Single Nucleotide Polymorphisms in G Protein Signaling Pathway Genes in Preeclampsia

Anne Stine Kvehaugen, Øyvind Melien, Oddgeir Lingaas Holmen, Hannele Lavuori, Pål Øian, Alice Beathe Andersgaard, Ralf Dechend, Anne Cathrine Staff

Abstract—Preeclampsia is a pregnancy specific disorder and a risk factor for later cardiovascular disease. The cause and detailed pathophysiology remains unknown. G protein signaling is involved in a variety of physiological processes, including blood pressure regulation. We assessed whether distributions of 3 single nucleotide polymorphisms in genes coding for components of G protein signaling pathways that have been associated with hypertension differ between women with preeclampsia and normotensive pregnant women; the G protein β3 subunit gene (GNB3) C825T polymorphism (rs5443), the angiotensin II type 1 receptor gene (AGTR1) 3′UTR A1166C polymorphism (rs5186), and the regulator of G protein signaling 2 gene (RGS2) 3′UTR C1114G polymorphism (rs4606). Two separate Norwegian study populations were used; a large population based study and a smaller, but clinically well-described pregnancy biobank. A descriptive study of 43 women with eclampsia was additionally included. In the population-based study, an increased odds of preeclampsia (odds ratio, 1.21; [95% confidence interval, 1.05–1.40]; P=0.009) and recurrent preeclampsia (odds ratio, 1.43; [95% confidence interval, 1.06–1.92]; P=0.017) was found in women carrying the rs4606 CG or GG genotype. In early-onset preeclamptic patients with decidual spiral artery biopsies available (n=24), the rs4606 CG or GG genotype was more frequent in those with acute atherosclerosis (resembling early stage of atherosclerosis) compared with those without: odds ratio, 15.0; (95% confidence interval, 2.02–111.2); P=0.004. No association was found between preeclampsia and the rs5443 or the rs5186. The genotype distribution in eclamptic women was not different from preeclamptic women. In conclusion, RGS2 rs4606 may affect the risk and progression of preeclampsia. (Hypertension. 2013;61:655-661.) • Online Data Supplement

Key Words: angiotensin II ■ G proteins ■ hypertension ■ polymorphism ■ preeclampsia

Preeclampsia affects ∼4% to 8% of all pregnancies and is characterized by onset of hypertension and proteinuria after 20 weeks of gestation.1 Preeclampsia may further develop into eclampsia, a severe and potential life-threatening complication characterized by seizures.1 A history of preeclampsia is also associated with an increased risk for hypertension and cardiovascular disease later in life.2 The detailed pathophysiology of preeclampsia and the association to future cardiovascular risk is unknown, but both genetic and environmental factors may play a role.

G protein signaling is involved in a variety of physiological processes,3 including the regulation of vascular smooth muscle cell contractility, and thus blood pressure homeostasis.4 Likewise, G proteins are involved in a variety of diseases, including cardiovascular disease,5 and could also possibly play a role in preeclampsia. G proteins, or heterotrimeric guanine nucleotide-binding proteins, consisting of an α, β, and γ subunit, are localized directly downstream of their membrane-bound receptors and play a key role in intracellular signaling cascades as transducers of the receptor signal.5,6 The G proteins may as well exert receptor-independent functions.5 The β-3 subunit of G proteins, encoded by the gene GNB3, is ubiquitously expressed in tissues.7 The C825T polymorphism of GNB3 (rs5443) is associated with a splice variant, named Gβ3s, and an increased signal transduction compared with unmodified Gβ3 protein.7 This single nucleotide polymorphism (SNP) has been associated with overweight and hypertension,7 but the evidence for an association between this SNP and preeclampsia is scarce.8-10

The renin–angiotensin system is dysregulated in preeclampsia,11 but the mechanisms are incompletely understood. Angiotensin II (Ang II) exerts most of its effects through binding to the Ang II receptor type 1 (AGTR1),12 which is coupled to G proteins. A SNP in AGTR1, 3′UTR A1166C polymorphism

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The regulators of G protein signaling (RGS) are a family of multifunctional proteins that were first identified as modulators of G protein signaling.\textsuperscript{17,18} The subtype RGS2 negatively regulates G protein signaling initiated by vasoconstrictor ligands, such as Ang II,\textsuperscript{19} and several observations point to a role for RGS2 in the control of blood pressure.\textsuperscript{18,19} Recently, the 3'UTR C1114G polymorphism in RGS2 (rs4606), has been associated with hypertension and reduced RGS2 expression.\textsuperscript{20} To our knowledge, the rs4606 in RGS2 has not previously been studied in preeclampsia. The aim of the present study was to analyze the distributions of these 3 SNPs with reference to preeclampsia in a case-control setting in 2 independent Norwegian study populations, including a large population-based cohort and a smaller, but clinically well-defined pregnancy biobank study. We also wanted to explore whether any of these SNPs were associated with clinical phenotype, such as early-onset preeclampsia and placental pathology. Additionally, we analyzed the genotype distribution of these SNPs in a small study population of women with eclampsia.

\textbf{Methods}

\textbf{Study Populations and DNA}

\textbf{Study Population 1}

All women in this study were retrospectively recruited from the phase 2 of the Nord-Trøndelag Health Study (HUNT2). HUNT2 is a large multipurpose health survey conducted in 1995 to 1997, where all residents ≥20 years old living in Nord-Trøndelag county, Norway, were invited, and a total of 65 258 (71.2\%) participated.

For the present study, the HUNT2 data set was linked to the medical birth registry of Norway (MBRN). Cases were selected if they had a DNA sample in HUNT2 and had been registered in the MBRN database with a diagnosis of preeclampsia (n=1281). MBRN registers all deliveries in Norway since 1967 by compulsory notification, and the diagnosis of preeclampsia in the MBRN is based on the report of this from the delivering units. Delivery units in Norway use the American College of Obstetricians and Gynecologists criteria for a diagnosis of preeclampsia (defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg) that occurs after 20 weeks of gestation in a previously normotensive woman, combined with proteinuria (urinary excretion of ≥20.3 g protein in a 24-hour urine specimen).\textsuperscript{1}

The control cases (2:1 controls per case) were any woman with a DNA sample in HUNT2 registered in MBRN without a diagnosis of preeclampsia in any of their pregnancies (n=2559). The control group was matched for age with the index preeclampsia group. For the control group, clinical information was derived from the last registered delivery in the MBRN, whereas for the preeclampsia group the corresponding clinical information from the MBRN was derived from the preeclampsia index pregnancy. As a consequence, despite of age matching during the selection process, mean age as registered in MBRN seems higher in the control group compared with the preeclampsia group. After this main selection of cases and controls, 44 preeclamptic women and 100 controls were excluded because of a diagnosis in MBRN of chronic hypertension, chronic renal disease, heart disease, diabetes mellitus, or registered non-proteinuric gestational hypertension. Furthermore, 88 women with preeclampsia and 130 controls were excluded owing to missing data regarding pregnancy duration at delivery. Thus, the final study population consisted of 3478 women with a DNA sample from the HUNT2 study; 1149 with preeclampsia (1093 singleton, 55 duplex, and 1 triplet) and 2329 control pregnancies (2285 singleton and 44 duplex).

\textbf{Study Population 2}

The HUNT2 study did not have detailed clinical information from the index pregnancy (as registered in the MBRN). Therefore, DNA from a clinically well-defined pregnancy research biobank from Oslo University Hospital was included to study associations between the SNPs and clinical characteristics. Pregnant women were included at or close to delivery during 2001 to 2008: preeclampsia; n=100 (including 3 women with the hemolysis, elevated liver enzymes, and low platelets [HELLP] syndrome) and uncomplicated pregnancies (controls, n=82), mostly delivered by cesarean section. Only women with singleton pregnancies were included, and none had preexisting hypertension. Preeclampsia was defined according to the American College of Obstetricians and Gynecologists criteria.\textsuperscript{1}

Placental decidual samples had previously been analyzed with immunohistochemistry for acute atherosclerosis, resembling early stages of atherosclerosis,\textsuperscript{21} in 36 of the preeclamptic women\textsuperscript{22} in the current SNP study. Because subjects were differently recruited (included during pregnancy: the Oslo study, versus retrospectively linked to the MBRN: the HUNT2 study) and obtained from different regions of Norway, we present results for these 2 study populations separately.

\textbf{Study Population 3}

We additionally performed a descriptive study of the genotype distribution of the 3 SNPs in women that had suffered eclampsia. These women were obtained from a Scandinavian cohort study of eclampsia, which have been described in more detail previously.\textsuperscript{23} DNA was available from 43 Norwegian and Swedish patients with singleton pregnancies.

The Regional Committees of medical research ethics in Norway approved the studies (middle Norway and eastern Norway for the HUNT and Oslo study populations, respectively), and informed written consent was obtained from each woman in both study populations. The study of eclampsia was approved in all the Scandinavian countries, and women provided informed written consent.

\textbf{SNP Analyses}

DNA was extracted at HUNT biobank or Oslo University Hospital, from whole blood or blood clot from the 3 population groups using standard methodology (Puregene kit; Gentra Systems, Minneapolis, MN, or MagNAPure LC, Roche Diagnostics GmbH, Mannheim, Germany). The TaqMan-based system 7900HT Fast RealTime PCR System (Applied Biosystems, Foster City, CA) was used to analyze the polymorphisms rs5443 in GNB3 (assay ID: C\textunderscore 2184734\textunderscore 10), rs5186 in AGTR1 (assay ID: C\textunderscore 3187716\textunderscore 10), and rs4606 in RGS2 (assay ID: C\textunderscore 2498717\textunderscore 10). Genotyping success rates were 98.6\% for rs5443, 99.1\% for rs5186, and 98.4\% for rs4606.

\textbf{Statistical Analyses}

Data were analyzed using the Statistical Package for the Social Sciences (SPSS-PC), version 15.0. Differences in continuous variables between diagnosis groups were tested by the Mann–Whitney U test or independent samples t test, where appropriate. Categorical data were compared between groups by cross-tabulation and \(\chi^2\) statistics, or logistic regression. Risk estimates are given as odds ratios (OR) with 95\% confidence intervals (CI). Homogeneity of ORs between the Oslo and the HUNT2 study populations was tested by the Breslow–Day test. Significance level was set at 5\% except for the SNP analyses, where a probability level of <0.015 was considered statistically significant owing to multiple comparisons (3 SNPs studied, and the regular 5\% level of significance was divided by 3 according to Bonferroni correction; 0.05/3=0.0167=0.015).
Hardy–Weinberg Equilibrium Testing

All SNPs were tested for possible deviations from the Hardy–Weinberg equilibrium using the Hardy–Weinberg equilibrium calculator of the Genetic Online Encyclopedia (http://www.oege.org/software/hwe-mr-calc.shtml). All SNPs were found to be in Hardy–Weinberg equilibrium for all study populations and diagnosis groups (control, preeclampsia, and eclampsia).

Results

Basic clinical characteristics for study population 1 (preeclampsia and controls) are presented in Table 1. Clinical variables are presented for study population 2 and 3 in Tables S1 and S2 in the online-only Data Supplement.

**Table 1. Clinical Characteristics (Mean and SD) of Preeclamptic Women and Controls**

<table>
<thead>
<tr>
<th>Study Variables</th>
<th>Preeclampsia (n=1149)</th>
<th>Control (n=2329)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y†</td>
<td>26.6 (5.57)</td>
<td>29.6 (5.07)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Birthweight, gms</td>
<td>3184 (830)</td>
<td>3607 (601)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gestational age at delivery, wk</td>
<td>38.7 (2.9)</td>
<td>39.7 (2.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Delivery &lt;37 wk, %</td>
<td>16.3</td>
<td>4.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Delivery &lt;34 wk, %</td>
<td>6.2</td>
<td>1.6</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Statistically significant, P<0.005. †For the control group, the clinical information for the women was collected from the last registered delivery in the Medical Birth Registry of Norway, ensuring that these selected women did not have a history of preeclampsia. Therefore, mean age in the control group seems higher than mean age in the preeclampsia group, although the study groups were initially matched by age.

SNPs and Pregnancy Outcome

**Association to Preeclampsia (HUNT2 Study Population)**

The genotype distribution of rs5443 in GNB3 and rs5186 in AGTR1 did not differ significantly between preeclamptic women and controls, or between early- and later-onset preeclampsia (Table 2).

The genotypes heterozygous or homozygous for the G allele of rs4606 in RGS2 were significantly more frequent among preeclamptic women compared with controls (Table 2); OR, 1.21; (95% CI, 1.05–1.40); P=0.009. A linear-by-linear association was found when comparing the genotype distribution in women with recurrent preeclampsia (n=193; CC, 44.6%; CG, 45.1%; GG, 10.4%) to controls (genotype distribution displayed in Table 2), and this was statistically significant (P=0.014). The higher proportion of women having either the CG or GG genotype in the recurrent preeclampsia group was borderline significant as compared with controls (P=0.017), and the corresponding OR, 1.43; (95% CI, 1.06–1.92) was higher than the OR for preeclampsia in general. The higher proportion of women having either the CG or GG genotype in early-onset preeclampsia compared with later-onset preeclampsia or controls (Table 2) did, however, not reach statistical significance.

No significant differences were found between the preeclampsia group and the control group regarding combinations of SNPs.

**Association to Clinical Characteristics (Oslo Study Population)**

In the present study, we found that the rs4606 CG or GG genotype of RGS2 was more frequent in a small group of...
Table 3. Regulator of G protein Signaling 2 gene (RGS2) 3’UTR C1114G Polymorphism (rs4606) in Relation to Acute Atherosclerosis in Decidual Spiral Arteries in Preeclampsia

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>RGS2 rs4606 Genotype, % (n)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC</td>
<td>CG+GG†</td>
</tr>
<tr>
<td>Preeclampsia, disregarding week of delivery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute atherosclerosis (n=15)</td>
<td>33.3 (5)</td>
<td>66.7 (10)</td>
</tr>
<tr>
<td>No acute atherosclerosis (n=21)</td>
<td>66.7 (14)</td>
<td>33.3 (7)</td>
</tr>
<tr>
<td>Preeclampsia, delivery &lt;37 wk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute atherosclerosis (n=14)</td>
<td>28.6 (4)</td>
<td>71.4 (10)</td>
</tr>
<tr>
<td>No acute atherosclerosis (n=19)</td>
<td>68.4 (13)</td>
<td>31.6 (6)</td>
</tr>
<tr>
<td>Preeclampsia, delivery &lt;34 wk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute atherosclerosis (n=11)</td>
<td>18.2 (2)</td>
<td>81.8 (9)</td>
</tr>
<tr>
<td>No acute atherosclerosis (n=13)</td>
<td>76.9 (10)</td>
<td>23.1 (3)</td>
</tr>
</tbody>
</table>

Study population: 2; Oslo pregnancy biobank. Statistics: Pearson χ². *Statistically significant acute atherosclerosis vs no acute atherosclerosis; P<0.015 (after correction for multiple analyses). †Only 1 woman had the GG genotype (this woman had acute atherosclerosis and a delivery <34 wk), therefore merging of CC and GG genotypes.

pre-eclamptic women with acute atherosclerosis compared with pre-eclamptic women without acute atherosclerosis in decidual spiral arteries (Table 3). This difference was statistically significant when the women delivered before week 34 (Table 3); OR for acute atherosclerosis in women with early-onset preeclampsia having either the CG or GG genotype compared with women with early-onset preeclampsia with the CC genotype: 15.0 (95% CI, 2.02–111.2). We did not find any statistical significant association between any of the 3 SNPs and other clinical data, such as blood pressure or maternal anthropometrics, nor fetal weight or weight percentile (data not shown).

The genotype distribution for the Oslo study population is given in Table S3 in the online-only Data Supplement. Briefly, no significant differences in genotype distribution of any of the SNPs were found between pre-eclamptic women and controls. No heterogeneity was, however, found between the Oslo and the HUNT2 study population (P>0.05), and pooled estimates (merging the 2 study populations) were in agreement with the main conclusions obtained from the HUNT2 study separately, including a significant association between rs4606 CG or GG genotype and preeclampsia (Mantel–Haenszel common OR, 1.201; [95% CI, 1.046–1.380]; P=0.010).

Association to Eclampsia (Scandinavian Study Population)

The genotype distribution in the eclampsia group is given in Table S4 in the online-only Data Supplement. The proportion of women having a genotype either heterozygous or homozygous for the rs5443 T allele of GNB3: the rs5186 C allele of AGTR1; and the rs4606 G allele of RGS2, respectively, were as follows: 44.2%; 41.9%; and 58.1%. Only 9 women had been delivered before gestational week 34 in the eclampsia group, but 7 (78%) of these women carried the rs4606 G allele of RGS2.

Discussion

In the present study, we analyzed 3 SNPs in genes coding for components of G protein signaling pathways at the levels of receptor rs5186, G protein rs5443, and regulators rs4606, respectively, with reference to preeclampsia. SNPs were selected that have been reported to be associated with hypertension. We found a modestly, but statistically significant, higher risk of preeclampsia in women carrying either the rs4606 CG or GG genotype of RGS2 in our large population-based study (the HUNT2 study).

One of many potential mechanistic pathways for this association could be that the rs4606 CG and GG genotype of RGS2 may be involved in the pathophysiology of preeclampsia through dysregulated Ang II signaling. As with the majority of vasoconstrictors, Ang II signals via G protein-coupled receptors that are coupled to G protein to increase intracellular calcium and subsequent responses, such as vasoconstriction.3,25 Ang II may also exert some of its effects via G protein signaling.30 RGS2 acts as a GTPase activating protein, negatively regulating both Gαq as well as Gαi proteins by enhancing the termination of the activated G protein state.16,20,27 Additionally, it has been suggested that RGS2 may act as a downstream effector of the NO-cGMP pathway, resulting in vascular relaxation by attenuating vasoconstrictor signaling, whereas suppression of this mechanism may facilitate development of hypertension.23 In this context, Semplicini et al demonstrated an association between the rs4606 G allele and RGS2 expression in peripheral blood mononuclear cells in hypertensive patients, with the lowest values in GG homozygotes.30 In a smaller number of subjects from the same study (cases and controls), RGS2 gene and protein expression were also reduced in fibroblasts from those carrying either the CG or GG genotype. Ang II-stimulated intracellular Ca²⁺ increase and ERK1/2 phosphorylation in fibroblasts were likewise higher in those with either the CG or GG genotype compared with CC genotype.20 We therefore hypothesize that women with the rs4606 CG or GG genotype of RGS2, which may potentially result in lower RGS2 protein, would have a reduced function in mediating the negative regulation of G protein signaling activated by vasoconstrictor ligands, such as Ang II. A resulting decrease in the termination control of Ang II-exerted signaling, caused by the presence of the G allele, could be an underlying mechanism for the previously described augmented Ang II sensitivity seen in preeclampsia, and that is believed to be of importance for the clinical presentation of the maternal syndrome.11,28 Gant et al11, who studied the pressor response to Ang II throughout primigravid pregnancy, found that ≈90% of women requiring >8 ng/kg per min of infused Ang II and ≈90% of women requiring ≤8 ng/kg per min of infused Ang II, on tests done between week 28 and 32, remained normotensive and developed preeclampsia, respectively. Because the odds of preeclampsia and recurrent preeclampsia with the CG or GG genotype in our study was increased by only ≈20% and 40%, respectively, compared with women carrying the CC genotype, the presence of the CG or GG genotype alone probably cannot explain the augmented Ang II sensitivity in all cases of pre-eclamptic women. However, the percentage of pre-eclamptic women with augmented Ang II sensitivity remains to be determined in large studies. Two recent studies extended the finding on increased Ang II sensitivity in women with preeclampsia to postpartum.29,30 These data underscore
that a dysregulation of the renin–angiotensin system in this population could be based on a genetic background. Other mechanisms, that have been proposed to result in increased Ang II responsiveness in preeclampsia include activating autoantibodies against the AT1-receptor,20 heterodimerization of AT1- and Bradykinin-receptors,31 and a redox state of angiotensinogen.32 The presence of the rs4606 CG or GG genotype of RGS2 could possibly result in interactions with these, or other yet unknown, mechanisms.

Women with a history of preeclampsia have an augmented risk of cardiovascular morbidity and mortality and the association strengthens with more severe preeclampsia, including early-onset, recurrent disease, intrauterine growth restriction, and low birthweight of the offspring; the 2 latter indicative of placental dysfunction.2,21 Also, offspring of a preeclampsia pregnancy have an increased risk of hypertension later in life.21 In preeclampsia, lipid deposition in walls of the maternal uterine arteries leading to the placenta, named spiral arteries, regularly occurs.22 These vascular lesions resemble early stages of atherosclerosis and are called acute atherosclerosis.21 In the present study, the rs4606 CG or GG genotype of RGS2 was found to be more common not only in preeclamptic women compared with controls but also in the group of preeclamptic women diagnosed with acute atherosclerosis in decidual spiral arteries compared with preeclamptic women without this phenotype, with the strongest association among those with early-onset preeclampsia. A link between acute atherosclerosis in pregnancy and adult atherosclerotic disease is not unlikely because the 2 phenotypes may have a common pathophysiological background.21 Accordingly, we speculate that the potential downregulation of RGS2 in individuals carrying the rs4606 G allele may increase their risk of both vascular atherosclerotic lesions in pregnancy (expressed as acute atherosclerosis) and atherosclerosis later in life, but future and larger studies would be needed to address any such relationship and the mechanistic pathway needs to be elucidated. Foam cell formation is a common step of early lesions in atherosclerosis development and the acute atherosclerosis process of preeclamptic pregnancy.21 Interestingly, Lee et al17 showed that toll-like receptor 4 stimulation decreased RGS2 mRNA expression and induced foam cell formation in macrophages.31 In a subsequent study, the same group showed that RGS2 reduced toll-like receptor 2-mediated NADPH oxidase 1 (Nox1) transcription by negatively regulating STAT3. Toll-like receptor–mediated expression of Nox1 is important for the generation of reactive oxygen species by macrophages.17 This in turn is necessary for macrophage transformation to foam cells during the innate immune response.17 Thus, macrophage activation could potentially represent the mechanism associating acute atherosclerosis and atherosclerotic disease in the women with the rs4606 G allele.

We did not find evidence in the present study for an increased risk of preeclampsia with the rs5186 in AGTR1 or the rs5443 in GNB3. The rs5186 in relation to preeclampsia has previously been described in some smaller studies conducted among different populations.16 In line with our present and larger study, the majority of these studies did not find evidence of a direct relationship to preeclampsia. Previous reports on the association between the rs5443 and preeclampsia have been conflicting.8–10 Our present study, which is much larger than these previous reports, does not support an association between preeclampsia and this polymorphism in a Norwegian population.

A strength of our study is the large sample size of the HUNT2 study. When assuming a preeclampsia prevalence of 3.4%,24 and a relative risk of 1.3 for genotypes heterozygous or homozygous for the variant allele, the HUNT2 study had a power >0.80 when α <0.01 to detect association of studied sequence variants with preeclampsia. Another strength is that our study populations may be ethnically more homogenous than those of many previous preeclampsia studies. HUNT2 is a large health survey conducted in Nord-Trøndelag County, Norway, where the population is ethnically homogeneous (<3% of subjects are of non-white ethnicity), making it, especially, suitable for epidemiological genetic research. We have used country of origin as a proxy of ethnicity in the Oslo study population, and most of the women were born in Norway (79% and 83% of preeclampsia and controls, respectively).

A limitation of the present study is that our finding of a significant association between the rs4606 CG and GG genotype of RGS2 and preeclampsia in the HUNT2 study was not replicated in the clinically well-defined Oslo pregnancy biobank study. No heterogeneity between the HUNT2 and the Oslo study populations were, however, detected. Because the association between the RGS2 rs4606 and preeclampsia in the much larger population-based HUNT2 study was only modest, the lack of a statistical significant association in the Oslo study population was probably rather the result of a smaller sample size. The odds of having either the CG or GG genotype versus CC genotype was also higher in the recurrent preeclampsia group (with assumedly larger genetic risk), compared with controls. In addition, the OR between the 2 groups was higher than the corresponding OR between the total preeclampsia group (disregarding recurrence or not) and controls. These findings lend further support for a true association between this SNP and development of preeclampsia. Additionally, in our small study population of women with eclampsia, representing a severe form of preeclampsia,1 we found a similar or even slightly higher proportion of women having either the rs4606 CG or GG genotype of RGS2, compared with the proportion of women having either the CG or GG genotype in the 2 preeclampsia groups of the present study. No separate control group was, however, available from the same population, and we therefore cannot conclude as to risk of eclampsia versus normotensive pregnancy for women with the CG or GG genotype.

Detailed clinical information from the index pregnancy (as registered in MBRN) was lacking for the large HUNT2 study population, reducing opportunities to study associations between genes of interest and clinical phenotype before and during pregnancy. However, we saw no clear pattern of differences in the clinically well-described Oslo study population in relation to body mass index, blood pressure, or birthweight percentile of the offspring in the preeclampsia group or in the control group. However, this latter study population was small, and we cannot exclude that some true associations may not have been detected.

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Another limitation could be that we included both singleton and multiple pregnancies from the HUNT2 study population. Adjustment for multiple versus singleton pregnancies did not alter the main conclusions (data not shown).

The selection of only 3 SNPs also represents a limitation of the present study. The selection was made on the basis of previous findings associating these particular polymorphisms to hypertension, as well as their influence on G protein pathways at different levels. An examination of polymorphisms in additional components of G protein signaling cascades would have required a comprehensive strategy, including a number of G protein subunit genes as well as other proteins potentially involved in these pathways. The observations derived from the present study, suggesting that G protein dependent signaling may be involved in the molecular mechanisms related to preeclampsia, raise questions of possible contributions also from other components of these pathways, which may be explored in future studies.

Perspectives
Our data encourage further research exploring the role of G protein-mediated signaling pathways in preeclampsia and placental pathologies. The results need to be validated in other population-based studies outside Scandinavia to assess whether these genotype distributions predispose for pregnancy complications, such as preeclampsia, in other populations. Longitudinal studies could reveal whether the presence of the rs4606 CG and GG genotype of RGS2 alone or in combination with preeclampsia and placental acute pathologies. The results need to be validated in other populations. Longitudinal studies could reveal whether the presence of the rs4606 CG and GG genotype of RGS2 alone or in combination with preeclampsia and placental acute atherosis (as compared with women without these features)

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Disclosures
None.

References


Novelty and Significance

**What Is New?**

- The rs4606 CG or GG genotype of RGS2 is more common among preeclamptic women compared with normotensive pregnant controls.
- The rs4606 CG or GG genotype of RGS2 is more common in women with preeclampsia and acute atherosis in uteroplacental spiral arteries, resembling early stage of atherosclerosis, compared with preeclamptic women without placental acute atherosis.

**Summary**

The presence of the rs4606 CG or GG genotype of RGS2 may contribute to the pathogenesis of preeclampsia and possibly to the risk of future cardiovascular disease.

**What Is Relevant?**

- The rs4606 CG and GG genotypes of RGS2 could possibly contribute to preeclampsia, placental dysfunction, and cardiovascular disease development, one of several potential mechanisms could be by altered angiotensin II signaling.
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SINGLE NUCLEOTIDE POLYMORPHISMS IN G PROTEIN SIGNALING PATHWAY GENES IN PREECLAMPSIA

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Anne Stine Kvehaugen, Øyvind Melien, Oddgeir Lingaas Holmen, Hannele Laivuori, Pål Øian, Alice Beathe Andersgaard, Ralf Dechend, Anne Cathrine Staff

From the Department of Obstetrics and Department of Gynecology, Oslo University Hospital, Ulleval and Faculty of Medicine, University of Oslo, Norway (A.C.S., A.S.K), The Norwegian Directorate of Health (ØM), HUNT Research Centre, Department of Public Health and General Practice, Norwegian University of Science and Technology, Levanger, Norway (OLH), Haartman Institute, Medical Genetics and Research Programs Unit, Women’s Health, University of Helsinki, Finland (HL), Department of Clinical Medicine, University of Tromsø, Norway (PØ), South-Eastern Norway Regional Health Authority (ABA), Experimental and Clinical Research Center, a joint cooperation between the Charite Medical Faculty and the Max-Delbrueck Center for Molecular Medicine and Helios Clinic Berlin-Buch, Berlin, Germany (RD).

Short title: G protein pathway SNPs in preeclampsia.

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S1: Clinical characteristics (median, interquartile range) of preeclamptic women and controls

Study population 2; Oslo pregnancy biobank

<table>
<thead>
<tr>
<th>Study variables</th>
<th>Preeclampsia n=100</th>
<th>Controls n=82</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>31.0 (28.0-34.0)</td>
<td>32.5 (28.0-35.0)</td>
<td>0.295</td>
</tr>
<tr>
<td>Birthweight, grams</td>
<td>2199 (1478-3039)</td>
<td>3528 (3222-3786)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Gestational age at delivery, weeks</td>
<td>34.2 (31.3-37.4)</td>
<td>38.7 (38.4-39.0)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Delivery &lt; 37 weeks, %</td>
<td>70</td>
<td>3.7</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Delivery &lt; 34 weeks, %</td>
<td>44</td>
<td>2.4</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

*Statistically significant; P<0.05.
S2. Clinical characteristics (median, interquartile range) of eclamptic women
Study population 3; Scandinavian eclampsia study

<table>
<thead>
<tr>
<th>Study variables</th>
<th>Eclampsia n=43</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>31.0 (26.0-34.0)</td>
</tr>
<tr>
<td>Birthweight, grams</td>
<td>2880 (1924-3710)</td>
</tr>
<tr>
<td>Pregnancy duration at delivery, weeks*</td>
<td>37.0 (34.0-40.0)</td>
</tr>
<tr>
<td>Delivery &lt;37 weeks*, %</td>
<td>41.9</td>
</tr>
<tr>
<td>Delivery &lt;34 weeks*, %</td>
<td>20.9</td>
</tr>
</tbody>
</table>

*Pregnancy duration at delivery was considered equal to the week in pregnancy in which eclampsia developed.
### S3. Genotype distribution in preeclamptic women and controls

**Study population 2; Oslo pregnancy biobank**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Diagnosis</th>
<th>n</th>
<th>CC</th>
<th>CT</th>
<th>TT</th>
<th>CT or TT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GNB3 C825T polymorphism (rs5443), % (n)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Preeclampsia</td>
<td>97</td>
<td>37.1 (36)</td>
<td>50.5 (49)</td>
<td>12.4 (12)</td>
<td>62.9 (61)</td>
</tr>
<tr>
<td></td>
<td>Early onset</td>
<td>43</td>
<td>37.2 (16)</td>
<td>44.2 (19)</td>
<td>18.6 (8)</td>
<td>62.8 (27)</td>
</tr>
<tr>
<td></td>
<td>Later onset</td>
<td>54</td>
<td>37.0 (20)</td>
<td>55.6 (30)</td>
<td>7.4 (4)</td>
<td>63.0 (34)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>79</td>
<td>43.0 (34)</td>
<td>43.0 (34)</td>
<td>13.9 (11)</td>
<td>57.0 (45)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Diagnosis</th>
<th>n</th>
<th>AA</th>
<th>AC</th>
<th>CC</th>
<th>AC or CC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AGTR1 A1166C polymorphism (rs5186), % (n)</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Preeclampsia</td>
<td>96</td>
<td>51.0 (49)</td>
<td>41.7 (40)</td>
<td>7.3 (7)</td>
<td>49.0 (47)</td>
</tr>
<tr>
<td></td>
<td>Early onset</td>
<td>44</td>
<td>65.9 (29)</td>
<td>29.5 (13)</td>
<td>4.5 (2)</td>
<td>34.1 (15) *</td>
</tr>
<tr>
<td></td>
<td>Later onset</td>
<td>52</td>
<td>38.5 (20)</td>
<td>51.9 (27)</td>
<td>9.6 (5)</td>
<td>61.5 (32) *</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>81</td>
<td>50.6 (41)</td>
<td>45.7 (37)</td>
<td>3.7 (3)</td>
<td>49.4 (40)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Diagnosis</th>
<th>n</th>
<th>CC</th>
<th>CG</th>
<th>GG</th>
<th>CG or GG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RGS2 C1114G polymorphism (rs4606), % (n)</strong></td>
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<tr>
<td></td>
<td>Preeclampsia</td>
<td>98</td>
<td>50.0 (49)</td>
<td>41.8 (41)</td>
<td>8.2 (8)</td>
<td>50.0 (49)</td>
</tr>
<tr>
<td></td>
<td>Early onset</td>
<td>44</td>
<td>45.5 (20)</td>
<td>50.0 (22)</td>
<td>4.5 (2)</td>
<td>54.5 (24)</td>
</tr>
<tr>
<td></td>
<td>Later onset</td>
<td>54</td>
<td>53.7 (29)</td>
<td>35.2 (19)</td>
<td>11.1 (6)</td>
<td>46.3 (25)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>82</td>
<td>51.2 (42)</td>
<td>40.2 (33)</td>
<td>8.5 (7)</td>
<td>48.8 (40)</td>
</tr>
</tbody>
</table>

* A higher proportion of women with later-onset preeclampsia compared to early-onset preeclampsia carried either the AC or CC genotype of the AGTR1 rs5186 (P=0.007). Neither the early-onset or late-onset group differed from controls in this genotype distribution.
S4. Genotype distribution in eclamptic women
Study population 3; Scandinavian eclampsia study

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>n</th>
<th>CC</th>
<th>CT</th>
<th>TT</th>
<th>CT or TT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eclampsia</td>
<td>43</td>
<td>55.8 (24)</td>
<td>37.2 (16)</td>
<td>7.0 (3)</td>
<td>44.2 (19)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diagnosis</th>
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<th>AA</th>
<th>AC</th>
<th>CC</th>
<th>AC or CC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eclampsia</td>
<td>43</td>
<td>58.1 (25)</td>
<td>34.9 (15)</td>
<td>7.0 (3)</td>
<td>41.9 (18)</td>
</tr>
</tbody>
</table>

<table>
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<tr>
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<th>CG</th>
<th>GG</th>
<th>CG or GG</th>
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<td>51.2 (22)</td>
<td>7.0 (3)</td>
<td>58.1 (25)</td>
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