Protective Low-Frequency Variants for Preeclampsia in the Fms Related Tyrosine Kinase 1 Gene in the Finnish Population

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Abstract—Preeclampsia is a common pregnancy-specific vascular disorder characterized by new-onset hypertension and proteinuria during the second half of pregnancy. Predisposition to preeclampsia is in part heritable. It is associated with an increased risk of cardiovascular disease later in life. We have sequenced 124 candidate genes implicated in preeclampsia to pinpoint genetic variants contributing to predisposition to or protection from preeclampsia. First, targeted exomic sequencing was performed in 500 preeclamptic women and 190 controls from the FINNPEC cohort (Finnish Genetics of Preeclampsia Consortium). Then 122 women with a history of preeclampsia and 1905 parous women with no such history from the National FINRISK Study (a large Finnish population survey on risk factors of chronic, noncommunicable diseases) were included in the analyses. We tested 146 rare and low-frequency variants and found an excess (observed 13 versus expected 7.3) nominally associated with preeclampsia ($P<0.05$). The most significantly associated sequence variants were protective variants rs35832528 (E982A; $P=2.49E-4$; odds ratio=0.387) and rs141440705 (R54S; $P=0.003$; odds ratio=0.442) in Fms related tyrosine kinase 1. These variants are enriched in the Finnish population with minor allele frequencies 0.026 and 0.017, respectively. They may also be associated with a lower risk of heart failure in 11 257 FINRISK women. This study provides the first evidence of maternal protective genetic variants in preeclampsia. **(*Hypertension*. 2017;70:365-371. DOI: 10.1161/HYPERTENSIONAHA.117.09406.)** • **Online Data Supplement**

Key Words: cardiovascular diseases ■ Finland ■ heart failure ■ preeclampsia ■ proteinuria

Preeclampsia is a common vascular disorder that affects 3% of pregnant women.[1] Worldwide, it annually accounts for ≈50 000 maternal and 900 000 perinatal deaths.[2,3] The clinical characteristics are diverse, and the course of the disease is unpredictable. Both a preeclamptic mother and a child born from a preeclamptic pregnancy are at higher risk for later-life cardiovascular diseases and type 2 diabetes mellitus.[4]

Angiogenesis is tightly involved in the pathophysiology of preeclampsia.[5] A high ratio of sFLT1 (soluble Fms related tyrosine kinase 1) to placental growth factor is among the most promising biomarkers for predicting the onset of the disease.[6] Several susceptibility loci for preeclampsia have been identified in genome-wide linkage studies.[7,8] However, linkage and candidate gene studies have been plagued with poor reproducibility.

The population of Finland is genetically unique in Europe.[9] The repeated bottleneck events that caused strong founder effects and geographic isolation over centuries have resulted in the enrichment of variants that are rare or absent in other populations.[10] These features provide an opportunity

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to overcome many of the obstacles in studying rare enriched variants that contribute to the risk of complex diseases.[11]

We designed a targeted gene sequencing protocol to screen the coding and splicing areas of genes of interest within angiogenic and vascular pathways and other putative candidate genes in a sizeable cohort of preeclampsia cases and controls from Finland. Here, we explore the potential causal role of variation in candidate genes in preeclampsia.

Methods

Diagnostic Criteria and Patient Cohorts

The study design is outlined in Figure. At stage 1, we studied 500 nonobese (body mass index <30 kg/m^2) women with preeclamptic pregnancies and 190 nonpreeclamptic controls that were matched geographically, in age, and in body mass index from the FINNPEC cohort (Finnish Genetics of Preeclampsia Consortium), a case–control cohort recruited from the 5 Finnish University Hospitals.[12] Nulliparous or multiparous women with a singleton pregnancy were eligible for the study. Preeclampsia was defined as hypertension and proteinuria occurring after 20 week's gestation. Hypertension was defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg after 20 weeks of gestation. Proteinuria was defined as the urinary excretion of ≥0.3 g protein in a 24-hour specimen, or 0.3 g/L, or 2 ≥1+ readings on dipstick in a random urine determination with no evidence of urinary tract infection. All diagnoses were independently verified by a research nurse and a physician. Seven preeclamptics and 1 control were excluded because of a failure in genotyping, and further 9 cases were excluded because of non-Finnish ethnicity and ovum donation pregnancy. The characteristics of the study participants are presented in Table S1 in the online-only Data Supplement. All women provided a written informed consent, and the FINNPEC study protocol was approved by the coordinating Ethics Committee of the Hospital District of Helsinki and Uusimaa.

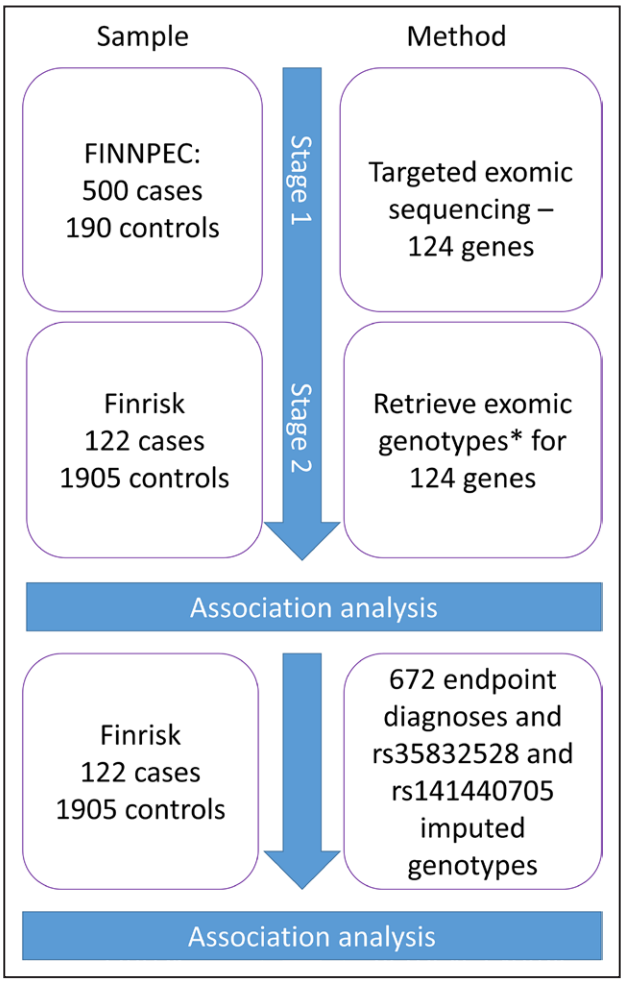

Figure. Methods and samples of study design. *Genotyping performed by Agilent 1.1 refseq (60 cases and 4 controls), Illumina coding v1 (162 cases and 10 controls), and Nimblegen SeqCap EZ VCRome (1682 cases and 109 controls) platforms. FINNPEC indicates Finnish Genetics of Preeclampsia Consortium.

At stage 2, we included in the analyses whole-exome sequencing from an additional 122 women with a history of preeclampsia and 1905 parous women with no such history from the national FINRISK study cohort (FINRISK license 8/2016).[13] For identifying preeclampsia and eclampsia cases in the FINRISK study cohort, we used following Finnish *International Classification of Disease* (*ICD*) codes in the comprehensive National Hospital Discharge Register covering years 1992 to 2007: *ICD-10* (in use since 1996): O14.0, O14.1, O14.9, O15.0, O15.1, O15.2, O15.9; *ICD-9* (in use from 1987 to 1996): 6424 to 6426, 6427A; and *ICD-8* (in use from 1968 to 1986): 637.03, 637.04, 637.09, 637.10, 637.99. Controls from FINRISK were all women who had given birth at least once and did not have any of these diagnoses recorded. In the final association analyses, we included genotypes of FINNPEC and FINRISK which combined totaled 609 cases and 2092 controls.

All polymorphic sites in the stage 1–targeted sequencing were queried from the population cohort exomes. All of the National FINRISK study methodology and ethical approvals are available online: https://www.thl.fi/en/web/thlfi-en/research-and-expertwork/population-studies/the-national-finrisk-study.

Preparation of Genomic DNA

Genomic DNA was extracted from whole blood using the NucleoSpin Blood XL DNA extraction kit (Macherey-Nagel GmbH & Co.) or the Chemagic Magnetic Separation Module I–machine (Chemagen) and subsequently stored at −20°C.

Targeted Sequencing and Capture Enrichment

Genes implicated in human biomarker studies were chosen as targeted candidate genes as were their signaling partners and proteases that alter their function and levels. Specific single nucleotide polymorphisms (SNPs) and any neighboring or associated gene that have been enriched in preeclamptic women based on meta-analysis, genome-wide association studies, or functional studies were included. This list of existing candidate genes was based on review of literature in January 2013, and it included genes involved in angiogenesis, such as *FLT1*, and pregnancy, such as pregnancy-zone protein, among other relevant functional pathways. The studied genes are listed in Table S2.

Libraries from genomic DNA were prepared in-house (Washington University School of Medicine).[14] Enzymes were purchased from Enzymatics (Beverly, MA). Briefly, the ends of sheared genomic DNA fragments were repaired by treatment with T4 DNA polymerase and T4 DNA polynucleotide kinase, which phosphorylates the 5′ hydroxyl group. An adenosine was then added to the 3′ position at each end of the DNA fragment with Taq Polymerase. Illumina adapters with an overhanging T were ligated onto the DNA fragment followed by bead-based size selection procedure to remove adapter dimers and fragments below the desired size. A unique index sequence was added by polymerase chain reaction by targeting the 2 ligated universal adapters on each fragment end.

Sequence capture by hybridization was performed according to the manufacturer's recommendations with modifications (Roche SeqCap Hybridization and Wash Kit No. 05634261001). We used longer blocking oligos containing an additional 7 bp inosine segment for promiscuous pairing with different index sequences. After hybridization, captured DNA was washed and eluted according to the manufacturer's instructions.

Sequencing and Analysis

Sequencing was performed on an Illumina HiSeq 2000 at the Washington University Genome Technology Access Center using 2×101 bp, 2×135 bp, and 2×150 bp reads. We aligned sequencing to GRCh37 using bwa aln (v0.6.1-r104) and genotyped the samples using the Genome Analysis ToolKit (v2.5.2-gf57256b) Unified Genotyper.[15–17]

Statistical Analyses

Quality control before meta-analysis included removal of singleton and monomorphic variants, removal of sites with >10% missing data in the targeted sequencing, or a significant departure from Hardy–Weinberg equilibrium in controls (P<0.001). The combined data set was analyzed using Fisher exact test that served as our primary association test. The analysis was divided into 2 classes: low frequency and rare variants (minor allele frequency [MAF] <10%) or common variants (MAF>10%). After quality control, 622 variants (443 variants with MAF<10% and 179 variants with MAF>10%) were advanced to the combined primary analysis. Of these, 201 were in putatively functional categories (missense, nonsense, or splice region variants) and 421 were in likely benign categories (synonymous, intronic, and intergenic).

The 2 SNPs with strongest associations were further compared with 672 predefined epidemiological diagnoses derived from national healthcare registries using imputed genotypes of 11 257 FINRISK women. Genotypes were imputed using a combined panel of Finnish whole-genome sequences and 1000 Genomes phase I reference with a high-imputation confidence (info metric >0.97).[18,19] Associations were calculated using logistic regression (SNPTEST v2.5.2, EM algorithm) and then further confirmed with Fisher exact test given the rarity of variants and several diagnoses. Because of the explorative nature of this part of the analysis, no multiple testing corrections to estimates were performed. Heart failure was identified using codes I50, 4289X, and 42700 in Finnish *ICD-10*, *ICD-9*, and *ICD-8*, respectively.

Data were analyzed using PlinkSeq, Plink,[20] and R. Kaviar,[21] and VEP Build 37 was used in additional annotations.[22] Loss of function (LoF) analyses were conducted for each gene with associating variants in silico by the LoF tool (https://github.com/ensembl-variation/VEP_plugins/blob/master/LoFtool.pm). The following annotations were calculated: LoF score <0.2 is probably damaging, LoF score 0.2 to 0.7 is possibly damaging, LoF score <0.7 is benign. In addition to appropriate statistical probability tests, odds ratios (OR) with 95% confidence intervals were calculated for all variants.

Results

Results of the association analyses are listed in Table. For these studies, genotypes from stage 1 (FINNPEC) were combined with a population cohort in stage 2 (FINRISK) for joint analysis. Forty-one variants demonstrated nominal association (P<0.05; 34 uncommon and 7 common). The tail of the P value distribution of benign variants was as expected (0 results with P<0.001; expected ≤1 and 2 results with P<0.01; expected ≤4.2), suggesting that the overall study design and quality control were successful. Among the 201 putatively functional variants, one had P<0.001 (α=0.05) compared with the 0 of 421 observed among benign variants. The lack of inflated P values points out that confounders, such as stratification, are not causing false positives.

Focusing on 146 putatively functional (missense or truncating) rare and low-frequency variants, we observed an excess (observed 13, expected 7.3) of nominally associated variants (P<0.05). The strongest associations observed were in *FLT1*, with 2 missense variants associated to preeclampsia: NM_002019.4:p.E982A (rs35832528; P=2.5E-4; OR=0.387, withstands correction for multiple testing for the 146 rare and low-frequency coding variants) and NM_001159920.1:p.R54S (rs141440705; P=0.0027; OR=0.44). These variants are enriched in the Finnish population with MAFs being 0.026 (1000 Genomes MAF=0.0014) and 0.017 (1000 Genomes MAF=0.0008), respectively (www.sisuproject.fi).

E982A and R54S were examined across registry-derived disease end points. Both of the assessed SNPs protected from heart failure (Fisher exact P=0.007 for both, E982A: OR=0.368; 95% confidence interval=0.164–0.830; cases/controls=301/10 956; minor allele [G] count in cases/controls=6/593; R54S: OR=0.340; 95% confidence interval=0.140–0.826; cases/controls=297/10 960; minor allele [G] count in cases/controls=5/543).

Discussion

We discovered low-frequency protective genetic variants in *FLT1* that contributed to lower preeclampsia risk. We also found associating genetic variants in known candidate genes using a targeted sequencing approach. These *FLT1* variants may also be associated with lower risk of heart failure.

FLT1 codes for VEGFR1 (vascular endothelial growth factor receptor 1). It consists of 7 immunoglobulin-like domains in an extracellular ligand-binding region, a transmembrane segment, and a cytoplasmic region containing a tyrosine kinase domain.[23] VEGFR1 is essential for survival by negatively regulating the levels of endogenous vascular endothelial growth factor. Internalization and signaling of functional VEGFRs will enhance angiogenic growth of blood vessels.[24] Sustaining angiogenesis is necessary for circulation, and antiangiogenic treatment causes cardiovascular morbidity.[25] There are multiple isoforms of VEGFR1 of which the soluble forms have been implicated in preeclampsia. The soluble forms of VEGFR1 only contain the extracellular parts of the protein encoded by the first 13 of 30 exons. In preeclampsia, an excess of VEGFR1 of placental origin has been recorded.[26] Furthermore, after healthy endothelium is restored, elevated levels of soluble VEGFR1 are observed in women with a history of preeclampsia.[27]

Increased levels of sFLT1/sVEGFR1 have also been indicated in peripartum cardiomyopathy where high levels of sFLT1 correlate with the symptoms' severity.[28] Similarly, heart failure after myocardial infarction independent of pregnancy is reflected in extreme levels of sFLT1.[29] Rhee et al report extreme sFLT1 levels in at least the 95th percentile in a cases of heart failure during pregnancy.[30] Although further research is required to assess the effect of underlying risk factor profile in preeclamptic women's increased risk of heart failure in later life, it was recently shown in a meta-analysis of results from 7 studies that 3.6-fold increase in risk of heart failure is associated with preeclampsia, particularly during the time period of 1 to 10 years after preeclampsia.[31] Preeclampsia increases the risk of peripartum cardiomyopathy, and it has been suggested that sFLT1 may be toxic to the heart. Also the driver of the cardiac dysfunction in susceptible preeclamptics is likely mediated by antiangiogenic factors.[32] Our results indicate that *FLT1* variants that protect from preeclampsia may also protect from heart failure, thereby adding to the growing body of evidence that imbalance of angiogenic factors may be the link between preeclampsia

Table. The Observed Variants With Significant Associations to Preeclampsia

RSID Number	Gene Name	*P* Value*	Odds Ratio (95% Confidence Interval)	MAF in Total Sample	Count With Minor Allele $n_{Cases}/n_{Controls}$ FINNPEC Minor Allele Count in Parentheses (Cases/Controls)	HWE	Consequence (Distance From Exon, Base Pairs)	LoF Tool
rs35832528	*FLT1*	2.49E-4	0.387 (0.205–0.678)	0.010	14/122 (7/6)	1	Missense variant, E982A	0.463
rs141440705	*FLT1*	0.003	0.442 (0.233–0.779)	0.010	14/106 (8/5)	1	Missense variant, R54S	0.463
rs61758484	*CORIN*	0.003	2.658 (1.320–5.261)	0.013	17/22 (16/1)	1	Noncoding transcript exon variant, E10K	0.201
rs34106916	*ANGPTL1*	0.006	3.186 (1.325–7.597)	0.008	12/13 (8/3)	1	Synonymous variant, Q103Q	0.658
rs61759670	*CORIN*	0.010	2.082 (1.147–3.693)	0.016	21/35 (18/4)	1	Missense variant, Y907T	0.201
rs80338240	*JAG1*	0.010	0.256 (0.051–0.806)	0.004	3/40 (2/4)	1	Intron variant (–11)	0.006
rs147998709	*GPR98*	0.011	13.757 (1.360–675.490)	0.003	4/1 (3/0)	0.004	Intron variant (+24)	0.977
rs2290843	*ADAM12*	0.011	0.726 (0.561–0.932)	0.071	83/383 (63/33)	1	Synonymous variant, T326T	0.320
rs61760500	*CORIN*	0.011	2.550 (1.179–5.382)	0.010	14/19 (11/3)	1.001	Intron variant (+13)	0.201
rs147942437	*ADAM28*	0.012	5.181 (1.226–24.998)	0.004	6/4 (5/0)	1.002	Missense variant, L449P	0.994
rs13406336	*ACVR1*	0.013	2.423 (1.129–5.062)	0.011	14/20 (13/2)	1.003	Missense variant, A15G	0.138
rs142436579	*ADAM28*	0.014	0.366 (0.129–0.852)	0.005	6/56 (4/3)	1.004	Missense variant, R219S	0.994
rs140437272	*INHBE*	0.014	0.477 (0.236–0.881)	0.013	12/85 (11/6)	1.005	Missense variant, P27L	0.786
rs201756397	*FLT4*	0.014	Inf (1.283–Inf)	0.001	3/0 (2/0)	1.006	Synonymous variant, E926E	0.023
rs80069610	*GPR98*	0.016	2.499 (1.122–5.411)	0.010	13/18 (11/2)	1.007	Synonymous variant, V1101V	0.977
rs139608664	*INHA*	0.018	5.716 (1.110–36.859)	0.003	5/3 (4/0)	1.008	Synonymous variant, S225R	0.046
rs4556933	*ACVR1C*	0.019	0.847 (0.735–0.976)	0.323	1157/381 (314/120)	0.113	Synonymous variant, F38F	0.076
rs41302834	*GPR98*	0.022	3.451 (1.031–11.557)	0.007	7/7 (7/2)	1.009	Missense variant, D1944N	0.977
rs3736061	*FLT4*	0.023	0.803 (0.665–0.973)	0.135	485/175 (143/39)	0.868	Synonymous variant, L252L	0.023
rs3741849	*PZP*	0.039	1.309 (1.004–1.696)	0.068	88/235 (71/21)	1	Synonymous variant, K563K, splice region variant	0.988
rs34307240	*LCT*	0.026	2.201 (1.038–4.515)	0.010	14/22 (12/2)	1.011	Missense variant, D106E	0.571
rs36032184	*INHA*	0.027	1.906 (1.058–3.346)	0.017	21/38 (20/3)	1.012	Synonymous variant, G109G	0.046
rs2228048	*TGFBR2*	0.029	0.672 (0.459–0.963)	0.030	38/191 (27/13)	0.450	Synonymous variant, N354N	0.060
rs138819536	*INHBA-AS1*	0.032	4.311 (0.926–21.758)	0.005	5/4 (4/1)	1	Missense variant, R229Q	0.043
rs1466360	*ADAM12*	0.033	1.152 (1.010–1.315)	0.414	1852/504 (404/152)	0.691	Intron variant (+30)	0.320
rs56133834	*TEK*	0.034	3.450 (0.921–12.934)	0.004	6/6 (6/0)	1	Synonymous variant, E986E	0.046
rs140593977	*TREX1*	0.034	3.448 (0.920–12.928)	0.004	6/6 (6/0)	1	Downstream gene variant (–44)	0.824
rs1466361	*ADAM12*	0.034	1.152 (1.010–1.316)	0.414	1810/501 (404/152)	0.691	Intron variant (–46)	0.320
rs148671842	*EHD3*	0.035	2.372 (0.992–5.459)	0.007	11/16 (8/2)	1	Synonymous variant, E188E	0.139

(*Continued*)

Table. Continued

RSID Number	Gene Name	*P* Value*	Odds Ratio (95% Confidence Interval)	MAF in Total Sample	Count With Minor Allele $n_{Cases}/n_{Controls}$ FINNPEC Minor Allele Count in Parentheses (Cases/Controls)	HWE	Consequence (Distance From Exon, Base Pairs)	LoF Tool
rs115734907	*KDR*	0.035	0.695 (0.483–0.979)	0.029	42/205 (28/11)	1	Intron variant (+12)	0.196
rs1554286	*IL10*	0.037	1.203 (1.009–1.438)	0.153	761/191 (145/60)	0.231	Intron variant (+18)	0.538
rs2453040	*NOTCH2*	0.037	0.818 (0.676–0.992)	0.141	482/172 (144/45)	0.423	Intron variant (–45)	0.016
rs116951780	*LCT*	0.038	10.333 (0.829–541.218)	0.001	3/1 (2/0)	1	Synonymous variant, NMD transcript variant, A921A	0.571
rs138894008	*TEK*	0.038	10.333 (0.829–541.218)	0.002	3/1 (3/0)	1	Missense variant, R479H	0.046
rs148588802	*FLT1*	0.038	10.328 (0.829–540.960)	0.002	3/1 (3/0	1	Intron variant (+40)	0.463
rs200071734	*FLT4*	0.040	10.114 (0.811–529.805)	0.002	3/1 (3/0)	1	Missense variant, V157M	0.023
rs150123876	*ANGPT4*	0.042	0.348 (0.090–0.968)	0.007	4/39 (3/7)	1	Missense variant, R25H	0.773
rs368518386	*FLT4*	0.046	5.581 (0.799–61.819)	0.003	4/2 (4/0)	1	Intron variant (–43)	0.023
rs3736062	*FLT4*	0.046	1.547 (0.988–2.374)	0.024	33/74 (26/6)	1	Synonymous variant, Y531Y	0.023
rs7830	*NOS3*	0.049	0.877 (0.769–1.002)	0.434	1635/525 (411/172)	0.346	Intron variant (+11)	0.817
rs61763183	*FLT1*	0.050	1.653 (0.967–2.754)	0.020	24/50 (20/7)	1	Downstream gene variant (+73)	0.463

Counts of minor alleles are given for the combined data; 615 cases and 2094 controls, and per FINNPEC data set (in parentheses); 487 of 500 women with preeclampsia and 187 of 190 control women passed quality control. FINNPEC indicates Finnish Genetics of Preeclampsia Consortium; HWE, Hardy–Weinberg Equilibrium; LoF, loss of function; MAF, minor allele frequency; and RSID, identifying number.

*Nonadjusted *P* values.

and cardiovascular morbidity in later life in susceptible individuals. Novel therapeutic options for these individuals may include blocking sFLT1 production or neutralizing antibodies against angiogenic proteins.[33,34]

From the observed associating variants in *FLT1*, rs141440705 causing R54S is more likely of functional importance. This is because it results in a polar change (pos→neutral) within the immunoglobulin-like domain 1, a functional part of the protein.[35] Rs141440705 is located in the last nucleotide of an exon. Therefore, the variant may affect splicing, as well as the coded amino acid sequence.

Epidemiological studies with many of the protective associations from severe cardiovascular diseases are strongest for R54S, supporting the suggestion of functional significance. The other variant resulting in the amino acid substitution E982A is not included in the sFLT1 or the VEGFR1 isoform 2, which is the dominant isoform in the placenta. Even so, because VEGFR1 isoform 1 (canonical sequence) is expressed in vascular endothelium, E982A might have an important role in pregnancy in mediating decidual blood flow and in remodeling the spiral arteries.

We also found support for the role of several other candidate genes in preeclampsia. *Corin* has been previously indicated in preeclampsia with population-specific variants.[36] Stepanian et al[36] report similar ORs of ≈2.5 at 2 intronic SNPs, rs2271036 and rs2271037 to our finding at rs61759670 and rs61760500. Rs13406336 and rs4556933 in activin A receptor type 1C have been previously listed as a candidate SNPs although no significant association to preeclampsia was established in a Norwegian population.[37] Interestingly, rs7830 features in numerous studies that pinpoint the association of nitric oxide synthase 3 to a spectrum of multifactorial diseases. Most relevantly, a haplotype including rs7830 was found to protect women from pregnancy hypertension and preeclampsia.[38] Rs1554286 in interleukin 10 has been shown to belong to an intronic haplotype, which strongly predisposes women of Bahraini Arab population to idiopathic recurrent miscarriages.[39] Our findings support the hypothesis that common immunologic pathogenesis may be shared between recurrent miscarriages and preeclampsia.[40,41]

Perspectives

To our knowledge, this study provides the first evidence that maternal *FLT1* sequence variants associate with lower preeclampsia susceptibility. Further research is required to pinpoint the mechanism of protection from preeclampsia and heart failure and the effect of *FLT1* variants on gene transcription. Genetic associations may open new avenues of drug development once the functional consequences of our findings are further deciphered. Genetic protection from preeclampsia because of *FLT1* variants may also protect these women from heart failure in later life.

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Disclosures

None.

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Novelty and Significance

What Is New?

- We have established a genetic association between preeclamptic mothers and the *FLT1* (Fms related tyrosine kinase 1) gene.
- The same variants may also protect Finnish women from heart failure in later life.

What Is Relevant?

- Preeclampsia is a common hypertensive disorder of the pregnancy.
- Identification of protective variants in *FLT1*, a known candidate gene, opens an opportunity for drug development to target the women most at risk of the disease.

Summary

Genetic variants within the maternal *FLT1* protect Finnish women from preeclampsia.

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Protective Low-Frequency Variants for Preeclampsia in the Fms Related Tyrosine Kinase 1 Gene in the Finnish Population

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PROTECTIVE LOW FREQUENCY VARIANTS FOR PREECLAMPSIA IN THE *FLT1* GENE IN THE FINNISH POPULATION

Online supplement

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Table S1. Clinical characteristics of targeted exome sequencing participants*.

Characteristic	PE women (n=487)†	Controls (n=187)	P-value‡
Age, years; median (25, 75 percentiles)	29 (26, 32)	29 (26, 32)	0.993
Body mass index before pregnancy; median (25, 27 percentiles)	22.6 (20.9, 24.8)	22.5 (20.6, 25)	0.450
Proteinuria, g/l; median (25, 75 percentiles)	3.31 (1.59, 6.04)	na	na
Blood pressure: systolic, mmHg; median (25, 75 percentiles)	164 (152, 177)	125 (118, 133)	<0.001
Blood pressure: diastolic, mmHg; median (25, 75 percentiles)	108 (103, 114)	82 (78, 88)	<0.001
Gestation weeks; median (25, 75 percentiles)	38 (35, 39)	40 (39, 41)	<0.001
Mode of birth (vaginal deliveries n; %)	279; 57.3	160; 85.6	<0.001
Baby sex (% female)	52.9	49.7	0.450
Baby weight; g median (25, 75 percentiles)	2820 (2210, 3270)	3540 (3260, 3850)	0.119
Baby weight; 5% percentile cut-off value; g, n	1132, 24	2905.4, 9	
Placenta weight, g; median (25, 75 percentiles)	500 (405, 600) n=464	590 (500, 670) n=181	0.139

Pre-pregnancy smoking %	21.2, n=467	19.7, n=177	0.582
First gravidity (n; %)	328; 67.4	144; 77	0.030
Primipara (n; %)	450; 92.4	187; 100	0.005
Region of origin: Helsinki region (n; %)	251; 51.5	100; 53.5	0.652
Region of origin: Eastern Finland (n; %)	67; 13.8	23; 12.3	0.618
Region of origin: Northern Finland (n; %)	83; 17	29; 15.5	0.632
Region of origin: Central Finland (n; %)	39; 8	22; 11.2	0.188
Region of origin: Southwestern Finland (n; %)	47; 9.7	14; 7.4	0.381

* 97.4% of 500 women with PE and 98.4% of 190 control women passed quality control and their data is reported here and in the association analyses.

† 15.6 % of the preeclamptic women gave birth <34 weeks of gestation and 72.7% had severe preeclampsia

‡ Chi^2

BMI = mass (kg) / $height^2$ (m); na, not available (no proteinuria in controls).

Table S2. The candidate genes and intronic or near-gene loci

Candidate genes					Candidate loci	
					Gene	SNP
ACE	AGTR1	ERAP2	JAG1	ROCK2	AGT	rs699
ACVR1	AGTR2	ESRRG	KDR	SOD1	AGT	rs4762
ACVR1B	ANGPT1	F13A1	KIAA1239	SOD2	AGTR1	rs5186
ACVR1C	ANGPT2	FLT1	KIAA1462	STOX1	APOE	rs429358
ACVR2A	ANGPT3	FLT4	LCT	STOX2	CTLA4	rs231775
ACVR2B	ANGPT4	FN1	LIPA	SWAP70	ESRRG	rs17686866
ACVRL1	ANTXR1	GPR98	LMCD1	TGFB1	IFLTD1	rs10743565
ADAM10	Cdkn1c	HEY1	LPL	TGFB2	IL10	rs1800896
ADAM12	COMMD7	HEY2	LRRFIP1	TGFB3	KIAA1239	rs1426409
ADAM15	COMT	IFLTD1	MAGI1	TGFBR1	LMCD1	rs9831647
ADAM17	CORIN	IL10	MME	TGFBR2	LPL	rs1800590
ADAM19	CTLA4	INHA	NODAL	TGFBRAP1	LPL	rs268
ADAM28	DEF6	INHBA	NOS3	TIE1	near IL-10	rs1800871
ADAM8	DGKE	INHBB	NOTCH2	TIE2	near IL-10	rs1800896
ADAM9	EDN1	INHBC	PDGFD	TNF	NOS3	rs61722009
ADAMTS7	EDN2	INHBE	PDXDC1	VEGFA	NOS3	rs2070744
ADM	EHD3	IP6K1	PGF	VEGFB	NOS3	rs1799983
ADM2	EHD4	ITGA2	PSG11	VEGFC	VEGFA	rs3025039
AGT	ENG	ITGB1	ROCK1			