High aldosterone-to-renin variants of CYP11B2 and pregnancy outcome

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Abstract

Background. Increased aldosterone concentrations and volume expansion of normal pregnancies are hallmarks of normal pregnancies and blunted in pre-eclampsia. Accordingly, we hypothesized an active mineralocorticoid system to protect from pre-eclampsia.

Methods. In pregnant women (normotensive n = 44; pre-eclamptic n = 48), blood pressure, urinary tetrahydro-aldosterone excretion and activating polymorphisms (SF-1 site and intron 2) of the aldosterone synthase gene (CYP11B2) were determined; 185 non-pregnant normotensive individuals served as control. Amino acid-changing polymorphisms of the DNA- and agonist-binding regions of the mineralocorticoid receptor were evaluated by RT-PCR, SSCP and sequencing.

Results. Urinary tetrahydro-aldosterone excretion was reduced in pre-eclampsia as compared to normal pregnancy (r = 0.99, P < 0.05). It inversely correlated with blood pressure (r = 0.99, P < 0.04). Homozygosity for activating CYP11B2 polymorphisms was preferably present in normotensive as compared to pre-eclamptic pregnancies, identified (intron 2, P = 0.005; SF-1 site, P = 0.016). Two mutant haplotypes decreased the risk of developing pre-eclampsia (RR 0.16; CI 0.05–0.54; P < 0.001). In contrast, intron 2 wild type predisposed to pre-eclampsia (P < 0.0015). No functional mineralocorticoid receptor mutant has been observed.

Conclusions. High aldosterone availability is associated with lower maternal blood pressure. In line with this observation, gain-of-function variants of the CYP11B2 reduce the risk of developing pre-eclampsia. Mutants of the mineralocorticoid receptor cannot explain the frequent syndrome of pre-eclampsia.

Keywords: aldosterone synthase; aldosterone; arterial hypertension; pre-eclampsia; pregnancy outcome

Introduction

Pre-eclampsia (PE), as defined by maternal arterial hypertension and proteinuria in pregnancy beyond the 20th week of gestation, causes severe morbidity often in previously healthy individuals [1]. In addition to the immediate effects of the disease that may include cerebral accidents, liver disease and renal failure, PE is associated with remote cardiovascular and metabolic disease [2,3]. Thus, the ability to predict PE, especially with tests based on pathophysiological mechanisms, would be an important step towards eventually preventing and managing the disease.

Extracellular and plasma volume expansion of normal pregnancy is linked to changes in renal haemodynamics and of circulating levels of several steroid hormones including markedly increased circulating aldosterone concentrations [4–10]. Following production by the enzyme aldosterone synthase (CYP11B2), aldosterone acts in target tissues via the mineralocorticoid receptor (MR).

Catalyzed by CYP11B2, aldosterone is synthesized from deoxycorticosterone in a three-step reaction with 18-methyl oxidation being rate limiting. In a group of pre-eclamptic women, we recently observed a reduced CYP11B2 activity characterized by a compromised 18-methyl oxidation step due to a loss-of-function mutation of CYP11B2 [11].

In non-pregnant subjects, essential hypertension with a high aldosterone-to-renin ratio, suggesting a high activity of CYP11B2, has previously been attributed to gain-of-function mutants representing biallelic gene polymorphisms of the CYP11B2. This is a -344C/T exchange located in the putative steroidogenic binding factor-1 (SF-1) within the 5′ regulatory region and an intron 2 conversion [Int2 (c)] resulting from the replacement of this region with that of the neighbouring gene encoding CYP11B1 [12].

With respect to our initial finding of an increased prevalence of loss-of-function mutants of CYP11B2 in PE, such gain-of-function alleles might protect from developing PE. Several functional relevant mutants of the aldosterone effector, the MR, are known to lead either to a loss or to a gain of function, which could as such further affect the aldosterone response [13].

We hypothesized that in pregnancy, gain-of-function mutants of the CYP11B2 gene in the absence of loss-of-function polymorphisms of the MR sustain a lower blood pressure and reduce the risk of developing PE.
Table 1. Characteristics of the pregnant women studied

<table>
<thead>
<tr>
<th>Type of pregnancy</th>
<th>Normotensive</th>
<th>Pre-eclampsia</th>
<th>P &lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Information from present pregnancy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>44</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>33.2 ± 1.3</td>
<td>n.s.</td>
<td>31.2 ± 0.7</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At delivery (kg/m²)</td>
<td>27.4 ± 0.9</td>
<td>n.s.</td>
<td>29.0 ± 0.9</td>
</tr>
<tr>
<td>Before pregnancy (kg/m²)</td>
<td>23.0 ± 0.8</td>
<td>0.04</td>
<td>26.3 ± 1.3</td>
</tr>
<tr>
<td>Blood pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic (mmHg)</td>
<td>117 ± 3</td>
<td>0.001</td>
<td>159 ± 16</td>
</tr>
<tr>
<td>Diastolic (mmHg)</td>
<td>75 ± 2</td>
<td>0.001</td>
<td>97 ± 2</td>
</tr>
<tr>
<td>Fetal birth weight (g)</td>
<td>3266 ± 98</td>
<td>0.001</td>
<td>1774 ± 179</td>
</tr>
<tr>
<td>Birth weight percentiles</td>
<td>42.3 ± 4.8</td>
<td>0.01</td>
<td>26.2 ± 4.1</td>
</tr>
<tr>
<td>corrected for gestation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low birth weight &lt;2.5 kg (%)</td>
<td>7</td>
<td>n.s.</td>
<td>0.0001</td>
</tr>
<tr>
<td>SGA &lt;10th birth weight percentile (%)</td>
<td>21</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Gestation at time of sampling (days)</td>
<td>221.9 ± 9.1</td>
<td>n.s.</td>
<td>224.7 ± 4.9</td>
</tr>
<tr>
<td>Proteinuria (g/day)</td>
<td>n.d.</td>
<td>0.002</td>
<td>2.3 ± 0.5</td>
</tr>
<tr>
<td>Time to delivery (day)</td>
<td>44 ± 6</td>
<td>0.001</td>
<td>6 ± 4</td>
</tr>
<tr>
<td>Information from previous pregnancies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total number of pregnancies (n/woman)</td>
<td>1.8 ± 0.2</td>
<td>n.s.</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>Primiparous (%)</td>
<td>53</td>
<td>n.s.</td>
<td>57</td>
</tr>
<tr>
<td>Multiparous (%)</td>
<td>47</td>
<td>n.s.</td>
<td>43</td>
</tr>
<tr>
<td>History of pre-eclampsia (%)</td>
<td>0</td>
<td>0.001</td>
<td>22</td>
</tr>
<tr>
<td>History of HELLP syndrome (%)</td>
<td>0</td>
<td>n.s.</td>
<td>4</td>
</tr>
<tr>
<td>Fetal loss (n/woman)</td>
<td>0</td>
<td>0.02</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Premature birth (n/woman)</td>
<td>0</td>
<td>0.001</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>Normal birth weight (n/woman)</td>
<td>1.8 ± 0.2</td>
<td>0.001</td>
<td>0.4 ± 0.1</td>
</tr>
</tbody>
</table>

n.d. = not detectable on either dipstick urinalysis or quantitative assessment of proteinuria, n.s. = not significant, SGA = small for gestational age.

Material and methods

Patients

Ninety-two pregnant women, 44 with normal pregnancies and 48 with PE, and 185 normotensive control subjects (115 males and 70 females) were investigated. Approval by the appropriate hospital ethics review committee and informed consent were obtained from all individuals. Basic demographic data, including blood pressure, information on concurrent diseases and the present medication, were obtained. Women with pre-existing arterial hypertension, diabetes mellitus or renal disease were excluded from the study. Patients were included in the PE group if blood pressure was ≥140/90 mmHg and proteinuria > 0.3 g/day in the absence of urinary tract infection beyond the 20th week of gestation (Table 1) [14]. Healthy pregnant women were selected as case controls according to the gestational age at initial presentation of pre-eclamptic women. Both groups of women were followed to delivery.

Collection of urine and blood samples and isolation of genomic DNA

Twenty-four-hour urine samples were collected from all women with PE at initial presentation. These samples were matched with those from women with a normal pregnancy at the same gestational age (± 2 weeks); however, analysis was postponed until delivery in order to establish the presence of an uncomplicated pregnancy. Ten millilitres of blood samples were drawn into tubes containing EDTA, mixed and stored at 4°C. Genomic DNA was extracted from peripheral blood leukocytes following the protocol supplied by the nucleon BACC3 kit (Bioscience; Piscataway, USA). Purified DNA was stored at 4°C in ddH₂O.

Screening of genomic DNA for the gain-of-function mutants of CYP11B2 ([SF1–344CT] and Int2 (ns)(c)) and functionally relevant mutants of the MR (Figure 1)

Polymerase chain reaction (PCR) for SF-1 and Int2 polymorphisms of CYP11B2 was performed using the primers and conditions reported by Davies et al. [16]. The region of DNA containing the HaeIII/SF-1 polymorphism was amplified by PCR by use of conditions similar to those previously described. A PCR product of 228 bp was amplified. This was digested with HaeIII and subjected to electrophoresis in 3% MetaPhor agarose. The 228 bp ampiclon contains two HaeIII restriction enzyme sites (GG CC). The presence of a C–T transition at position -344 (GG CT) removes one of these sites. After digestion, individuals homozygous for the transition (TT) produce two bands of 175 and 53 bp, individuals homozygous for the wild-type (CC) three bands of 104, 71 and 53 bp, and heterozygous individuals (TC) four bands.

The Int2 conversion was genotyped by using two separate PCR reactions, one that amplifies the normal gene (WT) and one that amplifies the conversion. The size of the amplicon in each reaction is ~418 bp.

The functionally important domains of the MR for DNA and agonist binding, exons 3 to 9, respectively, were amplified by using the following primers: forward 5'-TGT GCA GCA TGT TAA ATG CTC-3', 5'-TTC AAG GCA GGA AAG TAG-3', 5'-TGG CCT CAT CAT TAA TGG TTT C-3', 5'-TTC CTT CTG GAA TGT AAT ATG C-3', 5'-CAG TCT GGG TTT GAT AAT GAG-3', 5'-CCT GGA AAT TTA CAT GTC TTT C-3' or 5'-AAA GTC AGC AGG CAG AGG GAT C-3', respectively. As reverse primers served: 5'-AAC ACC CAC ATG AAC ATC TAC C-3', 5'-TTC TGT TGC ATT ACC TAT TAA C-3', 5'-TGC AGC CTG TGA AAG GAG AG-3', 5'-AAA TCA TAA CGC ATA ACT CTC-3', 5'-TAG AAA CAG...
Follows: no mutated haplotype [SF-1 C/C, Int2 (w)/(w)], one mutated haplotype for each allele contributing, for each gene, and by grouping haplotypes as needed for each gene. Fisher’s exact test were used, taking the appearance of PE as threshold. For comparison of genotype distributions, the chi-square test and t-test were used for continuous variables by nonparametric analysis and the Wilcoxon or Kruskal-Wallis test for variables that were not normally distributed. Linear regression analysis was performed for the continuous parameters (birth weight and systolic/diastolic blood pressure as related to urinary tetrahydro-aldosterone/creatinine). Standardized calculations of relative risk (RR) and confidence intervals (CI) were performed with log transformations and a normal approximation used for the CI. For comparison of genotype distributions, the chi-square test and Fisher’s exact test were used, taking the appearance of PE as threshold. Comparisons for the number of mutated haplotypes were performed for each allele contributing, for each gene, and by grouping haplotypes as follows: no mutated haplotype [SF-1 C/C, Int2 (w)/(w)], one mutated haplotype of both genes [SF-1 344C/T and Int2 (w)/(c)], and two mutated haplotypes [SF-1 T/T and Int2 (c)/(c)]. Significance was assigned at P < 0.05. All statistical analyses were performed using either SYSTAT version 10 (SPSS Inc., Chicago, IL, USA) or GraphPad Prism version 4.01 for Windows (GraphPad Software, San Diego, CA, USA).

Results

Patients characteristics

Basic demographic, clinical and biochemical data of the 92 pregnant women, dichotomized as to the occurrence of PE, are reported in Table 1. There was no difference in age and body mass index in the two groups of women. The clinical status was confirmed to be uneventful within the current and all former pregnancies in the 44 women included as normal pregnancies. PE was diagnosed in 48 women by the occurrence of the pregnant women and the controls studied were in Hardy–Weinberg equilibrium. Normotensive pregnancies more likely took place in the presence of homozygosity for Int2 (c)/(c) (P = 0.0046), but less frequently with Int2 (w)/(w) (P = 0.015). Homozygosity for SF-1 T/T was associated with reduced occurrence of hypertensive pregnancies (P = 0.016, Figure 3), yet heterozygosity did not protect from PE (P = 0.012). Combining alleles associated with high aldosterone-to-renin ratios indicated an increased frequency of these alleles in normotensive pregnancies complicated by fetal losses, premature births and low birth weight of their children (Table 1). Antihypertensive treatment in PE consisted of labetalol, α-methylldopa and nifedipine.

Statistics

All data are presented as means ± SEM. To test for statistically significant differences, Student’s t-test was used for continuous variables by nonparametric analysis and the Wilcoxon or Kruskal-Wallis test for variables that were not normally distributed. Linear regression analysis was performed for the continuous parameters (birth weight and systolic/diastolic blood pressure as related to urinary tetrahydro-aldosterone/creatinine). Standardized calculations of relative risk (RR) and confidence intervals (CI) were performed with log transformations and a normal approximation used for the CI. For comparison of genotype distributions, the chi-square test and Fisher’s exact test were used, taking the appearance of PE as threshold.

Comparisons for the number of mutated haplotypes were performed for each allele contributing, for each gene, and by grouping haplotypes as follows: no mutated haplotype [SF-1 C/C, Int2 (w)/(w)], one mutated haplotype of both genes [SF-1 344C/T and Int2 (w)/(c)], and two mutated haplotypes [SF-1 T/T and Int2 (c)/(c)]. Significance was assigned at P < 0.05. All statistical analyses were performed using either SYSTAT version 10 (SPSS Inc., Chicago, IL, USA) or GraphPad Prism version 4.01 for Windows (GraphPad Software, San Diego, CA, USA).

Genotype and haplotype analysis of MR and CYP11B2 gene polymorphisms (Figure 1)

A PCR-based screening for mutations in the DNA and steroid-binding domains of the MR was performed, and no amino acid-changing mutation leading to structural or functional changes was identified.

The CYP11B2 genotype distribution within the population of the pregnant women and the controls studied were in Hardy–Weinberg equilibrium. Normotensive pregnancies more likely took place in the presence of homozygosity for Int2 (c)/(c) (P = 0.0046), but less frequently with Int2 (w)/(w) (P = 0.015). Homozygosity for SF-1 T/T was associated with reduced occurrence of hypertensive pregnancies (P = 0.016, Figure 3), yet heterozygosity did not protect from PE (P = 0.012). Combining alleles associated with high aldosterone-to-renin ratios indicated an increased frequency of these alleles in normotensive pregnancies.
when compared to pre-eclamptic pregnancies (2.6 ± 0.3 versus 1.9 ± 0.2 alleles; \( P = 0.023 \)). So far, these results point towards a relationship between pregnancy outcome and the number of mutant haplotypes. The presence of two, yet not one, mutant haplotypes for each gene locus was highly significantly linked to uneventful pregnancies (\( P < 0.001 \), Figure 4). The relative risk of developing PE was not reduced with one mutant haplotype, but with two (compared to no and one mutant haplotype RR 0.16; CI 0.05–0.54).

### Discussion

This study shows an association of maternal blood pressure and fetal birth weight with aldosterone availability. As proposed, in normal pregnancies a high aldosterone production, determined via the measurement of urinary tetrahydro-aldosterone excretion, the most sensitive marker of circadian aldosterone production, was related to low maternal blood pressure levels [19]. Confirming our hypothesis, the number of high aldosterone-to-renin ratio alleles was increased in normal pregnancies. The absence of a high aldosterone-to-renin ratio allele of intron 2 was associated with an augmented frequency of PE. Furthermore, the presence of both mutants significantly favoured normal pregnancies.

The clarification of mechanisms of PE and hypertension in pregnancy is of practical relevance for multiple reasons with preventing the disease being the most important one [20–25]. If high aldosterone levels and the increased circulating plasma volume found in normal pregnancies represent a physiological adaptation crucial to support an adequate circulating fluid volume in pregnancy, two observations should be possible: first, a phenotypical adaptation in response to the aldosterone available, and second, mechanisms within the mineralocorticoid system either leading to or mimicking high aldosterone availability should prove beneficial to avoid PE [4–9,26].

If the primary impact of sufficient aldosterone availability would be to benefit pregnancy by avoiding PE, a genetic predisposition to produce high quantities of aldosterone or to appropriately respond to a given aldosterone concentration via the MR would be reasonable. Such a predisposition has been recognized by two gene loci of Mendelian forms of the CYP11B2 gene, the promoter SF-1 single nucleotide polymorphism -344C/T and a conversion in intron 2, which...
are variably linked to arterial mineralocorticoid hypertension with increased aldosterone-to-renin ratios in the non-pregnant population [12]. With these findings we stay in line with our initial finding of a compromised aldosterone synthesis being associated with PE [11].

In our study, the most pronounced differences between normal pregnancies and PE could be observed, if either an increased number of high ARR alleles or homozygosity for the mutant form were present. The RR to develop PE was reduced by >80%, if women were homozygous for both mutant alleles. Thus, combinations of activating polymorphisms seem to add to an uneventful pregnancy outcome, most likely due to an amplified aldosterone production as has been demonstrated during postural changes, when the maximal aldosterone response was found in subjects with two mutant haplotypes [12]. This observation is in line with our results and supports the view that variations in CYP11B2 may well lead to an intermediate phenotype of aldosterone excess supporting arterial hypertension in non-pregnant subjects and normotension during pregnancy due to an improved utero-placental perfusion.

One of these polymorphisms, the SF–1–344C/T conversion, had been studied in a pre-eclamptic population from Turkey with a lack of association [27]. A direct comparison of these data with our current findings is limited by the missing information on primiparity, which was high in our study group, and the ethnic divergence between the study groups. Also, we investigated a group of pregnant women as controls without any abnormalities in their for-

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Conflict of interest statement. None to be declared.

(See related article by M. D. Lindheimer and P. August. Aldosterone, maternal volume status and healthy pregnancies: a cycle of differing views. Nephrol Dial Transplant 2009; 24: 1712–1714.)

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