Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research
EVOLUTION AND TECHNOLOGY

Effects of *ABCB1* 3435C>T genotype on serum levels of cortisol and aldosterone in women with normal menstrual cycles

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Genet. Mol. Res. 8 (2): 397-403 (2009)
Received January 15, 2009
Accepted March 2, 2009
Published April 7, 2009

ABSTRACT. ABCB1, also known as MDR1/P-glycoprotein, can transport cortisol and aldosterone. We examined the effects of *ABCB1* polymorphisms on serum levels of cortisol and aldosterone among different phases of the normal menstrual cycle in 51 non-pregnant healthy Japanese female volunteers (22 ± 1 years old). The menstrual cycle was divided into three phases: premenstrual phase (14 days preceding the onset of menstruation, N = 22; menstrual phase, N = 11, and postmenstrual phase, N = 18). *ABCB1* -129T>C, 1236C>T, 2677G>A/T, and 3435C>T genotypes were determined. Serum levels of cortisol, aldosterone, estradiol, progesterone, and testosterone were measured. The serum levels of estradiol in the pre- and post-

menstrual phases and of progesterone in the premenstrual phase were significantly increased when compared to their serum levels in the menstrual phase ($P < 0.005$). In the postmenstrual phase, the mean serum cortisol level in subjects with the 3435CT and 3435TT genotype was 7.6 ± 3.4 µg/dL (mean ± SD, N = 7), which was significantly lower than in women with the 3435CC genotype (9.9 ± 1.8 µg/dL, N = 11) ($P = 0.037$). The opposite effect was observed in the serum aldosterone level during the postmenstrual phase (97.2 ± 23.4 and 141.2 ± 48.5 pg/mL for 3435CC and 3435CT + 3435TT, respectively; $P = 0.041$). These findings suggest that *ABCB1* 3435C>T genotype can influence serum levels of cortisol and aldosterone during the postmenstrual phase of the normal menstrual cycle.

Key words: MDR1; P-glycoprotein; Genetic polymorphism; Cortisol; Aldosterone; Menstrual cycle

INTRODUCTION

The transmembrane transporter ATP-binding cassette, sub-family B, member 1 (ABCB1, also known as MDR1/P-glycoprotein) functions as an energy-dependent efflux pump to confer multidrug resistance in tumor cells (Marzolini et al., 2004; Pauli-Magnus and Kroetz, 2004; Sakaeda et al., 2004). ABCB1 is also expressed in various normal tissues, such as the kidney, adrenal gland, liver, blood-brain barrier, placenta, and testis, and it plays a physiologically important role in the distribution of hormones, toxins, and physiological metabolites (Marzolini et al., 2004; Pauli-Magnus and Kroetz, 2004; Sakaeda et al., 2004). To date, *in vitro* studies have demonstrated that cortisol and aldosterone are transported via ABCB1 (Ueda et al., 1992; van Kalken et al., 1993; Bello-Reuss et al., 2000); in mice, ABCB1 can modulate plasma and tissue distribution of these two hormones (Uhr et al., 2002; Parker et al., 2006).

During the last decade, pharmacogenetic approaches have identified more than 40 single nucleotide polymorphisms in the *ABCB1* gene (Marzolini et al., 2004; Pauli-Magnus and Kroetz, 2004; Sakaeda et al., 2004). Among these, the 3435C>T polymorphism is common in ethnically diverse populations and has attracted a great deal of attention due to its influence on the expression level and function of various tissues (Marzolini et al., 2004; Pauli-Magnus and Kroetz, 2004; Sakaeda et al., 2004), presumably affecting the kinetics of cortisol and aldosterone. *ABCB1* 3435C>T genotype-dependent angiotensin II has been reported to increase serum aldosterone levels in male subjects (Zolk et al., 2007). Individuals with the *ABCB1* haplotype, including 3435C>T polymorphism, are reportedly predisposed to mood disorders, presumably due to alteration of the plasma cortisol level, which is dependent on ABCB1 activity (Qian et al., 2006). However, it is not known whether *ABCB1* genetic polymorphisms influence serum cortisol and aldosterone levels in female subjects and whether this influence varies across the menstrual cycle.

We examined the effects of *ABCB1* polymorphisms on serum cortisol and aldosterone levels during three different phases of the normal menstrual cycle.

MATERIAL AND METHODS

Subjects

Fifty-one unrelated healthy Japanese female volunteers living in Kobe city and neighboring areas, ranging in age from 21-23 years old (mean ± SD = 22.1 ± 0.5), were enrolled in our study. Their mean body weight was 51.0 ± 5.7 kg (range 38-67 kg) and their mean body mass index was 20.1 ± 2.0 kg/m^2 (range 16.7-29.0 kg/m^2). The subjects reported the first day of the last menstrual period and the usual length of their menstrual cycle, and they had not been taking oral contraceptives or other medications. The menstrual cycle phase was determined using the self-reported date of the last menstrual period and the usual length of the menstrual cycle. The 51 subjects were divided into three groups based on their menstrual phases. The 14 days preceding the onset of menstruation were defined as the premenstrual phase (N = 22). Days 8-14 of the menstrual cycle were defined as the postmenstrual phase (N = 11) and days 1-7 as the menstrual phase (N = 18). Menstrual dates were standardized to a 28-day cycle. The aims of the study were fully explained to all subjects, and written informed consent was obtained. The research protocol was approved by the Institutional Review Board of Kobe University Hospital, Kobe University, Japan.

Blood sampling

Peripheral venous blood samples (5 mL) from each subject were drawn into two sampling tubes; this was always done at 18:00 h, to minimize confounding influence from circadian variation in serum cortisol and aldosterone levels, which peak early in the morning and decline within several hours and then remain relatively stable in the evening (Parry et al., 2000; Hurwitz et al., 2004). The subjects were cautioned not to eat or drink anything for 3 h before blood sampling. Each of the peripheral venous blood (5 mL) samples drawn from patients was split into two aliquots. One was immediately centrifuged (10 min at 2,500 rpm), giving a serum sample for the determination of serum levels of cortisol and aldosterone by radioimmunoassay and estradiol, progesterone and testosterone serum levels by electrochemiluminescence immunoassay. The other was used for *ABCB1* genotyping. These whole-blood and serum samples were stored at -20°C until the assays were run.

Genotyping of *ABCB1* polymorphisms

Genomic DNA was extracted from 0.2 mL whole blood using a QIAamp DNA Blood mini Kit (QIAGEN, Hilden, Germany), as previously described (Horinouchi et al., 2002). The genotypes of *ABCB1* - 129T>C (rs3213619), 1236C>T (rs1128503), 2677G>A/T (rs2032582), and 3435C>T (rs1045642) polymorphisms were determined using the TaqMan® MGB probe and primer, as previously described (Komoto et al., 2006).

Statistical analysis

Data are reported as means ± SD. Statistical analysis was performed using the statistical package SPSS, version 14.0. Statistical significance of differences between the medians of

two different samples was calculated using the Mann-Whitney U-test. Multiple comparisons were performed by the Kruskal-Wallis analysis, followed by the Mann-Whitney U-test with Bonferroni's correction. For all tests, P values less than 0.05 were considered to be significant.

RESULTS

Sociodemographic variables and serum hormone levels during a normal menstrual cycle

Age, body weight, height, and body mass index were similar among the three groups, classified by the menstrual phases of the normal menstrual cycle (Table 1). In the menstrual phase, the serum level of estradiol was significantly lower than those in the pre- and post-menstrual phases (P = 0.001 and 0.005, respectively), and the serum progesterone level in the menstrual phase was lower than that in the premenstrual phase (P = 0.006). Similar differences in the baseline level of aldosterone in serum were observed among the three menstrual phases, although they were not significant (P = 0.061). There were no significant differences in the serum levels of cortisol and testosterone among the three groups.

Table 1. Sociodemographic variables and serum hormone levels during normal menstrual cycle.

Steroid hormone	Premenstrual phase	Menstrual phase	Postmenstrual phase
Number	22	11	18
Age (years)	22.1 ± 0.5	22.3 ± 0.5	21.9 ± 0.4
Weight (kg)	50.9 ± 5.6	50.7 ± 4.3	51.2 ± 6.6
Height (m)	159.3 ± 5.3	159.9 ± 6.9	159.1 ± 6.4
Body mass index (kg/m^2)	20.1 ± 1.7	19.9 ± 1.6	19.5 ± 2.6
Cortisol (µg/dL)	7.6 ± 3.1	10.2 ± 5.2	8.5 ± 3.1
Aldosterone (pg/mL)	126.3 ± 34.0	98.0 ± 29.7	124.1 ± 45.4
Estradiol (pg/mL)	96.3 ± 81.8	36.2 ± 9.2*,+	100.1 ± 92.0
Progesterone (ng/mL)	4.10 ± 5.02	0.57 ± 0.19*	3.32 ± 5.86
Testosterone (ng/mL)	0.61 ± 0.21	0.48 ± 0.14	0.54 ± 0.18

Data are reported as means ± SD. *P < 0.05, compared with data in the premenstrual phase. $^+$P < 0.05, compared with data in the postmenstrual phase.

Effects of *ABCB1* polymorphisms on serum hormone levels during the menstrual cycle

The *ABCB1* genetic polymorphisms -129T>C, 1236C>T, 2677G>A, 2677G>T, and 3435C>T were found at allele frequencies of 3/102, 74/102, 13/102, 44/102, and 41/102, respectively. Their frequencies were similar to what we found in a previous study of healthy subjects (Komoto et al., 2006). In the postmenstrual phase, the mean serum cortisol level in subjects with 3435CT and 3435TT genotypes was 7.6 ± 3.4 µg/dL, significantly lower than in those with 3435CC (Table 2), whereas the opposite tendency was observed in serum aldosterone levels (Table 2). Serum levels of cortisol in the premenstrual and menstrual phases and that of aldosterone in the menstrual phase also tended to be lower in the 3435CT + 3435TT genotype group, compared with those in the 3435CC group, although no significant differences were found between the groups. There was no significant effect of the *ABCB1* 3435C>T

genotype on serum levels of estradiol, progesterone and testosterone throughout the menstrual cycle. The effects of other *ABCB1* genotypes on basal hormone levels in serum were not examined, because the sample size for each genotype group was not large enough to compare.

Table 2. Effects of *ABCB1* 3435C>T genotype on serum hormone levels during the menstrual cycle.

Steroid hormone	Genotype	
	3435CC	3435CT + 3435TT
Cortisol (µg/dL)		
Premenstrual phase	8.7 ± 3.8 (N = 7)	7.1 ± 2.7 (N = 15)
Menstrual phase	11.4 ± 6.5 (N = 5)	9.2 ± 4.2 (N = 6)
Postmenstrual phase	9.9 ± 1.8 (N = 7)	7.6 ± 3.4* (N = 11)
Aldosterone (pg/mL)		
Premenstrual phase	118.2 ± 21.2	130.1 ± 38.7
Menstrual phase	113.7 ± 34.5	84.9 ± 18.8
Postmenstrual phase	97.2 ± 23.4	141.2 ± 48.5*
Estradiol (pg/mL)		
Premenstrual phase	106.6 ± 126.2	91.5 ± 56.0
Menstrual phase	35.3 ± 8.9	37.0 ± 10.2
Postmenstrual phase	70.5 ± 38.7	119.0 ± 111.7
Progesterone (ng/mL)		
Premenstrual phase	2.64 ± 3.03	5.13 ± 5.74
Menstrual phase	0.63 ± 0.15	0.52 ± 0.22
Postmenstrual phase	1.75 ± 3.28	4.32 ± 7.00
Testosterone (ng/mL)		
Premenstrual phase	0.77 ± 0.28	0.54 ± 0.13
Menstrual phase	0.46 ± 0.11	0.50 ± 0.17
Postmenstrual phase	0.57 ± 0.12	0.53 ± 0.21

Data are reported as means ± SD. *$P < 0.05$, compared with the 3435CC genotype group.

DISCUSSION

Cortisol and aldosterone are synthesized in the adrenals and then released into the blood circulation (Müller, 1995); ABCB1, which is highly expressed in the adrenal glands, has the potential to efflux these hormones out of cells (Sugawara et al., 1988; Ueda et al., 1992; van Kalken et al., 1993; Bello-Reuss et al., 2000). We found that the *ABCB1* 3435C>T genotype affected baseline serum levels of cortisol and aldosterone during the postmenstrual phase of the normal menstrual cycle (Table 2). We hypothesize that ABCB1-mediated transport is associated with the efflux of cortisol and aldosterone from the adrenal tissues and that its function is influenced by 3435C>T polymorphisms.

Although C to T substitution at position 3435 of the *ABCB1* gene does not induce changes in amino acids, instability of ABCB1 mRNA derived from the 3435T allele in human liver samples and transfected Chinese hamster ovary cells has been reported (Wang et al., 2005). If this also happens in adrenal tissue, subjects with 3435CT and 3435TT genotypes would have lower serum levels of cortisol and aldosterone than those with the 3435CC genotype, probably due to the relatively lower ABCB1-mediated transporting activity. Indeed, a lower serum cortisol level was observed throughout the menstrual cycle in subjects harboring the 3435T allele, compared to the 3435CC group; a similar tendency was observed in the serum aldosterone level during the menstrual phase (Table 2). However, the 3435C>T geno-

type-dependent effect on serum aldosterone levels inverted during the pre- and postmenstrual phases; this may be due to changes in sex hormones during the menstrual cycle.

In women with a normal menstrual cycle, luteal and follicular phases are characterized by increased and decreased production, respectively, of estradiol and/or progesterone; the baseline serum levels of aldosterone, but not cortisol, differ between these two phases (Parry et al., 2000; Chidambaram et al., 2002; Pechère-Bertschi et al., 2002; Szmuilowicz et al., 2006). We found that higher serum levels of aldosterone accompanied increased serum estradiol and progesterone levels in pre- and postmenstrual phases, although the differences were not quite significant (P = 0.061); these two phases apparently reflect the luteal phase. In addition, urinary excretion of aldosterone in the luteal phase was increased compared to that of the follicular phase, as serum levels were elevated (Szmuilowicz et al., 2006). Our findings lead to speculation that in pre- and postmenstrual phases, the *ABCB1* 3435C>T genotype results in greater urinary than adrenal excretion of aldosterone. However, our study had several limitations. Urine samples were not collected, which would be necessary to objectively evaluate urinary excretion of aldosterone. Also, blood samples were collected from each participant only once, so we were not able to determine if there were diurnal variations of steroid hormones among the participants. Further investigations with more subjects should be undertaken to confirm our preliminary hypotheses.

Recently, Zolk et al. (2007) reported that the *ABCB1* 3435C>T genotype affects the serum aldosterone level stimulated by angiotensin II; they found that the increase in serum aldosterone levels was greater in male subjects with the 3435TT genotype than in those with the 3435CC genotype. They speculated that this effect is due to reduced ABCB1-mediated tubular secretion of aldosterone and increased reabsorption from the tubule lumen, which could be caused by reduced renal expression of ABCB1 in subjects with the 3435TT genotype (Siegsmund et al., 2002). Our findings were consistent with the hypothesis of Zolk et al. (2007), but it remains unclear whether this *ABCB1* genotype affects urinary levels of aldosterone in both males and females, and whether angiotensin II is associated with this effect. The baseline serum aldosterone levels in the report of Zolk et al. (2007) were similar across the *ABCB1* 3435 genotypes, although they did not report the baseline serum concentrations of cortisol, estradiol and progesterone. In healthy female subjects, during the menstrual phase, serum levels of these three hormones are similar to those in males, whereas during the luteal phase, increased aldosterone production is partly attributed to increased serum angiotensin II levels (Müller, 1995; Chidambaram et al., 2002; Szmuilowicz et al., 2006). Szmuilowicz et al. (2006) also suggested that progesterone, but not estradiol, directly contributes to increased luteal phase aldosterone production, independent of the renin-angiotensin system, based on data from an *in vitro* study. In our study, during the postmenstrual phase, when there are significant changes in the serum level of estradiol, but not of progesterone, serum aldosterone levels differed between 3435CC and 3435CT + 3435TT genotypes; the contribution of the progesterone-aldosterone system, independent of the renin-angiotensin system, to these *ABCB1* genotype-dependent differences appears to be small, although further studies are warranted to appropriately investigate these influences.

ACKNOWLEDGMENTS

We acknowledge partial support from a grant-in-aid for scientific research from the Japanese Ministry of Education, Culture, Sports, Science, and Technology.

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