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Identification of complement factor H variants that predispose to preeclampsia: a genetic and functional study

AI Lokki[1,2,*], **Z Ren**[3,*], **M Triebwasser**[4], **E Daly**[5], **FINNPEC**[6], **M Perola**[7], **K Auro**[7], **R Burwick**[8,9], **JE Salmon**[10], **M Daly**[11,12], **H Laivuori**[6,12,13,14], **JP Atkinson**[15], **A Java**[16,*], **S Meri**[1,17,*]

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

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

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

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

[5]Hospital and Harvard Medical School, Boston, MA, 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, CA, USA

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

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

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

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

Corresponding authors: A. Inkeri Lokki, PhD; Seppo Meri, MD, PhD, Department of Bacteriology and Immunology, Haartmaninkatu 3 FIN-00014 University of Helsinki, tel. +358503546009 (AIL), +358505812462 (SM), fax. + 358294126382, inkeri.lokki@helsinki.fi, seppo.meri@helsinki.fi; Anuja Java, MD, Division of Nephrology, Department of Medicine, Washington University School of Medicine, St. Louis, MO 63110, Tel: 314-362-8351, ajava@wustl.edu.

*Equal contribution

Contribution to authorship

AIL performed the genetic studies with MT, ED and MD, and wrote the first draft of the manuscript. RZ and MT performed the functional studies for the discovered variants. FINNPEC provided samples for the study and board members and RB reviewed and approved the manuscript. MP and KA provided samples from the Finrisk cohort. JES designed the original genotyping platform with JPA and MT. HL is the principal investigator responsible for FINNPEC samples and data and designed the genetic study with JPA. JPA, AJ and SM supervised the data acquisition and helped to draft the manuscript and figures. All co-authors read and approved the manuscript.

Ethics statement

A 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://www.thl.fi/documents/10531/1921702/2015+FINRISK+description_for_researchers_final.pdf/fc952cba-86f6-4ef5-8ef2-fa13c23173c3.

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

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

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

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

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

Abstract

Setting: Preeclampsia 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 remains unproven.

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

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

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

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

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 preeclampsia. 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 synthesized, 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 pathophysiological mechanisms underlying severe preeclampsia.

Short abstract:

Rare functional variants in complement factor H are associated with severe pre-eclampsia.

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Introduction

Preeclampsia (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 characterized by new-onset hypertension associated with proteinuria and/or end-organ dysfunction after 20 weeks of gestation. Disease manifestations include HELLP (Hemolysis, 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 how homeostatic balance and control over invasive placental trophoblast cellscould 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-opsonized 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 hemolytic uremic syndrome (aHUS), C3 glomerulopathy (C3G), paroxysmal nocturnal hemoglobinuria (PNH), and age-related macular degeneration (AMD) [11]. The process in PE appears most analogous to that seen in aHUS, a complement-mediated TMA, characterized by endothelial injury leading to kidney failure, stroke and cardiovascular complications. aHUS 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 revolutionized the management of PNH and aHUS. In susceptible women, pregnancy is a known trigger for aHUS [14]. Preeclampsia may also have a genetic basis, considering the strong epidemiological evidence that PE is partially inherited [15–17].

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Due to 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].

Materials and Methods

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-hour 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 5g/d, 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 (Supplementary Table 1) [20, 19]. 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 Caucasian. 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 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-hour 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 license # 8/2016)[21] National FINRISK study description and ethical approvals are available online: https://www.thl.fi/

documents/10531/1921702/2015+FINRISK+description_for_researchers_final.pdf/fc952cba-86f6-4ef5-8ef2-fa13c23173c3. 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].

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 μL portions of a monospecific mouse antibody against FH (196X, 5 μg/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 μL 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 (horse radish 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 μL 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.

Functional studies

The functional consequences of rare and unique variants observed in *CFH* were characterized 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 to 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) by [27], and Figure 3C is based on structure of FH CCP19-20 in complex with C3d (PBD:2XQW) [28].

Statistics

Sequence data were analyzed in PLINK/Seq, Plink [29] and R programs. Analyses of the significant associations were performed by the Fisher's exact test. P-values < 0.05 were considered as 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 (CI95) were calculated for all variants.

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Study approval

All subjects provided a 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 or funders were not involved in study planning, data analysis or reporting of the results.

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 were 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 (Figures 1B and 1C). Western Blotting for the lysates for both variants demonstrated intracellular accumulation, thus establishing that the proteins were synthesized 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 to normal human serum, which was used as reference (patient levels: approx. 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 a guanine to adenine (that 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 programs.

Serum for the patient carrying the C1077S variant was not available, however, structural analysis for C1077S pointed out 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 compared to the wild type (WT) CCP1-4 but showed ~10-fold reduced binding (64.4 vs 6.8 μM) to C3b (Figures 2A and 2B). The reduced binding to C3b leads to decreased cofactor activity, compared to the WT (Figure 2C). This

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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 notable difference between WT and R166Q was the pattern of cleavage of C3b to iC3b (Figures 2A and 2B). 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 (Figures 2D and 2E). 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% confidence interval=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 high sFlt1/PlGF ratio of 447 and 520.

Discussion

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

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 that 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 to healthy pregnancy and 1.2% prevalence of *CFH* variants in PE or HELLP syndrome [39,40]. We describe

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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 as well as 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 twenty short consensus repeats (SCRs) that make up the FH 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 etiology 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 6 submissions listed in the ClinVar database, among which 4 cases were related to complement dysregulatory disorders. 2 in AMD, 1 in aHUS, and 1 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 a MAF of 0.025% in gnomAD is reported in a pregnant woman with SLE who developed PE. Despite extensive investigations, no functional defect was shown [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 warrants further investigation.[22,41]

Notably, among the seven patients with *CFH* rare variants, the incidence of HELLP syndrome (a rare life-threatening complication of PE) was 2 out of 7 (28%) compared to 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 pointing towards an underlying kidney disease.

Conclusion

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 and thereby validate a potential subphenotype 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 linkto the FH signal peptide variants, but the functional mechanisms are yet to be revealed. Furthermore, replication in a second population is needed preferably including non-European patients. Additionally, the role of fetal complement genes in predisposition to PE also remains to be explored.

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Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Disclosure of interests

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 in Advisory Boards of UCB, Inc.; SciRhom GmbH; Realta Life Sciences. RB is the recipient of research grants and/or speakers' fees from Alexion, AstraZeneca Rare Disease and is in Clinical Advisory Board - Comanche Biopharma, UCB Biosciences.

The Finnish Genetics of Pre-eclampsia Consortium (FINNPEC) Core Investigator Group

Principal investigator: Hannele Laivuori, M.D., Ph.D.

Members: Seppo Heinonen, M.D., Ph.D. Obstetrics and Gynecology, University of Helsinki and Helsinki University Hospital; Eero Kajantie, M.D., Ph.D.PEDEGO Research Unit, Medical Research Center Oulu, Oulu University Hospital and University of Oulu Public. Health Promotion Unit, National Institute for Health and Welfare; Juha Kere, M.D., Ph.D. Department of Biosciences and Nutrition, Karolinska Institutet, Huddinge, Sweden. Folkhälsan Research Center, Helsinki, Finland School of Basic & Medical Biosciences, King's College London, London, United Kingdom; Katja Kivinen, Ph.D. Institute for Molecular Medicine Finland, Helsinki Institute of Life Science, University of Helsinki, Helsinki, Finland; Anneli Pouta, M.D., Ph.D. Department of Government Services, National Institute for Health and Welfare, Helsinki

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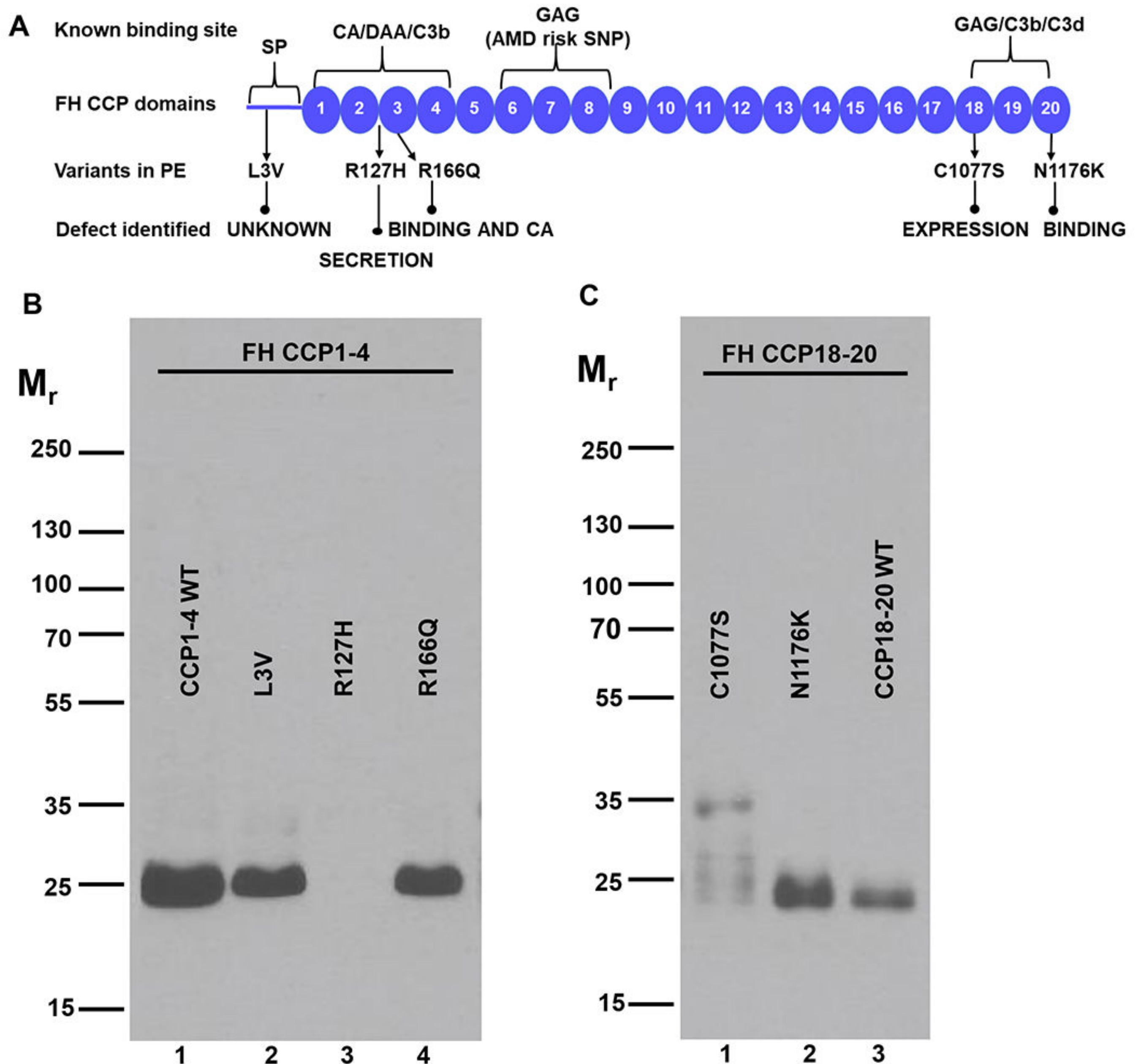

Figure 1.

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 linker between CCP2 and CCP3, CCP3, CCP18 and CCP20. The defect associated with each variant is indicated.

Factor H, FH wild type, WT; CCP, complement control protein (repeats). 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 μg/ml) and R166Q (6.3 ± 1.0 μg/ml) were comparable with the WT CCP1-4 (5.3 ± 1.1 μg/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 μg/ml) was comparable with the WT CCP18-20 (3.4 ± 0.53 μg/ml) ($P = 0.6984$, SEM 0.534). This experiment was repeated three times with similar results.

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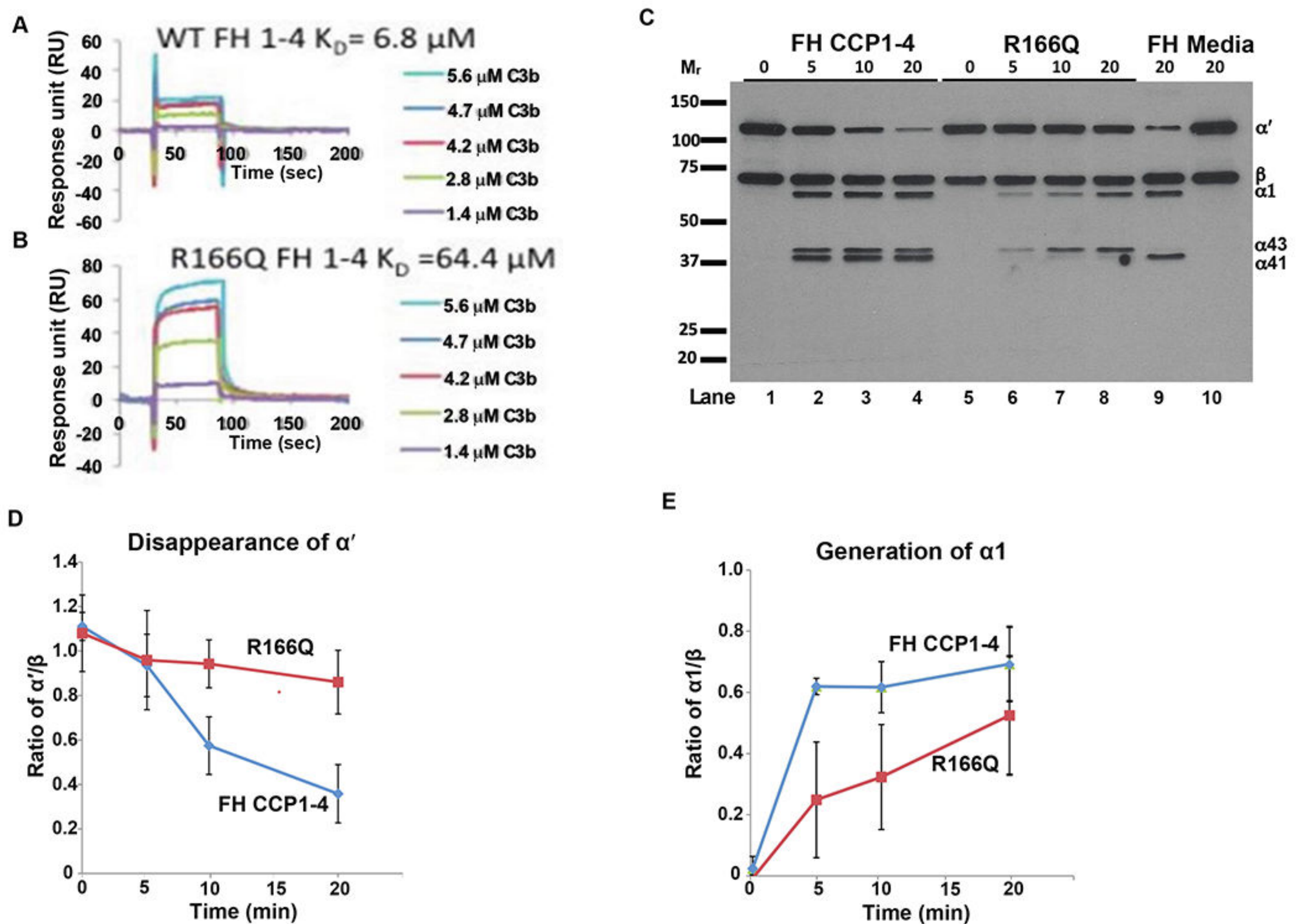

Figure 2.

Functional evaluation of the WT FH CCP1-4 and the variant R166Q. (A) and (B) Affinity of R166Q for C3b by surface plasmon resonance (SPR). R166Q had decreased affinity for human purified C3b in comparison to the WT CCP1-4. (C), (D) and (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 compared to the WT FH CCP1-4. The percentage of the α′ chain remaining and the generation of α1 fragment indicate cleavage of C3b to iC3b. The kinetic analyses of cofactor activity were conducted at 0, 5, 10 and 20 mins. Lane 9 represents a positive control employing purified human FH and Lane 10 represents a negative control employing concentrated cell media both in the presence of purified human FI and C3b. The cleavage rate was measured by densitometric analysis of α′ chain remaining and by the generation of α1 relative to the β chain. This assay was repeated 3 times. Factor H, FH wild type, WT; CCP, complement control protein (repeats). Residue numbering includes the 18 amino acids of the signal peptide (SP).

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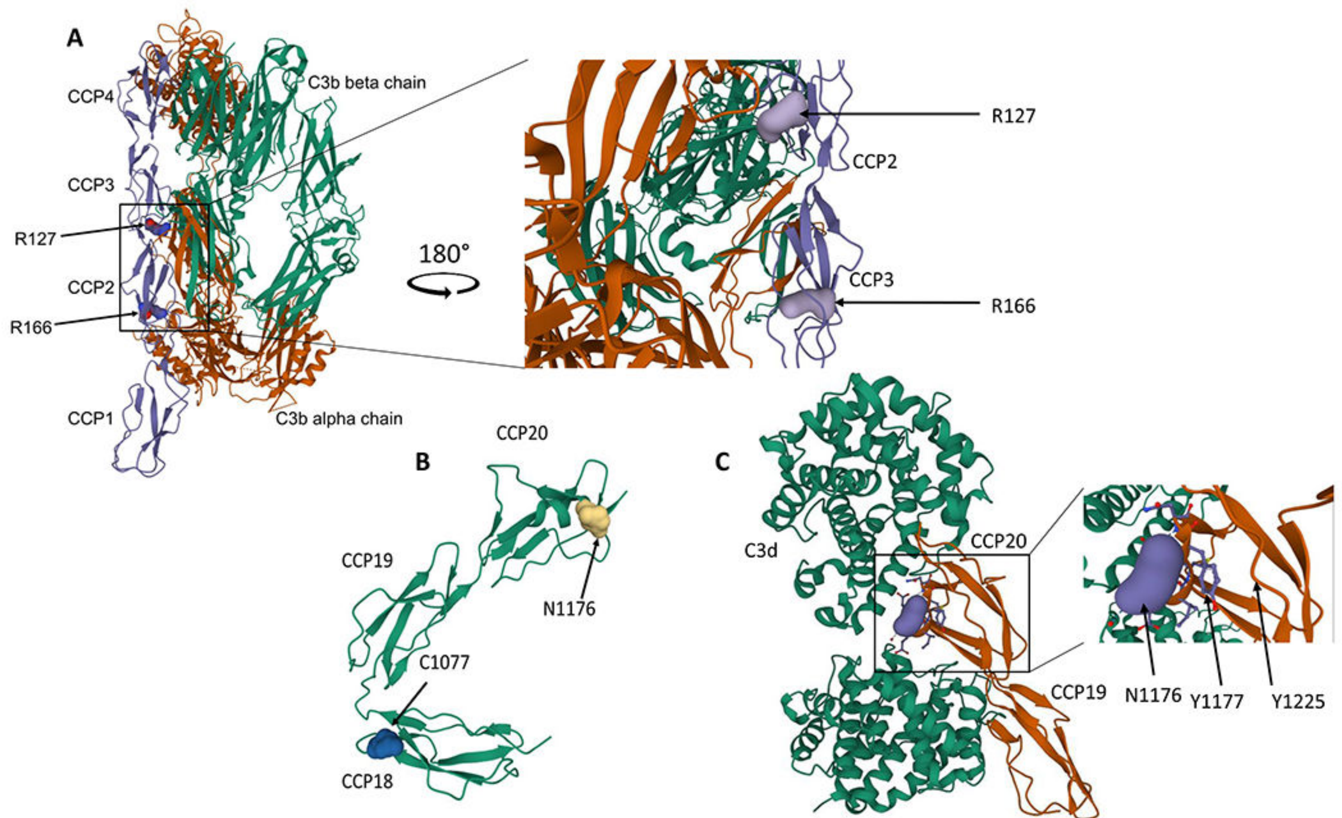

Figure 3.

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 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. Factor H, FH wild type, WT; CCP, complement control protein (repeats). Residue numbering includes the 18 amino acids of the signal peptide (SP).

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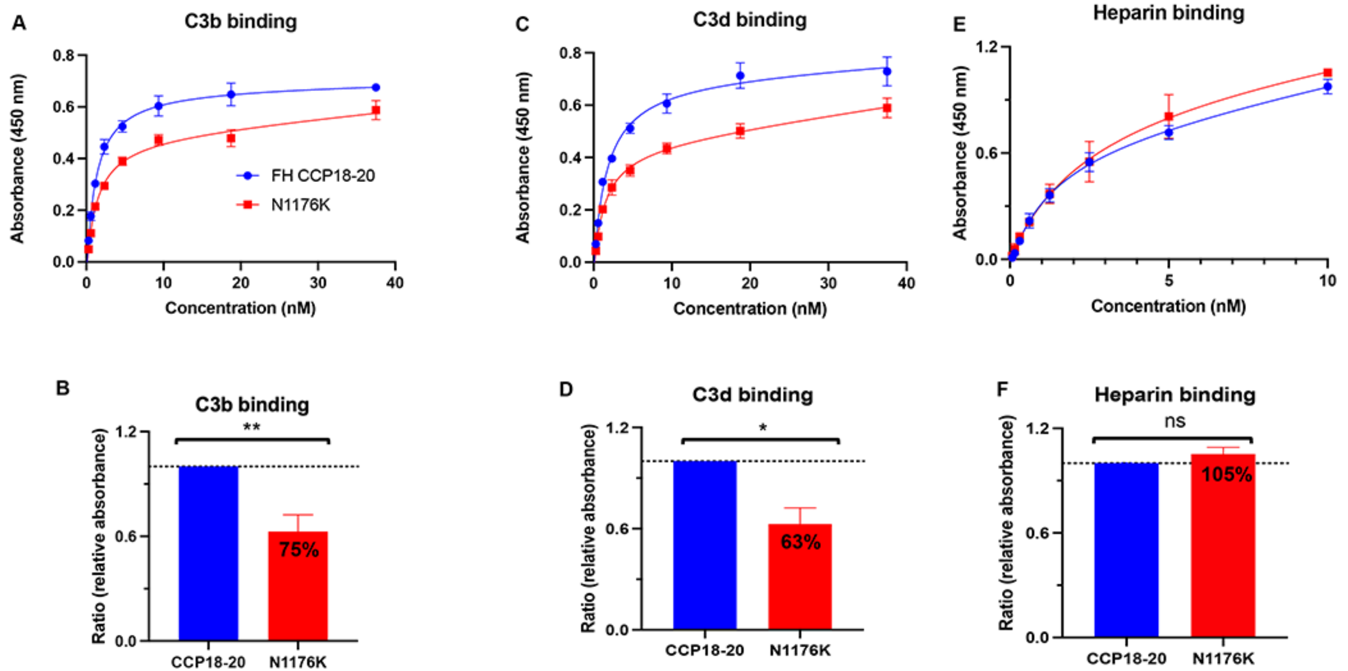

Figure 4:

ELISA of FH CCP 18-20 and variant N1176K binding to C3b, C3d and heparin. **(A)**, **(C)** and **(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 microtiter plates. Mean ± SEM of three independent experiments shown. **(B)**, **(D)** and **(F).** Each bar represents the Mean ± SEM of relative absorbance detected in WT CCP18-20 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 to WT were 0.001 for C3b and 0.022 for C3d binding. For heparin binding, the *P*-value for the difference of N1176K compared to WT was 0.31. The horizontal dash line represents 1.0. Standard error of mean, SEM; ns, not significant, $*P < 0.05$; $**P < 0.01$. Factor H, FH wild type, WT; CCP, complement control protein (repeats). Residue numbering includes the 18 amino acids of the signal peptide (SP).

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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

RSID, variant identifier; G/P, gravidity/parity; early-onset PE, diagnosis < 34 weeks of gestation; PE, preeclampsia; SGA, small for gestational age; GDM, gestational diabetes mellitus managed by nutritional therapy; FET, frozen embryo transfer; HELLP, hemolysis, elevated liver enzyme and low platelet count; SP, signal peptide