Published in final edited form as:

Eur J Clin Pharmacol. 2013 September ; 69(9): 1733–1735. doi:10.1007/s00228-013-1538-0.

The use of allelic imbalance to ascertain *cis*-regulation for human *UGT2B7* *in vivo*

Pin-Yi Wang¹, Dezheng Huo², Chang Sun^{1,3,*}, and Olufunmilayo I. Olopade^{3,4,*}

¹Laboratory for Conservation and Utilization of Bio-Resources, and Key Laboratory of Microbial Diversity in Southwest China, Ministry of Education, Yunnan University, Kunming, Yunnan 650091, P. R. China

²Department of Health Studies, University of Chicago, Chicago, IL 60637

³Department of Human Genetics, University of Chicago, Chicago, IL 60637

⁴Center for Clinical Cancer Genetics, Department of Medicine, University of Chicago, Chicago, IL 60637

Dear Editor,

Glucuronidation plays an important role in clearing numerous endobiotics and xenobiotics from the human body, including steroid hormones, bile acids, carcinogens and clinical drugs and is catalyzed by UDP-glucuronosyltransferases (UGT) [1–4]. Considering this and the fact that steroid hormones play a central role in breast cancer risk, it has long been proposed that UGTs are involved in breast cancer susceptibility [5,6]. Based on the chromosome location and homology, this gene family can be classified into two major subfamilies, UGT1 and UGT2, the latter of which can be further divided into UGT2A and UGT2B [3]. Among UGT2B family, UGT2B7 is most relevant due to its high expression level [7–9] and activity [10], and wide substrate spectrum [10,11]. As a consequence, the genetic variants in this gene, especially those that can affect the regulation of gene expression or alter the protein sequence and further enzyme activity, have received much attention in attempt to understand the inter-individual differences in drug response and susceptibility to breast cancer. Recently, by transient transfection, it has been shown that –900G/A (relative to translation start; rs7438135) could influence promoter activity in liver cell line in both basal [12] and oxidative stress conditions [13] *in vitro*. However, it still remains unclear whether this polymorphism can affect the regulation of *UGT2B7* expression *in vivo*. Interestingly, two adjacent coding SNPs, 801A/T (rs7438284) and 802T/C (rs7439366, causing 268Y/H amino acid substitution), were found to be in complete linkage disequilibrium (LD) with this putative regulatory SNP, –900G/A, and were used to categorize two major haplotypes, *UGT2B7**1 and *2 [12,14–16]. Therefore, if this SNP indeed controls *UGT2B7* expression, differential allelic expression (DAE; or allelic imbalance, AI) should be observed between two haplotypes.

To this end, we retrieved 31 normal liver (3 European American [CA] and 1 African American [AA], 27 unknown) tissue samples. All liver tissues came from biospecimen bank of University of Chicago and this study was approved by ethics committee (approval ID

*Corresponding author: Olufunmilayo I. Olopade, Center for Clinical Cancer Genetics, Department of Medicine, University of Chicago, 5841 S Maryland Avenue, Chicago, IL 60637, Phone: (773) 702-6149, Fax: (773) 702-0963, folopade@medicine.bsd.uchicago.edu Or Chang Sun, Yunnan University, sunchang@ynu.edu.cn.

Conflict of interest

The authors declare that they have no conflict of interest.

10760B). RNA and DNA were isolated by RNeasy Lipid Tissue and QIAamp DNA Mini Kit (Qiagen, Valencia, CA), respectively. cDNA was synthesized by High Capacity Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Genotype of *UGT2B7* haplotype was determined by a Taqman genotyping assay with primers GGAAAGCTGACGTATGGCTTATT and AAAGCCAACAAAATAAAACCAACA, and probes 6FAM-TCAGTTTCCTCATCCAC and VIC-TCAGTTTCCATATCCAC (target in bold; Applied Biosystems) according to the manufacturer's protocol. Among all tissues, 11 liver samples were heterozygous for the two haplotypes. To evaluate the allelic imbalance in 801 and 802 positions, allele-specific real time PCR was performed in cDNA from heterozygous individuals by the Taqman Assay with primers GACAATGGGGAAAGCTGACG and GGAGTCCTCCAACAAAATCAACA and abovementioned probes. For normalization, a heterozygous genomic DNA sample was serially diluted as standard. All real time PCR assays were performed in triplicate for each sample and the AI ratio was expressed as $\text{Type2}_{\text{quantity}}/\text{Type1}_{\text{quantity}}$, in which type 1 and type 2 indicated 801T-802C and 801A-802T, and were in LD with haplotype 1 and 2, respectively. All real time PCR and genotyping reading were performed on a StepOne Plus Realtime PCR System (Applied Biosystems).

To make sure that the two alleles had the same efficiency in our allele-specific real time PCR and that our AI assay was accurate, we first quantified the gDNA mixes by type 1 and 2 with known ratios (4:1, 2:1, 1:1, 1:2, and 1:4). The observed ratio presented a highly linear relation with the expected one ($r^2=0.991$, $P<10^{-17}$; result not shown), thus confirming that our assay was sensitive enough to differentiate two alleles, at least in the 0.25–4 ratio interval, which was consistent with our recent study [17].

As shown in Figure 1, nearly all heterozygous samples presented an excess of type 1 over type 2, with a significant deviation from 1:1 ratio (mean $\pm$ standard deviation [SD], 0.81 ± 0.11 ; 95% confidence interval [CI] 0.73–0.88; t -test, $P<0.001$). This result showed that –900G/A can indeed influence *UGT2B7* gene expression in liver. However, the proportion of haplotype 1 was only 13.6%–37.0% higher than that of haplotype 2 on average *in vivo* (see Fig 1), which was much lower than previous result (around one fold increasing) *in vitro* [12,13]. This discrepancy might hint the existence of one or more *cis*-regulatory variants that can hamper the effect of –900G/A and the importance of using both *in vivo* and *in vitro* approaches to investigate gene expression regulation. Moreover, the limited difference in gene expression *in vivo* might interpret the lack of association between haplotype and multiple drugs metabolism [18–20], especially when no enzyme activity difference on these drugs was observed between H and Y proteins. Indeed, considering the remarkable difference in promoter activity between two haplotypes *in vitro*, it could be easily deduced that the regulatory SNP in promoter should have some functional consequence in drug metabolism. However, multiple studies failed to obtain any difference between two haplotypes [18–20]. This result could be explained by our observation that the promoter activity difference *in vivo* is not as great as that *in vitro*. Moreover, the diverse metabolism efficiency on some drugs between two haplotypes in previous study [21–23] was more likely to be attributed to enzyme activity instead of promoter.

Acknowledgments

This research was supported by National Basic Research Program of China (2013CB127500), National Institute of Health grant CA125183, and National Natural Science Foundation of China (No. 31260266).

References

- Guillemette C. Pharmacogenomics of human UDP-glucuronosyltransferase enzymes. *Pharmacogenomics J.* 2003; 3:136–158. [PubMed: 12815363]

2. King CD, Rios GR, Green MD, Tephly TR. UDP-glucuronosyltransferases. *Curr Drug Metab.* 2000; 1:143–161. [PubMed: 11465080]
3. Mackenzie PI, Bock KW, Burchell B, Guillemette C, Ikushiro S, Iyanagi T, Miners JO, Owens IS, Nebert DW. Nomenclature update for the mammalian UDP glycosyltransferase (UGT) gene superfamily. *Pharmacogenet Genomics.* 2005; 15:677–685. [PubMed: 16141793]
4. Tukey RH, Strassburg CP. Human UDP-glucuronosyltransferases: metabolism, expression, and disease. *Annu Rev Pharmacol Toxicol.* 2000; 40:581–616. [PubMed: 10836148]
5. Desai AA, Innocenti F, Ratain MJ. UGT pharmacogenomics: implications for cancer risk and cancer therapeutics. *Pharmacogenetics.* 2003; 13:517–523. [PubMed: 12893990]
6. Nagar S, Rummel RP. Uridine diphosphoglucuronosyltransferase pharmacogenetics and cancer. *Oncogene.* 2006; 25:1659–1672. [PubMed: 16550166]
7. Ohno S, Nakajin S. Determination of mRNA expression of human UDP-glucuronosyltransferases and application for localization in various human tissues by real-time reverse transcriptase-polymerase chain reaction. *Drug Metab Dispos.* 2009; 37:32–40. [PubMed: 18838504]
8. Nakamura A, Nakajima M, Yamanaka H, Fujiwara R, Yokoi T. Expression of UGT1A and UGT2B mRNA in human normal tissues and various cell lines. *Drug Metab Dispos.* 2008; 36:1461–1464. [PubMed: 18480185]
9. Izukawa T, Nakajima M, Fujiwara R, Yamanaka H, Fukami T, Takamiya M, Aoki Y, Ikushiro S, Sakaki T, Yokoi T. Quantitative analysis of UDP-glucuronosyltransferase (UGT) 1A and UGT2B expression levels in human livers. *Drug Metab Dispos.* 2009; 37:1759–1768. [PubMed: 19439486]
10. Turgeon D, Carrier JS, Levesque E, Hum DW, Belanger A. Relative enzymatic activity, protein stability, and tissue distribution of human steroid-metabolizing UGT2B subfamily members. *Endocrinology.* 2001; 142:778–787. [PubMed: 11159850]
11. Coffman BL, Rios GR, King CD, Tephly TR. Human UGT2B7 catalyzes morphine glucuronidation. *Drug Metab Dispos.* 1997; 25:1–4. [PubMed: 9010622]
12. Duguay Y, Baar C, Skorpen F, Guillemette C. A novel functional polymorphism in the uridine diphosphate-glucuronosyltransferase 2B7 promoter with significant impact on promoter activity. *Clin Pharmacol Ther.* 2004; 75:223–233. [PubMed: 15001974]
13. Nakamura A, Nakajima M, Higashi E, Yamanaka H, Yokoi T. Genetic polymorphisms in the 5'-flanking region of human UDP-glucuronosyltransferase 2B7 affect the Nrf2-dependent transcriptional regulation. *Pharmacogenet Genomics.* 2008; 18:709–720. [PubMed: 18622263]
14. Sun C, Southard C, Huo D, Hernandez RD, Witonsky DB, Olopade OI, Di Rienzo A. SNP discovery, expression and cis-regulatory variation in the UGT2B genes. *Pharmacogenomics J.* 2012; 12:287–296. [PubMed: 21358749]
15. Innocenti F, Liu W, Fackenthal D, Ramirez J, Chen P, Ye X, Wu X, Zhang W, Mirkov S, Das S, Cook E Jr, Ratain MJ. Single nucleotide polymorphism discovery and functional assessment of variation in the UDP-glucuronosyltransferase 2B7 gene. *Pharmacogenet Genomics.* 2008; 18:683–697. [PubMed: 18622261]
16. Holthe M, Rakvag TN, Klestad P, Idle JR, Kaasa S, Krokan HE, Skorpen F. Sequence variations in the UDP-glucuronosyltransferase 2B7 (*UGT2B7*) gene: identification of 10 novel single nucleotide polymorphisms (SNPs) and analysis of their relevance to morphine glucuronidation in cancer patients. *Pharmacogenomics J.* 2003; 3:17–26. [PubMed: 12629580]
17. Sun C, Southard C, Witonsky DB, Olopade O, Di Rienzo A. Allelic imbalance (AI) identifies novel tissue specific cis-regulatory variation for human *UGT2B15*. *Hum Mutat.* 2010; 31:99–107. [PubMed: 19847790]
18. Kagaya H, Inoue K, Miura M, Satoh S, Saito M, Tada H, Habuchi T, Suzuki T. Influence of UGT1A8 and UGT2B7 genetic polymorphisms on mycophenolic acid pharmacokinetics in Japanese renal transplant recipients. *European journal of clinical pharmacology.* 2007; 63:279–288. [PubMed: 17211619]
19. Holthe M, Klestad P, Zahlsten K, Borchgrevink PC, Hagen L, Dale O, Kaasa S, Krokan HE, Skorpen F. Morphine glucuronide-to-morphine plasma ratios are unaffected by the *UGT2B7* H268Y and *UGT1A1**28 polymorphisms in cancer patients on chronic morphine therapy. *European journal of clinical pharmacology.* 2002; 58:353–356. [PubMed: 12185559]

20. Chung JY, Cho JY, Yu KS, Kim JR, Lim KS, Sohn DR, Shin SG, Jang IJ. Pharmacokinetic and pharmacodynamic interaction of lorazepam and valproic acid in relation to *UGT2B7* genetic polymorphism in healthy subjects. *Clin Pharmacol Ther.* 2008; 83:595–600. [PubMed: 17687269]
21. Thibaudeau J, Lepine J, Tojcic J, Duguay Y, Gelletier G, Plante M, Brisson J, Tetu B, Jacob S, Perusse L, Belanger A, Guillemette C. Characterization of common *UGT1A8*, *UGT1A9*, and *UGT2B7* variants with different capacities to inactivate mutagenic 4-hydroxylated metabolites of estradiol and estrone. *Cancer Res.* 2006; 66:125–133. [PubMed: 16397224]
22. Gall WE, Zawada G, Mojarrabi B, Tephly TR, Green MD, Coffman BL, Mackenzie PI, Radomska-Pandya A. Differential glucuronidation of bile acids, androgens and estrogens by human *UGT1A3* and *2B7*. *J Steroid Biochem Mol Biol.* 1999; 70:101–108. [PubMed: 10529008]
23. Djebli N, Picard N, Rerolle JP, Meur YL, Marquet P. Influence of the *UGT2B7* promoter region and exon 2 polymorphisms and comedications on acyl-MPAG production in vitro and in adult renal transplant patients. *Pharmacogenet Genomics.* 2007; 17:321–330. [PubMed: 17429314]

Fig 1.

The ratio of Type 2/Type 1 in liver. Each bar in x-axis indicates one individual while the height of each bar denotes the AI ratio, which is expressed as mean $\pm$ standard deviation (SD). Type 1 and 2 represent 801T-802C and 801A-802T, respectively.