Maternal-Fetal Flow, Negative Events, and Preeclampsia
Role of ACE I/D Polymorphism

Giorgio Mello, Elena Parretti, Francesca Gensini, Elena Sticchi, Federico Mecacci, Gianfranco Scarselli, Maurizio Genuardi, Rosanna Abbate, Cinzia Fatini

Abstract—The risk for an adverse pregnancy outcome is markedly higher in women with history of preeclampsia. This may stem from impaired placentation in early gestation and from high impedance to flow in uteroplacental circulation. The renin-angiotensin system is one of the mediators of the remodeling of spiral arteries throughout pregnancy. The D allele of the Insertion/Deletion (I/D) polymorphism in the ACE gene has been associated with higher ACE activity, accounting for 47% of the total phenotypic variance of serum enzyme levels. To investigate whether the ACE I/D polymorphism affects maternal uteroplacental and fetal umbilical circulation and the pregnancy outcome in women with a history of preeclampsia, 106 women underwent Doppler examination of uterine arteries resistance index and umbilical artery pulsatility index at the 16th, 20th, and 24th weeks of gestation and were genotyped for the I/D polymorphism. This study found a difference in genotype distribution \((P=0.0002)\) and allele frequency \((P<0.0001)\) between women with and those without preeclampsia recurrence and fetal growth restriction as well as an association \((P=0.0007)\) between DD genotype and risk of recurrent preeclampsia or fetal growth restriction. At the 16th, 20th, and 24th weeks, uterine artery resistance indexes were significantly lower in II, higher in DD, and intermediate in ID genotype carriers, whereas the umbilical artery pulsatility index values were significantly higher in the DD group in comparison to ID and II genotypes. The current study shows that the ACE I/D polymorphism affects uteroplacental and umbilical flows and the recurrence of an adverse pregnancy outcome in women with history of preeclampsia. \(\textit{(Hypertension. 2003;41:932-937.)}\)

Key Words: angiotensin-converting enzyme I/D polymorphism preeclampsia pregnancy

The risk for an adverse pregnancy outcome in women who have previously had preeclampsia is markedly higher (from 20% to 40%)\(^1,2\) in comparison to women with history of normal pregnancy, but the mechanisms involved have not yet been identified. The maternal syndrome of preeclampsia (PE) and the fetal syndrome of fetal growth restriction (FGR) yet been identified. The maternal syndrome of preeclampsia affects normal pregnancy, but the mechanisms involved have not been characterized. Deficient placentation is characterized by inadequate trophoblast invasion into the maternal spiral arteries and a failure to develop low-resistance uteroplacental circulation.\(^3\) Doppler ultrasonographic studies of uteroplacental\(^5\) and fetal umbilical circulation\(^6\) have shown that high impedance to flow is associated with subsequent PE, FGR, and related complications.

Previous experimental studies\(^7\) suggested that the “physiological remodeling” of spiral arteries throughout pregnancy is mediated by the renin-angiotensin system (RAS), which is one of the main factors regulating blood pressure, and fluid and electrolyte balance.\(^8\) Throughout normal pregnancy, the RAS is stimulated: plasma renin activity, angiotensinogen, angiotensin II, and aldosterone levels are all increased.\(^9\) At the same time, pregnancy induces a refractoriness to the pressor effects of angiotensin II.\(^10\)

In patients with PE, a significant association between the T235 molecular variant of the angiotensinogen (AGT) gene, previously associated with essential hypertension,\(^11\) and abnormal physiological change of uterine spiral arteries in first-trimester decidua\(^12\) has been found. Moreover, recent data have shown that upregulation of angiotensin II type 1 (AT1) receptor subtype in the syncytiotrophoblasts could play a pathophysiological role in patients with PE.\(^13\) Moreover, PE is characterized by the loss of this physiological refractoriness to angiotensin II.\(^10\)

Angiotensin II levels are modulated by ACE, whose plasma levels have been associated with the insertion/deletion (I/D) polymorphism in intron 16 of the ACE gene.\(^14\) The ACE I/D polymorphism accounted for 47% of total phenotypic variance of serum ACE, contributing much to the variability of ACE level.\(^14\) A marked difference in serum ACE levels was observed between subjects in each of the 3 genotype
classes: the DD is associated with higher tissue and plasma ACE levels, whereas the II is associated with lower levels; the ID genotype is associated with intermediate levels.\(^1\) Moreover, it has been assumed that the I allele has a sequence similar to a silencer sequence, which might explain why the D allele is associated with higher ACE levels than the I allele.\(^1\)

ACE activity and ACE I/D polymorphism were not found to be associated with PE in previous studies,\(^2\)\(^3\)\(^4\) but no information is available about the effect of this polymorphism on maternal-fetal hemodynamics in women at high risk of PE.

In this study, women at high risk of PE for a previous PE, without other known risk conditions, were studied in order to investigate whether the ACE I/D polymorphism affects maternal uteroplacental and fetal umbilical circulation and pregnancy outcome.

Methods

Study Subjects

Between January 1998 and December 2000, we enrolled 249 consecutive white women with a history of PE. All patients were from central Italy (Tuscany) and were referred to the Maternal-Fetal Medicine/High Risk Pregnancies Unit of the University of Florence for preconception counseling. A detailed history, including demographic profile, social background, and a summary of past obstetric and medical data, was obtained from all women.

Previous PE was defined as the presence of blood pressure values \(>140/90\) mm Hg at least twice in a 24-hour period and of proteinuria \(>300\) mg/24 hours after the 20th week of pregnancy in a previously normotensive and nonproteinuric woman.\(^1\) Eighty-five (35%) subjects positive for at least one thrombophilic factor (activated protein C resistance, factor V Leiden and factor II 20210A variants, hyperhomocysteinemia, protein C, protein S and antithrombin deficiency, antcardiolipin antibodies, and lupus anticoagulant) were also excluded from the analysis.

Three women were excluded because of spontaneous fetal loss before the 12th week of pregnancy and 2 because of twin pregnancies diagnosed before the 13th week. The study group included 106 women. Of these, 57 (54%) had, in their previous pregnancy, an early onset of severe PE and FGR. None of the recruited women was taking drugs, drank alcohol, or smoked. All of them received iron and vitamin supplements during pregnancy.

Gestational age was calculated according to the date of the last menstrual period and confirmed by first-trimester ultrasound examination.

The outcome variables analyzed were (1) PE with or without FGR; (2) FGR without PE (defined as birth weight less than the 10th percentile for the reference chart\(^9\) in the absence of chromosome or congenital anomalies); (3) gestational age at delivery; and (4) birth weight. A noncomplicated outcome was defined as the delivery at term of an appropriately grown fetus, with no evidence of maternal hypertension.

The onset of obstetric complications such as FGR and PE took place after the 28th week of gestation. The study was approved by an institutional review committee, and the subjects gave informed consent.

Doppler Ultrasound Examination

Women underwent transabdominal color flow/pulsed Doppler examination of both uterine arteries and the umbilical artery at the 16th (quartiles 16 to 17), 20th (quartiles 20 to 21), and 24th (quartiles 24 to 25) week of pregnancy, by means of a 3.5- or 5-MHz convex probe with a 100-Hz filter (ESAOTE AU5EPI, Genoa, Italy).

The results were not available to the clinicians, and no clinical information was communicated to the ultrasonographer.

Molecular Diagnosis

Peripheral venous blood samples were collected from the antecubital vein in Vacutainer tubes containing 0.129 mol/L sodium citrate, the final blood/anticoagulant ratio being 9:1.

Genomic DNA was extracted from leukocytes using a QIAmp Blood Kit (QIAGEN, Hilden, Germany). The ACE I/D polymorphism was genotyped according to Rigat et al.\(^1\) (Figure). DNA was amplified at an annealing temperature of 60°C in the presence of 5% dimethylsulfoxide (DMSO) to reduce the incidence of mistyping ID as DD. Moreover, each DD genotype was subjected to a second PCR amplification without 5% DMSO at an annealing temperature of 67°C and by using a primer pair that recognizes the insertion-specific sequence. These modifications were made to reduce underestimation of heterozygotes.

Statistical Analysis

Statistical analysis was performed with Stata 6.0 software for Microsoft Windows (Stata Corporation). The ACE polymorphism allele frequency was obtained by using a direct count. The Hardy-Weinberg equilibrium for genotype distribution and allele frequency was estimated by the \(\chi^2\) test. Descriptive statistics was used to obtain median and range and mean and standard deviation.

One-factor ANOVA was used to compare the means of continuous variables that followed a normal distribution. When significant differences were found by using variance analysis, pairwise comparisons were performed with the use of the least significant differences test. For data that did not follow a normal distribution and demonstrated different variances, nonparametric Kruskal-Wallis 1-way AVOVA was performed. We used simple regression analysis to test for an association between the ACE I/D polymorphism and a risk of adverse outcome recurrence. Statistical significance was at a level of \(P<0.05\).

Results

The characteristics of the study population are shown in Table 1. The ACE I/D polymorphism genotype distribution and allele frequency were compatible with Hardy-Weinberg equilibrium. Thirty-seven (35%) DD homozygous, 46 (43%) ID heterozygous, and 23 (22%) II homozygous women were
found. Eighty-three of 106 patients (78%) carried the D allele; the ACE D allele frequency was 0.57. Among the 57 (54%) women with early onset of severe preeclampsia and FGR, a significantly higher D allele frequency (0.77) and DD genotype percentage (60%) was observed in comparison to the 49 (46%) women in whom obstetric complications took place after the 34th week of pregnancy (D allele frequency 0.33 and DD genotype percentage 6%; \( P<0.0001 \)).

There were no differences in maternal age, parity, gravidity, and body mass index among the groups classified according to ACE I/D genotypes.

**Clinical Pregnancy Outcome**

Forty-eight (45%) women with documented complications (PE or FGR) were identified. In this subgroup, the ACE D allele frequency was 0.73. The onset of severe PE and FGR occurred early in 9 women who had early onset also in the previous pregnancy. A significant difference in ACE genotype distribution and allele frequency between women with and without PE recurrence and/or FGR was observed (Table 2). A significant association between ACE DD genotype and risk of PE or FGR was also found (OR DD versus ID+II=4.17; CI 95%, 1.78 to 9.76; \( P=0.0007 \)).

The percentage of cases with PE progressively increased from 4.3% in women with the II genotype to 43.2% in women with DD genotype (\( P<0.0002 \)) (Table 3). In addition, 12 of 16 women with the DD genotype who had FGR required delivery before the 34th week of pregnancy. The 9 women with early-onset severe PE and FGR in both the previous and successive pregnancy carried the D allele. In this group, the D allele prevalence was 0.89.

A significantly lower gestational age at delivery was observed in women with the DD genotype (Table 3). Birth weight was significantly lower in the DD group than in the ID group, which in turn showed a lower birth weight compared with the II group (Table 3).

**Maternal Uteroplacental and Fetal Umbilical Circulation**

The mean of resistance indexes of both uterine arteries in women with uncomplicated pregnancy outcomes showed a significant progressive decrease from the 16th to the 24th week of pregnancy (Table 4). This pattern was not documented in women with a complicated outcome. In 9 women with both PE and FGR, the mean resistance index of uterine arteries increased from the first to the third testing (16 weeks, 0.65 ± 0.11; 20 weeks, 0.68 ± 0.18; 24 weeks, 0.73 ± 0.2; ANOVA \( P<0.01 \)). At the 16th, 20th, and 24th weeks, the mean of the resistance indexes of uterine arteries in women with PE or FGR was significantly higher than that of uncomplicated pregnancies (Table 4).

The pulsatility indexes of the umbilical artery showed a significant progressive decrease from the 16th to the 24th week of pregnancy in all 3 groups, but the decrease was smaller in PE and FGR groups, whereas the umbilical indexes were significantly higher than those in uncomplicated pregnancies (Table 4).

With regard to maternal uteroplacental and fetal umbilical circulations in relation to ACE I/D polymorphism, the uterine artery resistance indexes showed a significant progressive decrease from the 16th to the 24th week of pregnancy the ACE II genotype. Such decrease was not observed in DD and ID genotypes (Table 5). At the 16th week, the mean of the resistance indexes for the uterine arteries of the ACE DD genotype was significantly higher with respect to the other 2 genotypes. At the 20th and 24th weeks, the uterine indexes in the ACE DD group were significantly higher than those in the ID group, which were, in turn, higher than those in the II group (Table 5).

The pulsatility indexes for the umbilical artery at the 20th and 24th weeks were significantly higher in the ACE DD genotype than in ID and II women (Table 5).
TABLE 3. Clinical Pregnancy Outcomes in Relation to the ACE I/D Polymorphism

<table>
<thead>
<tr>
<th>Clinical Pregnancy Outcomes</th>
<th>DD (n=37)</th>
<th>ID (n=46)</th>
<th>II (n=23)</th>
<th>ANOVA Group Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE, n (%)</td>
<td>9 (24.3)</td>
<td>7 (15.2)</td>
<td>1 (4.3)</td>
<td>P&lt;0.01 DD vs ID vs II</td>
</tr>
<tr>
<td>FGR, n (%)</td>
<td>16 (43.2)</td>
<td>13 (28.3)</td>
<td>2 (8.7)</td>
<td>P&lt;0.002 DD vs ID vs II</td>
</tr>
<tr>
<td>Gestational age at delivery, wk: median (range)</td>
<td>36 (29–39)</td>
<td>39 (36–40)</td>
<td>39 (37–41)</td>
<td>P&lt;0.02 DD vs ID, II</td>
</tr>
<tr>
<td>Birth weight, g: median (range)</td>
<td>2540 (1420–3150)</td>
<td>2880 (2250–3520)</td>
<td>3349 (2850–3850)</td>
<td>P&lt;0.001 DD vs ID vs II</td>
</tr>
</tbody>
</table>

Discussion

The current study provides novel evidence that the ACE I/D polymorphism affects uteroplacental and umbilical flows and the recurrence of an adverse obstetric outcome in women with a history of PE. At the 16th week, the mean resistance indexes of uterine arteries in women with the ACE DD genotype were significantly higher with respect to the other 2 genotypes. In addition, a D allele dose-dependent effect was found at the 20th and 24th weeks.

Our results, which underscore the modulatory role of the RAS on uteroplacental flow, are in keeping with studies reporting that the AGT T235 allele predisposes pregnant women to abnormal development of uteroplacental circulation, potentially initiating the cascade of events that leads to PE.12

All the components of the vascular RAS are expressed in and around the remodeling spiral arteries.7 Moreover, local RAS generates angiotensin II,7,22 so possibly causing medial hyperplasia23 and/or angiogenesis.24 RAS components are involved in the pathogenesis of PE,29 and second, its role in hemostasis, which includes the regulation of tissue plasminogen activator production and of glycoprotein IIb/IIIa complex on the platelet surface.30

Interestingly, this study documents the influence of the ACE DD genotype on the pregnancy outcome in women with a history of PE. The high prevalence of the D allele in the general white population (from 50% to 62%)31,32 and the multifactorial pathogenesis of PE do not presently allow to attribute a predictive value to this factor. However, our findings, if confirmed, could be used for counseling women with PE in the previous pregnancy regarding the risk of hypertensive complications in future pregnancies and for selecting those requiring more accurate dating and assessment of fetal growth.

In this study, when the overall group is considered, the ACE genotype distribution is not different from that observed in our previous studies in the general population31 and in women with a history of normal pregnancy,31 thus indicating

TABLE 4. Maternal Uteroplacental and Fetal Umbilical Circulation Indices According to Pregnancy Complications

<table>
<thead>
<tr>
<th>Maternal-Fetal Circulation Indices</th>
<th>A (n=17)</th>
<th>B (n=31)</th>
<th>C (n=58)</th>
<th>ANOVA Group Differences by LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean uterine artery (RI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 wks</td>
<td>0.64±0.12</td>
<td>0.66±0.2</td>
<td>0.57±0.13</td>
<td>P&lt;0.02 A, B vs C</td>
</tr>
<tr>
<td>20 wks</td>
<td>0.65±0.16</td>
<td>0.67±0.19</td>
<td>0.53±0.19</td>
<td>P&lt;0.003 A, B vs C</td>
</tr>
<tr>
<td>24 wks</td>
<td>0.65±0.12</td>
<td>0.67±0.25</td>
<td>0.48±0.24</td>
<td>P&lt;0.002 A, B vs C</td>
</tr>
<tr>
<td>ANOVA NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>P&lt;0.001</td>
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<tr>
<td>Group differences by LSD</td>
<td>16 vs 20 vs 24</td>
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| Umbilical artery (PI)             |          |          |          |                              |
| 16 wks                            | 1.69±0.11| 1.71±0.17| 1.68±0.17| NS                           |
| 20 wks                            | 1.59±0.15| 1.63±0.19| 1.49±0.23| P<0.01 A vs B vs C           |
| 24 wks                            | 1.47±0.18| 1.57±0.24| 1.27±0.17| P<0.01 A vs B vs C           |
| ANOVA                            | P<0.01   | P<0.02   | P<0.002  |                              |
| Group differences by LSD          | 16 vs 20 vs 24 | 16 vs 20 vs 24 | 16 vs 20 vs 24 |

Data are mean±SD. A indicates preeclampsia; B, fetal growth restriction; C, uncomplicated; LSD, least significant difference test; RI, resistance index; PI, pulsatility index.
that the ACE DD genotype is not associated with the risk of PE in the first pregnancy. Finding an association between the DD genotype and a negative outcome in the successive pregnancy suggests that the DD genotype can be related to PE and FGR only when nulliparity is no more present. Epidemiologic and clinical studies well document that nulliparity per se is a risk factor for PE, being induced by mechanisms that are not present in successive pregnancies. In addition, immunologic mechanisms may play a role in a negative outcome, possibly related to paternal factors. Moreover, the observation that patients with PE are at increased risk for chronic hypertension in life indicates that a preeclamptic status during the first pregnancy may induce persistent and latent functional alterations that strengthen the D allele–dependent angiotensin II effect during the second pregnancy.

In conclusion, the results of our study suggest that the ACE I/D polymorphism is involved in the modulation of maternal uteroplacental and fetal umbilical flows and provide the rationale for investigating this polymorphism in a larger sample population to define its role as a new susceptibility rationale for investigating this polymorphism in a larger sample population to define its role as a new susceptibility

Perspectives

The results of this study show, for the first time, an association between the ACE DD genotype and a recurrent pregnancy negative outcome after PE in a previous pregnancy. These findings might bear implications for a more accurate management of pregnancy in high-risk DD genotype women. The clinical relevance of these results deserves to be further evaluated in larger studies, which, if positive, will provide an additional marker for risk assessment of patients with a history of PE.

References


TABLE 5. Maternal Uteroplacental and Fetal Umbilical Circulation Indices in Relation to the ACE I/D Polymorphism

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<td>DD vs ID, II, P&lt;0.01</td>
</tr>
<tr>
<td>20 wks</td>
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<td>0.61±0.27</td>
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</tr>
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</tr>
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<tr>
<td>ANOVA</td>
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