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ORIGINAL ARTICLE

THE ANGIOTENSIN-CONVERTING ENZYME GENE POLYMORPHISM IS ASSOCIATED WITH PREGNANCY MISCARRIAGE AND PLACENTAL INSUFFICIENCY

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ABSTRACT

Research of past decades has shown that pregnancy miscarriage (PM) and placental insufficiency (PI) underlie severe obstetric pathologies that result in complications of fetal development. The occurrence of PI developed during previous PMs varied from 47.6 to 77.3%. Pregnancy miscarriage can be considered to be the clinical manifestation of PI, which is a common complication in women with a previous history of PM. Endothelial dysfunction in the mother and in the fetoplacental complex are basic causes of PI. The angiotensin-converting enzyme (ACE) plays a key role in maintenance of proper balance between vasoconstriction and vasodilatation of blood vessels, and thus, in blood pressure regulation. DNA samples were genotyped for insertion/deletion (I/D) polymorphism of the *ACE* gene by polymerase chain reaction (PCR) assay. This study shows that the frequency of allele I of the *ACE* gene in DNA samples from placentae of the women with PM and PI (group 4) is twice as high than in the placentae of women with a PM history but without PI in the current pregnancy (group 3) (64.6 and 30.4%, respectively, $p < 0.001$). Odds ratio (OR) analyses showed that the D/D genotype in placentae increases by 3-fold the risk of PI in women negative for PM history. The presence of the I/I genotype in patients positive for PM history increases the risk of PI 2.6-fold. It is concluded that the D/D genotype in placentae correlates with increased risk of PI in women negative for PM history, whereas the I/I genotype is a risk factor for PI in patients positive for PM history.

Key words: Pregnancy miscarriage (PM); Placental insufficiency (PI); *ACE* gene; Angiotensin-converting enzyme (ACE)

INTRODUCTION

Numerous studies have shown that pregnancy miscarriage (PM) and placental insufficiency (PI) underlie many kinds of severe obstetric pathology that result in complications of pregnancy and of fetal development. The occurrence of PI developed during previous PMs varied from 47.6 to 77.3% [1]. Pregnancy miscarriage can be considered to be the clinical manifestation of PI. Secondly, PI is a common complication in women with a former history of PM. Compensated and subcompensated forms of PI manifest as threatened spontaneous abortion or threatened premature birth, while decompensated PI may be complicated by early or late spontaneous abortion, delayed miscarriage and premature birth [2]. These clinical complications are considered to be forms of PM. Thus, PI and PM are closely interconnected, but they can also be treated as independent entities.

The etiology of PM and PI includes: hormonal insufficiency, abnormal vascularization of the uterus, endometrium lesions due to previous curettages, genital infantilism, detrimental inflammatory or

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vascular reactions and thrombophilic complications provoked by autoimmune processes. Consequently, PI in patients with PM is usually treated as a primary abnormality [3]. Placental insufficiency often occurs as a secondary consequence of uterine blood-flow abnormalities resulting from, for example, hypo- or hypertension, heart attack, partial detachment of the placenta or inflammatory reaction to genital infection in the mother [4,5]. Thus, abnormalities of placental blood circulation, including microcirculation failure that leads to defects of efficient metabolic exchange between maternal and fetal tissues, constitute the basic mechanism of PI etiology.

Recent clinical findings attribute a major role, in PI and in PM, to endothelial dysfunction in the mother, the fetoplacental complex and in umbilical cord arteries [6]. The angiotensin-converting enzyme (ACE) occupies a key position in maintenance of a proper balance between vasoconstriction and vasodilatation of blood vessels, and thus in blood pressure regulation. The enzyme converts angiotensin I into angiotensin II. Angiotensin II has vasoconstrictor properties, and consequently participates in regulation of vascular tone [7-10]. Changes in the concentration of angiotensin II may be very important in regulation of functions of the fetoplacental complex and of blood circulation in the placenta. Information on the association of the *ACE* gene polymorphisms and PM/PI is rather scanty [11,12]. The regulation of a vascular tone by ACE is mainly attributed to insertion/deletion (I/D) of a 287 bp Alu-repeat polymorphism in intron 16 of the *ACE* gene which affects expression of the *ACE* gene [6,13,14]. The presence of the D allele correlates with increase of ACE concentration in blood plasma. The I allele is functionally less active than the D allele [13,15]. The present study focuses on the analysis of allele and genotype frequencies of the I/D polymorphism of the *ACE* gene in placentae from parturient women with a history of PI and PM.

MATERIALS AND METHODS

Samples of placentae were obtained from 137 parturient women from the Maternity Department at the Institute of Obstetrics and Gynecology D.O. Otta of the Russian Academy of Medical Science, Saint Petersburg, Russia. Approval for this study was granted by the local Human Institutional Investigation Committee. They were classified into four groups as follows: group 1 (controls) was composed of 36 parturient women negative for both PM and PI in the present pregnancy (supported by clinical-laboratory, ultrasonic, Doppler and morphological methods), group 2 comprised 54 parturient women negative for PM in their obstetric history but positive for PI in the current pregnancy, group 3 composed 23 parturient women positive for PM (with one or more spontaneous abortions, and/or premature birth in their obstetric history) but negative for PI in the present pregnancy, and group 4 comprised 24 parturient women with PM in their obstetric history and positive for PI in the current pregnancy.

DNA extraction from placental tissue was carried out according to the technique by Sambrook *et al.* [16] with some modifications. The I/D polymorphism of the *ACE* gene was determined by a polymerase chain reaction (PCR) method. Amplification was carried out in a total volume 25 μL that contained 15 nM of each primer (ACE F: 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3', ACE R: 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3'), 67 mM Tris-HCl (pH 8.8), 16.6 mM of ammonium sulfate, 6.7 mM $MgCl_2$, 6.7 μM EDTA, 10 mM mercaptoethanol, 170 mg BSA, 1.0 mM of each dNTP and one unit of Taq-polymerase ('Bion', Moscow, Russia). The PCR was performed under the following conditions: denaturation at 94°C for 7 min., followed by 30 cycles of amplification at 94°C for 40 seconds, 60°C for 40 seconds, 72°C for 1 min. and 72°C for 5 min. The DNA fragments were separated in a 7% polyacrylamide gel and subsequently stained with ethidium bromide and visualized under ultraviolet light.

A standard chi-square test was used for comparing the genotype and allele frequencies. The relative risk was estimated by odds ratio (OR) and p values of <0.05 were considered to be significant. All confidence intervals (CIs) were calculated at the 95% level. Statistical analysis of results was performed using 'STATISTICA v5.5a' software.

RESULTS

Obstetric history data and some clinical features of pregnancy in the four groups are shown in Table 1. The average age of patients in group 4 was 31.67 ± 4.57 years which was slightly higher than in the

Table 1. Obstetric history data and current pregnancy parameters in the patients of studied groups.

Parameters	Group 1: PM– and PI–	Group 2: PM– and PI+	Group 3: PM+ and PI–	Group 4: PM+ and PI+
Number of patients	36	54	23	24
Age (years)	27.9±6.2	27.6±7.2	29.7±5.2[a]	31.7±4.6[a]
Menstrual cycle abnormalities (%)	48.8±7.8	61.2±7.0	51.3±9.7	71.4±9.9
Temporary sterility (%)	9.8±4.6	10.2±4.3	11.5±4.3	33.3±10.3[a]
Number of pregnancies	2.0±1.3	2.1±1.6	4.1±2.7	3.8±1.7
Cardiovascular diseases (%)	24.4±6.7	30.6±6.6	38.5±9.5	47.6±10.9
Urogenital infections in current pregnancy (%)	53.7±7.8	66.2±7.0	76.9±8.3[a]	52.4±10.9
Nephropathy in current pregnancy (%)	24.4±6.7	44.9±7.1[a]	26.9±8.7	33.3±10.3
Term of the present delivery (weeks)	39.0±1.7	37.7±3.4	38.8±2.1	36.7±3.1

Values are presented as mean ± SEM (standard error of mean).

[a] $p < 0.05$ (difference between group 1 and others).

other groups. Patients of groups 2 and 4 had a 1.5-fold higher frequency of abnormalities of menstrual function compared to those in group 1. One-third of the women in group 4 suffered temporary sterility.

Cardiovascular disease was common in the patients of all groups. Half the women in group 4 had hypertension or hypotension and varix in their obstetric data. Urogenital infection was present in about one-half of those in groups 1 and 4 (66.2%) and of those in groups 2 and 3 (76.9%), respectively.

Nephropathy as a complication of the current pregnancy was most frequent in women in group 2 (44.9%) (Table 1). Obstetric history of the women in group 4 was more complicated by extragenital pathology and nephropathy than in the women of group 3.

Characteristics of the newborns in four groups are summarized in Table 2. Their average birth weight was 2840.31 ± 809.31 in group 2 and 2664.52 ± 772.67g in group 4. This discrepancy was primarily due to fetus hypotrophy at the term of pregnancy as well as to premature birth. The average birth length of newborns in groups 2 and 4 was 47.92 ± 6.25 and 47.01 ± 4.95 cm, respectively (Table 2). It should be noted that intrauterine growth restriction of fetus confirmed at birth was diagnosed for 39% in group 2 and for 50% of newborns in group 4.

The frequencies of alleles and genotypes of the *ACE* gene in the four studied groups are shown in Table 3. The frequency of the D allele of the *ACE* gene in group 1 (51.4%) and that in group 2 (61.3%) did not differ significantly ($p > 0.05$) and was comparable to that in a population from northwest Russia (64.0%) [17]. However, the distribution of *ACE* genotypes was significantly different in groups 1 and 2 [$p < 0.01$; degree of freedom (df) = 2]. The frequency of the D/D genotype was essentially lower (22.2%) in group 1 compared to 46.4% in group 2. The relative risk of PI in the placenta of the D/D genotype from the patients without PM (group 2) was increased 3-fold compared to the patients of group 1 (OR: 3.01, CI: 1.18-7.76). Moreover, the

Table 2. Phenotypic features of the newborns in the four groups.

Parameters	Group 1: PM– and PI–	Group 2: PM– and PI+	Group 3: PM+ and PI–	Group 4: PM+ and PI+
Male/Female	15/21	30/24	14/9	8/16
Apgar scale (points)	7.8±0.5	7.3±1.1	7.3±1.5	7.15±1.6[a]
Birth weight (g)	3364.5±515.8	2840.3±809.3[a]	3332.3±650.9	2664.5±772.7[a]
Birth length (cm)	50.7±2.4	47.9±6.3[a]	50.4±3.54	47.0±5.0[a]
Intrauterine growth restriction of the fetus (%)	–	38.9±6.6	–	50.0±10.2

Values are presented as mean ± SEM.

[a] $p < 0.05$ (difference between group 1 and others).

Table 3. Frequency distribution of alleles and genotypes of the ACE gene in 4 groups of placentae from parturient women.

Alleles/Genotypes	Group 1: PM– and PI–	Group 2: PM– and PI+	*p*	Group 3: PM+ and PI–	Group 4: PM+ and PI+	*p*
Alleles: • D • I	 51.4% (37) 48.6% (35)	 61.3% (65) 38.7% (41)	0.18	 69.6% (32) 30.4% (14)	 35.4% (17) 64.6% (31)	0.001
Co-dominant model: • D/D • I/D • I/I	 22.2% (8) 58.3% (21) 19.5% (7)	 46.4% (25) 29.6% (15) 24.0% (13)	0.01	 47.8% (11) 43.5% (10) 8.7% (2)	 16.6% (4) 37.5% (9) 45.9% (11)	0.009
Dominant model: • D/D • I/D+I/I	 22.2% (8) 77.8% (28)	 47.2% (25) 52.8% (28)	0.02	 47.8% (11) 52.2% (12)	 16.7% (4) 83.3% (20)	0.02
Recessive model: • D/D+I/D • I/I	 80.6% (29) 19.4% (7)	 75.5% (40) 24.5% (13)	0.57	 91.3% (21) 8.7% (2)	 54.2% (13) 45.8% (11)	0.004

Number of cases are in parentheses.

frequency of the I/D genotype of placentae in group 1 was 2-fold more when compared to that in group 2 (58.3 and 29.6%, respectively) (Table 3).

In the anamnesis of women with PM, the frequencies of alleles of the *ACE* gene were also significantly different in the groups of placental samples. The frequency of the I allele in group 3 (PM+ and PI–) was 2-times less frequent compared to group 4 (PM+ and PI+) (30.4 and 64.6%, respectively, $p < 0.001$) (Table 3). The distribution of the genotypes between groups 3 and 4 was also significantly different ($p < 0.01$; df = 2). The frequency of the I/I genotype in group 3 (8.7%) was decreased 5-times compared to group 4 (45.9%). Thus, the I/I genotype of the *ACE* gene in placentae increases 7-times the risk of PI development in PM+ women (OR: 7.33, CI: 1.86-28.9).

Further analyses included comparison of all the studied groups. Distribution of *ACE* gene genotypes (D/D, I/D and I/I) varied significantly in four studied groups ($p<0.01$; df=6). The frequency of the D/D genotype in group 2 (46.4%) was significantly higher than in group 4 (16.6%) (Table 3). Also, the frequency of the D/D genotype was 2- times lower in group 1 (PM– and PI–) compared to group 3(PM+ and PI–) (22.2 and 47.8%, respectively). It should be stressed that the I/I genotype of the *ACE* gene dominated in placentae from group 4 (45.9%) compared to all other groups, whereas the I/D genotype prevailed in group 1 (58.3%). Thus, the D/D genotype of the *ACE* gene in placentae is associated with a 3-fold increased risk of PI development in PM– women, whereas the I/I genotype correlates with a 6-fold risk of development of both pathologies.

DISCUSSION

According to some recent data, PI accompanies practically all pregnancy complications [18]. Despite modern techniques of complex treatment and preventive maintenance of PI, the risk of PI in PM remained high and varied between 47.6 and 77.3% [1]. The delicate relation between mother and placenta/fetus operates during progression of the pregnancy. Former spontaneous abortions and endometritis could provoke receptor abnormalities in the endometrium, including hormone receptors, and thus initiate unfavorable hormonal and metabolic shifts as a prerequisite of PI at the present pregnancy [19].

Some authors classify PI as a display of "disadaption disease" [3]. The modern concept of physiological adaptation of the maternal organism and her fetus during pregnancy formulated by Wallenburg [20]. To a great extent, the favorable outcome of pregnancy in the case of PI depends on adequate adaptation of various components of cardiovascular systems of the mother and fetus. Recent investigations have shown that endothelial dysfunction in mother, in fetoplacental complex and in arteries of the umbilical cord is one of the

leading factors in pathogenesis of PM and PI [6]. Angiotensin-converting enzyme, as a basic component of the renin-angiotensin system, is responsible for conversion of angiotensin I into angiotensin II and for inactivation of bradykinin, and plays a key role in the origin and progression of endothelial dysfunction and vasoconstriction [21]. Changes in vascular metabolites affect the functions of the fetoplacental complex and may induce abnormalities of blood circulation in the placenta [7-10]. The I/D polymorphism of the *ACE* gene is functionally significant as it may influence the whole renin-angiotensin system that participates in regulation of vascular tone [13]. The I allele is associated with decreased ACE production compared to the D allele. An association of the D allele of with cardiovascular disease, in particular, with hypertension [22,23] and with some kinds of preeclampsia [24], has been reported. Since ACE is responsible for processes of vasoconstriction and vasodilatation in placental tissue, it is logical to assume an association between its allelic variants and development of PI. The results of the present study demonstrate the association between allelic variants of the *ACE* gene and PI development, and indicate that the D allele may be a risk factor for PI in women without PM in the anamnesis, whereas the I allele may be a risk factor for development of PI on a PM background.

At first sight, these results are rather inconsistent. However, the causes of PI in women without PM– and with PM in their obstetric history could be quite different. Placental insufficiency is mostly primary in patients with PM. Placental insufficiency during early pregnancy develops on a background of hormone abnormalities, inflammatory processes and changes in ednometrium receptors. Patients without PM have secondary PI, which occurs on the background of a pregnancy complicated by nephropathy, premature placental separation and extragenital pathology. Thus, we were dealing with rather different groups of patients.

In a case of 'pure' PI (group 3), it's clear-cut correlation with the D/D genotype of the *ACE* gene was assessed. The same allele is known to be associated with risk of myocardial infarction at a young age, ischemic heart disease, *etc.* [10,23]. It can be suggested that the D/D genotype in placentae results in an increase of placental infarctions and partial detachment of the placenta, and thus it could contribute to abnormalities of blood circulation in the placenta and to development of PI.

The pathogenesis of PI in conjunction with a PM case history could be quite different. Hormonal insufficiency, abnormalities of vascularization and endometrium reception owing to previous curettages, genital infantilism, and the presence of inflammatory and vascular reactions, thrombophilic complications at autoimmune processes are the most important causes of PI [25]. In PM cases, morphological and functional changes result in abnormalities of placenta maturation, involutive-dystrophic and inflammatory abnormalities of placental tissue [1,2,4]. Owing to this pathogenic background, PI in patients with PM should be treated as a primary character. Homozygosity for the I allele could decrease ACE concentration in plasma, and thus decrease the rate of bradykinin inactivation in the placenta. Reduction of bradykinin level may increase vessel permeability, and modulate inflammatory reaction, provoking premature interruption of pregnancy.

The differences in frequencies of the *ACE* alleles between groups 3 and 4 with PI could mainly be due to the complexity of the PI syndrome including pathological changes in the fetus and placenta joined by similar clinical manifestations, but varied in the cause of complications and in a number of development pathways.

Determining which genetic factors influence development of PI is of great importance for understanding the pathophysiological mechanisms of this disorder. Identification of candidate genes that participate in PM and PI has great practical value for prevention of this frequent obstetric pathology. Our data show that products of the *ACE* gene in placenta participate in PI development. The D/D genotype is associated with a 3-fold increased risk of PI development in women without PM. The I/I genotype increases by 2.6-fold the risk of PI development in patients positive for PM in obstetric history. Testing for the I/D polymorphism of the *ACE* gene in chorionic villus cells in the first trimester of pregnancy could be very important for prediction of PI. However, it is still unknown whether the maternal I/D polymorphism may influence development of PM and PI. Identification of genetic markers of PI is still in its infancy but could provide new insight into molecular mechanisms of pathology in obstetrics, gynecology, and clinical medicine as a whole.

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