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 placentaion 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 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. (Hypertension. 2003;41:932-937.)

Key Words: angiotensin-converting enzyme • 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) during the latter half of pregnancy are believed to result from impaired placentation 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. (Hypertension. 2003;41:932-937.)

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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. More- 
over, 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.

ACE activity and ACE I/D polymorphism were not found to be associated with PE in previous studies 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. Thirty-five (14%) women with kidney disease, cardiovascular pathology other than hypertension, and preexisting diabetes were excluded from the study. One hundred three (41%) 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, anticardiolipin 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 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 hypertensive.

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 (ESAOITE AUSEPI, Genoa, Italy).

**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 24.3% in women with the DD genotype (\( P<0.01 \)) (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</th>
<th>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</td>
<td>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</td>
<td>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</td>
<td>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</td>
<td>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 increased circulating angiotensin II levels27; however, in hypertensive complications in future pregnancies and for identifying 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>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</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Group differences by LSD</td>
<td>16 vs 20 vs 24</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

| 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 factor to a negative pregnancy outcome in women with a history of PE. Further studies are required to investigate the interaction among different genetic polymorphisms, including the ACE I/D polymorphism, other RAS (AGT and AT1R) variants, and other well-known metabolic and thrombophilic factors predisposing to PE, which we have not considered in our study population.

**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**


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