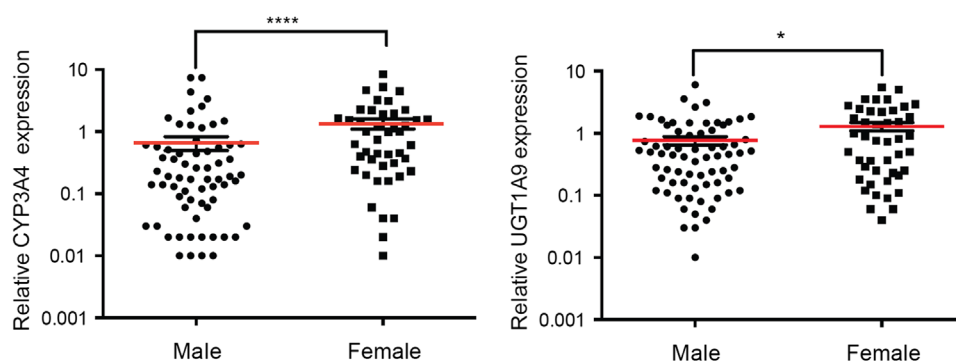


Figure 4 Relative mRNA expression levels of CYP3A4 and UGT1A9 are higher in women than in men. mRNA expression of CYP3A4 and UGT1A9 in the human liver tissues ($n=115$) were measured by qRT-PCR, and normalized by the levels in an arbitrarily chosen liver tissue. The red and error bars represent the mean and standard error, respectively. * $P < 0.05$; **** $P < 0.0001$.

showed insignificant differences in UGT1A9 mRNA levels between E2- and vehicle-treated hepatocytes (data not shown), in part consistent with a previous report of relatively poor inducibility of UGTs in primary hepatocytes¹⁴. This likely indicates a limitation of human hepatocytes as a model to study regulation of UGT expression. Together, our data provide mechanistic support for the clinical findings of UGT-mediated metabolism and suggest important roles of estrogen in determining UGT1A9 expression and activity.

The mode of ER α action in UGT1A9 upregulation appears complex. Results from deletion and mutation assays revealed that the putative ERE within $-2262/-1987$ of UGT1A9 plays a key role in promoter activation by E2. Also, studies using ER α mutants revealed that ER α enhances UGT1A9 promoter activity *via* direct DNA binding. Interestingly, however, the results from EMSA showed that ER α did not directly bind to a DNA probe harboring the putative ERE of UGT1A9. This may reflect weak interaction between the DNA and ER α , and the need for binding of additional transcription factor(s) to the regulatory region of UGT1A9. It has been reported that imperfect EREs or half EREs that are located in the proximity of each other significantly enhance ER α binding to the DNA^{15,16}. Three half-EREs are found at 9-, 20- and 27-bp away from the putative ERE of UGT1A9, and it appears possible that these half-EREs enhance the ER α binding to the regulatory region of UGT1A9.

UGT1A9-mediated drug metabolism in humans exhibits large inter-individual variability^{17–19}. We also found that UGT1A9 expression exhibited >300 -fold difference in our human liver tissues. Several factors are known to alter UGT1A9 expression, including genetic polymorphisms of UGT1A9 and differential expression levels of transcriptional factors^{20,21}. Our results indicating the regulatory role of estrogen in UGT1A9 expression suggest sex as an additional potential contributor to UGT1A9 variability. Indeed, UGT1A9 expression in human liver tissues was found to be higher in women than in men, similarly to CYP3A4, in our study. Notably, although the ethnicity of the donors and circulating estrogen levels are not known for the tissue samples analyzed, our finding is consistent with the proposed regulatory role of estrogen. The extent of contribution by sex to UGT1A9 variability, as compared to that by other known factors that alter UGT1A9 expression, remains to be determined.

In conclusion, we demonstrated that E2 up-regulates UGT1A9 expression *via* ER α . This provides mechanistic insights into previously reported increases in UGT1A9-mediated drug metabolism in pregnant women or oral contraceptive users. This finding

may also shed a light on identifying sources for inter-individual variability in UGT1A9-mediated drug metabolism.

Acknowledgments

This work was supported by the U. S. National Institute of Health (Grants HD065532 and GM112746).

References

- de Wildt SN, Kearns GL, Leeder JS, van den Anker JN. Glucuronidation in humans. Pharmacogenetic and developmental aspects. *Clin Pharmacokinet* 1999;**36**:439–52.
- Mackenzie PI, Bock KW, Burchell B, Guillemette C, Ikushiro S, Iyanagi T, et al. Nomenclature update for the mammalian UDP glycosyltransferase (UGT) gene superfamily. *Pharmacogenet Genom* 2005;**15**:677–85.
- Rowland A, Miners JO, Mackenzie PI. The UDP-glucuronosyltransferases: their role in drug metabolism and detoxification. *Int J Biochem Cell Biol* 2013;**45**:1121–32.
- Albert C, Vallee M, Beaudry G, Belanger A, Hum DW. The monkey and human uridine diphosphate-glucuronosyltransferase UGT1A9, expressed in steroid target tissues, are estrogen-conjugating enzymes. *Endocrinology* 1999;**140**:3292–302.
- Barbier O, Villeneuve L, Bocher V, Fontaine C, Torra IP, Duhem C, et al. The UDP-glucuronosyltransferase 1A9 enzyme is a peroxisome proliferator-activated receptor α and γ target gene. *J Biol Chem* 2003;**278**:13975–83.
- Watanabe Y, Nakajima M, Yokoi T. Troglitazone glucuronidation in human liver and intestine microsomes: high catalytic activity of UGT1A8 and UGT1A10. *Drug Metab Dispos* 2002;**30**:1462–9.
- Choong E, Loryan I, Lindqvist M, Nordling A, el Bouazzaoui S, van Schaik RH, et al. Sex difference in formation of propofol metabolites: a replication study. *Basic Clin Pharmacol Toxicol* 2013;**113**:126–31.
- Kulo A, Van Calsteren K, van de Velde M, Mulabegovic N, Verbesselt R, de Hoon JN, et al. Weight, pregnancy and oral contraceptives affect intravenous paracetamol clearance in young women. *Eur Rev Med Pharmacol Sci* 2014;**18**:599–604.
- Miners JO, Robson RA, Birkett DJ. Paracetamol metabolism in pregnancy. *Br J Clin Pharmacol* 1986;**22**:359–62.
- Li W, Ning M, Koh KH, Kim H, Jeong H. 17 β -Estradiol induces sulfotransferase 2A1 expression through estrogen receptor α . *Drug Metab Dispos* 2014;**42**:796–802.
- Kuiper GG, Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S, et al. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β . *Endocrinology* 1997;**138**:863–70.

12. Chen H, Yang K, Choi S, Fischer JH, Jeong H. Up-regulation of UDP-glucuronosyltransferase (UGT) 1A4 by 17 β -estradiol: a potential mechanism of increased lamotrigine elimination in pregnancy. *Drug Metab Dispos* 2009;**37**:1841–7.
13. Wolbold R, Klein K, Burk O, Nussler AK, Neuhaus P, Eichelbaum M, et al. Sex is a major determinant of CYP3A4 expression in human liver. *Hepatology* 2003;**38**:978–88.
14. Soars MG, Petullo DM, Eckstein JA, Kasper SC, Wrighton SA. An assessment of udp-glucuronosyltransferase induction using primary human hepatocytes. *Drug Metab Dispos* 2004;**32**:140–8.
15. Gruber CJ, Gruber DM, Gruber IM, Wieser F, Huber JC. Anatomy of the estrogen response element. *Trends Endocrinol Metab* 2004;**15**:73–8.
16. Klinge CM, Bodenner DL, Desai D, Niles RM, Traish AM. Binding of type II nuclear receptors and estrogen receptor to full and half-site estrogen response elements *in vitro*. *Nucleic Acids Res* 1997;**25**:1903–12.
17. Court MH. Interindividual variability in hepatic drug glucuronidation: studies into the role of age, sex, enzyme inducers, and genetic polymorphism using the human liver bank as a model system. *Drug Metab Rev* 2010;**42**:209–24.
18. Loryan I, Lindqvist M, Johansson I, Hiratsuka M, van der Heiden I, van Schaik RH, et al. Influence of sex on propofol metabolism, a pilot study: implications for propofol anesthesia. *Eur J Clin Pharmacol* 2012;**68**:397–406.
19. Jiao Z, Ding JJ, Shen J, Liang HQ, Zhong LJ, Wang Y, et al. Population pharmacokinetic modelling for enterohepatic circulation of mycophenolic acid in healthy Chinese and the influence of polymorphisms in UGT1A9. *Br J Clin Pharmacol* 2008;**65**:893–907.
20. Aueviriyavit S, Furihata T, Morimoto K, Kobayashi K, Chiba K. Hepatocyte nuclear factor 1 α and 4 α are factors involved in inter-individual variability in the expression of *UGT1A6* and *UGT1A9* but not *UGT1A1*, *UGT1A3* and *UGT1A4* mRNA in human livers. *Drug Metab Pharmacokinet* 2007;**22**:391–8.
21. Carlini LE, Meropol NJ, Bever J, Andria ML, Hill T, Gold P, et al. *UGT1A7* and *UGT1A9* polymorphisms predict response and toxicity in colorectal cancer patients treated with capecitabine/irinotecan. *Clin Cancer Res* 2005;**11**:1226–36.