BJOG | An International Journal of Obstetrics and Gynaecology

Identification of complement factor H variants that predispose to pre-eclampsia: A genetic and functional study

A. Inkeri Lokki[1,2] | Zhen Ren[3] | Michael Triebwasser[4] | Emma Daly[5] | FINNPEC[6] |
Markus Perola[7] | Kirsi Auro[7] | Richard Burwick[8,9] | Jane E. Salmon[10] | Mark Daly[11,12] |
Hannele Laivuori[6,12,13,14] | John P. Atkinson[15] | Anuja Java[16] | Seppo Meri[1,17]

[1]Immunobiology Research Program, Bacteriology and Immunology, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

[2]Heart and Lung Centre, Helsinki University Hospital, Helsinki, Finland

[3]Division of Clinical Immunology and Allergy, Department of Medicine, Washington University School of Medicine, St. Louis, Missouri, USA

[4]Division of Pediatric Hematology and Oncology, Department of Pediatrics, University of Michigan, Ann Arbor, Michigan, USA

[5]Hospital and Harvard Medical School, Boston, Massachusetts, USA

[6]Medical and Clinical Genetics, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

[7]Department of Health, National Institute for Health and Welfare, Helsinki, Finland

[8]Maternal Fetal Medicine, San Gabriel Valley Perinatal Medical Group, Pomona Valley Hospital Medical Center, Pomona, California, USA

[9]Department of Obstetrics and Gynecology, Cedars-Sinai Medical Center, Los Angeles, California, USA

[10]Hospital for Special Surgery, Weill Medical College of Cornell University, New York, New York, USA

[11]Analytic and Translational Genetics Unit, Massachusetts General Hospital, Boston, Massachusetts, USA

[12]Institute for Molecular Medicine Finland, Helsinki Institute of Life Science, University of Helsinki, Helsinki, Finland

[13]Department of Obstetrics and Gynaecology, Tampere University Hospital, Tampere University, Tampere, Finland

[14]Centre for Child, Adolescent, and Maternal Health Research, Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland

[15]Division of Rheumatology, Department of Medicine, Washington University School of Medicine, St. Louis, Missouri, USA

[16]Division of Nephrology, Department of Medicine, Washington University School of Medicine, St. Louis, Missouri, USA

[17]HUSLAB Diagnostic Centre, Helsinki University Hospital, Helsinki, Finland

Correspondence
A. I. Lokki and S. Meri, Department of
Bacteriology and Immunology, University
of Helsinki, Haartmaninkatu 3, FIN-00014
Helsinki, Finland.
Email: inkeri.lokki@helsinki.fi and seppo.
meri@helsinki.fi

A. Java, Division of Nephrology, Department
of Medicine, Washington University School of
Medicine, St. Louis, MO 63110, USA.
Email: ajava@wustl.edu

Funding information
Academy of Finland, Grant/Award Number:
121196 and 278941; Alfred Kordelinin
Säätiö; Jane ja Aatos Erkon Säätiö; National
Institutes of Health, Grant/Award Number:
F30 HL103072, U54 HL112303, R01GM099111
and R35GM136352; Sigrid Juséliuksen Säätiö,
Grant/Award Number: 4708373; Finnish

Abstract

Objective: The objective of the study was to investigate the role of genetic variants in complement proteins in pre-eclampsia.

Design: In a case–control study involving 609 cases and 2092 controls, five rare variants in complement factor H (*CFH*) were identified in women with severe and complicated pre-eclampsia. No variants were identified in controls.

Setting: Pre-eclampsia is a leading cause of maternal and fetal morbidity and mortality. Immune maladaptation, in particular, complement activation that disrupts maternal-fetal tolerance leading to placental dysfunction and endothelial injury, has been proposed as a pathogenetic mechanism, but this remains unproven.

Population: We genotyped 609 pre-eclampsia cases and 2092 controls from FINNPEC and the national FINRISK cohorts.

Methods: Complement-based functional and structural assays were conducted in vitro to define the significance of these five missense variants and each compared with wild type.

A. I. Lokki and Z. Ren contributed equally to this study.

A. Java and S. Meri contributed equally to this study.

See Acknowledgements for the members of FINNPEC.

Main outcome measures: Secretion, expression and ability to regulate complement activation were assessed for factor H proteins harbouring the mutations.

Results: We identified five heterozygous rare variants in complement factor H (L3V, R127H, R166Q, C1077S and N1176K) in seven women with severe pre-eclampsia. These variants were not identified in controls. Variants C1077S and N1176K were novel. Antigenic, functional and structural analyses established that four (R127H, R166Q, C1077S and N1176K) were deleterious. Variants R127H and C1077S were synthesised, but not secreted. Variants R166Q and N1176K were secreted normally but showed reduced binding to C3b and consequently defective complement regulatory activity. No defect was identified for L3V.

Conclusions: These results suggest that complement dysregulation due to mutations in complement factor H is among the pathophysiological mechanisms underlying severe pre-eclampsia.

KEYWORDS
complement system, factor H, genetics, pre-eclampsia: basic science

1 | INTRODUCTION

Pre-eclampsia (PE) occurs in approximately 3% of pregnancies,[1] accounting annually for over 50 000 maternal and 900 000 perinatal deaths worldwide.[2,3] It is characterised by new-onset hypertension associated with proteinuria and/or end-organ dysfunction after 20 weeks of gestation. Disease manifestations include HELLP (Haemolysis, Elevated Liver enzymes, Low Platelets), kidney failure, seizures (eclampsia), stroke and cardiovascular complications. Despite it being a common pregnancy complication, the molecular basis for PE is incompletely understood and there is no treatment other than delivery.

As pregnancy represents an allogeneic-type mismatch between the fetus and the maternal immune system, multiple mechanisms are needed to maintain tolerance. In particular, the process of self-recognition must be carefully regulated during placentation to allow for fetal extravillous trophoblast invasion through the maternal decidua and myometrium to enable endothelial remodelling and vasodilation of the spiral arteries.[4] The involvement of complement in adaptive immune response as well as the interaction between complement and natural killer (NK) cells are potential ways in which homeostatic balance and control over invasive placental trophoblast cells could be achieved.[5] During pregnancy, complement assists in the clearance of placental fragments that enter the maternal circulation as a result of syncytiotrophoblast turn-over.[6] Integrin-type complement receptors 3 (CR3; CD11b/18) and 4 (CR4; CD11c/18) play important roles in the phagocytosis of complement iC3b-opsonised particles.[7] One prevailing hypothesis is that improper clearance of such components, driven by an inadequately regulated complement cascade, may lead to deposition of debris in tissues and vascular walls, leading to an overly exuberant inflammatory response.[8] Such unwarranted complement activation could provoke a maladaptive maternal immune attack against fetoplacental structures, leading to microthrombi and endothelial damage in PE, characteristic of a thrombotic microangiopathy (TMA).[9] Complement activation at the maternal–fetal border may thereby result in disordered maternal-fetal tolerance. Lack of tolerance may be a basis for inadequate spiral artery remodelling, a key contributor to the pathogenesis of early-onset PE (diagnosis before 34 weeks of gestation).[10]

An overly exuberant inflammatory response driven by a dysregulated complement cascade has been implicated in a number of other diseases such as atypical haemolytic uremic syndrome (aHUS), C3 glomerulopathy (C3G), paroxysmal nocturnal haemoglobinuria (PNH) and age-related macular degeneration (AMD).[11] The process in PE appears most analogous to that seen in aHUS, a complement-mediated TMA, characterised by endothelial injury leading to kidney failure, stroke and cardiovascular complications. Atypical HUS occurs due to loss-of-function mutations in complement regulators factor H, factor I or membrane cofactor protein (CD46) or gain-of-function mutations in complement proteins C3 or factor B.[12,13] Understanding the genetic basis has led to the development of anticomplement therapies that have revolutionised the management of PNH and aHUS. In susceptible women, pregnancy is a known trigger for aHUS.[14] Pre-eclampsia may also have a genetic basis, considering the strong epidemiological evidence that PE is partially inherited.[15–17]

Because of the similarity between PE and aHUS and the potential of complement overactivation to cause PE in a subset of high-risk patients, we investigated the role of genetic variants in one of the major complement regulators, factor H (FH) in a cohort of Finnish patients with PE.[9] An advantage of examining the Finnish founder population to study variants in complex diseases is that it is a more homogeneous population and thus disease-causing variants may be population-specific and occur at a higher frequency than in other, related European populations.[18]

2 | METHODS

2.1 | Subjects and sequencing protocol

The Finnish Genetics of Pre-eclampsia Consortium (FINNPEC) study consisted of 1377 family trios (mother, father and infant) affected by PE or non-PE pregnancy, where biological samples and comprehensive clinical data were available to allow for in-depth analyses.[19]

In the FINNPEC cohort, PE was defined as hypertension and proteinuria occurring after 20 weeks of gestation. Hypertension was defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg. Proteinuria was defined as the urinary excretion of ≥0.3 g protein in a 24-h specimen, or 0.3 g/l, or two ≥1$^+$ readings on a dipstick in a random urine determination with no evidence of a urinary tract infection. The control group consisted of pregnant women who did not meet the criteria for PE. Early onset PE was defined as presentation of symptoms before 34 weeks of gestation and severe PE was diagnosed if at least one of the following criteria were fulfilled: blood pressure ≥160 mmHg systolic and/or ≥110 mmHg diastolic, proteinuria ≥5 g/day, other findings or symptoms indicating multi-organ and/or placental involvement, including headache and upper gastric pain. HELLP syndrome was diagnosed when at least two of the following criteria were met: lactate dehydrogenase ≥235 U/l, alanine aminotransferase ≥70 U/l, aspartate aminotransferase ≥70 U/L, platelets ≤100 E9/L. The subjects and methodology of this study have been described in detail previously (Table S1).[19,20] For the current study, nulliparous or multiparous women with a singleton pregnancy and no history of chronic hypertension, diabetes or renal disease were considered eligible. All patients were self-declared white women. A jury consisting of a midwife and an obstetrician independently confirmed the diagnosis of PE. For the diagnosis, hypertension and proteinuria occurring after 20 weeks of gestation were required. Hypertension was defined as a systolic blood pressure of ≥140 mm Hg and/or a diastolic blood pressure of ≥90 mm Hg after 20 weeks of gestation. Proteinuria was defined as the urinary excretion of ≥0.3 g protein in a 24-h specimen or >0.3 g/L of urine, or two positive dipstick readings in the absence of a urinary tract infection. In Stage 1, we enrolled 487 non-obese (body mass index <30 kg/m^2) women with PE pregnancies and 187 non-PE controls from the FINNPEC cohort who fulfilled the required diagnostic criteria and had DNA available for genetic sequencing.[19] In Stage 2, we enrolled 122 women with a history of PE (based on a clinician's diagnosis obtained through the comprehensive national Hospital Discharge Register), and 1905 parous controls with no such history from the national FINRISK study (FINRISK licence # 8/2016).[21] National FINRISK study description and ethical approvals are available online: https://thl.fi/documents/3287543/5808577/FINRISK_readmefirst_220920.pdf/aafab52b-a53b-d23c-73d7-15c5581b24fa?t=1600769319413. For the association studies, genotypes from Stage 1 individuals were combined with the population cohort in Stage 2, providing a total sample size of 609 PE cases (487 + 122) and 2092 non-PE controls (187 + 1905).

In a previously described custom-made targeted exomic sequencing protocol, we combined Illumina sequencing libraries for capture and sequencing with Nimblegen sequence capture.[20,22]

2.2 | Factor H quantification

ELISA was used to determine FH concentrations in third trimester patient sera. To quantify FH, Nunc Maxisorp plates were first coated with 100 microl portions of a monospecific mouse antibody against FH (196X, 5 microg/ml). After an overnight incubation at 4°C, the plates were washed with phosphate-buffered saline (PBS) containing 0.05% Tween 20 and blocked with 200 microl of PBS-Tween for 2 h. Duplicate serum samples in varying dilutions were added and incubated for 2 h at 37°C, whereafter the plates were washed with PBS. Polyclonal rabbit anti-human FH diluted at 1:2000 in PBS was added. After washing, IgG-HRP (horseradish peroxidase)-conjugated anti-rabbit IgG was added. After an incubation at 37°C for 1 h, the plates were washed with PBS and o-phenylenediamine dihydrochloride (OPD) substrate was added. The reaction was stopped with 120 microl of 0.5 M H_2SO_4 and a spectrophotometer was used to measure the optical density of samples at 492 nm wavelength. FH concentrations were determined from comparison to standard samples with known amounts.

2.3 | Functional studies

The functional consequences of rare and unique variants observed in *CFH* were characterised using 293T cells transfected under serum-free conditions.[23] Functional studies and surface plasmon resonance (SPR) interaction analyses with the Biacore X100 and Biacore 2000 instruments (GE Healthcare) were performed as previously described.[22,24] Variants in CCP19 and CCP20 were purified as described, with the exception that they were eluted from a HisTrap column (GE Healthcare) at 285 mM NaCl as compared with variants in CCP2 and 3, which were eluted at 95 mM.[24] Numbering of variants includes the 18-amino acid signal peptide. Structural analyses were done in RCSB PDB.[25] Figure 3A is based on FH domains 1–4 in complex with C3b (PBD:2WII),[26] Figure 3B is based on structure of FH domains 18–20 (PBD:3SW0),[27] and Figure 3C is based on structure of FH CCP19-20 in complex with C3d (PBD:2XQW).[28]

2.4 | Statistics

Sequence data were analysed in PLINK/Seq, Plink[29] and R programmes. Analyses of the significant associations were

performed using Fisher's exact test. p-Values <0.05 were considered significant. Kaviar[30] and VEP Build 37 were used in additional annotations.[31] In addition to an appropriate statistical probability test, odds ratios (OR) with 95% confidence intervals (95% CI) were calculated for all variants.

2.5 | Study approval

All subjects provided written informed consent for the study. The FINNPEC study protocol was approved by the coordinating Ethics Committee of the Hospital District of Helsinki and Uusimaa (permit number 149/E0/07). Patients and funders were not involved in study planning, data analysis or reporting of the results.

3 | RESULTS

Gene-based burden testing for rare and unique variants (minor allele frequency [MAF] <1%) in the FINNPEC cohort revealed that variation in *CFH* was associated with PE (non-adjusted P-value = 0.035, Bonferroni corrected P-value = 0.0003).[22] We identified seven women with severe PE who carried five heterozygous rare variants in *CFH* (L3V, R127H, R166Q, C1077S and N1176K) (Table 1) among 609 cases. According to the registry-based health data, none of these patients developed aHUS during <10-year follow-up.

Two of these variants were novel (C1077S and N1176K), having not been described in the literature before. None of these five variants was identified in the 2092 controls. Of the seven women carrying *CFH* variants, six were diagnosed with early-onset PE. Two mothers developed HELLP syndrome (Figure 1A). Three of the seven were delivered by caesarean section. The mean pre-pregnancy body mass index (BMI) was in the normal range at 23.5 kg/m^2. All seven

women delivered infants that were small for gestational age (SGA, birthweight <−2 SD).

As assessed by western blotting, the variants R127H and C1077S were not expressed (Figure 1B,C). Western blotting for the lysates for both variants demonstrated intracellular accumulation, thus establishing that the proteins were synthesised but secretion in these heterozygous patients was reduced by 50% (not shown). These results were consistent with the reduced serum FH antigenic level in the patient carrying R127H, which showed that the FH level was decreased by 57.5% compared with normal human serum, which was used as reference (patient levels: approximately 0.21 g/L; normal range = 0.15–0.45 g/L). R127H has been described previously in two brothers with membranoproliferative glomerulonephritis (MPGN)[32] and was shown to be associated with low antigenic levels. This variant has also been reported in a family with advanced AMD and was shown not to be secreted.[33,34] The substitution of guanine to adenine (which results in the change from an R to H at the 127th amino acid) is predicted to be deleterious to the protein structure according to in silico prediction software programmes.

Serum for the patient carrying the C1077S variant was not available; however, structural analysis for C1077S indicated that the likely reason for decreased production was a lack of formation of a disulphide bridge, with a consequent change in the folding of the CCP18 domain (Figure 3B).

The variant R166Q has been previously reported in an AMD patient.[35] R166Q was expressed in an equivalent amount to the wild type (WT) CCP1-4 but showed a ~10-fold reduced binding (64.4 versus 6.8 microM) to C3b (Figure 2A,B). The reduced binding to C3b leads to decreased cofactor activity, compared with the WT (Figure 2C). This decreased function is probably due to the loss of positive charge (owing to substitution of positively charged arginine by uncharged glutamine) in CCP3, which is critical to C3b binding (Figure 3A). Another

TABLE 1 Clinical and pathological characteristics of patients with rare missense mutations in *CFH*.

RSID	Factor H protein domain	Amino acid substitution	Clinical characteristics	Delivery (pregnancy weeks+days)	G/P	Age of mother at delivery (years)
rs139254423	SP	L3V	Severe early-onset PE, SGA	32^{+5}	7+/5+	43
rs139254423	SP	L3V	Severe early-onset PE, SGA	35^{+2}	5/3	25
rs139254423	SP	L3V	Severe early-onset PE, SGA, HELLP	29^{+2}	1/0	35
rs121913058	2	R127H	Severe early-onset PE	35	1/0	20
Novel variant 1	3	R166Q	Severe early-onset PE, SGA, GDM	30^{+5}	2/0	35
Novel variant 2	18	C1077S	Severe early-onset PE, SGA, HELLP	33^{+6}	1/0	27
Novel variant 3	20	N1176K	PE, SGA, FET	38^{+4}	3/0	43

Note: Early-onset PE, diagnosis <34 weeks of gestation.

Abbreviations: FET, frozen embryo transfer; G/P, gravidity/parity; GDM, gestational diabetes mellitus managed by nutritional therapy; HELLP, haemolysis, elevated liver enzyme and low platelet count; PE, pre-eclampsia; RSID, variant identifier; SGA, small for gestational age; SP, signal peptide.
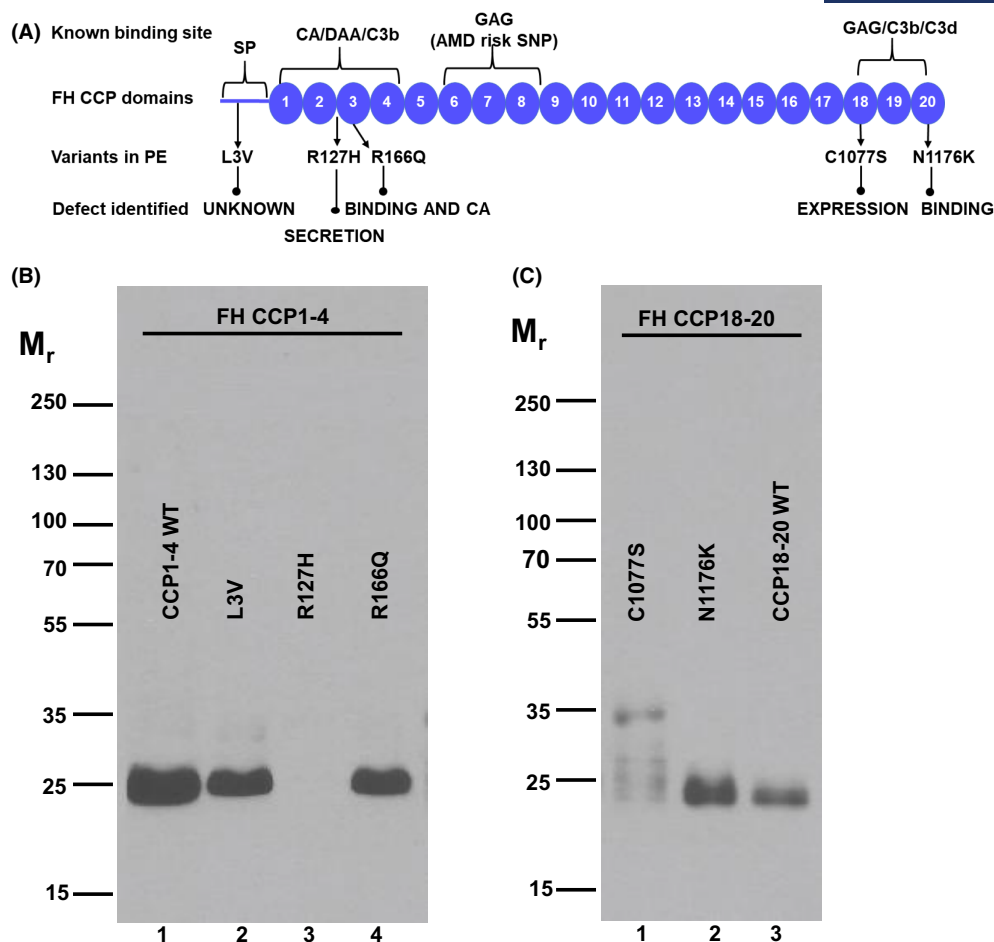
FIGURE 1 Western blots of supernatants from transfected WT FH and variants. (A) Schematic structure of FH. The protein consists of 20 CCP domains with binding sites for complement components. Variants associated with PE were discovered in the signal peptide, the link between CCP2 and CCP3, CCP3, CCP18 and CCP20. The defect associated with each variant is indicated. CCP, complement control protein (repeats); FH, factor H; WT, wild type. Residue numbering includes the 18 amino acids of the signal peptide (SP). CA, cofactor activity; DAA, decay-accelerating activity; C3b, C3b binding site; C3d, C3d binding site; GAG, glycosaminoglycan binding site. (B) Recombinant expression of L3V, R127H and R166Q in the WT CCP1-4 construct. R127H was not secreted. Secretion of L3V (6.7 ± 1.3 microg/ml) and R166Q (6.3 ± 1.0 microg/mL) were comparable with the WT CCP1-4 (5.3 ± 1.1 microg/ml) ($P = 0.8057$). (C) Recombinant expression of C1077S and N1176K in the WT CCP18-20 construct. C1077S was barely secreted. Secretion of N1176K (3.1 ± 0.29 microg/ml) was comparable to the WT CCP18-20 (3.4 ± 0.53 microg/ml) ($P = 0.6984$, SEM 0.534). This experiment was repeated three times with similar results.

notable difference between WT and R166Q was the pattern of cleavage of C3b to iC3b (Figure 2A,B). Normal cofactor activity produces a 41-kDa α-chain fragment and releases a 2-kDa fragment (the C3f fragment). The R166Q variant did not cleave the 2-kDa C3f fragment, thereby producing an incompletely cleaved α-chain fragment of 43 kDa (Figure 2C). Thus, both the efficiency and type of cleavage of the α-chain was affected by this mutation (Figure 2D,E). Structural analysis for R166Q demonstrated that this variant is located in a unique hypervariable loop of FH, which is significant for C3b binding.

The variant N1176K was expressed equivalently to WT FH CCP18-20 (Figure 1C) and western blotting of the lysates did not show intracellular accumulation (not shown). Structural analysis for N1176K established that N1176 on the surface of FH CCP20 is adjacent to the C3d fragment. The change to 1176K by a missense mutation might alter the C3d binding surface (Figure 3C). By competing with Y1225 further across from N1176, the positively charged N1176K could

form a new cation-π interaction with Y1177. Thereby, the replacement of this missense mutation N1176K might alter the FH C-terminal C3d binding surface. Indeed, the expressed recombinant protein demonstrated reduced binding to C3b and C3d (Figure 4), thus establishing that N1176K has decreased functional activity.

Variant L3V (rs139254423) was identified in three individuals and significantly associated with PE ($P = 0.01$, odds ratio 24.1150, 95% CI 1.25–467.20; MAF in cases = 0.25). However, the expression of variant L3V was comparable to WT (Figure 1A) and no functional defect was observed (not shown). These results were as consistent with normal serum FH antigenic level in two of three patients carrying this variant (serum was not available from the third patient). Soluble FLT1 (sFlt1) and placental growth factor (PlGF) levels in third trimester patient sera were available for two of the cases with the L3V variant. Both patients had a significantly elevated sFlt1 levels with a high sFlt1/PlGF ratio of 447 and 520.
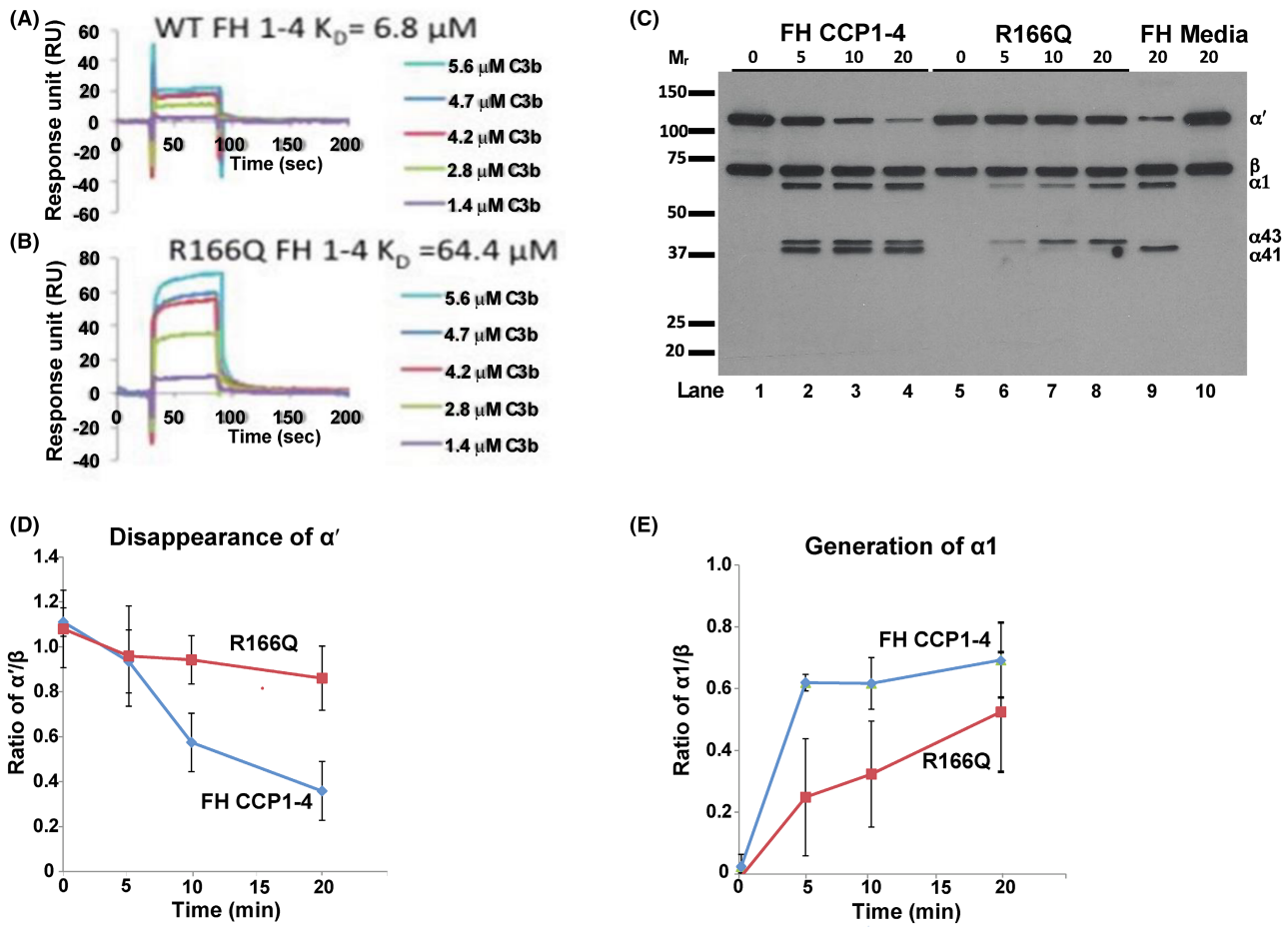
FIGURE 2 Functional evaluation of the WT FH CCP1-4 and the variant R166Q. (A,B) Affinity of R166Q for C3b by surface plasmon resonance (SPR). R166Q had decreased affinity for human purified C3b in comparison with the WT CCP1-4. (C–E) The fluid-phase C3b cofactor activity of the variant R166Q with purified human Factor I (FI) was assessed by cleavage of purified C3b to iC3b and was compared with the WT FH CCP1-4. The percentage of the α′ chain remaining and the generation of the α1 fragment indicate cleavage of C3b to iC3b. The kinetic analyses of cofactor activity were conducted at 0, 5, 10 and 20 min. Lane 9 represents a positive control employing purified human FH and Lane 10 represents a negative control employing concentrated cell media in the presence of both purified human FI and C3b. The cleavage rate was measured by densitometric analysis of the remaining α′ chain and by the generation of α1 relative to the β chain. This assay was repeated three times. CCP, complement control protein (repeats); FH, factor H; WT, wild type. Residue numbering includes the 18 amino acids of the signal peptide (SP).

4 | DISCUSSION

Pre-eclampsia is a heterogeneous disease with multiple genetic and environmental risk factors. Although complement activation has been implicated in PE,[33,36,37] the aetiology of an overactive system has not been clearly delineated.

4.1 | Main findings

Gene-burden testing approach allows for association analysis between cases and controls by grouping rare protein-altering variants within a single gene. Using this method, we found factor H variants to be linked to severe pre-eclampsia.[22] Our results identify maternal genetic variants in complement FH, the chief regulator of the alternative pathway of complement, in 1.4% of patients with PE. Four of these variants resulted in a dysfunctional FH, which in turn led to a dysregulated complement system.[38] This is in line with recent findings of decreased

serum levels of FH in PE in comparison with healthy pregnancy and 1.2% prevalence of *CFH* variants in PE or HELLP syndrome.[39,40] We describe four missense variants within *CFH* gene that cause either a quantitative defect (decreased secretion as in R127H and C1077S) or a qualitative defect (normal secretion but defective function, as in R166Q and N1176K). Of note, the variant R127H has also been described in kidney and eye diseases (aHUS and AMD, respectively) known to be associated with complement overactivation.[32,33] The variant R166Q, reported once in AMD, leads to an aberrant cofactor activity which is expected to affect both soluble C3b and C3b bound to a surface.[22,35] The variant N1176K has not been described before and demonstrates reduced binding for both C3b and C3d, which likely affects the cleavage of C3b bound to self surfaces coated with sialic acids or polyanionic glycosaminoglycans. A rare variant within the signal peptide of FH (L3V) was also identified in three patients with severe PE. L3V alters the signal peptide sequence before the first 20 short consensus repeats (SCRs) that make up the FH
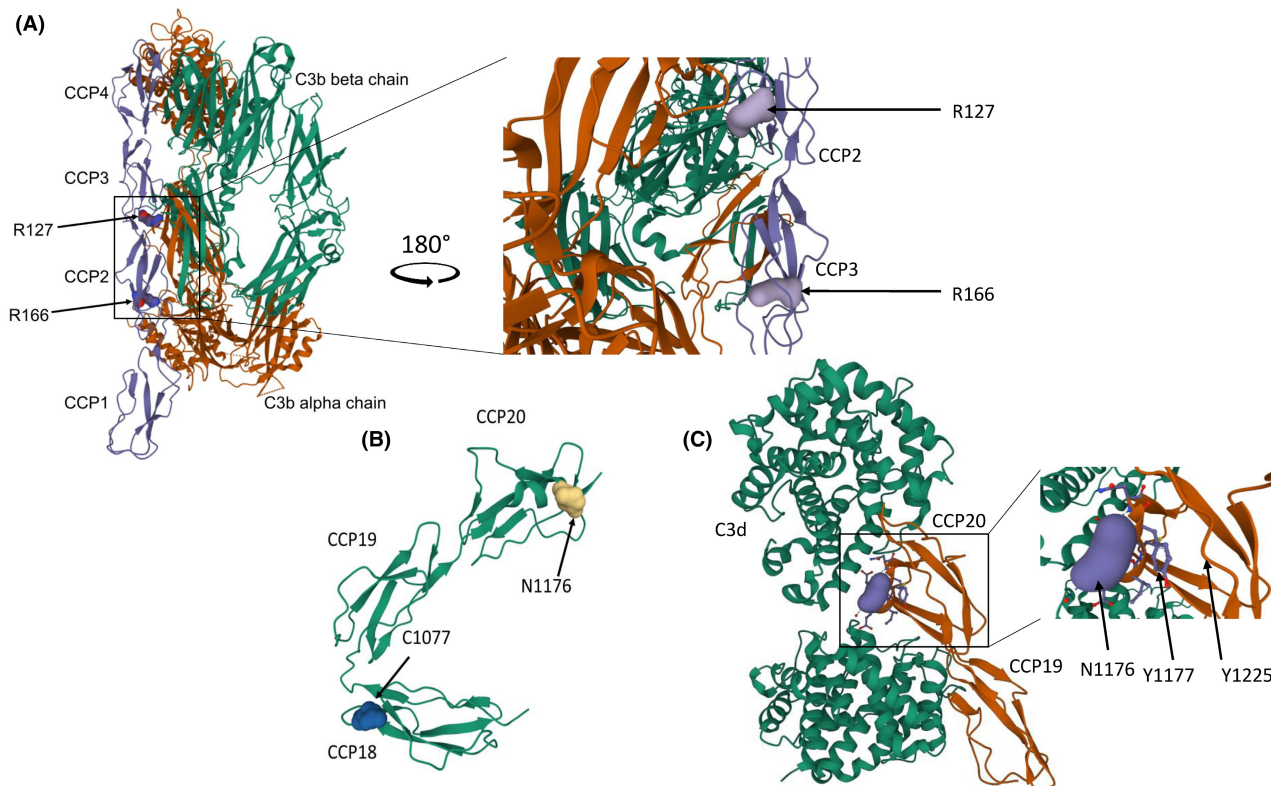
FIGURE 3 Structural analysis of FH variants. (A) Structural analysis of R127H and R166Q. Complement C3b (brown and green) in complex FH CCP1-4 (purple) demonstrating where variants R127H and R166Q are located. R166 is in the unique hypervariable loop of domain 3 of FH, which has been shown to interact with C3b. (B) Factor H CCP18-20 with variants C1077S and N1176K highlighted. (C) Factor H CCP19-20 (orange) in complex with C3d (green) shown in cartoon. N1176 (purple) is on the surface of FH CCP20 and is adjacent to the C3d fragment. CCP19-20 has a contiguous binding surface for C3d including Y1177. Y1225 is spatially on the opposite side of N1176 and forms a cation-p interaction with Y1177. CCP, complement control protein (repeats); FH, factor H; WT, wild type. Residue numbering includes the 18 amino acids of the signal peptide (SP).

molecule. However, the 3V protein appeared to be of the correct size with normal functional activity and the level of FH in two of our three patients with an available serum sample were normal, therefore the aetiology of severe PE in patients carrying this variant remains unclear.[4] L3V is a rare variant classified as a variant of unknown significance with a minor allele frequency (MAF) of 0.024% in the genome aggregation database (gnomAD). There are six submissions listed in the ClinVar database, four of which were related to complement dysregulatory disorders: two in AMD, one in aHUS and one in membranoproliferative glomerulonephritis (MPGN). Our study, however, demonstrated that the L3V variant has a normal secretory profile and no apparent functional defects. Similarly, rare variant S58A in CFH with an MAF of 0.025% in gnomAD has been reported in a pregnant woman with SLE who developed PE. Despite extensive investigations, no functional defect was found.[22,41] These data strongly point to rare variants in FH predisposing pregnant women to an increased risk of developing PE. However, the underlying mechanisms remain unclear and warrant further investigation.[22,41]

Notably, among the seven patients with *CFH* rare variants, 28% had HELLP syndrome (a rare life-threatening complication of PE) compared with 7.4% in patients with PE in the FINNPEC cohort.[42] Pre-existing kidney disease or autoimmune conditions were not reported for any of the patients and proteinuria levels were typical for severe PE. Therefore, patients with deleterious *CFH* variants represent a TMA-like severe PE phenotype, rather than an underlying kidney disease.

Our results establish that regulation of the maternal complement system by FH is essential for a healthy pregnancy, and mutations affecting this process predispose to PE. These patients likely represent a high-risk group who present with severe PE and HELLP syndrome, validating a potential sub-phenotype of PE with TMA features.[9] These data also lay the groundwork for future studies to assess the association of PE with defects in complement proteins in other cohorts and provide further support for treatment of selected severe PE/HELLP cases with complement-specific therapies such as eculizumab. Our results support a link to the FH signal peptide variants, but the functional mechanisms have yet to be revealed. Furthermore, replication in a second population is needed, preferably including non-European patients. The role of fetal complement genes in the predisposition to PE also remains to be explored.

AUTHOR CONTRIBUTIONS

AIL performed the genetic studies with MT, ED and MD, and wrote the first draft of the paper. ZR and MT performed the functional studies for the discovered variants.
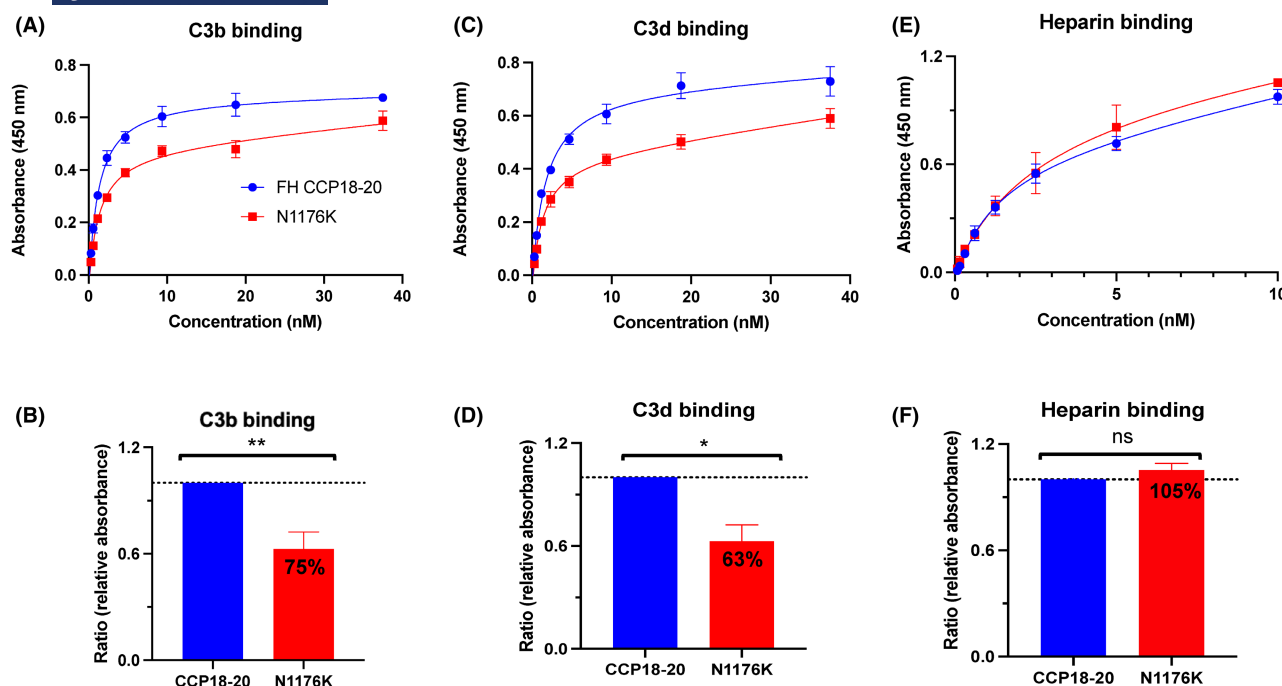
FIGURE 4 ELISA of FH CCP 18–20 and variant N1176K binding to C3b, C3d and heparin. (A,C,E) Interaction between serial dilutions of purified FH CCP 18–20 and variant N1176K with C3b (37.5 nM), C3d (37.5 nM) and heparin (10 nM) deposited on microtitre plates. Mean ± SEM of three independent experiments is shown. (B,D,F) Each bar represents the mean ± SEM of relative absorbance detected in WT CCP 18–20 and variant N1176K at a concentration of C3b (4.69 nM), C3d (4.69 nM) and heparin (5 nM), respectively. The P-values for the percentage difference of N1176K compared with WT were 0.001 for C3b and 0.022 for C3d binding. For heparin binding, the P-value for the difference of N1176K compared with WT was 0.31. The horizontal dash line represents 1.0. SEM, standard error of mean; ns, not significant; $*P < 0.05$; $**P < 0.01$. CCP, complement control protein (repeats); FH, factor H; WT, wild type. Residue numbering includes the 18 amino acids of the signal peptide (SP).

CONFLICT OF INTEREST STATEMENT
AJ serves on the scientific advisory boards of Alexion, AstraZeneca Rare Disease and Novartis International AG,

and serves as a consultant for Chinook Therapeutics. She is also a Principal Investigator for Apellis Pharmaceuticals. JES is a Consultant or is on Advisory Boards of UCB, Inc., SciRhom GmbH and Realta Life Sciences. RB is the recipient of research grants and/or speakers' fees from Alexion, AstraZeneca Rare Disease and is on a Clinical Advisory Board of Comanche Biopharma, UCB Biosciences.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS APPROVAL

Written informed consent was provided by all subjects. The FINNPEC study protocol was approved by the coordinating Ethics Committee of the Hospital District of Helsinki and Uusimaa (permit number 149/E0/07). The National FINRISK cohort was accessed by FINRISK licence # 8/2016. National FINRISK Study description and ethical approvals are available online: https://thl.fi/documents/3287543/58085 77/FINRISK_readmefirst_220920.pdf/aafab52b-a53b-d23c-73d7-15c5581b24fa?t=1600769319413.

ORCID

A. Inkeri Lokki https://orcid.org/0000-0003-4397-8415

REFERENCES

1. Lisonkova S, Joseph KS. Incidence of pre-eclampsia: risk factors and outcomes associated with early- versus late-onset disease. Am J Obstet Gynecol. 2013;209(6):544.e1–12.
2. van Lerberghe W, Manuel A, Matthews Z, Cathy W. The World health report 2005 – make every mother and child count. Geneva: World Health Organization; 2005 [cited 2019 Apr 20]. Available from: https://eprints.soton.ac.uk/40785/
3. Duley L. The global impact of pre-eclampsia and eclampsia. Semin Perinatol. 2009;33(3):130–7.
4. Guzin K, Tomruk S, Tuncay YA, Naki M, Sezginsoy S, Zemheri E, et al. The relation of increased uterine artery blood flow resistance and impaired trophoblast invasion in pre-eclamptic pregnancies. Arch Gynecol Obstet. 2005;272(4):283–8.
5. Girardi G, Lingo JJ, Fleming SD, Regal JF. Essential role of complement in pregnancy: from implantation to parturition and beyond. Front Immunol. 2020;31(11):1681.
6. Teirilä L, Heikkinen-Eloranta J, Kotimaa J, Meri S, Lokki AI. Regulation of the complement system and immunological tolerance in pregnancy. Semin Immunol. 2019;45:101337.
7. Lokki A, Teirilä L, Triebwasser M, Daly E, Bhattacharjee A, Uotila L, et al. Dysfunction of complement receptors CR3 (CD11b/18) and CR4 (CD11c/18) in preeclampsia: a genetic and functional study. BJOG. 2021;128(8):1282–91.
8. Regal JF, Burwick RM, Fleming SD. The complement system and pre-eclampsia. Curr Hypertens Rep. 2017;19(11):87.
9. Lokki AI, Heikkinen-Eloranta J. Pregnancy induced TMA in severe preeclampsia results from complement-mediated thromboinflammation. Hum Immunol. 2021;82(5):371–8.
10. Khodzhaeva ZS, Kogan YA, Shmakov RG, Klimenchenko NI, Akatyeva AS, Vavina OV, et al. Clinical and pathogenetic features of early- and late-onset pre-eclampsia. J Matern Fetal Neonatal Med. 2016;29(18):2980–6.
11. Liszewski MK, Java A, Schramm EC, Atkinson JP. Complement dysregulation and disease: insights from contemporary genetics. Annu Rev Pathol. 2017;12:25–52.
12. Bu F, Maga T, Meyer NC, Wang K, Thomas CP, Nester CM, et al. Comprehensive genetic analysis of complement and coagulation genes in atypical hemolytic uremic syndrome. J Am Soc Nephrol. 2014;25(1):55–64.
13. Java A, Atkinson J, Salmon J. Defective complement inhibitory function predisposes to renal disease. Annu Rev Med. 2013;64:307–24.
14. Fakhouri F, Scully M, Ardissino G, Al-Dakkak I, Miller B, Rondeau E. Pregnancy-triggered atypical hemolytic uremic syndrome (aHUS): a Global aHUS Registry analysis. J Nephrol. 2021;34(5):1581–90.
15. Harrison GA, Humphrey KE, Jones N, Badenhop R, Guo G, Elakis G, et al. A genomewide linkage study of preeclampsia/eclampsia reveals evidence for a candidate region on 4q. Am J Hum Genet. 1997;60(5):1158–67.
16. Arngrimsson R, Siguroardottir S, Frigge ML, Bjarnadottir RI, Jonsson T, Stefansson H, et al. A genome-wide scan reveals a maternal susceptibility locus for pre-eclampsia on chromosome 2p13. Hum Mol Genet. 1999;8(9):1799–805.
17. Salonen Ros H, Lichtenstein P, Lipworth L, Cnattingius S. Genetic effects on the liability of developing pre-eclampsia and gestational hypertension. Am J Med Genet. 2000;91(4):256–60.
18. Lim ET, Würtz P, Havulinna AS, Palta P, Tukiainen T, Rehnström K, et al. Distribution and medical impact of loss-of-function variants in the Finnish founder population. PLoS Genet. 2014;10(7):e1004494.
19. Jääskeläinen T, Heinonen S, Kajantie E, Kere J, Kivinen K, Pouta A, et al. Cohort profile: The Finnish Genetics of Pre-eclampsia Consortium (FINNPEC). BMJ Open. 2016;6(11):1–8.
20. Lokki AI, Daly E, Triebwasser M, Kurki MI, Roberson EDO, Häppölä P, et al. Protective low-frequency variants for preeclampsia in the Fms related tyrosine kinase 1 gene in the Finnish population. Hypertension. 2017;70(2):365–71.
21. Borodulin K, Vartiainen E, Peltonen M, Jousilahti P, Juolevi A, Laatikainen T, et al. Forty-year trends in cardiovascular risk factors in Finland. Eur J Public Health. 2015;25(3):539–46.
22. Triebwasser M. Excessive complement activation due to genetic haploinsufficiency of regulators in multiple human diseases. Washington University in St.Louis, Arts & Sciences Electronic Theses and Dissertations; 2015 [cited 2022 Jan 12]. Available from: http://openscholarship.wustl.edu/art_sci_etds/427
23. Ren Z, Perkins SJ, Love-Gregory L, Atkinson JP, Java A. Clinicopathologic implications of complement genetic variants in kidney transplantation. Front Med. 2021;8:775280.
24. Yu Y, Triebwasser MP, Wong EKS, Schramm EC, Thomas B, Reynolds R, et al. Whole-exome sequencing identifies rare, functional CFH variants in families with macular degeneration. Hum Mol Genet. 2014;23(19):5283–93.
25. Sehnal D, Bittrich S, Deshpande M, Svobodová R, Berka K, Bazgier V, et al. Mol* Viewer: modern web app for 3D visualization and analysis of large biomolecular structures. Nucleic Acids Res. 2021;49(W1):W431–7.
26. Wu J, Wu YQ, Ricklin D, Janssen BJC, Lambris JD, Gros P. Structure of complement fragment C3b–factor H and implications for host protection by complement regulators. Nat Immunol. 2009;10(7):728–33.
27. Morgan HP, Mertens HDT, Guariento M, Schmidt CQ, Soares DC, Svergun DI, et al. Structural analysis of the C-terminal region (modules 18-20) of complement regulator factor H (FH). PLoS One. 2012;7(2):e32187.
28. Morgan HP, Schmidt CQ, Guariento M, Blaum BS, Gillespie D, Herbert AP, et al. Structural basis for engagement by complement factor H of C3b on a self surface. Nat Struct Mol Biol. 2011;18(4):463–71.
29. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007;81(3):559–75.
30. Glusman G, Caballero J, Mauldin DE, Hood L, Roach JC. Kaviar: an accessible system for testing SNV novelty. Bioinformatics. 2011;27(22):3216–7.
31. McLaren W, Pritchard B, Rios D, Chen Y, Flicek P, Cunningham F. Deriving the consequences of genomic variants with the Ensembl API and SNP effect predictor. Bioinformatics. 2010;26(16):2069–70.

32. Dragon-Durey MA, Fremeaux-Bacchi V, Loirat C, Blouin J, Niaudet P, Deschenes G, et al. Heterozygous and homozygous factor H deficiencies associated with hemolytic uremic syndrome or membranoproliferative glomerulonephritis: report and genetic analysis of 16 cases. J Am Soc Nephrol. 2004;15(3):787–95.

33. Wagner EK, Raychaudhuri S, Villalonga MB, Java A, Triebwasser MP, Daly MJ, et al. Mapping rare, deleterious mutations in factor H: association with early onset, drusen burden and lower antigenic levels in familial AMD. Sci Rep. 2016;6(1):1–11.

34. Albuquerque JAT, Lamers ML, Castiblanco-Valencia MM, dos Santos M, Isaac L. Chemical chaperones curcumin and 4-phenylbutyric acid improve secretion of mutant factor H R 127 H by fibroblasts from a factor H-deficient patient. J Immunol. 2012;189(6):3242–8.

35. Triebwasser MP, Roberson EDO, Yu Y, Schramm EC, Wagner EK, Raychaudhuri S, et al. Rare variants in the functional domains of complement factor H are associated with age-related macular degeneration. Invest Ophthalmol Vis Sci. 2015;56(11):6873–8.

36. Rhim MS, Meddeb S, Kaabia O, Jalloul M, Sakouhi M, Jrzad BB, et al. C3F gene mutation is involved in the susceptibility to pre-eclampsia. Arch Gynecol Obstet. 2015;291(5):1023–7.

37. Hoffman MC, Rumer KK, Kramer A, Lynch AM, Winn VD. Maternal and fetal alternative complement pathway activation in early severe preeclampsia. Am J Reprod Immunol. 2014;71(1):55–60.

38. Velickovic I, Dalloul M, Wong KA, Bakare O, Schweis F, Garala M, et al. Complement factor B activation in patients with preeclampsia. J Reprod Immunol. 2015;109:94–100.

39. Dijkstra DJ, Lokki AI, Gierman LM, Borggreven NV, van der Keur C, Eikmans M, et al. Circulating levels of anti-C1q and anti-factor H autoantibodies and their targets in normal pregnancy and preeclampsia. Front Immunol. 2022;13:842451.

40. Burwick RM, Feinberg BB. Complement activation and regulation in preeclampsia and hemolysis, elevated liver enzymes, and low platelet count syndrome. Am J Obstet Gynecol. 2022;226(2):S1059–70.

41. Jokiranta TS, Hellwage J, Koistinen V, Zipfel PF, Meri S. Each of the three binding sites on complement factor H interacts with a distinct site on C3b. J Biol Chem. 2000;275(36):27657–62.

42. Salmon JE, Heuser C, Triebwasser M, Liszewski MK, Kavanagh D, Roumenina L, et al. Mutations in complement regulatory proteins predispose to preeclampsia: a genetic analysis of the PROMISSE cohort. PLoS Med. 2011;8(3):e1001013.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.