Biochemical Pharmacology 79 (2010) 921–925

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Modulation of human placental P-glycoprotein expression and activity by *MDR1* gene polymorphisms

Sarah J. Hemauer, Tatiana N. Nanovskaya, Sherif Z. Abdel-Rahman, Svetlana L. Patrikeeva, Gary D.V. Hankins, Mahmoud S. Ahmed *

Department of Obstetrics & Gynecology, University of Texas Medical Branch, 301 University Boulevard, Galveston, TX, 77555-0587, USA

ARTICLE INFO

Article history:
Received 28 August 2009
Accepted 29 October 2009

Keywords:
Placenta
Membrane vesicle
p-Glycoprotein
MDR1
Polymorphism

ABSTRACT

The ABC transporter P-glycoprotein is a product of the *MDR1* gene and its function in human placenta is to extrude xenobiotics from the tissue thus decreasing fetal exposure. The goal of this investigation was to examine the effect of three polymorphisms in the *MDR1* gene on the expression and activity of placental P-gp. In 199 term placentas examined, the C1236T variant was associated with 11% lower P-gp protein expression than wild-type, while the C3435T and G2677T/A variants each were associated with a 16% reduction ($p < 0.05$). Homozygotes for the C1236T and C3435T variant allele (TT) were associated with 42% and 47% increase in placental P-gp transport activity, respectively ($p = 0.04$ and $p = 0.02$) of the prototypic substrate, [^{3}H]-paclitaxel. These findings indicate that the C3435T and G2677T/A SNPs in *MDR1* are significantly associated with decreased placental P-gp protein expression, while the C1236T and C3245T homozygous variants are significantly associated with an increase in its efflux activity.

1. Introduction

The placental ABC transporter P-glycoprotein (P-gp) decreases fetal exposure to exogenous or endogenous compounds present in the maternal circulation, by extruding those that are substrates from the fetal-to-maternal direction. A recent investigation in our laboratory utilized inside–out vesicles from human placental brush border membrane to determine P-gp activity as transport of the P-gp substrate, paclitaxel. In 200 human placentas examined, there was no correlation between P-gp protein expression and transport activity of paclitaxel [1]. Consequently, we hypothesized that genetic variation could contribute to discrepancies between observed protein expression and activity of placental P-gp.

The *MDR1* gene encoding P-gp displays considerable genetic variability, with specific single nucleotide polymorphisms (SNPs) occurring at high frequencies in certain populations [2]. More than 50 SNPs in the *MDR1* gene have been reported [3], and the most commonly found SNPs are C1236T, C3435T, and G2677T/A. The C1236T, C3435T, and G2677T/A SNPs are found in linkage disequilibrium in up to 49% of Chinese, Malay and Indian populations [4,5]. Additionally, the three SNPs are in linkage disequilibrium with allele frequency of 45–55% in Whites and 5–10% in African Americans [4,5].

Consensus on the functional consequences of *MDR1* genetic variation, specifically the effect of SNPs on P-gp protein expression and transport activity in the placenta, remains unclear. In Japanese women, the G2677T/A polymorphisms were associated with lower placental P-gp expression [6]. In German mothers of Caucasian ethnicity, significantly lower P-gp expression in placentas carrying the G2677T and C3435T polymorphisms was reported [7]. Homozygous carriers of the C3435T variant allele (TT) have both reduced P-gp expression and efflux activity in human intestine and leukocytes [8,9]. However, a study by Mölsä et al., demonstrated that the presence of C3435T and G2677T/A polymorphism did not alter the transplacental transfer of the P-gp substrate saquinavir [10]. Moreover, a meta-analysis of studies containing 1036 patients did not demonstrate a correlation between the C3435T SNP and altered pharmacokinetics of the P-gp substrate cyclosporine [11]. Several reports have implicated the C3435T and G2677T/A variant alleles with increased P-gp activity *in vivo* [12–14]. Studies on the consequences of the C1236T polymorphism in humans are scarce; however the TT genotype is associated with increased plasma concentrations of the P-gp substrate irinotecan in cancer patients [15]. These inconclusive findings indicate that direct measurements of P-gp transport activity and the correlation between genotype and protein expression need further investigations.

Therefore, the aim of this investigation was to determine the relationship between C1236T, C3435T, and G2677T/A

* Corresponding author at: Departments of Obstetrics & Gynecology and Pharmacology & Toxicology, University of Texas Medical Branch, 301 University Blvd., Galveston, TX, 77555-0587, USA. Tel.: +1 409 772 0977; fax: +1 409 747 1669.
E-mail addresses: sjhemaue@utmb.edu (S.J. Hemauer), tnnanovs@utmb.edu (T.N. Nanovskaya), sabdelra@utmb.edu (S.Z. Abdel-Rahman), svpatrik@utmb.edu (S.L. Patrikeeva), ghankins@utmb.edu (Gary D.V. Hankins), maahmed@utmb.edu (M.S. Ahmed).

0006-2952/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2009.10.026

polymorphisms and P-gp protein expression and transport activity. The health implication of this investigation is that P-gp protein expression and polymorphisms in *MDR1* could constitute significant contributing factors to P-gp transport activity, consequently affecting placental transfer and fetal exposure to xenobiotics that are P-gp substrates.

2. Material and methods

2.1. Chemicals

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise mentioned. Paclitaxel and paclitaxel [*o*-benzamido-^{3}H] (38 Ci/mmol) were purchased from Moravek Biochemicals, Inc. (Brea, CA). The anti-P-gp murine monoclonal antibodies (mAb C219) were purchased from Signet Laboratories (Dedham, MA). Actin (C-2) mouse monoclonal antibodies and goat antimouse horseradish peroxidase-conjugated antibody were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

2.2. Clinical material

A staff of trained research nurses was responsible for transporting the placentas immediately after delivery to our laboratory according to a protocol approved by the Institutional Review Board of UTMB. Along with placenta, a data sheet was provided that included the following: maternal age, maternal ethnicity, health conditions (if any), gestational age, and type of delivery. *Exclusion criteria*: documented drug abuse during pregnancy or infections with HIV or hepatitis. *Inclusion criteria*: term placentas (38–41 weeks) obtained from uncomplicated pregnancies.

2.3. Preparation of placental brush border membrane vesicles

Placental brush border membrane vesicles were prepared using a protocol modified from Ushigome et al. [16]. Tissue was cut from the maternal side, washed two times in 0.9% NaCl. All of the following steps were carried out at 4 °C. The cut tissue was transferred to sucrose-HEPES-Tris (SHT) buffer (250 mM sucrose, 10 mM HEPES-Tris, pH 7.4), and stirred for 1 h to disrupt brush border membrane. The tissue plus buffer was filtered through two layers of woven cotton gauze, and tissue was discarded. The filtrate was centrifuged at 800 × *g*: 10 min, to remove blood and cell debris. The supernatant was combined with 20 mM $MgCl_2$ (1:1 ratio) and stirred for 10 min. The solution was centrifuged at 10,500 × *g*, 10 min. The pellet was discarded and the supernatant was centrifuged at 20,000 × *g*, 20 min. The pellet, containing brush border membranes, was re-suspended in SHT buffer with a 26-gauge needle.

To maximize the proportion of IOVs, affinity chromatography was used to separate ROVs according to a previously reported method [1,17]. The ratio of IOVs (approximately 75% oriented inside–out) was determined by the activity of acetylcholinesterase [1].

2.4. Determination of P-gp protein levels by Western blot

The brush–border membranes were prepared as described above. The total protein concentration in all samples was determined by detergent-compatible Bradford protein assay. Briefly, Bradford Reagent (Bio-Rad Laboratories, Hercules, CA, USA) was diluted 4:1 with water. Samples of brush border membranes (10 μL) were added to the reagent (5 mL) and determined in triplicate using a Genesys 20 spectrophotometer (Thermo Scientific, Waltham, MA) at 595 nm. Total protein concentration in vesicle preparations was determined using bovine serum albumin (10–100 μg total protein) as a standard.

Western blot quantification of P-gp protein expression was carried out using 7.5% SDS/polyacrylaminde gel electrophoresis. The amount of total placental apical membrane protein loaded on each well was 10 μg. At the end of electrophoresis, the gel was electroblotted on nitrocellulose membranes overnight at 4 °C and a constant potential of 25 V. Blots were probed with anti-P-gp murine monoclonal antibodies (mAb C219) diluted 1:200 and secondary goat anti-mouse horseradish peroxidase-conjugated antibodies diluted 1:1000. Detection of the protein bands was carried out by spot densitometry and digital imaging of the enhanced chemiluminescence spots. The amount of expressed β-actin was used to normalize the amount of P-gp in each loaded sample on the gel. A positive control consisted of human P-gp membranes (Gentest Corporation).

The operator interpreting the results was blinded to the genotype of the subject. Samples from wild-type and variant were run together in mixed batches, and 10% of the samples were randomly selected and subjected to repeat analysis.

2.5. Uptake by membrane vesicles

P-gp transport activity was determined by the uptake of its radiolabeled prototypic substrate [^{3}H] paclitaxel (38 Ci/mmole, 83 dpm/fmole), in placental IOVs according to a previously reported protocol [15]. Each reaction was carried out in SHT buffer (250 mM sucrose, 10 mM HEPES-Tris) containing 4 mM $MgCl_2$, 10 mM creatine phosphate, 100 μg/mL creatine phosphokinase, either 2 mM ATP or 3 mM NaCl, and placental IOVs at a concentration of 0.05 μg/μL (7 μg total protein). The reaction was initiated by the addition of [^{3}H] paclitaxel, at a final concentration ~70 nM unless otherwise indicated. The reaction was terminated after 1 min by the addition of 1 mL ice cold buffer, and vesicles were isolated using rapid filtration by a Brandel Cell Harvester. The amount of [^{3}H]-paclitaxel retained on the filter was determined by liquid scintillation analysis. Active transport was calculated as the difference between the amount of [^{3}H]-paclitaxel in the presence and absence of ATP and expressed as pmol/mg protein min. P-gp-specific transport of paclitaxel was confirmed by its inhibition with P-gp monoclonal antibody C219 and the P-gp inhibitor, verapamil.

2.6. TaqMan genotyping

Placental nuclear fractions (n = 199) were isolated using subcellular fractionation. DNA was extracted from the placental nuclear fraction using the Qiagen DNeasy Blood & Tissue kit (Qiagen, Inc., Valencia, CA). Genotyping of the SNPs at position 1236 and 3435 (rs1128503 and rs1045642, respectively) was performed in all samples using TaqMan® Drug Metabolism Genotyping Assays (Applied Biosystems, Inc., Foster City, CA). The triallelic SNP at position 2677 of *MDR1* (rs2032582) in 104 of the 199 samples was genotyped using two different Custom TaqMan® SNP Gynotyping Assays (ABI): one for G/A genotyping, and one for G/T genotyping [18]. The reactions consisted of 2× Taqman Universal Master Mix, 20× or 40× Genotyping Assay Mix, DNase-free water, and at least 10 ng of genomic DNA in a final volume of 10 μL per reaction. The PCR amplification was performed under the following conditions: 10 min at 95 °C followed by 40 cycles at 92 °C for 15 s and 60 °C for 1 min. Allelic discrimination was determined after the amplification by performing an end-point read.

2.7. PCR-RFLP-based genotyping

PCR-RFLP-based genotyping assay [6] was used for the determination of the G2677T/A polymorphism in 95 of the 199

placental samples. Briefly, the forward primer FP 5′-TACCCATCATTGCAATAGCAG-3′, and the reverse primer RP 5′-TTTAGTTTGACTCACCTTGCTAG-3′, were used to generate a 107 base-pair fragment. The PCR reaction mixture (50 μL) consisted of ~50 ng of genomic DNA, 200 μM dNTPs, 1×-PCR buffer solution, 1.0 mM $MgCl_2$, 5 pmol of each primer, and 1 U of Taq DNA polymerase (Promega, Madison, WI). The PCR conditions consisted of an initial melting step of 94 °C for 5 min, followed by 35 cycles of melting at 94 °C for 30 s, annealing at 50 °C for 45 s, and extension at 72 °C for 60 s. A final extension step at 72 °C for 5 min terminated the process. A 20 μL amplicon was digested at 37 °C overnight with 2 U of Nhel restriction enzyme, which recognizes the wild-type G allele. The digested product was run on a 2% agarose gel at 85 V for 1 h and the genotypes were identified according to the banding pattern observed. For quality control, representative samples of both the reference and the variant alleles were confirmed by direct sequencing. The G allele was classified as wild-type (WT), and the A and T minor alleles were classified together as variant (V) genotype.

2.8. Statistical analysis

Hardy–Weinberg equilibrium of determined allele frequencies was assessed using the χ^2-test. For comparison of protein expression and uptake studies between groups, statistical significance was determined using a paired Student's *t*-test. A probability of $p < 0.05$ was considered to indicate statistical significance.

3. Results

3.1. Genotype distribution and allele frequency

Placentas from 199 term deliveries were analyzed for the C1236T, C3435T and G2677T/A triallelic polymorphism using TaqMan® Genotyping Assay and PCR-RFLP-base genotyping assay. The frequency of variant allele of the C1236T and G2677T/A SNPs differed significantly between Caucasian, African American, and Hispanic mothers. The frequency of the variant allele for the C3435T polymorphism was similar in Caucasian and Hispanic mothers, yet was significantly lower in African American mothers than in either Hispanic or Caucasian. All allele frequencies were found to be in Hardy–Weinberg equilibrium (*p* values of 0.79, 0.23, and 0.29 for the 1236, 3435, and 2677 SNPs, respectively). Allele frequencies are listed in Table 1.

3.2. Effect of MDR1 polymorphisms on placental P-gp expression

P-gp protein expression was quantified using Western Blot analysis of brush border membrane isolated from human placenta ($n = 199$), as represented in Fig. 1. The effect of *MDR1* polymorphisms on levels of P-gp protein expression in the placenta was evaluated using a dominant model. Placentas with the homozygous and heterozygous variant alleles were grouped together and compared to the referent homozygous wild-type group.

Table 1
Observed genotype frequencies of the *MDR1* polymorphism at positions 1236, 3435, and 2677 using TaqMan® Genotyping Assay (all SNPs) and RFLP analysis (G2677T/A only).

	1236		3435		2677	
Ethnicity	wt	var	wt	var	wt	var
Caucasian	0.58	0.42	0.46	0.54	0.76	0.24
African American	0.77	0.23	0.74	0.26	0.86	0.14
Hispanic	0.39	0.61	0.48	0.52	0.5	0.5

Comparison	A	B	C	A	B	C	A	B	C
p value	<0.01	<0.01	<0.01	<0.01	<0.01	0.69	0.02	<0.01	<0.01

A—Caucasian vs. African American. B—African American vs. Hispanic. C—Caucasian vs. Hispanic.

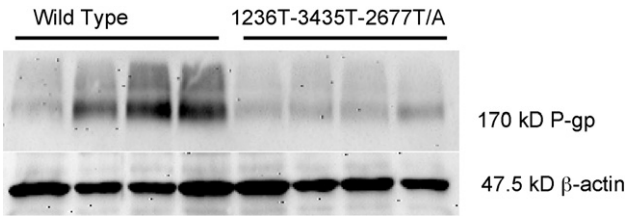

Fig. 1. Western blot of P-gp protein expression in human placental brush border membranes in representative wild-type and variants representing 1236T-3435T-2677T/A genotype (10 μg/lane). The immunoblot was probed with the P-gp monoclonal antibody C219. Immunoreactive protein bands detected by immunoblotting were analyzed by densitometry. P-gp expression was determined as a proportion of the total amount of β-actin present per lane.

The C1236T variant was associated with 11% lower P-gp protein expression than the CC wild-type (0.039 ± 0.002 vs. 0.045 ± 0.002 μg P-gp/μg total protein) (Fig. 2A). The C3435T and G2677T/A variants each were associated with a significant 16% reduction in placental P-gp protein expression compared with wild-type (0.039 ± 0.002 vs. 0.046 ± 0.003 μg P-gp/μg total protein, $p < 0.05$) (Fig. 2B and C). For each of the three polymorphisms examined, P-gp protein expression in heterozygotes was not different from homozygous variants.

3.3. Effect of MDR1 polymorphisms P-gp transport activity

The effect of the *MDR1* polymorphisms C1236T, C3435T, and G2677T/A, as determined by TaqMan Genotyping Assay, on P-gp transport activity was determined in 105 term placentas (Fig. 3). P-gp transport activity was determined by measuring the uptake of its prototypic substrate [^{3}H]-paclitaxel, in placental brush border membrane vesicles. P-gp-mediated active transport of paclitaxel was significantly greater in homozygous variant 1236T/T genotype (24 ± 5 pmol mg protein^{-1} min^{-1}) than in homozygous wild-type (C/C) (14 ± 2 pmol mg protein^{-1} min^{-1}, $p = 0.04$) (Fig. 3A). Similarly, homozygous variants of the 3435T/T genotype displayed significantly greater P-gp transport activity than homozygous wild-type (C/C) (27 ± 6 vs. 14 ± 2 pmol mg protein^{-1} min^{-1}, $p = 0.02$) (Fig. 3B). The G2677T/A variants (A/A, A/T, or T/T) exhibited a trend toward increased transport activity over homozygous wild-type (14 ± 2 vs. 21 ± 7 pmol mg protein^{-1} min^{-1}), but did not reach statistical significance ($p = 0.20$) (Fig. 3C). With all three SNPs examined, there was a gene-dose effect, where heterozygous placentas (wild-type/variant) had intermediate level of P-gp uptake activity compared to placentas with the wild-type (lowest uptake) and homozygous variants (highest uptake).

4. Discussion

The goal of this investigation was to examine the relationship between *MDR1* polymorphism, human placental P-gp protein expression and activity. A previous report from our laboratory [1] revealed that there was inter-individual variability and thus no correlation between the expression of P-gp protein and its activity in the transport of paclitaxel in 200 term human placentas. In order to elucidate the genetic factors contributing to the above finding, we investigated three common polymorphisms in the *MDR1* gene for their effect on placental P-gp protein expression and its activity in the transport of its prototypic substrate, paclitaxel.

Genotype frequencies of the C1236T, C3435T, and G2677T/A SNPs differ between Caucasian, African Americans, and Hispanic

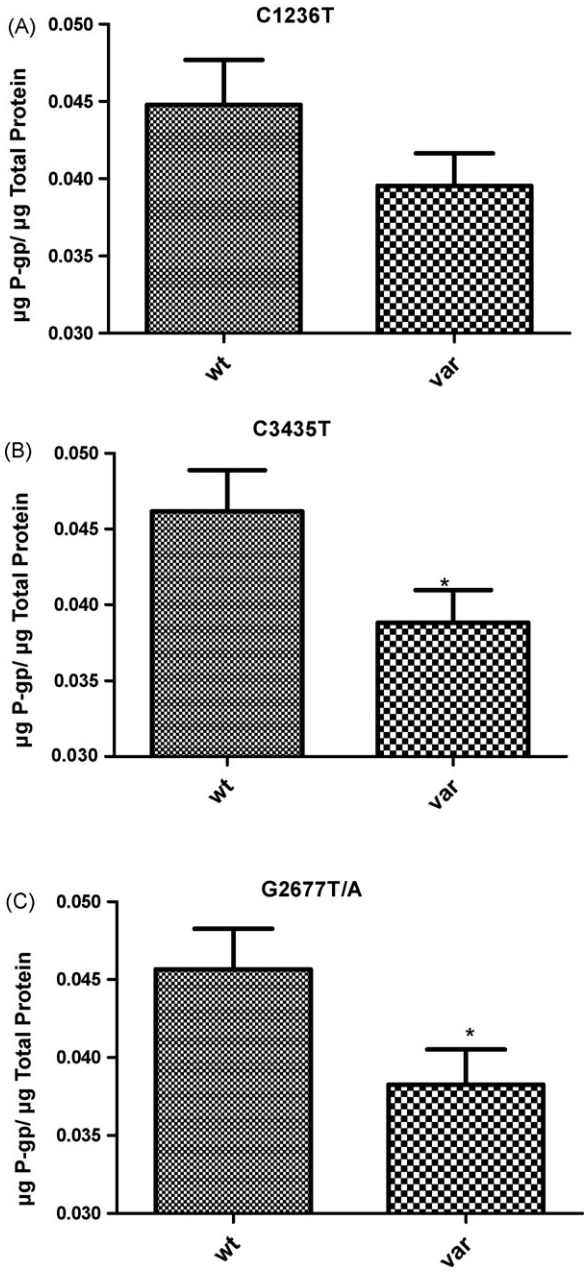

Fig. 2. Western blot analysis of P-gp protein expression in placental brush border membrane samples from wild-type (wt/wt) and variant (wt/var, var/var) genotypes (mean ± SEM of 199 individual samples). (A) C1236T Variants had 11% lower P-gp protein expression than wild-type. (B) C3435T and (C) G2677T/A variants each had a significant 16% reduction in placental P-gp protein expression compared with wild-type ($p < 0.05$).

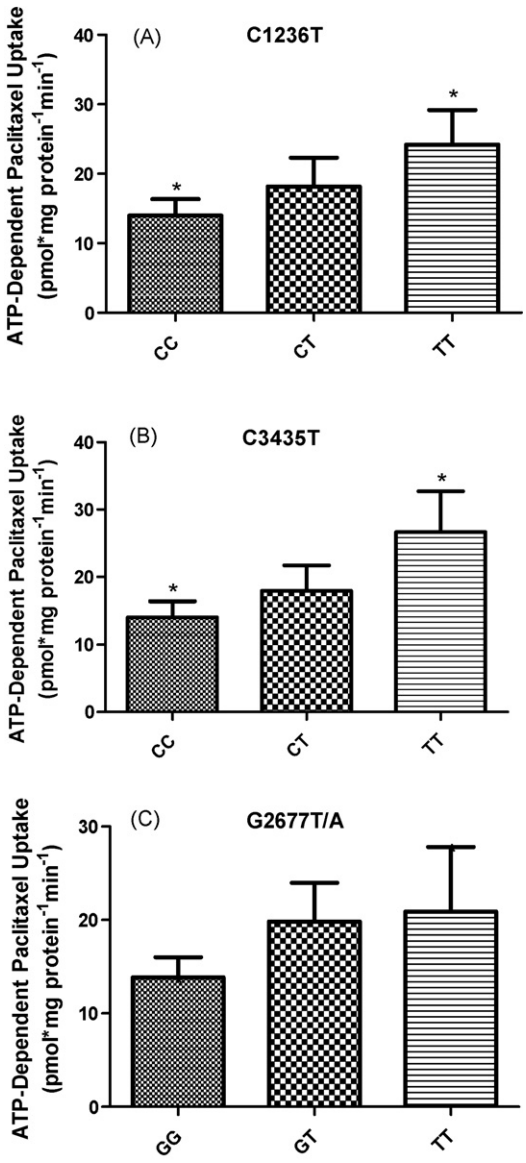

Fig. 3. P-gp transport activity was determined as the ATP-dependent uptake of P-gp substrate [^{3}H]-paclitaxel in vesicles prepared from placental brush border membrane (mean ± SEM of 105 individual samples). (A) P-gp-mediated active transport of paclitaxel was significantly greater in homozygotes for the variant 1236T/T genotype than in wild-type (C/C) homozygotes ($p = 0.04$). (B) Homozygous variants of the 3435T/T genotype displayed significantly greater P-gp transport activity than wild-type (C/C) homozygotes ($p = 0.02$). (C) G2677T/A variants (A/A, A/T, or T/T) had a trend toward increased P-gp transport activity over wild-type homozygotes.

Americans [4,5]. Similarly, in the patients delivering at our site, the allele frequencies for the three polymorphisms differed significantly between these maternal ethnic groups and are in agreement with those reported separately in Caucasian, Hispanic, and African Americans [19]. However, analysis of phenotype using segregation of maternal ethnicity may not be extrapolated to placental genotype, because the placental DNA is that of the fetus (maternal and paternal DNA). Therefore, the data on allele frequency presented are based on the assumption that maternal ethnicity is the same as fetal, which was not ascertained because the information on paternal ethnicity was not available in these patients.

The C3435T and G2677T/A alleles were associated with significantly reduced placental P-gp protein expression. Additionally, the C1236T variant was associated with a trend toward decreased placental P-gp expression. This finding is in agreement with other reports in which variants for the above alleles were associated with decreased P-gp protein expression in placental and non-placental tissues [6–9]. Similar association between variant allele and reduced placental protein expression has also been reported in ABC transporter Breast Cancer Resistance Protein (BCRP) [20]. Interestingly, the reduction in P-gp observed with each variant allele was associated with an increase in its transport of the prototypic substrate paclitaxel. This observation is consistent with reports demonstrating that variant alleles could be associated with increased P-gp activity *in vivo* [12–14]. On the other hand, this is the first investigation that compared genotype and protein expression with direct measurement of P-gp transport activity using membrane vesicles prepared from human placentas.

The synonymous SNPs (associated with no change in amino acid sequence) C1236T and C3435T were associated with altered functional activity of P-gp. There are several plausible explanations for this finding. First, the finding could be due to C1236T and C3435T occurring in linkage disequilibrium with the functional non-synonymous G2677T/A polymorphism. Alternatively, the synonymous SNPs have been hypothesized to introduce a rare codon, which affects the timing of cotranslational folding and insertion of P-gp into the membrane, thereby altering the structure of substrate and inhibitor interaction sites [21]. In fact, the synonymous polymorphisms result in P-gp with altered conformation, drug and inhibitor interactions despite similar mRNA and protein levels [21]. Finally, it is possible that both linkage to the functional G2677T/A polymorphism and the introduction of a rare codon with altered conformation and substrate interaction contribute to the relationship between synonymous SNPs and P-gp function.

Taken together, the data obtained in this investigation demonstrate that the variant alleles for the three SNPs examined are associated with decreased protein expression and an increase in the transport activity of placental P-gp. The observed inverse relationship between protein expression and activity with the variant alleles could – in addition to inter-individual variability observed between placentas – contribute to the lack of correlation between P-gp protein expression and transport activity reported in vesicle preparations of human placenta [1]. The mechanism by which protein expression is reduced yet activity is increased in variants remains unknown. However, it should be noted that there are several substrates of P-gp that are transported and are also inducers of *MDR1* gene expression [22]. It is plausible that in variants with increased P-gp-mediated efflux of a substrate/inducer, there is lower intracellular concentration and thus less induction of *MDR1* gene expression by that particular substrate/inducer leading to lower P-gp protein expression. Therefore, it is possible that the decrease in P-gp protein expression is secondary to an increase in its transport activity in variants.

A second explanation for the lack of positive correlation between protein expression and activity is that the total P-gp protein expression level vs. the amount of P-gp actively transporting across the placental brush border membrane may be regulated independently. A previous report from our laboratory and others have demonstrated that P-gp protein expression declines with advancing gestation [23–25]. However, there was no significant decrease in P-gp transport activity associated with increasing gestational age (unpublished). Therefore, there could be regulation of an "inactive pool" of expressed P-gp protein in placental tissue that is yet to be elucidated. Furthermore, subcellular localization of P-gp within organelles as demonstrated in non-placental tissue [26,27] may also be present in placenta and contribute to protein expression disproportionate with activity. A role of the C1236T, C3435T, and G2677T/A polymorphisms in the subcellular localization and/or membrane insertion of expressed P-gp could contribute to total P-gp efflux activity and cannot be ruled out at this time.

In summary, variants for the C1236T, C3435T, and G2677T/A alleles in the *MDR1* gene encoding P-gp are associated with reduced protein expression and increased transport of P-gp selective substrate, paclitaxel, in human term placenta. The implication of these findings is that populations associated with decreased placental P-gp efflux activity may be at greater risk of potential fetal exposure to xenobiotics in the maternal circulation.

Acknowledgements

The authors thank the Perinatal Research Division for their assistance, and the Publication, Grant, & Media Support of the UTMB Department of Obstetrics and Gynecology. Supported by the National Institute on Drug Abuse Grant DA13431 (M.S.A.).

References

[1] Hemauer SJ, Patrikeeva SL, Nanovskaya TN, Hankins GD, Ahmed MS. Opiates inhibit paclitaxel uptake by P-glycoprotein in preparations of human placental inside-out vesicles. Biochem Pharmacol)2009;(July) [Epub ahead of print].
[2] Ambudkar SV, Kimchi-Sarfaty C, Sauna ZE, Gottesman MM. P-glycoprotein: from genomics to mechanism. Oncogene 2003;22(47):7468–85.
[3] Saito S, Iida A, Sekine A, Miura Y, Ogawa C, Kawauchi S, et al. Three hundred twenty six genetic variations in genes encoding nine members of ATP-binding cassette subfamily B (ABCB/MDR/TAP), in the Japanese population. J Hum Genet 2002;47(1):38–50.
[4] Pauli-Magnus C, Kroetz DL. Functional implications of genetic polymorphisms in the multidrug resistance gene MDR1 (ABCB1). Pharmacol Res 2004; 21(6):904–13.
[5] Kroetz DL, Pauli-Magnus C, Hodges LM, Huang CC, Kawamoto M, Johns SJ, et al. Sequence diversity and haplotype structure in the human ABCB1 (MDR1, multidrug resistance transporter) gene. Pharmacogenetics 2003;13(August (8)):481–94.
[6] Tanabe M, Ieiri I, Nagata N, Inoue K, Ito S, Kanamori Y, et al. Expression of P-glycoprotein in human placenta: relation to genetic polymorphism of the multidrug resistance (MDR)-1 gene. J Pharmacol Exp Ther 2001;297(3):1137–43.
[7] Hitzl M, Schaeffeler E, Hocher B, Slowinski T, Halle H, Eichelbaum M, et al. Variable expression of P-glycoprotein in the human placenta and its association with mutations of the multidrug resistance 1 gene (MDR1, ABCB1). Pharmacogenetics 2004;14(5):309–18.
[8] Hoffmeyer S, Burk O, von Richter O, Arnold HP, Brockmoller J, Johne A, et al. Functional polymorphisms of the human multidrug-resistance gene: Multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity *in vivo*. P Natl Acad Sci USA 2000;97(7):3473–8.
[9] Hitzl M, Drescher S, van der Kuip H, Schaffeler E, Fischer J, Schwab M, et al. The C3435T mutation in the human MDR1 gene is associated with altered efflux of the P-glycoprotein substrate rhodamine 123 from CD56+ natural killer cells. Pharmacogenetics 2001;11:293–8.
[10] Mölsä M, Heikkinen T, Hakkola J, Hakala K, Wallerman O, Wadelius M, et al. Functional role of P-glycoprotein in the human blood-placental barrier. Clin Pharmacol Ther 2005;78(2):123–31.
[11] Jiang Z, Wang Y, Xu P, Liu R, Zhao X, Chen F. Meta-analysis of the effect of MDR1 C3435T polymorphism on cyclosporine pharmacokinetics. Basic Clin Pharmacol 2008;103(5):433–44.
[12] Taubert D, von Beckerath N, Grimberg G, Lazar A, Jung N, Goeser T, et al. Impact of P-glycoprotein on clopidogrel absorption. Clin Pharmacol Ther 2006;80(5):486–501.
[13] Kwan P, Wong V, Ng PW, Lui CH, Sin NC, Poon WS, et al. Gene-wide tagging study of association between ABCB1 polymorphisms and multidrug resistance in epilepsy in Han Chinese. Pharmacogenomics 2009;10(5):723–32.
[14] Gréen H, Söderkvist P, Rosenberg P, Mirghani RA, Rymark P, Lundqvist EA, et al. Pharmacogenetic studies of Paclitaxel in the treatment of ovarian cancer. Basic Clin Pharmacol 2009;104(2):130–7.
[15] Mathijssen RH, Marsh S, Karlsson MO, Xie R, Baker SD, Verweij J, et al. Irinotecan pathway genotype analysis to predict pharmacokinetics. Clin Cancer Res 2003;9(9):3246–53.
[16] Ushigome F, Koyabu N, Satoh S, Tsukimori K, Nakano H, Nakamura T, et al. Kinetic analysis of P-glycoprotein-mediated transport by using normal human placental brush-border membrane vesicles. Pharm Res 2003;20(1):38–44.
[17] Awasthi S, Singhal S, Srivastava SK, Zimniak P, Bajpai KK, Saxena M, et al. Adenosine triphosphate-dependent transport of doxorubicin, daunomycin, and vinblastine in human tissues by a mechanism distinct from the P-glycoprotein. J Clin Invest 1994;93(3):958–65.
[18] Morita A, Nakayama T, Doba N, Hinohara S, Mizutani T, Soma M. Genotyping of triallelic SNPs using TaqMan® PCR. Mol Cell Probe 2006;21:171–6.
[19] Sherry ST, Ward MH, Kholodov M, Baker J, Phan L, Smigielski EM, et al. dbSNP: the NCBI database of genetic variation. Nucleic Acids Res 2001;29 (1):308–11.
[20] Kobayashi D, Ieiri I, Hirota T, Takane H, Maegawa S, Kigawa J, et al. Functional assessment of ABCG2 (BCRP) gene polymorphisms to protein expression in human placenta. Drug Metab Dispos 2005 Jan;33(1):94–101.
[21] Kimchi-Sarfaty C, Oh JM, Kim IW, Sauna ZE, Calcagno AM, Ambudkar SV, et al. A "silent" polymorphism in the MDR1 gene changes substrate specificity. Science 2007;315(5811):525–8.
[22] Wang X, Meng M, Gao L, Liu T, Xu Q, Zeng S. Permeation of astilbin and taxifolin in Caco-2 cell and their effects on the P-gp. Int J Pharm 2009;378(1–2):1–8.
[23] Nanovskaya TN, Nekhayeva IA, Hankins GD, Ahmed MS. Transfer of methadone across the dually perfused preterm human placental lobule. Am J Obstet Gynecol 2008;198(1):126.e1–4.
[24] Gil S, Saura R, Forestier F, Farinotti R. P-glycoprotein expression of the human placenta during pregnancy. Placenta 2005;26(2):268–70.
[25] Sun M, Kingdom J, Baczyk D, Lye SJ, Matthews SG, Gibb W. Expression of the multidrug resistance P-glycoprotein (ABCB1 glycoprotein) in the human placenta decreases with advancing gestation. Placenta 2006;27(6–7):602–9.
[26] Molinari A, Calcabrini A, Meschini S, Stringaro A, Crateri P, Toccacieli L, et al. Subcellular detection and localization of the drug transporter P-glycoprotein in cultured tumor cells. Curr Protein Pept Sci 2002;3(6):653–70.
[27] Maraldi NM, Zini N, Santi S, Scotlandi K, Serra M, Baldini N. P-glycoprotein subcellular localization and cell morphotype in MDR1 gene-transfected human osteosarcoma cells. Biol Cell 1999;91(1):17–28.