Interindividual Differences in Placental Expression of the *SLC22A2* (*OCT2*) Gene: Relationship to Epigenetic Variations in the 5′-Upstream Regulatory Region

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ABSTRACT: Organic cation transporters (OCTs) mediate the transport of organic cations and some drugs (e.g., metformin and cimetidine). *OCT1*, *OCT2*, and *OCT3* are located in the imprinting cluster of the insulin-like growth factor 2 receptor. It has been reported that *OCT1* and *OCT3* show a biallelic expression, whereas *OCT2* undergoes maternal imprinting in the human placenta; however, a loss of the imprinting of *OCT2* has recently been reported in some placental samples. This study investigated whether epigenetic mechanisms are involved in interindividual differences in the placental expression of *OCT2*. Because OCT2 mRNA levels were higher in biallelic samples than that in monoallelic samples, we compared the DNA methylation and chromatin modifications in the promoter regions. There was no remarkable difference in DNA methylation between the mono allelic samples and biallelic samples. In contrast, histone H3 acetylation (H3Ac) was increased in the biallelic samples. A significant negative correlation was observed between the trimethylation of lysine-9 on histone H3 (H3K9me3) and the OCT2 mRNA levels. Our results suggest that H3Ac plays a role in the allelic expression of *OCT2*. In addition, H3K9me3 in the *OCT2* promoter may explain the interindividual differences in placental OCT2 mRNA levels. © 2011 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 100:3875–3883, 2011

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INTRODUCTION

Genomic imprinting is an epigenetic phenomenon in which only a single allele is expressed in a parent-of-origin-dependent manner.[1,2] Most genes are expressed equally from both parental alleles, whereas imprinted genes are expressed exclusively or preferentially from either the paternal or the maternal allele. In imprinted genes, the epigenetic information that is transmitted independently of the DNA sequence is conveyed through alterations in nucleosomal structure, resulting from covalent DNA methylation and histone modifications.[3] A small number of human disorders have been shown to be associated with imprinting defects.[4] One of the first imprinted genes to be identified, insulin growth factor 2 (*Igf2*), was found to be expressed from the paternal allele and exhibited placental stunting and fetal growth retardation.[5] Overexpression of *Igf2* via loss of imprinting through deletion of imprinting control region can cause placentomegaly as well as fetal overgrowth.

Imprinted genes often cluster in large chromosomal regions, forming imprinted domains that are regulated by a *cis*-acting imprinting control region.[6–8] The organic cation transporter (OCT) 1, 2, and 3 genes (*OCT1-3*/SLC22A1-3) encode transporters of organic cations and some substrate drugs (e.g., metformin and cimetidine) and are located in the insulin-like growth factor 2 receptor (*IGF2R*) imprinting cluster

Additional Supporting Information may be found in the online version of this article. Supporting Information

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on human chromosome 6.[9] *IGF2R* is imprinted maternally in rodents, marsupials, and artiodactyls, but is expressed in a biallelic manner in humans.[10,11] In mice, *Igf2r* encodes two reciprocally imprinted transcripts, each of which is associated with a differentially methylated DNA region (DMR).[12] The first DMR includes the promoter for the sense *Igf2r* transcript, whereas the second DMR (region 2), which is located within the second intron of the gene, includes the promoter for an antisense transcript noncoding RNA, *Airn*.[9] The paternally expressed *Airn* suppresses the expression of the sense *Igf2r* as well as *Oct2* and *Oct3* on the paternal chromosome, depending on the methylation of region 2.[13] Deletion or premature termination of *Airn* leads to a loss of *Igf2r* locus imprinting.[14] Nagano et al.[15] showed that *Airn* interacts with the *Oct3* promoter chromatin and the H3K9 (histone H3 lysin-9) histone methyltransferase G9a in the placenta. Genetic ablation of G9a and/or truncation of *Airn* are associated with the biallelic transcription of *OCT3*.[15] Imprinting status of *OCT* genes is quite different between human and mice. *Oct2* and *Oct3* are maternally imprinted genes in mice placenta.[9] In contrast, *OCT1* and *OCT3* show a biallelic expression (*OCT3* exhibited monoallelic expression in the first trimester placenta), whereas *OCT2* is a maternal imprinting gene in the human placenta.[12] The human *IGF2R*, *OCT2*, and *OCT3* genes show a polymorphic expression in placenta in approximately 40% of informative samples, which may be responsible for the interindividual difference in mRNA levels.[16] Recently, human *AIRN*, a noncoding RNA (ncRNA), was identified by Yotova et al.[17] Although its function has not yet been elucidated, human *AIRN* is suggested to regulate the allelic expression of *IGF2R*, *OCT2*, and *OCT3* by a *cis*-acting mechanism, similar to that in mice. In mice, *Airn* is mostly transcribed as an unspliced mature form. The unspliced *Airn* is highly unstable and degrades rapidly. On the contrary, spliced *Airn* is stable.[17,18] Spliced *Airn* functions through specific interaction with chromatin and mediates targeted recruitment of a repressive histone-modifying enzyme to epigenetically silence transcription, suggesting that the spliced form has an epigenetic repressive function in the *IGF2R* imprinting cluster in mice.

In this study, we focused on the interindividual differences in the allelic expression of *OCTs* and evaluated whether the status of covalent DNA methylation and chromatin modifications affects polymorphic allelic expression and individual mRNA expression. Furthermore, we tried to detect the AIRN transcript in human placental samples and analyzed the relationship between AIRN expression and histone H3K9 trimethylation in the promoter regions of *OCT1* and *OCT2*.

MATERIALS AND METHODS

Placentas and DNA Samples

Human placentas were obtained from patients who underwent a normal pregnancy at Tottori University Hospital (Nishimachi, Tottori, Japan). Placental samples for the extraction of RNA were frozen in liquid nitrogen immediately after delivery and were stored at −80°C. Each patient gave written informed consent to participate in the study, which was approved by the Tottori University Ethics Committee. Genomic DNA was isolated with a DNeasy kit (QIAGEN, Valencia, California).

Identification of DNA Samples Harboring a Marker Single Nucleotide Polyrmorphism as Heterozygosity

To analyze imprinting states, DNA samples harboring a marker single nucleotide polymorphism (SNP) as heterozygosity are necessary. For this purpose, we selected the following two marker SNPs due to their higher frequencies among Japanese (Supporting Information, Table S1): 1676G>A (rs316003) and 2327A>C (rs2450975). Polymerase Chain Reaction (PCR) primers were designed to amplify exon 10 and exon 11 for genotyping of 1676G>A and 2327A>C, respectively. Primer sequences were as follows: 1676G>A, 5′-CAAAAATAAGGGAAACTCTAA-3′ (forward) and 5′-CAAGTCTTTGATTTAGTTTGA-3’ (reverse); 2327A>C, 5′-TAAAGTTGTCCAGAATGTATG-3′ (forward) and 5′-ATTAGTAGAGGTGAAATAGGG-3′ (reverse). PCR was performed using genomic DNA with 20 nM of each primer and Taq DNA polymerase (Applied Biosystems, Foster City, California) in a total volume of 50 μL. The following amplification conditions were used: an initial denaturation step of 95°C for 9 min followed by 30–40 cycles of denaturation at 95°C for 40 s, annealing at 52°C for 45 s, extension at 72°C for 30 s, and a final extension at 72°C for 5 min. For 1676G>A, the PCR products were digested by *Afa*I, and then electrophoresed on a 3% agarose gel. Direct sequencing was applied to 2327A>C with BigDye Terminator version 3.1 (Applied Biosystems).

Complementary DNA Synthesis

Total RNA was isolated using an RNeasy Mini-kit (QIAGEN) according to the manufacturer’s direction. The RNA was treated with DNase I (RNase-Free DNase Set; QIAGEN) to eliminate genomic DNA. The first-strand complementary DNA (cDNA) synthesis was carried out using SuperScript II (Invitrogen, Carlsbad, California) and a random primer (Promega, Madison, Wisconsin). Reverse transcription (RT) reactions were always performed in the presence or absence of reverse transcriptase to ensure that genomic DNA did not contaminate the subsequent PCR.