Microsatellite Polymorphism in the Heme Oxygenase-1 Promoter Is Associated With Nonsevere and Late-Onset Preeclampsia

Tea Kaartokallio,* Miira M. Klemetti,* Anni Timonen, Jukka Uotila, Seppo Heinonen, Eero Kajantie, Juha Kere, Katja Kivinen, Anneli Pouta, Päivi Lakkisto, Hannele Laivuori

Abstract—Preeclampsia is a serious and phenotypically heterogeneous vascular pregnancy disorder. Heme oxygenase-1 (HO-1) is a stress response enzyme that may protect the maternal endothelium and facilitate adequate metabolic adaptation to pregnancy by its antioxidant and anti-inflammatory functions. HO-1 stress response is modulated by HO-1 gene (HMOX1) polymorphisms. Individuals with the long allele of a guanine-thymine (GT)n microsatellite repeat located in the promoter region of HMOX1 have a higher risk of cardiometabolic diseases compared with those with the short allele. We investigated whether the long GT allele of HMOX1 is associated with subtypes of preeclampsia. The GT repeat was genotyped in 759 patients and in 779 controls from the Finnish Genetics of Preeclampsia Consortium (FINNPEC) cohort using DNA fragment analysis. In subtype analyses, the long-long (LL) genotype was associated with nonsevere (additive model: odds ratio [OR], 1.94; 95% confidence interval [CI], 1.13–3.31; recessive model: OR, 1.39; 95% CI, 1.02–1.89) and late-onset (additive model: OR, 1.44; 95% CI, 1.02–2.05; recessive model: OR, 1.28; 95% CI, 1.02–1.59) preeclampsia and with preeclampsia without a small-for-gestational-age infant (recessive model: OR, 1.27; 95% CI, 1.02–1.58). The long allele was associated with nonsevere (OR, 1.35; 95% CI, 1.07–1.70) and late-onset (OR, 1.21; 95% CI, 1.03–1.42) preeclampsia and with preeclampsia without a small-for-gestational-age infant (OR, 1.19; 95% CI, 1.02–1.40). Moreover, both the LL genotype and the long allele were associated with preeclampsia in women who had smoked during pregnancy. In conclusion, the GTn long allele seems to predispose to late-onset, less severe form of preeclampsia. This finding supports the role of HO-1 in the pathogenesis of preeclampsia and suggests that the HO-1 pathway may provide a potential target for the treatment of preeclampsia. (Hypertension. 2014;64:00-00.) ● Online Data Supplement

Key Words: heme oxygenase-1 ■ preeclampsia ■ vascular diseases

Preeclampsia is a syndrome of hypertension, proteinuria, and generalized vasoconstriction, complicating ≈3% to 5% of all pregnancies and causing significant maternal and perinatal morbidity and mortality. The syndrome of preeclampsia is considered to include different subtypes, with possibly differing causes. Impaired placentation and placental ischemia as well as poor maternal adaptation to pregnancy are recognized as probable disease mechanisms. Oxidative stress, immunologic dysfunction, increased inflammation, and an imbalance between proangiogenic and antiangiogenic factors characterize the maternal disorder. Cardiovascular disease risk factors such as chronic hypertension, renal disease, and obesity predispose to preeclampsia, suggesting pre-existing vascular or endothelial dysfunction as a possible factor in the development of the disease.

Heme oxygenase (HO) is a rate-limiting enzyme, found in all tissues, responsible for the decomposition of heme into CO, ferrous iron, and biliverdin. The highly inducible isoform HO-1, encoded by the HMOX1 gene, is a stress response enzyme that, along with its byproducts, mediates antioxidant,
anti-inflammatory, vasodilatory, and angiogenic functions.\(^8,9\) HO-1 has been linked to insulin signaling\(^10\) and cardiometabolic disorders.\(^11\) as well as to some pregnancy disorders such as gestational diabetes mellitus\(^15\) and recurrent miscarriage.\(^16\)

Placental HO-1 expression is known to increase throughout gestation,\(^13\) but the exact role of HO-1 during pregnancy has not been established. It is possible that in normal pregnancies the HO-1 pathway protects the maternal endothelium facilitating metabolic adaptation to pregnancy, but in preeclampsia this mechanism is impaired. Reduced \(HMOX1\) expression and HO-1 protein levels have been documented in the placenta of women with preeclampsia,\(^17,18\) although contradictory results have also been obtained.\(^19\)

Furthermore, patients with preeclampsia have decreased concentrations of CO in their exhaled breath compared with healthy pregnant women, suggesting decreased HO-1 activity.\(^20\) Interestingly, HO-1 inhibits the release of antiangiogenic factors\(^22\) whose expression increases in preeclamptic women before the clinical onset of the disease.\(^22,23\)

The promoter region of \(HMOX1\) contains cis-regulatory variants, including a guanine-thymine (GT\(_n\)) microsatellite polymorphism. The short GT\(_n\) allele (≤25 repeats) is linked to significantly higher \(HMOX1\) expression compared with the long allele (≥25 repeats).\(^11,12,24\) We hypothesize that in pregnant women who develop preeclampsia, the capacity to upregulate \(HMOX1\) gene in response to placental and endothelial stress might be impaired. Our objective was to investigate whether the long GT\(_n\) allele of \(HMOX1\) is associated with an increased risk of preeclampsia with special emphasis placed on testing the association with subtypes of the disease.

### Methods

#### Study Subjects

We studied 760 patients with preeclampsia and 781 control women without preeclampsia from the Finnish Genetics of Preeclampsia Consortium (FINNPEC) cohort, collected prospectively during 2008 to 2011 at 5 Finnish university hospitals. The study participants were mainly of Finnish origin, and only a few represented other ethnicities. The exclusion criteria in the present study were multiple pregnancy; chronic inflammatory, autoimmune, hemolytic, and renal diseases; and, for multiparous control subjects, previous preeclampsia. All subjects provided written informed consent, and the FINNPEC study protocol was approved by the coordinating Ethics Committee of the Hospital District of Helsinki and Uusimaa. Methods for blood sample collection and genotyping of the GT\(_n\) repeat can be found in the online-only Data Supplement.

#### Obstetric and Perinatal Data

Information on maternal self-reported prepregnancy weight and height, smoking, blood pressure, and proteinuria during pregnancy, as well as perinatal outcomes, was obtained from patient records. Preeclampsia was defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg after 20 weeks of gestation in a previously normotensive woman, combined with new-onset proteinuria ≥0.3 g per 24 hours.\(^25\) Systolic blood pressure ≥160 mm Hg or diastolic blood pressure ≥110 mm Hg or proteinuria ≥5 g per 24 hours was classified as severe preeclampsia. Preeclampsia was defined as early onset when diagnosed before 34+0 weeks of gestation and late onset when diagnosed at 34+0 weeks of gestation or later. The definitions of body mass index, chronic hypertension, preterm delivery, and small for gestational age are presented in the online-only Data Supplement. Each diagnosis was ascertained based on hospital records and confirmed independently by a research nurse and a study physician.

### Statistical Methods

With a risk allele frequency of 0.66\(^26\) and a preeclampsia prevalence of 5\%, a sample size of 728 preeclampsia cases was estimated to be sufficient to detect an effect size of 1.2 for the short-long (SL) genotype and 1.5 for the LL genotype with power of 0.80 when α<0.05.

The Hardy–Weinberg equilibrium test was performed using a Web version of Genepop software (version 4.2).\(^27,28\) Because of skewed distributions, comparisons between continuous variables were performed using the Mann–Whitney \(U\) test. Categorical variables were analyzed using the \(χ^2\) test or the Fisher exact test. The association of the GT\(_n\) repeat alleles and genotypes with preeclampsia was tested using binary logistic regression. For the genotype analysis, both additive and recessive genetic models were applied. The association was tested in the whole data set as well as in several subgroups based on subtypes of preeclampsia or other classification criteria relevant for the disease. For all tests, a \(P\) value <0.05 was considered significant. Statistical analyses were performed with IBM SPSS statistics 20 software (IBM Corp).

### Results

#### Background Characteristics of the Study Population

Basic maternal and perinatal background characteristics of the study population are presented in Table 1. Women with preeclampsia had chronic hypertension more frequently, delivered on average earlier, and had infants with a lower birth weight compared with control subjects. The multiparous preeclampsia patients had a higher mean body mass index compared with the multiparous controls. Gestational and pregestational (type 1 or type 2) diabetes mellitus were also more common among multiparous patients with preeclampsia. Of the 91 multiparous preeclampsia patients, 81 had preeclampsia also in a previous pregnancy.

#### Allele Frequencies of the GT\(_n\) Repeat Polymorphism

The GT\(_n\) repeat polymorphism located in the promoter region of the \(HMOX1\) gene was successfully genotyped in 759 patients and in 779 control subjects. The length of the repeat ranged from 16 to 41 repeats, the most frequent alleles being 30 (46.3\%) and 23 (20.2\%). The distribution of allele frequencies in the study sample is shown in Figure S1 in the online-only Data Supplement. The GT\(_n\) alleles were further divided into short (≤25 repeats) and long (≥25 repeats) allele classes. Genotypes of the repeat polymorphism were in Hardy–Weinberg equilibrium in control subjects.

#### Association of the Long Allele of the GT\(_n\) Repeat With Preeclampsia or Subtypes of Preeclampsia

No association was found between the long allele of the GT\(_n\) repeat polymorphism of \(HMOX1\) and preeclampsia when all patients with preeclampsia and all control subjects were analyzed together (Table 2). When subtypes of preeclampsia were analyzed separately, the long allele was associated with nonsevere and late-onset preeclampsia (Table 2). In line with these findings, the long allele was associated with preeclampsia when women with small-for-gestational-age infants were excluded (Table 2). The association of the LL genotype was seen for nonsevere and late-onset preeclampsia both with additive and recessive genetic models and for preeclampsia not associated with small for gestational age when the recessive model was used (Figure; Table S1).

The long allele of the GT\(_n\) repeat was significantly associated with preeclampsia in women who had smoked during
Table 1. Maternal and Perinatal Background Characteristics of 760 Patients With Preeclampsia and 781 Control Subjects From the Finnish Genetics of Preeclampsia Consortium Cohort

<table>
<thead>
<tr>
<th>Maternal or Perinatal Characteristics</th>
<th>Preeclampsia</th>
<th>Control</th>
<th>Primipara</th>
<th>Multipara</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primipara (n=669)</td>
<td>Multipara (n=91)</td>
<td>Primipara (n=442)</td>
<td>Multipara (n=339)</td>
</tr>
<tr>
<td>Age, y</td>
<td>29 (25/32)</td>
<td>34 (29/37)</td>
<td>28 (25/32)</td>
<td>31 (28/34)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>23.5 (21.2/27.0)</td>
<td>24.5 (21.3/30.0)</td>
<td>23.2 (21.1/26.0)</td>
<td>23.0 (20.7/26.7)</td>
</tr>
<tr>
<td>Highest systolic blood pressure, mmHg</td>
<td>165 (153/177)</td>
<td>168 (157/178)</td>
<td>129 (121/138)</td>
<td>124 (118/134)</td>
</tr>
<tr>
<td>Highest diastolic blood pressure, mmHg</td>
<td>109 (103/115)</td>
<td>108 (104/114)</td>
<td>85 (80/90)</td>
<td>82 (78/88)</td>
</tr>
<tr>
<td>Proteinuria, g/24 h</td>
<td>4.3 (1.5/5.8)</td>
<td>4.0 (1.4/5.3)</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Smoking before pregnancy</td>
<td>132 (20.8)</td>
<td>13 (15.7)</td>
<td>109 (25.7)</td>
<td>46 (14.3)</td>
</tr>
<tr>
<td>Gestational hypertension</td>
<td>76 (11.4)</td>
<td>14 (15.4)</td>
<td>26 (5.9)</td>
<td>19 (5.6)</td>
</tr>
<tr>
<td>Gestational diabetes mellitus</td>
<td>81 (12.1)</td>
<td>23 (25.3)</td>
<td>39 (8.8)</td>
<td>29 (8.6)</td>
</tr>
<tr>
<td>Pregestational diabetes mellitus</td>
<td>8 (1.2)</td>
<td>5 (5.5)</td>
<td>4 (0.9)</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>Birth weight, g</td>
<td>2825 (2228/3310)</td>
<td>2940 (1940/3515)</td>
<td>3518 (3225/3850)</td>
<td>3695 (3340/4010)</td>
</tr>
<tr>
<td>Relative birth weight (SD)</td>
<td>−1.10 (−1.85/−0.40)</td>
<td>−0.84 (−1.64/−0.16)</td>
<td>−0.17 (−0.81/0.48)</td>
<td>0.17 (0.42/0.85)</td>
</tr>
<tr>
<td>Gestational age at birth, wk</td>
<td>37.9 (35.8/39.4)</td>
<td>38.0 (35.3/39.0)</td>
<td>40.5 (39.4/41.3)</td>
<td>40.3 (39.4/41.1)</td>
</tr>
</tbody>
</table>

Values for continuous variables are median (25th/75th percentile) and for categorical variables are frequencies (%). Number of subjects is shown in brackets if different.

Discussion

Our results show that the long allele of the GT<sub>n</sub> microsatellite polymorphism located in the promoter region of HMOX1 is associated with an increased risk of late-onset and nonsevere preeclampsia, as well as with preeclampsia not associated with small for gestational age. Overall, GT<sub>n</sub> long allele seems to be linked to preeclampsia with a less severe phenotype. We also identified an association of the GT<sub>n</sub> long allele with preeclampsia in women who had smoked during pregnancy.

To the best of our knowledge, the association of the GT<sub>n</sub> microsatellite polymorphism of HMOX1 with subtypes of preeclampsia has not been previously studied. The strengths of the study include a carefully characterized study cohort with comprehensive clinical and background information available from each study subject. All subtypes were independently ascertained by a clinician and a research nurse. The study population can be considered fairly representative of the Finnish population because it is composed of subjects recruited from all Finnish university hospitals with geographically based catchment areas. Furthermore, the distribution of allele frequencies of the GT<sub>n</sub> repeat polymorphism in our study population is similar to that reported in another Finnish study. A limitation of the study is the lack of data on serum concentrations of HO-1. In addition, although the sample size was estimated to be sufficient for the analysis of patients with preeclampsia, a larger sample size could have been more ideal for the analysis of smaller groups of patients with preeclampsia subtypes.

The genome-wide linkage study by Lachmeijer et al. suggested that the chromosome 22q region, which contains the HMOX1 gene, harbors a maternal susceptibility locus for preeclampsia. HO-1 is involved in several biological processes associated with the pathogenesis of preeclampsia, including implantation, placentation, and immune system regulation. The HO-1 pathway seems to be cytoprotective in the placenta, because the overexpression of HO-1 inhibits the production of antiangiogenic factors whose balance is distorted in preeclampsia. Increased expression of HO-1 protects the endothelium via antioxidant, anti-inflammatory, cytoprotective, and vasodilatory effects. The end products of heme breakdown, biliverdin and bilirubin, are powerful antioxidants. Another product of heme degradation, CO, mediates several of the beneficial effects of HO-1, including anti-inflammatory, antiapoptotic, and vasodilatory effects. Patients with preeclampsia have decreased concentrations of CO in their exhaled breath compared with healthy pregnant controls, suggesting decreased HO-1 activity.

In mice, placental HO-1 is a critical factor in blastocyst implantation, placentation, and fetal growth, and lack of HO-1 results in poor placental development, intrauterine growth restriction, and fetal death. A partial maternal HO-1 deficiency causes inadequate placental vascularization, probably via changes in the angiogenic profile. Moreover, pregnant mice heterozygous for HO-1 present with high diastolic blood pressure and elevated angiogenic plasma fms-like tyrosine kinase-1 (sFlt-1) levels, which also characterize preeclampsia in humans. Also, inhibiting HO activity in pregnant rats significantly increases maternal mean arterial pressure. Interestingly, HO-1 induction normalizes elevated blood pressure in pregnant rats continuously infused with sFlt-1, which suggests that HO-1 has beneficial effects also independent of its capacity to suppress the release of sFlt-1.

According to one hypothesis, abnormal placentation typically characterizes early-onset preeclampsia, whereas maternal metabolic risk factors, such as obesity and diabetes mellitus, may play a more significant role in the late-onset form of the

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Hypertension July 2014

Several of the metabolic abnormalities associated with preeclampsia resemble those seen in the metabolic syndrome and persist several years postpartum. In line with this, previous preeclampsia is a sign of increased risk of cardiovascular diseases and type 2 diabetes mellitus in women. In the present study, the GTn long allele was associated with late-onset, less severe form of preeclampsia, which is in agreement with the previous observations of associations of this polymorphism with type 2 diabetes mellitus and cardiovascular disorders. All these disorders share similar metabolic characteristics and risk factors. It is possible that the effect of the GTn repeat polymorphism on the HMOX1 expression may not be strong enough to modify the risk for early or severe preeclampsia. Yachie et al described the case of a woman heterozygous for complete loss of exon2 of HMOX1, who experienced 2 fetal deaths. This could suggest that the most serious placental complications emerge below a certain threshold of HO-1 expression.

Women with the short GTn allele and more powerful HO-1 stress response could be less prone to develop adverse outcomes because of the beneficial effects of HO-1 on the maternal endothelium. In contrast, women with severe preeclampsia subtype may be affected by a burden of severely impaired placental perfusion in conjunction with an abundance of predisposing genetic factors with a large impact. In the presence of these factors, the effect of increased HO-1 expression could be too modest to modulate disease susceptibility, and therefore, the association of the long GTn allele with preeclampsia could only be seen for the less severe subtype.

Our results indicate that the long allele of the GTn repeat, which decreases the expression of HO-1, is associated with preeclampsia in women who smoke during pregnancy. This is in line with previous studies that revealed that cigarette smoke induces HO-1 expression in trophoblast cells and decreases sFlt-1 release from placental villous explants. CO has been shown to have anti-inflammatory and antiapoptotic effects and to regulate placental angiogenesis in mice by increasing the expression of angiogenic factors including vascular endothelial growth factor and placental growth factor, and decreasing the expression of sFlt-1 and soluble endoglin. Low-dose CO breathing has been demonstrated to prevent growth...
restriction in a mouse intrauterine growth restriction model. Both the CO from cigarette smoke as well as the increased CO levels and the decreased production of antiangiogenic factors resulting from the induction of HO-1 expression may explain the protective effect of smoking on preeclampsia. Because the long allele of the GT repeat polymorphism decreases HO-1 expression, we speculate that smokers with the long repeat are not protected by these mechanisms induced by cigarette smoke as effectively as smokers with the short repeat.

In conclusion, we found that the long allele of the GT repeat polymorphism located in the promoter region of the HMOX1 gene is associated with nonsevere and late-onset preeclampsia. Our finding highlights the importance of careful subtyping of this heterogeneous pregnancy disorder and sheds light on the factors predisposing to the less severe subtypes of preeclampsia.

Perspectives

In this study, we show that the long allele of the GT repeat polymorphism in the HMOX1 promoter region is associated with certain subtypes of preeclampsia. This raises the intriguing possibility of preventive and therapeutic use of HO-1 and the end products of heme degradation, as well as molecules that stimulate HO-1 expression, such as statins, in preeclampsia. CO, biliverdin, and bilirubin have all shown therapeutic potential in vascular diseases in preclinical animal models. Statins, which are currently used in cardiovascular diseases, stimulate HO-1 expression and inhibit sFlt-1 release and are currently being studied as potential therapeutic agents in preeclampsia as well (www.controlled-trials.com; ISRCTN23410175). Our results underline the importance of identifying preeclampsia subtypes. The ability to accurately distinguish subtypes of this heterogeneous pregnancy disorder would facilitate the search for genetic factors that predispose to the disease as well as the development of preventive and therapeutic methods.
Hypertension July 2014


Novelty and Significance

• What is New?
  • This is the first study to address the association of an HMOX1 polymorphism with preeclampsia.
  • The long allele of a GT, microsatellite repeat located in the HMOX1 promoter region is associated with increased risk of late-onset late-stage preeclampsia.

• What Is Relevant?
  • This study supports the role of heme oxygenase-1 in the pathogenesis of preeclampsia.
  • Because the association in our study was found for certain subtypes of preeclampsia, the study encourages to identify preeclampsia subtypes.

• Our findings suggest a possibility for the therapeutic use of heme oxygenase-1, the end products of heme degradation, and molecules that stimulate heme oxygenase-1 expression, such as statins, in preeclampsia.

Summary

The long allele of the GT, repeat polymorphism located in the promoter region of the HMOX1 gene is associated with nonsevere and late-onset preeclampsia, preeclampsia not associated with small for gestational age, and preeclampsia in women who had smoked during pregnancy.
Microsatellite Polymorphism in the Heme Oxygenase-1 Promoter Is Associated With Nonsevere and Late-Onset Preeclampsia
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MICROSATELLITE POLYMORPHISM IN THE HEME OXYGENASE -1 PROMOTER IS ASSOCIATED WITH NON-SEVERE AND LATE-ONSET PREECLAMPSIA

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14 Minerva Institute for Medical Research, Helsinki, Finland
15 Department of Clinical Chemistry, University of Helsinki, Helsinki, Finland
Short title: GT_n repeat in HMOX1 is associated with PE subtypes

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SUPPLEMENTARY METHODS

**Obstetric and perinatal data.** Body mass index (BMI) was defined as the self-reported pre-pregnancy weight divided by height squared (kg/m²). Chronic hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg detected before 20 weeks of gestation. Preterm delivery was defined as delivery occurring before 37 weeks of gestation. Birth weights below −2.0 SD units were classified as small-for-gestational age (SGA) according to Finnish standards.1

**Collection of blood samples.** A venous blood sample (36mL) was drawn from all subjects. Genomic DNA was extracted from whole blood using the NucleoSpin Blood XL DNA extraction kit (Macherey-Nagel GmbH & Co.) or Chemagic Magnetic Separation Module I –machine (Chemagen) and subsequently stored at -20°C.

**Genotyping of the GTₙ repeat**
Size of the GTₙ repeat polymorphism in the promoter area of *HMOX1* was studied by fragment analysis. The repeat area was amplified by PCR using the FAM –labeled forward primer 5’-FAM-AGAGCCTGCAGCTTCTCAGA-3’ and reverse primer 5’-ACAAAGTCTGGCCATAGGAC-3’ 2 in PCR conditions of initial denaturation at 95°C for 10 min followed by 25 alternating cycles of denaturation at 95°C for 30 s and annealing and extension at 54°C for 75s. The sizes of the amplified fragments were determined in the FIMM Sequencing Laboratory, using an automated capillary sequencer ABI3730xl DNA Analyzer (Applied Biosystems). GeneScan 500 LIZ Size Standard (Applied Biosystems) was used to size the fragment data. The number of GTₙ repeats was determined with Gene Mapper v4.0 software (Applied Biosystems).

REFERENCES


### SUPPLEMENTARY TABLE

**Table S1.** Genotypic association results for the GT<sub>n</sub> repeat in *HMOX1* from binary logistic regression analysis.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Genotype count (frequency)</th>
<th>Additive model</th>
<th></th>
<th>Recessive model</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OR SL (95% CI)</td>
<td>p*</td>
<td>OR LL (95% CI)</td>
<td>p*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SS</td>
<td>SL</td>
<td>LL</td>
<td>Ref SS</td>
<td>Ref SS</td>
</tr>
<tr>
<td>PE subtypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PE (all)</td>
<td>97 (12.8)</td>
<td>336 (44.3)</td>
<td>326 (43.0)</td>
<td>1.07 (0.79-1.46)</td>
<td>0.658</td>
</tr>
<tr>
<td>Severe PE</td>
<td>78 (14.2)</td>
<td>243 (44.3)</td>
<td>228 (41.5)</td>
<td>0.96 (0.69-1.34)</td>
<td>0.830</td>
</tr>
<tr>
<td>Non-severe PE</td>
<td>19 (9.0)</td>
<td>93 (44.3)</td>
<td>98 (46.7)</td>
<td>1.52 (0.89-2.59)</td>
<td>0.129</td>
</tr>
<tr>
<td>Early-onset PE</td>
<td>32 (16.2)</td>
<td>90 (45.5)</td>
<td>76 (38.4)</td>
<td>0.87 (0.55-1.37)</td>
<td>0.551</td>
</tr>
<tr>
<td>Late-onset PE</td>
<td>65 (11.6)</td>
<td>246 (43.9)</td>
<td>250 (44.6)</td>
<td>1.17 (0.83-1.66)</td>
<td>0.369</td>
</tr>
<tr>
<td>Non-severe, late-onset PE</td>
<td>18 (9.1)</td>
<td>87 (43.9)</td>
<td>93 (47.0)</td>
<td>1.50 (0.86-2.59)</td>
<td>0.151</td>
</tr>
<tr>
<td>Control (all)</td>
<td>113 (14.5)</td>
<td>365 (46.9)</td>
<td>301 (38.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other phenotype subgroups</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primipara with PE</td>
<td>83 (12.4)</td>
<td>289 (43.3)</td>
<td>296 (44.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primipara control</td>
<td>61 (13.9)</td>
<td>212 (48.2)</td>
<td>167 (38.0)</td>
<td>1.00 (0.69-1.46)</td>
<td>0.992</td>
</tr>
<tr>
<td>Multipara with PE</td>
<td>14 (15.4)</td>
<td>47 (51.6)</td>
<td>30 (33.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multipara control</td>
<td>52 (15.3)</td>
<td>153 (45.1)</td>
<td>134 (39.5)</td>
<td>1.14 (0.58-2.24)</td>
<td>0.702</td>
</tr>
<tr>
<td>Condition</td>
<td>Genotype Counts</td>
<td>Genotype Frequencies (%)</td>
<td>OR (95% CI)</td>
<td>p-value</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>-----------------</td>
<td>--------------------------</td>
<td>-------------</td>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>PE and SGA infant</td>
<td>26 (15.7)</td>
<td>78 (47.0)</td>
<td>62 (37.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control with SGA infant</td>
<td>4 (36.4)</td>
<td>3 (27.3)</td>
<td>4 (36.4)</td>
<td>4.00 (0.84-19.06)</td>
<td>0.082</td>
</tr>
<tr>
<td>PE without SGA infant</td>
<td>71 (12.0)</td>
<td>258 (43.5)</td>
<td>264 (44.5)</td>
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<td></td>
</tr>
<tr>
<td>Control without SGA infant</td>
<td>109 (14.2)</td>
<td>362 (47.1)</td>
<td>297 (38.7)</td>
<td>1.09 (0.78-1.54)</td>
<td>0.603</td>
</tr>
<tr>
<td>PE patient who smoked during pregnancy</td>
<td>4 (8.0)</td>
<td>13 (26.0)</td>
<td>33 (66.0)</td>
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<td></td>
</tr>
<tr>
<td>Control who smoked during pregnancy</td>
<td>3 (4.8)</td>
<td>39 (62.9)</td>
<td>20 (32.3)</td>
<td>0.25 (0.05-1.27)</td>
<td>0.094</td>
</tr>
<tr>
<td>PE patient who smoked before pregnancy</td>
<td>20 (13.8)</td>
<td>59 (40.7)</td>
<td>66 (45.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control who smoked before pregnancy</td>
<td>21 (13.5)</td>
<td>85 (54.8)</td>
<td>49 (31.6)</td>
<td>0.73 (0.36-1.46)</td>
<td>0.373</td>
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<tr>
<td>PE and diabetes</td>
<td>7 (6.0)</td>
<td>59 (50.4)</td>
<td>51 (43.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control with diabetes</td>
<td>12 (16.4)</td>
<td>28 (38.4)</td>
<td>33 (45.2)</td>
<td>3.61 (1.28-10.17)</td>
<td>0.015</td>
</tr>
<tr>
<td>PE and BMI ≥ 25 kg/m²</td>
<td>34 (11.8)</td>
<td>137 (47.7)</td>
<td>116 (40.4)</td>
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<td></td>
</tr>
<tr>
<td>Control with BMI ≥ 25 kg/m²</td>
<td>43 (16.3)</td>
<td>122 (46.2)</td>
<td>99 (37.5)</td>
<td>1.42 (0.85-2.37)</td>
<td>0.179</td>
</tr>
</tbody>
</table>

Genotype counts and frequencies (%) in brackets are presented. Alleles are divided into short (S) (≤25 repeats) and long (L) (>25 repeats) allele classes.

* Binary logistic regression, PE or PE subgroup vs. all control subjects
† Binary logistic regression, PE vs. control subjects within phenotype subgroup
‡ Gestational diabetes, type 1 diabetes or type 2 diabetes
BMI = body mass index, CI= confidence interval, OR= odds ratio, SGA = small for gestational age
Figure S1. Allele frequencies of the HMOX1 GTn repeat