

Stanniocalcin-1 Hormone in Nonpreeclamptic and Preeclamptic Pregnancy: Clinical, Life-Style, and Genetic Modulators

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Context and Objectives: The study represents the first comprehensive analysis of Stanniocalcin-1 (STC1) hormone in human pregnancy, assessing clinical, lifestyle, and genetic determinants of circulating STC1 at term.

Design, Setting, and Participants: Participants included women with ($n = 50$) and without ($n = 316$) preeclampsia (PE) at delivery, recruited in the REPROgrammed fetal and/or maternal METAbolism (REPROMETA) study (2006–2011, Estonia). Genetic association analysis combined PE cases ($n = 597$) and controls ($n = 623$) from the REPROMETA and Finnish Genetics of Preeclampsia Consortium (2008–2011) studies.

Main Outcome Measure(s): Maternal postpartum plasma STC1 was measured by ELISA ($n = 366$) and placental *STC1* gene expression by TaqMan quantitative RT-PCR ($n = 120$). Genotyping was performed using Sequenom MassArray.

Results: Significantly higher STC1 plasma level was measured for the PE (median, 1952 pg/mL; 1030–4284 pg/mL) compared with non-PE group (median, 1562 pg/mL; 423–3781 pg/mL; $P = 3.7 \times 10^{-4}$, Mann-Whitney *U* test). Statistical significance was enhanced after adjustment for cofactors (linear regression, $P = 1.8 \times 10^{-6}$). STC1 measurements were negatively correlated with maternal smoking. Prepregnancy body mass index had a positive correlation with STC1 only among PE patients ($r = 0.45$; $P = .001$). The strongest genetic association with hormone concentrations was detected for *STC1* single nucleotide polymorphisms rs3758089 (C allele: minor allele frequency, 5%; linear regression: $\beta = 249.2$ pg/mL; $P = .014$) and rs12678447 (G allele: minor allele frequency, 7%; $\beta = 147.0$ pg/mL; $P = .082$). rs12678447 placental genotypes were significantly associated with *STC1* gene expression ($P = .014$). The REPROMETA/Finnish Genetics of Preeclampsia Consortium meta-analysis suggested an increased risk to develop late-onset PE for the rs12678447 G allele carriers ($P = .05$; odds ratio = 1.38 [0.98–1.93]).

Conclusions: Increased STC1 hormone represents a hallmark of late-onset PE. *STC1* gene variants modulate placental gene expression and maternal hormone levels. (*J Clin Endocrinol Metab* 101: 4799–4807, 2016)

Stanniocalcin (STC) is a glycoprotein hormone that was initially described as a regulator of calcium homeostasis in teleost fish (1), released in response to elevated serum calcium from the corpuscles of Stannius in kidneys. The human counterpart, Stanniocalcin-1 (STC1) protein,

was described in 1995 (2, 3). Studies in mammals indicated that STC1 (and its paralog STC2) exhibits high similarity with its teleost ortholog and has also an important role in regulating calcium and phosphate metabolism (4, 5). Compared with fish, the mammalian STC1 appears to

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Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate; EO-PE, early-onset PE; FINNPEC, Finnish Genetics of Preeclampsia Consortium; GDM, gestational diabetes mellitus; GWAS, genome-wide association studies; LD, linkage disequilibrium; LGA, large for gestational age; LO-PE, late-onset PE; MAF, minor allele frequency; NORM, normal birth weight newborn for its gestational age; OR, odds ratio; PAPP, pregnancy-associated plasma protein; PE, preeclampsia; REPROMETA, REPROgrammed fetal and/or maternal METAbolism; RT-qPCR, quantitative RT-PCR; SGA, small for gestational age; SNP, single nucleotide polymorphism; STC, stanniocalcin.

be involved in more diverse physiological and autocrine/paracrine functions. In female reproductive endocrinology, *STC1* modulates ovarian function (6, 7), implantation (8, 9), gestation, and lactation (6, 10–12). The expression of ovarian *STC1* increases 15-fold during murine gestation, it is further up-regulated in lactating mice, and the circulating hormone reaches detectable threshold only during pregnancy and lactation (6). Transgenic mice overexpressing human *STC1* exhibit reduced female reproductive competence and severely postnatal dwarfism (10). Overexpression of *STC1* inhibits normal skeletal development (13).

Despite considerable expression in the human placenta, there is little knowledge on *STC1* in human gestation. In vitro studies have associated *STC1* expression to angiogenesis (14) and remodeling of the spiral arteries within the maternal uterus (15), processes crucial for developing and maintaining normal pregnancy. Our seminal study showed that human *STC1* gene exhibits distinct placental gestational dynamics (11). Its placental transcript levels increase 5-fold between gestational days 40 and 140, peak at midpregnancy, and drop low at term. In term placentas, the *STC1* gene expression was significantly higher in cases of preeclampsia (PE), mainly originating from impaired placental function. A pilot analysis in 129 women revealed that the mothers with PE pregnancies were measured the highest postpartum circulating *STC1* levels. Genome-wide association studies (GWAS) have associated genetic variants near *STC1* with multiple renal traits, estimated glomerular filtration rate (eGFR) (16, 17), chronic kidney disease (18), and serum urate concentrations (19). This is consistent with the pathophysiology of PE, often accompanied with proteinuria and renal failure. Furthermore, a recent study demonstrated that *STC1* is a proteinase inhibitor of the pregnancy-associated plasma protein (PAPP)-A and PAPP-A2, modulating IGF signaling (12). Both encoding genes, *PAPPA* and *PAPPA2*, are among the most highly expressed genes in the human placenta (20), and their altered expression has been linked to PE (21, 22).

The current study aimed at the first comprehensive analysis of *STC1* hormone levels in human pregnancy at term. Specific objectives were: 1) to determine clinical and lifestyle-related covariates of circulating *STC1* levels; 2) to confirm the robustness of increased maternal *STC1* concentration in PE cases; 3) to screen the *STC1* gene for the variants modulating maternal hormone concentration

and placental *STC1* transcript levels; and finally 4) to test the association of these single nucleotide polymorphisms (SNPs) with the risk to develop PE. The study revealed increased *STC1* hormone as a hallmark of PE and identified maternal lifestyle and genetic variants modulating circulating *STC1* levels and, potentially, the risk to PE.

Materials and Methods

REPROgrammed fetal and/or maternal METAbolism (RERPOMETA) study

The REPROMETA study was approved by the Ethics Review Committee of Human Research of the University of Tartu, Estonia (permissions no. 146/18, 27.02.2006; 150/33, 18.06.2006; 158/80, 26.03.2007; and 180/M-15, 23.03.2009), and it was carried out in compliance with the Helsinki Declaration. A written informed consent to participate in the study was obtained from each family before recruitment. All study participants were recruited and the study material was collected at the Women's Clinic of Tartu University Hospital, Estonia in 2006–2011. All participants were of White European ancestry and living in Estonia.

The REPROMETA study represents family trios (mother, father, placenta) recruited before or shortly after delivery, and it covers well-defined pregnancy outcomes at term. In the current study, the analyzed REPROMETA study subjects ($n = 366$) were stratified into 2 subgroups: pregnancies with PE ($n = 50$) and without PE (non-PE, $n = 316$) (Table 1 and Supplemental Table 1). In case-control genetic association analysis, the PE group was subdivided based on the disease onset, as early-onset PE (EO-PE) and late-onset PE (LO-PE) cases tend to have different etiology (23). If the first symptoms appeared before 34 gestational weeks, PE was classified as EO-PE ($n = 25$), and if the symptoms started after 34 completed weeks, as LO-PE ($n = 25$) (24). Non-PE group was comprised of uncomplicated pregnancies with the delivery of a normal birth weight newborn for its gestational age (NORM) (10th–90th percentile; $n = 110$), pregnancies with newborns small for gestational age (SGA) (<10th percentile; $n = 64$) and large for gestational age (LGA) (>90th percentile; $n = 89$), and pregnancies accompanied with gestational diabetes mellitus (GDM) ($n = 53$). Details for clinical criteria applied for PE (25), GDM (26), and SGA and LGA (27) are provided in Supplemental Methods. Information on maternal smoking, somatometric data, and reproductive history was obtained from self-reported questionnaires and medical records.

Quantitative genetic association analysis with *STC1* hormone levels was performed with the maternal (blood samples) and placental genotypes of the full REPROMETA sample set ($n = 366$). Case-control association testing included the PE group ($n = 50$) and controls representing normal pregnancy with normal birth weight newborn, NORM group ($n = 110$) (Table 1).

Table 1. Parental and Offspring Characteristics of the Analyzed REPROMETA Study Groups

Parameter	PE (n = 50)	Non-PE (n = 316)	NORM (n = 110)
Maternal age (y)	27.9 ± 6.0	28.8 ± 5.9	28.3 ± 5.4
Prepregnancy BMI (kg/m ²)	24.4 ± 4.8	24.4 ± 5.3	23.5 ± 4.4
Nulliparity (n)	34 (68%)	136 (43%) ^a	48 (44%) ^a
Gestational age at birth (d)	245.2 ± 23.1	278.1 ± 12.3 ^a	278.9 ± 12.9 ^a
Newborn weight (g)	2198.1 ± 792.8	3790.5 ± 931.8 ^a	3640.8 ± 519.5 ^a
Newborn length (cm)	44.6 ± 4.6	50.8 ± 3.4 ^a	50.8 ± 2.2 ^a
Ponderal index (g/cm ³)	2.44 ± 0.29	2.82 ± 0.33 ^a	2.76 ± 0.25 ^a
Gender (male/female)	23/27	160/156	59/51
Gestational weight gain (kg)	14.9 ± 6.0	15.9 ± 6.5	15.3 ± 7.5
Smokers during pregnancy (n)	6 (12%)	59 (19%)	17 (15%)
Delivery mode (vaginal/C-section)	9/41	200/116 ^a	97/13 ^a
Placental weight (g)	402.7 ± 121.4	621.4 ± 180.8 ^a	571.5 ± 117.0 ^a

Data given as mean ± SD, except where indicated differently. Nulliparity, no previous childbirth; ponderal index, a ratio of body weight to length; non-PE, study group comprised of uncomplicated pregnancies with the delivery of NORM, SGA (<10th percentile), or LGA (>90th percentile) and pregnancies accompanied with GDM; NORM, a subset of non-PE group. Details on the SGA, LGA, and GDM subgroups are provided in Supplemental Table 1.

^a $P < .05$ compared with PE group, Student's t test (for quantitative variables) or χ^2 test (for binary variables).

Finnish Genetics of Preeclampsia Consortium (FINNPEC) study

Finnish PE patient and non-PE control samples originate from the FINNPEC study cohort and the Southern Finland PE study cohort. The study protocol was approved by the coordinating Ethics Committee of the Hospital District of Helsinki and Uusimaa. The FINNPEC study participants have been recruited in 5 university centers in Finland (Helsinki, Turku, Tampere, Kuopio, and Oulu) in 2008–2011. All participants were of White European ancestry and living in Finland. All subjects provided a written informed consent. Details of the FINNPEC study cohort are published elsewhere (28, 29). In the current genetic case-control study, the FINNPEC participants meeting the REPROMETA diagnostic criteria for PE and NORM groups (non-PE pregnancy with a normal birth weight newborn) were included (Supplemental Methods). Identically to the REPROMETA study, the FINNPEC PE patients were further subdivided into early-onset (EO-PE) and late-onset (LO-PE) disease groups.

In total, there were 547 FINNPEC patients with PE (EO-PE, $n = 165$; LO-PE, $n = 382$) and 513 controls. For 378 patients (EO-PE, $n = 88$; LO-PE, $n = 290$) and 496 controls, also fetal DNA was extracted from umbilical cord blood, and genotype/phenotype association was analyzed (Supplemental Table 2).

Measurement of STC1 protein expression in maternal plasma by ELISA

Plasma STC1 levels in 366 REPROMETA study participants were analyzed using ELISA implemented by DuoSet ELISA kit (DY2958; R&D Systems). The analyzed maternal postpartum plasma samples were collected on the day of delivery, aliquoted, and immediately stored at -80°C . The exact recorded time of blood sampling was available for 88 samples, and the median interval from the delivery to blood sampling was 2 hours and 21 minutes. Before ELISA analysis, the samples were thawed on ice and processed with STC1 ELISA kit according to manufacturer's protocol. The detailed ELISA workflow is described elsewhere (11). All the measurements were performed in duplicate, and every assay plate included a reference sample for intra-assay variability estimation. In this experiment, the average intra-assay

variability for processed 10 ELISA plates was 4% and interassay variability 7% accordingly.

Genotyping of the STC1 gene

Single nucleotide polymorphisms tagging the allelic association structure of *STC1* (tag-SNPs) were identified based on the genotype data derived from the 1000 Genomes Project (<http://www.internationalgenome.org/>) for UTAH residents with Northern and Western European ancestry (CEU) as an input for Haploview Tagger software (version 4.2) (30). Tag-SNPs were determined for the genic (12 893 bp) and 2672 bp of the 5' upstream regions, including gene promoter. The selected 13 tag-SNPs and 22 captured SNPs ($r^2 > 0.9$) are listed in Supplemental Figure 1. One of the selected SNPs, rs3758086, is in strong linkage disequilibrium (LD) ($r^2 > 0.9$) with rs10109414 and rs17786744 located 38.8 and 64.7 kb upstream of *STC1* and associated in GWAS with eGFR and serum urate levels, respectively (17, 19).

Genomic DNA extracted from REPROMETA maternal blood samples was genotyped for 13 SNPs ($n = 366$) (Table 1). REPROMETA placental DNA ($n = 366$) and FINNPEC samples (maternal blood, $n = 1060$; umbilical cord blood DNA, $n = 874$) (Supplemental Table 2) were genotyped for 3 SNPs (rs12678447, rs3758089, rs3758086). SNP genotyping was performed on Sequenom MassArray platform (Sequenom) according to standard protocol (Supplemental Methods, Supplemental Table 3).

Placental gene expression of the STC1 gene

Expression of the *STC1* gene in the placenta was assessed with TaqMan quantitative RT-PCR (RT-qPCR) assay for a subset of placental samples from the REPROMETA study subjects ($n = 120$). Placentas (stored at $+4^{\circ}\text{C}$) had been sampled within 1 hour after vaginal delivery or Caesarean section (C-section). Details for placental sampling, RNA extraction, cDNA synthesis, and qPCR are provided in Supplemental Methods. The assays of the target gene *STC1* (TaqMan ID 4331182) and reference gene ubiquitin C (*UBC*) (ID 4448484) were amplified in a biplex PCR. Relative *STC1* placental mRNA expression values were deter-

mined by comparative cycle of threshold (C_T) method that accounted for mean values of normalized expression calculated by averaging 3 independently measured normalized expression values of the triplicate. Q-Gene software was used for calculations and efficiency corrections (31).

Statistical analysis

Covariates modulating maternal hormone levels were assessed using univariate analysis between ELISA measurements of STC1 and clinical/lifestyle parameters of REPROMETA subgroups of PE ($n = 50$) and non-PE ($n = 316$) cases. The analyzed parameters included maternal age, prepregnancy body mass index (BMI), parity, gestational weight gain and smoking status, gestational age, placental weight and delivery mode, newborn gender, and birth weight/length. Statistical significance threshold after correction for the number of independent traits was estimated $\alpha = 0.05/8 = 6.25 \times 10^{-3}$.

All genetic tests were implemented in PLINK software, version 1.07 (32). Genetic association analysis between REPROMETA maternal postpartum STC1 measurements and maternal/placental genotypes ($n = 366$) was performed using linear regression analysis. The tests applied additive genetic model and were adjusted for predetermined covariates for circulating STC1 levels. All genetic association tests used natural logarithm transformed STC1 hormone ELISA measurements. Association of studied SNPs with the placental *STC1* gene expression level was tested using REPROMETA placenta samples ($n = 120$). Linear regression analysis was performed for logarithm transformed relative mRNA values and genotypes of the *STC1* genic SNPs, adjusted by the study group and delivery mode. Case-control analysis comparing PE patients and NORM in REPROMETA and FINNPEC studies for *STC1* genetic variants was assessed by logistic regression additive model, adjusted for maternal age, BMI, and parity as known risk factors for PE development (33). Analysis of the FINNPEC study data was additionally adjusted for the recruitment center. The results of the REPROMETA and the FINNPEC case-control analysis were combined in a meta-analysis under fixed effects model implemented in PLINK software. For SNP-STC1 hormone analysis, the stringent Bonferroni threshold was calculated $\alpha = 0.05/13$ (SNPs) = 3.8×10^{-3} , and for SNP-gene expression and case-control association testing $0.05/3 = 1.67 \times 10^{-2}$.

Results

Elevated maternal postpartum plasma STC1 in PE

In the REPROMETA study sample, significantly higher STC1 postpartum plasma level was measured in pregnancies complicated with PE ($n = 50$) compared with non-PE women ($n = 316$). Median STC1 was measured 1952 pg/mL in the PE group (range, 1030–4284 pg/mL; $n = 50$) and 1562 pg/mL in non-PE group (range, 423–3781 pg/mL; $n = 316$) ($P = 3.7 \times 10^{-4}$, Mann-Whitney U test; significant after Bonferroni correction) (Figure 1A).

Across all study subjects, a significantly higher median STC1 level was detected for the mothers who gave birth with normal vaginal delivery compared with C-section (median, 1663.5 vs 1446 pg/mL; $P = .013$) (Figure 1B). Notably,

STC1 was reduced among mothers who smoked during pregnancy compared with nonsmokers (1385 vs 1631 pg/mL; $P = .02$). Trends for higher STC1 levels among pregnancies with female compared with male newborns (1656 vs 1492 pg/mL; $P = .053$) and in cases with maternal nulliparity (1662 vs 1524 pg/mL; $P = .074$) were observed (Supplemental Figure 2, A and B). The prepregnancy BMI had a significant positive correlation with circulating STC1 only among PE patients ($r = 0.45$; $P = .001$, significant after Bonferroni correction), whereas women in the non-PE group showed a trend for reduced STC1 with increasing maternal age ($P = .050$) (Figure 1C). We were unable to find any statistically supported associations between maternal postpartum plasma STC1 measurements and gestational age at delivery, time of postpartum blood draw (within 24 h), placental weight, gestational weight gain, and newborn's anthropometric measurements at birth (Supplemental Figures 2, C–I, and 3).

Notably, when the identified clinical and lifestyle-related cofactors were incorporated into the analysis, statistical significance of the association of higher STC1 plasma levels in PE compared with non-PE pregnancies was enhanced by 2 orders of magnitude. The comparison of circulating STC1 in the 2 groups adjusted for delivery mode, smoking status, maternal age, and prepregnancy BMI resulted in highly significant difference (linear regression, $\beta = 500.8$ pg/mL; $P = 1.8 \times 10^{-6}$) (Figure 1A).

Genetic modulators of circulating STC1 hormone level

In order to assess potential genetic modulators of circulating STC1, we tested association between genetic variants in the *STC1* gene and hormone levels in maternal plasma of the REPROMETA participants ($n = 366$). From among the 13 analyzed tag-SNPs, the strongest genetic association was detected for 2 rare variants, rs3758089 (C allele: minor allele frequency [MAF], 5%) and rs12678447 (G allele: MAF 7%) (Table 2). SNP rs3758089, located approximately 1.2 kb upstream of the *STC1* gene transcription start site, exhibited the largest allelic effect (linear regression, $\beta = 249.2$ pg/mL; $P = .015$). Minor allele carriers of both rare SNPs had significantly higher median STC1 levels compared with wild-type homozygotes (Mann-Whitney U test; $P < .05$), rs3758089 CT/CC-compared TT-genotype carriers 1915.5 vs 1576 pg/mL and rs12678447 GA/GG-compared with AA-group 1873 vs 1562 pg/mL, respectively (Figure 2A).

None of the common SNPs in the *STC1* gene exhibited a statistically significant effect on circulating STC1 levels (Table 2 and Supplemental Table 4). A trend towards association was observed for rs3758086 (T allele: MAF, 46%) and

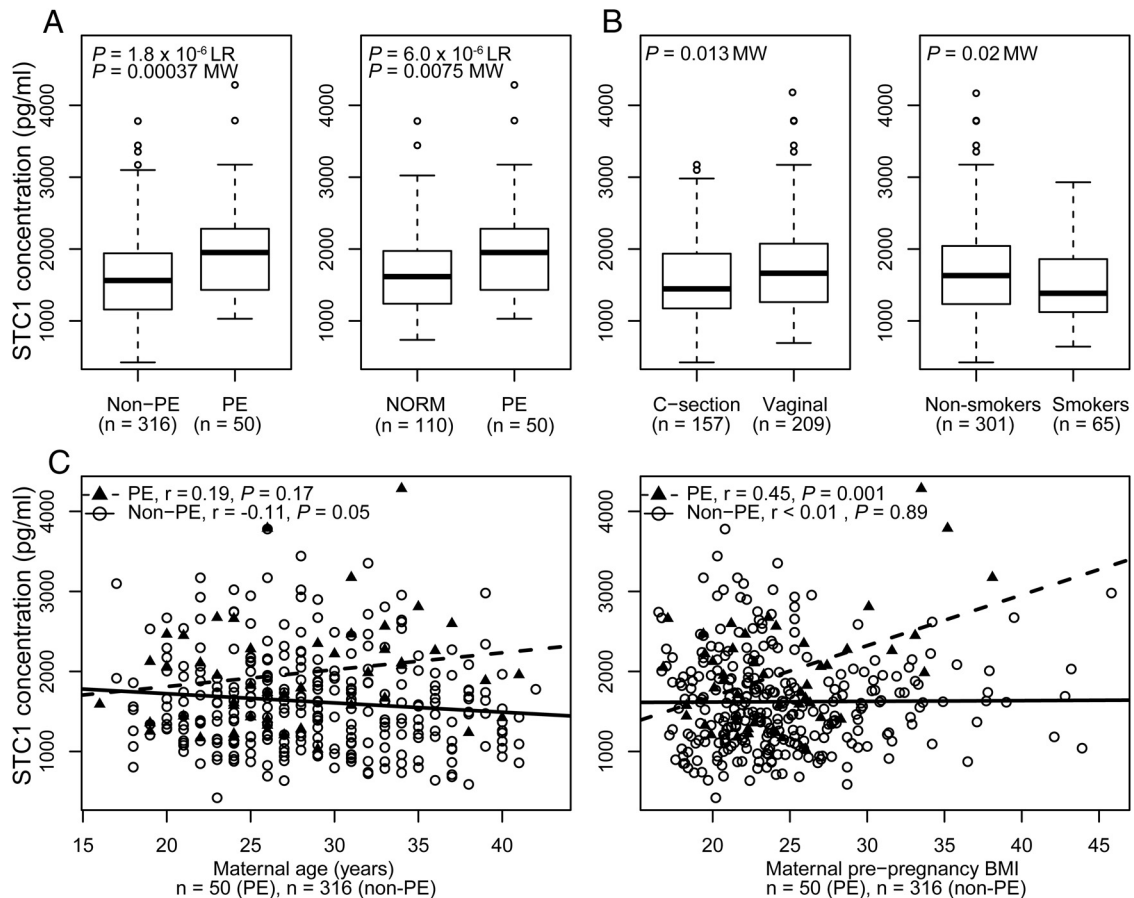


Figure 1. Maternal postpartum plasma STC1 hormone levels in the REPROD study sample subgrouped based on (A) the pregnancy outcome, (B) delivery mode and smoking status, and (C) maternal age and prepregnancy BMI. The boxes represent the 25th and 75th percentiles; median is denoted as the line that bisects the boxes. The whiskers are lines extending from each end of the box covering the extent of the data on 1.5x interquartile range; circles represent the outlier values. Statistical significance in comparisons of median STC1 values among groups was tested with Mann-Whitney *U* test (MW). Additionally, linear regression (LR) model was applied to adjust for the delivery mode, smoking status, maternal age, and prepregnancy BMI as covariates. Associations between STC1 and maternal age and prepregnancy BMI were assessed with LR. Regression line was plotted separately for PE ($n = 50$, discontinuous line) and non-PE ($n = 316$, continuous line) study groups, respectively. non-PE, all pregnancies without PE.

rs423432 (A allele: MAF, 48%). SNP rs3758086 is in strong LD with a reported GWAS hits rs10109414 and rs17786744 ($r^2 > 0.9$; at <38 -kb distance) for renal function parameters, eGFR, and serum urate concentration (17, 19) (Supplemental Figure 1).

Because the *STC1* gene is also expressed in the placenta (11), we assessed whether the composition of placental genotypes may further modulate maternal circulating STC1 levels. Three SNPs (rs12678447, rs3758089, rs3758086) highlighted in the analysis of maternal genotype-STC1 hormone associations (Table 2) were additionally genotyped for the respective placental DNA samples ($n = 366$). None of the analyzed associations between placental genotypes and maternal hormone measurements reached statistical significance.

Genetics of placental expression of the *STC1* gene

In the analysis of potential covariates for the placental *STC1* gene expression at term, none of the tested

parameters appeared to have a major effect (Supplemental Figure 4, A–N). We analyzed whether the SNPs highlighted in the association testing with plasma STC1 hormone levels may also represent genetic modulators of *STC1* transcript levels. Correlation between *STC1* mRNA expression and genotype data of 2 rare (rs12678447, rs3758089) and 1 common SNP (rs3758086) was tested for 120 placental samples. The strongest association was detected for the rare variant rs12678447 (linear regression, $P = .014$; significant after Bonferroni correction) (Table 2 and Figure 2B). Also, the other tested SNPs showed a trend for association ($P < .07$) and the same direction of effects as in previous analysis with the hormone level. We conclude that the highlighted SNPs indeed represent functional variants with the direct effect on placental expression of the *STC1* gene. The genetic effects in the placenta are consistent with the associations identified between maternal genotypes and circulating hormone levels.

Table 2. Genetic Association Tests for the Maternal Circulating STC1 Hormone and Placental *STC1* Gene Expression Levels

Genotyped Samples	Analyzed Phenotype	Unit	rs12678447 (A/G)		rs3758089 (T/C)		rs3758086 (C/T)	
			MAF (%)	<i>P</i> Value, β (SE)	MAF (%)	<i>P</i> Value, β (SE)	MAF (%)	<i>P</i> Value, β (SE)
REPROMETA mothers (n = 366)	Maternal STC1 hormone	pg/mL	7.0	8.23×10^{-2} 147.0 (82.8)	5.0	1.47×10^{-2} 249.2 (97.2)	46.0	1.48×10^{-1} -58.6 (41.8)
REPROMETA placentas (n = 366)	Maternal STC1 hormone	pg/mL	7.0	8.74×10^{-1} -12.8 (82.9)	4.0	2.38×10^{-1} 128.9 (107.6)	43.0	1.28×10^{-1} -60.2 (40.7)
	Placental <i>STC1</i> mRNA expression (n = 120)	log ₂ (RGE)	6.0	1.39×10^{-2}* 0.80 (0.32)	4.0	6.67×10^{-2} 0.72 (0.39)	47.0	6.14×10^{-2} -0.27 (0.15)

STC1 hormone levels in maternal plasma were measured using ELISA (DY2958; R&D Systems). Placental gene expression levels were determined using TaqMan RT-qPCR (assay ID 4331182). Statistical significance in SNP-trait association testing was assessed using linear regression additive model. In the table heading, the tested alleles for each SNP are shown as underlined in bold. Association testing between maternal or placental genotypes and maternal STC1 hormone levels were adjusted for the identified cofactors (Figure 1 and Supplemental Figure 2): study group (PE or non-PE), delivery mode (vaginal or C-section), smoking status, maternal age, and prepregnancy BMI. Association tests between placental genotypes and gene expression were adjusted for the group and delivery mode. Statistically significant nominal *P* values are highlighted in bold, and significant results after Bonferroni correction for multiple testing are indicated with an asterisk. RGE, relative gene expression.

STC1 genetic variants and risk to develop PE

We hypothesized that *STC1* gene variants modulating gene expression and circulating STC1 hormone levels may also represent risk factor for PE. Because PE arises from the malfunction of the mother and placenta, the conducted case-control study targeted both, maternal and fetal genotypes of *STC1* rare variants rs12678447 and rs3758089, as well as a common variant rs3758086. Due to limited size of the REPROMETA patient groups representing PE (n = 50) and NORM (n = 110), this pilot case-control genetic association study included additionally respective cases/controls (PE, n = 547; NORM, n = 513) from the FINNPEC study.

No associations from case-control genetic tests remained statistically significant after correction for multiple testing (Table 3). Still, meta-analysis across REPROMETA and FINNPEC data suggested an increased risk to develop LO-PE for the carriers of rs12678447 G allele (*P* = .05; OR = 1.38). Frequency of this variant in both, REPROMETA and FINNPEC, PE groups was 10%, whereas allele frequencies between 2 studies differed for the controls. In REPROMETA NORM group, the rs12678447 G allele was 6.4% and FINNPEC NORM group 8.8%. The latter is somewhat higher compared with ENSEMBL database, where prevalence of this variant among Finns is 7.6% and in other reported European populations 1.0%–4.7%.

In REPROMETA, also 2 other tested SNPs resulted in association results with LO-PE, supported by nominal *P* < .05.

We did not identify any significant associations between maternal *STC1* genetic variants and the onset EO-PE (Table 3). Fetal *STC1* allelic composition, determined from the placental tissue (REPROMETA) or umbilical cord blood (FINNPEC) genomic DNA, did not show any associations with maternal risk to develop PE in neither of the study samples nor meta-analysis (Supplemental Table 5).

Discussion

We conducted the first systematic analysis measuring circulating STC1

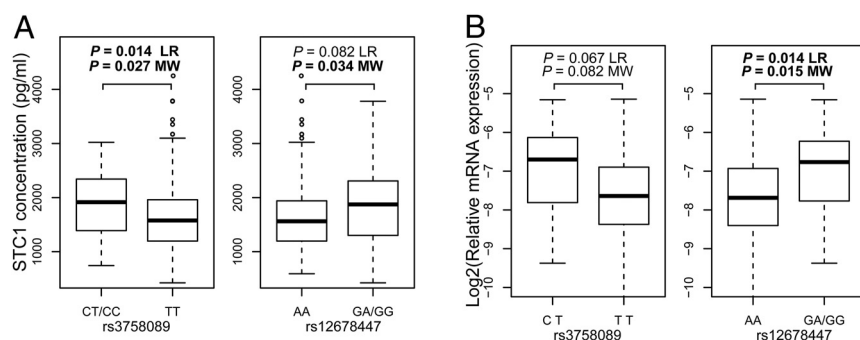


Figure 2. Genetic effects of the *STC1* gene polymorphisms in the REPROMETA study sample shown for (A) the maternal genomic variants on maternal plasma STC1 level and (B) the placental genomic variants on placental *STC1* gene expression. STC1 hormone levels in maternal plasma were measured using ELISA (DY2958; R&D Systems). Placental gene expression levels were determined using TaqMan RT-qPCR (assay ID 4331182). Boxes represent the 25th and 75th percentiles; median is denoted as the line that bisects the boxes. The whiskers are lines extending from each end of the box covering the extent of the data on 1.5x interquartile range; circles represent the outlier values. Statistical significance between the minor allele carriers and major allele homozygotes was tested using Mann-Whitney *U* test (MW) comparing median values of the distributions. In parallel, linear regression (LR) analysis was applied to adjust for possible confounding factors. Testing associations between genetic variants and circulating STC1 hormone levels were adjusted by the study group (PE or non-PE), delivery mode, maternal age, smoking status, and prepregnancy BMI. SNP gene expression association tests were adjusted for the study group and delivery mode.

Table 3. Case-Control Association Analysis Between *STC1* Genetic Variants and PE in REPROMETA and FINNPEC Studies

Analysis Group	Phenotype n, Case/Control	<u>rs12678447 (A/G)</u>		<u>rs3758089 (T/C)</u>		<u>rs3758086 (C/T)</u>		
		MAF (%) Case/ Control	P Value (OR) [95% CI]	MAF (%) Case/ Control	P Value (OR) [95% CI]	MAF (%) Case/ Control	P Value (OR) [95% CI]	
REPROMETA mothers	PE n = 50/110	9.0/6.4	5.14×10^{-2} (2.80) [0.99–7.85]	7.0/3.6	1.28×10^{-2} (4.70) [1.39–15.96]	38.0/47.0	1.24×10^{-1} (0.67) [0.40–1.11]	
	EO-PE n = 25/110	8.3/6.4	2.42×10^{-1} (2.24) [0.58–8.63]	6.0/3.6	8.98×10^{-2} (4.00) [0.80–19.88]	46.0/47.0	6.75×10^{-1} (0.87) [0.46–1.65]	
	LO-PE n = 25/110	10.0/6.4	1.05×10^{-1} (2.86) [0.80–10.20]	8.0/3.6	4.11×10^{-2} (4.54) [1.06–19.41]	30.0/47.0	2.13×10^{-2} (0.44) [0.21–0.88]	
	FINNPEC mothers	PE n = 547/513	9.0/8.8	4.29×10^{-1} (1.14) [0.82–1.56]	5.5/5.0	3.87×10^{-1} (1.19) [0.80–1.80]	43.0/45.0	3.12×10^{-1} (0.90) [0.75–1.10]
FINNPEC mothers	EO-PE n = 165/513	7.5/8.8	6.67×10^{-1} (0.90) [0.55–1.46]	5.0/5.0	7.34×10^{-1} (1.11) [0.61–2.00]	42.0/45.0	2.26×10^{-1} (0.84) [0.64–1.11]	
	LO-PE n = 382/513	10.0/8.8	1.29×10^{-1} (1.31) [0.92–1.85]	6.0/5.0	2.65×10^{-1} (1.28) [0.82–2.01]	44.0/45.0	3.21×10^{-1} (0.90) [0.73–1.10]	
	Meta-analysis	PE n = 597/623	n.a.	1.82×10^{-1} (1.23) [0.90–1.67]	n.a.	<u>1.08×10^{-1}</u> (1.37) [0.93–2.02]	n.a.	1.38×10^{-1} (0.87) [0.73–1.04]
	EO-PE n = 190/623	n.a.	9.92×10^{-1} (0.99) [0.63–1.57]	n.a.	3.64×10^{-1} (1.30) [0.74–2.25]	n.a.	2.01×10^{-1} (0.85) [0.66–1.09]	
LO-PE n = 407/623	n.a.	5.86×10^{-2} (1.38) [0.98–1.93]	n.a.	<u>9.60×10^{-2}</u> (1.43) [0.93–2.19]	n.a.	1.10×10^{-1} (0.85) [0.70–1.03]		

Case-control association testing was performed using logistic regression additive model, adjusted for maternal age, BMI, and parity in both studies. In FINNPEC analysis, recruitment center was included as an additional cofactor. *P* values for meta-analysis are combined under fixed effects model. In the table heading, the tested alleles for each SNP are shown as underlined in bold. Statistically significant nominal *P* values are indicated in bold, .05 < *P* ≤ .1 are underlined. n.a., not applicable.

concentrations in human postpartum plasma and assessing the modulatory effect of maternal, fetal and genetic determinants. The analysis confirmed a robust and statistically highly significant association of increased maternal hormone levels with the diagnosis of PE ($P = 1.8 \times 10^{-6}$; linear regression adjusted for cofactors). In PE compared with non-PE cases, the median measured *STC1* level was approximately 25% higher and a significant positive correlation was detected with maternal BMI. The exact roles of *STC1* in pregnancy and in the pathogenesis of PE are largely unknown. *STC1* was reported to inhibit the proteolytic activity of PAPP-A and PAPP-A2 in vitro (12). Reduced maternal serum PAPP-A levels refer to a high PE risk (21), whereas maternal sera and placental tissues from PE cases show, in contrast, elevated levels of PAPP-A2 (22). We speculate that increased circulating *STC1* in PE pregnancy may represent a feedback mechanism to high expression of PAPP-A2. A study in mice showed that PAPP-A represents a pregnancy-associated oncogene, whereby the high expression of *STC1* (and *STC2*) in the mammary glands during late pregnancy and lactation correlated with the reduced PAPP-A activity and a protective effect against carcinogenesis (34).

Interestingly, *STC1* measurements were negatively correlated with maternal smoking. Because a decreased risk of PE occurrence among smoking pregnant women has been shown (35), it is consistent with lower concentration of *STC1* detected in the non-PE group. Reduced *STC1* in

smokers may either reflect the overall effect of smoking on gestational metabolism and/or be involved in the PE protective effect.

As a second study outcome, we identified genetic variants affecting the expression of the *STC1* gene. We applied 2 independent genetic analyses, testing the association of maternal genotypes with measured interindividual variation in *STC1* hormone levels and testing the association of placental genotypes with term placental *STC1* transcript abundance. The analysis highlighted concordant results for 2 rare SNPs (rs3758089; MAF, 5% and rs12678447; MAF, 7%) with the effect on *STC1* gene expression and maternal hormone levels (Table 2 and Figure 2). SNP rs3758089 is located in the *STC1* promoter region and less than 200 bp from a strong H3K27Ac peak (a landmark for a regulatory element) identified in the Encyclopedia of DNA Elements (ENCODE) project (36). Because rs12678447 in the third intron of the *STC1* gene is partially in LD with rs3758089 (Supplemental Figure 1), we suggest that the causative, gene expression modulating variant is rather the latter. The study detected a trend for the association for a common variant rs3758086 (2.7 kb upstream from *STC1* transcription start site) with maternal hormone and placental *STC1* gene expression. This SNP is in strong LD with 2 GWAS hits for renal function-related parameters, located in distance 38–65 kb ($r^2 > 0.9$; rs10109414 and rs17786744). The role of our discovered variants in the *STC1* genomic region, rare SNPs

rs3758089 and rs12678447 in relation to the kidney function is still to be analyzed.

We performed a pilot case-control association testing of the identified *STC1* regulatory SNPs for the risk to develop PE. Although no robustly supported statistically significant associations we detected, the observed trends for the *STC1* variants as potential genetic risk factors for LO-PE warrant further investigations. Differently from EO-PE, which is considered to arise primarily due impaired placental development (23), LO-PE develops at the third trimester due to the limited capacity of maternal metabolism to cope with the needs of the growing placenta and fetus (37, 38). The pathophysiology of PE involves various mechanisms being strongly implicated by renal dysfunction and renin angiotensin system (14, 15, 39). We hypothesize that elevated *STC1* level during LO-PE might act as a compensatory mechanism to impair renal function and in some degree it might be modulated by the *STC1* genetic variants. Previous experiments with *STC1* transgenic mice suggest that *STC1* acts as a potent antiinflammatory and renal protective protein (40).

Regarding the study limitations, we consider modest sample size as an issue in our genetic analyses. Because the original REPROMETA study design was based on the recruitment at the delivery room, the maternal blood samples were drawn within 24 hours postpartum. However, the exact interval from the delivery to blood draw was available only for a subset of cases. The gestational and postpartum dynamics of *STC1* hormone, and the respective modulatory factors are still to be investigated in prospective studies.

In summary, our comprehensive analysis of pregnancy-related effectors of *STC1* in human gestation at term revealed a substantially increased hormone levels in PE patients. The analysis of tag-SNPs representing the *STC1* genic region discovered genetic variants modulating placental gene expression and maternal hormone levels. These SNPs have a potential to be further investigated as risk factors for LO-PE and for impaired renal function.

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