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Quantitative Alterations in Complement Alternative Pathway and Related Genetic Analysis in Severe Phenotype Preeclampsia

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Key Points

- Women with severe preeclampsia demonstrate abnormal levels of the complement alternative pathway components.
- Genetic variants in the complement alternative pathway are more prevalent in those with severe phenotype preeclampsia compared with the general population.
- Future studies should explore the role of medications that block the complement alternative pathway in treatment of preeclampsia.

Abstract

Background Preeclampsia and hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome share many clinical and biologic features with thrombotic microangiopathy syndromes caused by complement abnormalities. Our hypothesis was that similar functional and genetic alterations in the complement alternative pathway (CAP) are present in these disorders of pregnancy.

Methods We conducted quantitative analysis of proteins involved in CAP using ELISA and nephelometry on prospectively collected blood samples from patients with severe phenotype preeclampsia (defined as delivery ≤34 weeks due to preeclampsia), HELLP syndrome, or eclampsia, and matched normotensive controls (n=25 in each arm) between 2011 and 2016. Sequencing was performed to interrogate 14 genes encoding CAP components.

Results Both groups were similar in age, gravidity, parity, marital status, and race. The study group had a higher BMI (mean±SD, 32±8 versus 25±4 kg/m^2; P=0.002) and earlier gestational age at delivery (32.5±3.6 versus 40.3±1 weeks; P<0.001). Serologic studies demonstrated elevated Bb subunit (median [range], 1.2 [0.5–4.3] versus 0.6 [0.5–1] μg/ml; P<0.001), complement C5 concentration (28 [18–33] versus 24 [15–34] mg/dl; P=0.03), and sMAC (371 [167–761] versus 184 [112–249] ng/ml; P<0.001) concentrations in patients with preeclampsia. Two thirds of patients with preeclampsia had at least one nonsynonymous sequence variant in CAP genes.

Conclusion Patients with severe phenotype preeclampsia manifest functional alterations in CAP activation. Genetic variants in the CAP genes were detected in several patients, but a larger population study is necessary to fully evaluate genetic risk. Genetic screening and complement-targeted treatment may be useful in risk stratification and novel therapeutic approaches.

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Introduction

Preeclampsia is a progressive and potentially devastating multisystem disorder, representing one of the leading causes of both maternal and perinatal morbidity and mortality, and resulting in high health and economic burdens on society. Although multiple pathophysiologic theories have been proposed, the exact etiology of preeclampsia remains unknown.

Maternal-placental immune tolerance is a phenomenon that describes the immunologic changes that occur in pregnancy, resulting in enhancement of the innate immune system and suppression of the adaptive

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immune system (1,2). The complement cascade has three pathways that lead to a common terminal pathway (Figure 1). The classic pathway is triggered by the formation of an immune complex. A single IgM or two IgG molecules are sufficient to trigger complement activation. The lectin pathway is homologous to the classic pathway, but is activated by danger-associated molecular patterns and pathogen-associated molecular patterns, including carbohydrates on bacterial surfaces. The complement alternative pathway (CAP) is always active at low levels, playing a role in the innate immune system, which functions to sustain an immunologically vigilant defense. Therefore, to maintain an intact immune system, complement regulatory proteins are needed. The theory of immune maladaptation in preeclampsia is based on epidemiologic studies demonstrating that prior exposure to paternal antigens has a protective effect against developing preeclampsia (3,4).

Preeclampsia and hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome share similar clinical characteristics with complement-mediated hemolytic uremic syndrome, also known as atypical hemolytic uremic syndrome (aHUS), including microangiopathic hemolytic anemia, microvascular thrombosis, thrombocytopenia, and renal injury (4). It has been demonstrated that plasma elevation of a CAP activation fragment of Factor B (Bb) could be a potential biomarker for elevated risk of preeclampsia (5), and several human studies have reported the association of CAP activation in the placenta and in human blood samples with preeclampsia (6).

In this context, we conducted a multilevel study to determine if severe phenotype preeclampsia is associated with functional and quantitative complement abnormalities and genetic alterations. We hypothesized that the CAP is altered in severe phenotype preeclampsia and HELLP syndrome, and that complement-related genetic variants would be present in the cohort.

Materials and Methods

We prospectively collected blood samples from women who were preeclamptic and normotensive delivering at our institution between 2011 and 2016. We identified patients with severe phenotype preeclampsia, defined as preeclampsia with severe features necessitating delivery at or before 34 weeks; eclampsia; and HELLP syndrome. Severe features of preeclampsia were defined according to current criteria, including new onset of any of the following: platelets $<100 \times 10^3/\mu l$, serum creatinine of >1.1 mg/dl or doubling of serum creatinine concentration in the absence of other

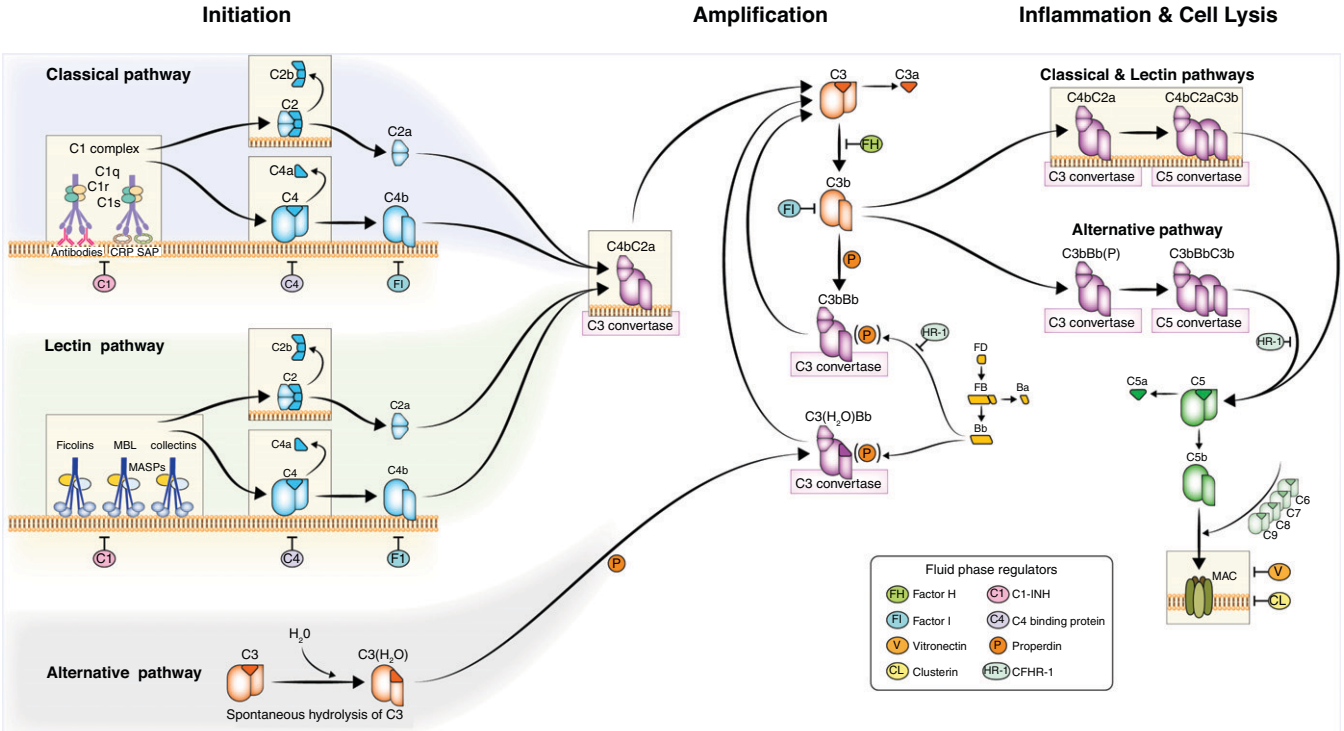

Figure 1. | **The complement cascade.** The complement cascade is made up of three pathways: the classic, the lectin, and the alternative pathways. An amplification loop of the initial cascade signals includes C3, and a terminal common pathway ensues. The alternative pathway is always active at low levels and may be self-activated after C3 hydrolysis. Without proper regulation with complement regulatory proteins (Factor H [FH], Factor I, complement factor H [CFH]–related proteins), an overactive pathway may result in damage to self. Factor B is cleaved into smaller fragments, the most notorious being Ba and Bb. Bb is added onto the alternative pathway C3 convertase to continue the C3 amplification loop cycle. Inflammation is led by the C3a and C5a, and the most visible effect of the terminal complement cascade is cell lysis, mediated by the C5b, C6, C7, C8, and C9 components, in a complex named C5b-9 or membrane attack complex (MAC). Adapted from Frazer Abel *et. al.*, *Adv. Clin. Chem.* 77:1-75, 2016, with permission from the Mayo Clinic, copyright owner. C1-INH, C1 inhibitor; CFHR-1, complement factor H receptor 1; H_2O, water. CRP, C-reactive protein; SAP, serum Amyloid P protein; MASPs, mannose binding lectin associated serine proteases; MBL, mannose binding lectin.

kidney disease, liver transaminases twice the upper limits of normal, presence of pulmonary edema, and cerebral or visual symptoms (new-onset and persistent headaches, blurred vision, flashing lights [7]). Chart review was performed by two clinicians (L.A. and M.L.G.S.) to ensure accurate diagnoses consistent with contemporary definitions of preeclampsia and HELLP syndrome, per the American College of Obstetricians and Gynecologists (ACOG) (8). Patients were matched with a normotensive control on the basis of maternal age and time of sample collection. We allowed a maximum of 6 months between the blood draw of a patient and her matched control. Samples were collected at time of delivery for both groups. All samples used in this study were obtained within 24 hours of delivery and stored at −80°C until time of analysis. The study was reviewed and approved by the Mayo Clinic Institutional Review Board. All participants gave written informed consent before inclusion in the study.

Complement component concentrations and function were measured in serum. EDTA plasma was used for measurement of complement activation fragments. All analyses were performed in the Mayo Clinic Protein Immunology Clinical Laboratory per standard techniques, using the same assays commercially available for routine patient testing. Laboratory personnel were blinded to the patient/control status of the samples. Briefly, samples were thawed on an ice bath only immediately before testing, and kept on ice water throughout the duration of the test (<3 hours). Total complement function was assessed using the liposome assay (Wako Diagnostics), and variations of this test were used for measurements of the functions of individual complement components (C1q, C2, C3, C4, C5, C6, C7, C8, C9). Complement component concentrations were measured by nephelometry using a Siemens BNII nephelometer. C3 reagents are approved by the US Food and Drug Administration (FDA) for Siemens, whereas C5, complement factor B (CFB), and complement factor H (CFH) are laboratory-developed tests. Activation fragments (Bb, soluble membrane attack complex [sMAC]) were measured by ELISA (Quidel Diagnostics).

DNA was extracted from the buffy coat and subsequently exome sequencing was performed. Genomic DNA was sonicated to an average size of 175 bp. The fragments were blunt ended, the "A" base was added to the 3′ end, and Illumina's sequencing adapters were ligated to the ends. The ligated fragments underwent amplification for eight cycles with primers that incorporate a unique indexing sequence tag. Indexed libraries underwent equimolar pooling in batches of five. Each pool was hybridized to biotinylated RNA oligos specific to the regions of interest (SureSelectXT Clinical Research Exome; Agilent Technologies) and selected from the remaining fragments using streptavidin beads. Enriched fragments were amplified for 11 cycles. The resulting libraries were sequenced using the Illumina HiSeq-3000 as paired-end reads extending 150 bases from both ends of the fragments.

The genetic analysis was performed using Ingenuity Variant Analysis (version 4.3.20170330; QIAGEN, Redwood City, CA). All variants filtered into the analysis had a call quality of at least 20.0 and an allele frequency of <0.05 in ExAC (version 0.3.1; http://exac.broadinstitute.org). Variants were included if they were frameshift, in-frame indel, stop codon change, missense, or canonical splice-site disrupting in the following genes involved in CAP: *complement factor I* (*CFI*), *CFH*, *CFB*, *C3*, *C5*, CFH-related 1 (*CFHR1*), *CFHR2*, *CFHR3*, *CFHR4*, *CFHR5*, complement C3a receptor 1 (*C3AR1*), complement C3d receptor 2 (*CR2*), complement C3b/C4b receptor 1 like (*CR1L*), membrane cofactor protein (*CD46*), and thrombomodulin (*THBD*). Variants were analyzed using the American College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology joint guidelines for sequence interpretation (9).

Statistical analyses for serum complement studies were performed using JMP Pro version 14.1.0 (SAS Institute, Inc.). Patients and controls were compared using Wilcoxon/Kruskal–Wallis tests (rank sums tests). Statistical significance was attributed when P values were <0.05.

Results

A total of 25 patients and 25 matched controls were included in the study. The preeclampsia group included 16 patients with preeclampsia with severe features that resulted in delivery at or before 34 0/7 weeks, eight patients with HELLP syndrome, and one patient with eclampsia. The two groups were similar in age, gravidity, parity, marital status, and race; however, the preeclampsia group had a higher body mass index (BMI; mean±SD, 32±8 versus 25±4 kg/m^2; P=0.002) and an earlier gestational age at delivery (32.5±3.6 versus 40.3±1 weeks; P<0.001). Table 1 describes the demographic characteristics of the two groups. Table 2 provides an overview of the quantitative versus genetic alterations of different components of the complement pathway that we interrogated. All genetic variants detected, including those classified as benign or likely benign, are included in the summary counts in Table 2. Details on the specific variants identified are listed in Supplemental Table 1 with the ACMG classification (9).

We found elevated Bb subunit when comparing patients with preeclampsia with controls (median [range], 1.2 [0.5–4.3] versus 0.6 [0.5–1] μg/ml; P<0.001), as seen in Figure 2. Additionally, Bb was also increased further in those with HELLP syndrome when compared with those with the early-onset preeclampsia with severe features (1.9 versus 1.2 μg/ml; P=0.3), as illustrated in Figure 3. C5 concentration (median [range], 28 [18–33] versus 24 [15–34] mg/dl; P=0.03) and sMAC (371 [167–761] versus 184 [112–249] ng/ml; P<0.001) were also increased in patients when compared with controls (Figure 4). Of note, although total C5 was slightly higher in patients than controls, C5 function showed similar activity in patients (49.4 U/ml) and controls (50.0 U/ml), P=0.43 (Supplemental Table 2).

We performed further analysis on the complement classic pathway and found no differences between the preeclampsia group when compared with the controls. C1q function was similar in both groups (median [range], 60 [34–71] versus 57 [47–65] U/ml; P=0.2), as was C2 function (46 [33–59] versus 44 [34–51] U/ml; P=0.3), C4 concentration (19 [9–108] versus 24 [12–102] mg/dl; P=0.2), and C4 function (54 [32–67] versus 56 [46–61] U/ml; P=0.7; Supplemental Table 2).

At the genetic level, six of 25 patients (24%) had at least one variant in CAP genes. Table 3 describes the specific variants

Table 1. Demographic and clinical characteristics (n=50)

Characteristic	Preeclampsia	Control	P Value
Age (yr), mean±SD	30.6±5.3	30.6±5.6	>0.99
Body mass index (kg/m^2), mean±SD	32.3±7.7	25.2±4.4	0.002
Race, *n* (%)			0.26
White	22 (88)	22 (88)	
Black	1 (4)	1 (4)	
Hispanic	2 (8)	0 (0)	
Asian	0 (0)	2 (8)	
Private insurance, *n* (%)	18 (72)	23 (92)	0.07
Married, *n* (%)	20 (80)	21 (84)	0.71
Gravida, median (range)	1 (1–6)	2 (1–10)	0.56
Parity, median (range)	0 (0–5)	1 (0–9)	0.20
Gestational age (wk), mean±SD	32.5±3.5	40.3±0.9	<0.001
Birth weight (g), mean±SD	1975±952.4	3540±359.4	<0.001

(excluding benign/likely benign) for each of the patients with accompanying relevant clinical features. All of the identified genetic variants were heterozygous and none were established pathogenic variants reported in aHUS or other disorders. Common variants in *CFH*, *CFHR1*, and *CFHR3* previously reported as risk factors for aHUS were also evaluated (10). Although the phase cannot be determined with the methods used here, the allele frequency for each variant was similar to that previously reported in the non-Finnish European population in Genome Aggregation Database (11), suggesting these variants are unlikely to increase risk of preeclampsia in this population.

The creatinine values for patients with preeclampsia at time of delivery are reported in Table 3. Most of the patients presented with a creatinine value <1 mg/dL, except for two patients: one had a creatinine value of 1.2, and the other a creatinine mg/dL value of 1.9 mg/dL with a history of chronic hypertension. Proteinuria in patients with preeclampsia varied from 300 mg up to 13 g in 24 hours, fulfilling the criteria of preeclampsia as per the ACOG definition (8). Healthy controls did not have creatinine measurements at time of delivery (Table 3).

Fetal and immediate perinatal outcomes for both groups are presented in Table 4. Neonates in the preeclamptic group

Table 2. Quantification, functionality, and genetics of factors in complement alternative pathway in patients with severe phenotype preeclampsia compared to matched normotensive controls

Complement Component	Reference Intervals	Median (range) PE (n=25)	Median (range) Control (n=25)	P Value	PE Patients with Genetic Variants (n=25), % (n)[a]	Allele Frequency in General Population, (%)[b]
Factor B, mg/dl	15.2–42.3	32 (20–56)	36 (24–48)	0.7	20 (5)	1.107–3.885
Bb, μg/ml[c]	≤1.6	1.24 (0.5–4.3)	0.6 (0.5–0.97)	<0.001[d]	—	—
Factor I (genetics only)	No variants	—	—	—	8 (2)	0.072–0.839
Factor H, mg/dl	18.5–40.8	30.6 (24.1–47.5)	31.5 (24.4–35.9)	0.7	12 (3)	1.0
CFHR1 (genetics only)					4 (1)	0.849
CFHR2 (genetics only)					8 (2)	0.397–0.770
CFHR4 (genetics only)					4 (1)	0.288
CFHR5 (genetics only)					4 (1)	1.621–1.777
MCP (*CD46*) (genetics only)	No variants	—	—	—	4 (1)	1.532
THBD (genetics only)	No variants	—	—	—	8 (2)	0.762
C3 concentration, mg/dl	75–175	140 (101–234)	143 (106–233)	0.4	12 (3)	0.001–0.336
C3AR1 (genetics only)		—	—	—	4 (1)	2.019
CR2 (genetics only)					8 (2)	0.000
CR1L (genetics only)					16 (4)	0.184–4.89
C5 concentration, mg/dl	10.6–26.3	28 (18–33)	24.1 (15–34)	0.03[d]	4 (1)	0.000
sMAC, ng/ml	≤250	371 (167–761)	184 (112–249)	<0.001[d]	—	—

PE, severe phenotype preeclampsia; CFHR, complement factor H related; MCP, membrane cofactor protein; THBD, thrombomodulin; C3AR1, C3a receptor 1; CR2, C3 receptor 2; CR1L, Complement C3b/C4b Receptor 1 Like; sMAC, soluble membrane attack complex.
[a]Encompasses all variants detected (including benign and likely benign).
[b]Population allele frequency as reported in Exome Aggregation Consortium (ExAC; http://exac.broadinstitute.org) and Genome Aggregation Database (gnomAD; http://gnomad.broadinstitute.org); accessed January 19, 2018. See Supplemental Table 1 for individual variants and allele frequencies.
[c]*Bb* is the activation fragment of complement factor B, an activator of the complement alternative pathway.
[d]P<0.05.

were delivered at earlier gestational ages, had corresponding lower birth weights compared with controls, and had lower Apgar scores. However, there were no cases of fetal demise in either group.

Discussion

This study demonstrates quantitative alterations of complement components specific to CAP in patients with preeclampsia compared with controls. Classic pathway complements showed no difference between the two groups, suggesting that only the alternative pathway led to the activation of the common pathway in our cohort. We found three specific components to be elevated in the preeclamptic cohort compared with controls: (*1*) Bb, the activation fragment of CFB, a marker exclusive to the CAP; (*2*) C5, which is a part of the terminal pathway; and (*3*) MAC (C5b-9), which is the terminal product of the complement cascade. Notably, MAC functions by attaching to cells to lyse them and has been associated with endothelial dysfunction, a hallmark of vascular injury in preeclampsia. Genetic analysis of CAP-related genes in patients revealed five heterozygous variants of unknown significance in four genes (*CR2*, *CFI*, *C3*, and *C5*), each in one individual. The remaining variants identified were classified as benign or likely benign by ACMG criteria (9). Of these, five individuals were found to carry three benign variants in *CFB*. However, these variants were classified according to criteria used for Mendelian disorders, and we cannot exclude the possibility that these variants may contribute to risk, either as low penetrance variants or in combination with environmental and/or other genetic variables. Our study adds to the current literature a comprehensive analysis of the complement pathway at the quantitative and functional levels, identifies the CAP as the primary pathway that is affected in severe preeclampsia, and provides preliminary data regarding variants in the CAP genes.

Complement dysregulation has been described in multiple pathologic conditions—most interestingly, thrombotic microangiopathy syndromes, many of which share clinical and biologic features with preeclampsia and HELLP syndrome (12–14). *In vitro* studies have shown that MAC, an important driver of inflammation, colocalizes to sites of placental villous injury in preeclampsia, associates with fibrin deposits, and increases apoptosis of cytotrophoblasts (15). Clinical studies have demonstrated that urinary excretion of MAC is present in the majority of patients with severe preeclampsia (96%) and in few healthy controls (8%) (16). MAC concentration can be indirectly quantified in plasma by measuring its soluble product, sMAC (17). The PROMISSE study found that pregnant patients with lupus and/or antiphospholipid antibodies had increased levels of Bb and sMAC detectable in early pregnancy (12–15 weeks of gestation) that remained elevated in the third trimester (31 weeks of gestation); elevated levels of sMAC were associated with adverse pregnancy outcomes compared with pregnant healthy controls (18). However, to date, a direct function of sMAC is yet to be identified. Our study also found elevated sMAC levels in patients with preeclampsia when compared with controls and is further supported by previous reports of elevated levels of CFB and Bb in preeclampsia (4,5,19–21). As for the C5 levels, some studies have reported increased C5 levels in severe preeclampsia (16,22), whereas others have shown no differences (23). We have also found elevated C5 levels, but we are interpreting this finding with caution for two reasons. First, our test cannot distinguish between intact C5 or fragmented/active C5 and, second, a subsequent C5 functional assay that we performed did not differ between patients and controls.

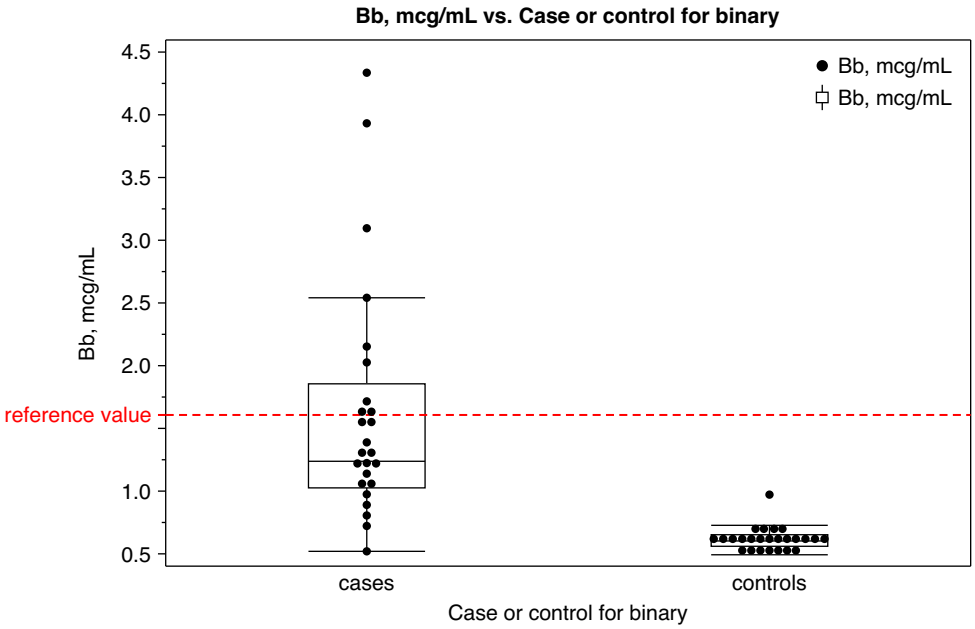

Figure 2. | **Bb level in patients with severe phenotype preeclampsia is elevated compared with controls.** Bb level in those with severe phenotype preeclampsia (including hemolysis, elevated liver enzymes, and low platelets [HELLP] syndrome) is increased when compared with normotensive matched controls ($P<0.001$).

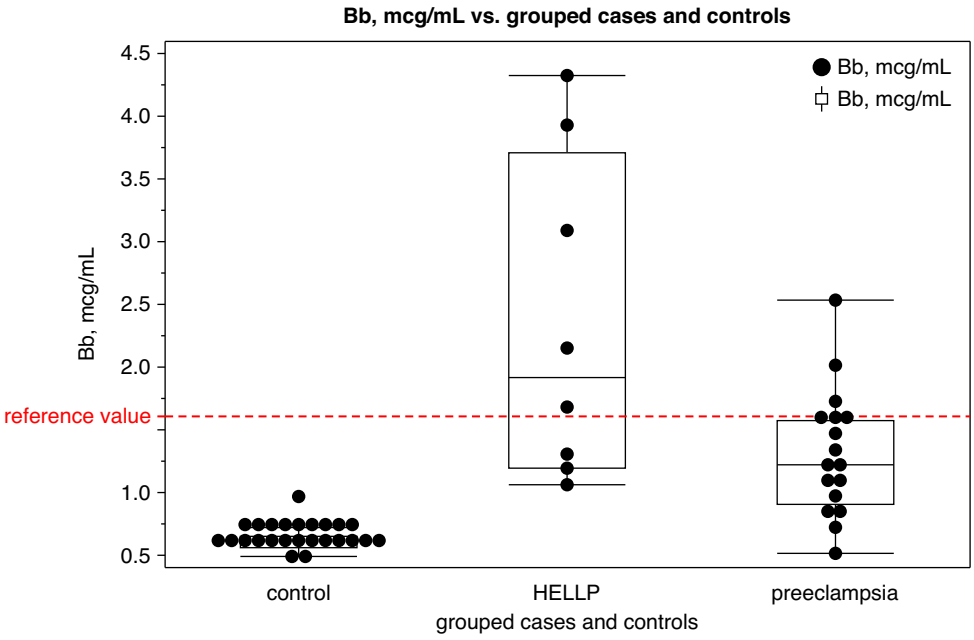

Figure 3. | **Compared to normotensive pregnancies, Bb levels are elevated in preeclampsia and further increased in HELLP syndrome.** Control versus HELLP, $P<0.001$; control versus preeclampsia, $P<0.001$; preeclampsia versus HELLP, $P=0.04$.

The diagnosis of aHUS is commonly supported by the presence of genetic alterations in complement components and regulatory factors. Normal complement factor serum levels do not exclude the diagnosis of aHUS, because the disease-causing complement activation occurs on the surface of endothelial cells, while the plasma concentrations of the complement components may remain normal (24,25). According to ACMG guidelines, genetic variants are categorized as benign, pathogenic, or variants of unknown significance (9). As knowledge of population-based genetics has expanded, the verbiage "genetic variant" rather than "mutation" is preferred (16). We used next-generation sequencing (exome sequencing) to identify variants in specific complement genes only for patients. Review of allele

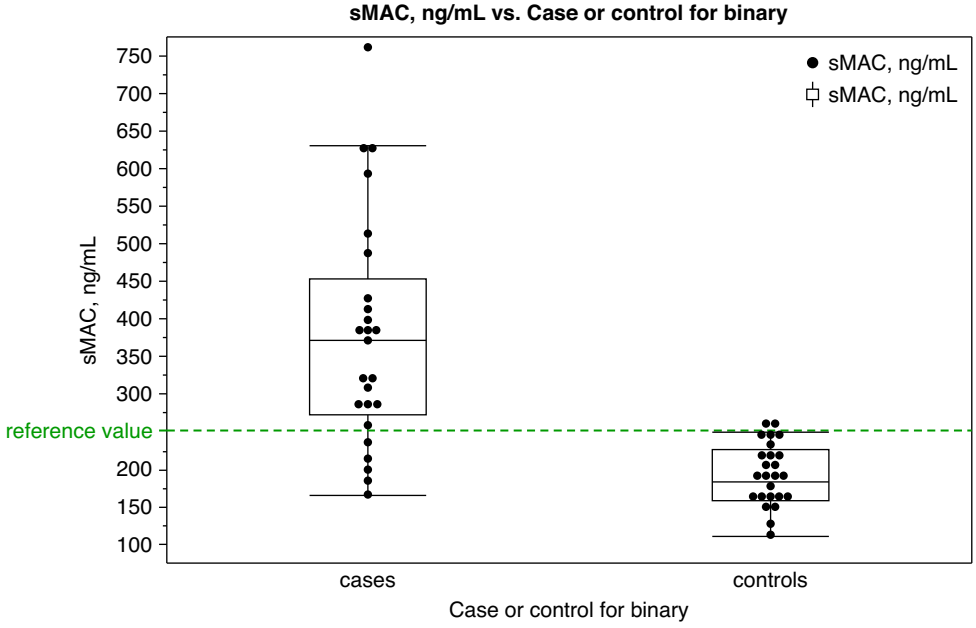

Figure 4. | **sMAC levels are increased in severe phenotype preeclampsia compared with control.** sMAC levels are significantly elevated in patients with severe phenotype preeclampsia when compared with normotensive controls ($P<0.001$). sMAC, soluble membrane attack complex.

Table 3. Patients with severe phenotype preeclampsia, relevant clinical findings, and genetic variants found

Patient	Diagnosis	GA (wk+d)	Age (yr)	P	BMI (kg/m^2)	Race	Platelets (mm^3 in 10^3)	AST (IU/L)	Cr (mg/dl)	Urine PC	Gene (variant)[a]
1	Eclampsia	39 + 4	25	0	33	Black	185	33	0.6	1.6	*C3* (c.3299T>C; p.L1100P); VUS *CFI* (c.1642G>C; p.Glu548Gln); VUS
2	HELLP	36 + 1	35	0	47	White	80	108	0.9	0.54	*C5* (c.2686G>T; p.V896L); VUS
3	HELLP	25 + 2	26	0	34	White	22	512	0.8	2.26	None
4	HELLP	33 + 3	31	2	33	White	324	170	0.7	318[b]	None
5	HELLP	31	31	0	23.4	White	62	241	0.7	2.2	None
6	HELLP	26 + 4	21	1	26	White	44	258	0.6	0.4	None
7	HELLP	38 + 5	24	0	25.5	White	46	254	0.9	368[b]	None
8	HELLP	29 + 3	40	0	30	Hispanic	71	377	1	796[b]	None
9	HELLP	38	31	0	31	White	48	532	0.9	n/a	None
10	Early PE	33 + 3	38	1	26	White	207	55	0.8	1.53	None
11	Early PE	26 + 5	22	0	42	White	180	18	1	867[b]	*CR2* (c.475A>G; p.M159V); VUS
12	Early PE	31 + 4	37	5	33	White	116	23	1	0.97	None
13	Early PE	30 + 4	39	4	29	White	148	27	0.7	937[b]	*CFI* (c.1642G>C; p.Glu548Gln); VUS
14	Early PE	34	33	0	34	White	238	24	0.6	1041[b]	None
15	Early PE	34	34	0	25	White	186	46	1	2	None
16	Early PE	32 + 1	27	0	30.8	White	206	26	0.7	633[b]	*CR2* (c.763C>T; p.R255W); VUS
17	Early PE	33 + 2	26	0	30.1	White	201	41	0.7	0.57	None
18	Early PE	32 + 1	35	0	37.8	White	65	345	0.8	0.74	None
19	Early PE	34	33	2	37.5	White	101	63	0.8	0.16	None
20	Early PE	34	28	0	23.5	White	125	59	1.2	500[b]	None
21	Early PE	33 + 4	35	3	23	White	159	33	0.7	308[b]	None
22	Early PE	27 + 6	27	0	40	White	152	44	1.2	1.22	None
23	Early PE	34	30	0	54	Hispanic	354	30	0.5	634[b]	*C3* (c.3299T>C; p.L1100P); VUS
24	cHTN SI early PE	29 + 1	26	1	24	White	164	45	1.9	13.6	None
25	cHTN SI early PE	33 + 5	31	0	34	White	172	36	0.8	2862[b]	None

GA, gestational age; P, parity; BMI, body mass index; AST, aspartate aminotransferase; Cr, creatinine; PC, protein-creatinine ratio; VUS, variant of uncertain significance; HELLP, hemolysis, elevated liver enzymes, and low platelets; early PE, early-onset preeclampsia with severe features; cHTN SI early PE, chronic hypertension with superimposed early-onset preeclampsia with severe features.

[a]Excludes benign/likely benign variants.

[b]24-hour urine protein (in milligrams).

frequency in Genome Aggregation Database allowed us to compare the frequency of variants identified in our cohort with a large population. This methodic approach is justified by the fact that our controls were recruited from, and thus are representative of, the general population. The correlation of genetic variants with clinical implications is challenging in non-Mendelian, multifactorial disorders. Indeed, our data were inconsistent when attempting to correlate specific genetic variants with abnormal quantities and function of complement components. Table 5 describes the five patients found to have genetic variants in *CFB*. One variant (c.26T>A, p.Leu9His) has a frequency of 5% in non-Finnish Europeans, and its frequency was at 20% in our cohort, suggesting it is possibly enriched in our study population. Preeclampsia is a disease of multifactorial etiology, and its genetic correlations are largely uncharacterized. Cognizant of this, in addition to evaluating rare variants, we are reporting several common genetic variants previously associated with an increased risk of aHUS. The presence of different variants in different genes across our study population, rather than rare known pathogenic variants in a single gene, suggests that preeclampsia/HELLP is multifactorial, consistent with other studies of complement-mediated disorders and polygenic risk scores (26). A multicenter study or larger dataset will be necessary to fully evaluate contributors to multifactorial inheritance. Because preeclampsia may affect up to 8% of all pregnancies (8), it is possible that other common variants, beyond those evaluated here, could increase risk for this disorder; however, a larger cohort will be required to better understand the role of common variants. As more information is obtained from genome-wide and proteomic-based studies in preeclampsia (21) and HELLP syndrome (27), the effects of these variants may be further clarified.

The strengths of our study include its case-control design, which allowed us to compare phenotypic and functional data. We limited our study to only severe phenotypes of

Table 4. Fetal and immediate perinatal outcomes

Fetal Outcomes	Preeclampsia	Control	*P* Value
Gestational age (wk), mean±SD	32.5±3.6	40.3±1.0	<0.001
Birth weight (g), mean±SD	1975±952.4	3540±359.4	<0.001
Fetal demise, *n* (%)	0 (0)	0 (0)	>0.99
5-min Apgar score <7, *n* (%)	6 (24)	0 (0)	<0.001

preeclampsia and HELLP syndrome, because this created a more homogeneous disease population postulated to have a different pathogenesis than milder and later onset forms of preeclampsia. Mild presentations of preeclampsia (without severe features) were not included in our study. The limitations of our study include its small sample size, which resulted in limitation of our statistical analysis to find stronger associations with clinically relevant genetic variants. Our samples were obtained within 24 hours of the time of diagnosis and, as such, no serial sampling and measurements of complement level trends were performed. An elevated BMI is known to be associated with a higher risk of preeclampsia (28). In addition, plasma levels of C3, CFH, and CFB have been found to positively correlate with higher BMI in patients with preeclampsia (29). The same may be true for complement breakdown components. Whereas our current data showed a higher BMI in the preeclampsia group when compared with controls, in future studies we plan to study longitudinal changes of complement factors in normotensive and preeclamptic pregnancies and correlate them with BMI changes with advancing gestational age.

For most patients, the use of quantitative and functional serologic complement assays for serologic complement testing are important tools to aid in diagnosis of aHUS (30). C5a, C3a, and sMAC are high in patients with aHUS (30) and, therefore, therapies to halt the complement activation are relevant. Eculizumab, a recombinant humanized monoclonal IgG antibody that blocks the cleavage and subsequent activation of C5, is currently approved by the FDA for the treatment of aHUS and paroxysmal nocturnal hemoglobinuria, both of which are diseases caused by dysregulation of the CAP. Treatment of a patient with HELLP syndrome with eculizumab at 26 weeks prolonged pregnancy by 17 days, suggesting a potential therapeutic role in the improvement of perinatal outcomes and a reduction in healthcare economic burden (31). Other reports of the use of eculizumab during pregnancy suggest no identifiable detrimental effects (32–35). More studies are needed to explore the potential role of eculizumab in the treatment of preeclampsia and HELLP syndrome; functional and genetic studies of the affected patients may identify those who are likely to respond to this medication.

In conclusion, patients with severe phenotype preeclampsia manifest functional alterations in CAP activation, as suggested by the presence of elevated levels of Bb and sMAC. Future research addressing genetic studies in a larger patient cohort and longitudinal changes in complement concentrations and activities and their association with disease severity are needed. Additional larger studies may show genetic screening and complement-targeted treatment to be useful in risk stratification and novel therapeutic approaches to a currently untreatable disease.

Disclosures

V.D. Garovic reports being the inventor of the technology "Markers for preeclampsia." The technology has not been licensed. M.L. Gonzalez Suarez reports receiving research funding from

Table 5. Clinical and quantitative complement assessment of patients with genetic variants in complement factor B

Patient	Diagnosis	GA (wk+d)	Age (yr)	P	BMI (kg/m^2)	Plt (mm^3 in 10^3)	AST (IU/L)	Cr (mg/dl)	Urine PC	Gene (variant)	Bb (μg/ml)[a]	C5 (mg/dl)[a]	sMAC (ng/ml)[a]
5	HELLP	31	31	0	23.4	62	241	0.7	2.2	*CFB* (p.L9H)	1.3	27.2	382.5
6	HELLP	26 + 4	21	1	26	44	258	0.6	0.4	*CR1L* (p.K88N) *CFB* (p.L9H) *C3AR1* (p.L333P)	1.2	21.2	235.9
17	Early PE	33 + 2	26	0	30.1	201	41	0.7	0.57	*CFB* (p.G252S, p.E566A)	1.2	28.6	487.3
12	Early PE	31 + 4	37	5	33	116	23	1	0.97	*CFHR2* (p.T71M) *CFHR5* (p.R356H) *CR1L* (p.V5001) *CFB* (p.L9H)	0.8	22.7	199.9
14	Early PE	34	33	0	34	238	24	0.6	1041[b]	*CFB* (p.L9H)	0.5	28.9	206.9

GA, gestational age; P, parity; BMI, body mass index; Plt, platelets; AST, aspartate aminotransferase; Cr, creatinine; PC, protein-creatinine ratio; sMAC, soluble membrane attack complex; HELLP, hemolysis, elevated liver enzymes, and low platelets; early PE, early-onset preeclampsia with severe features.

[a]Reference ranges in nonpregnant cohort: Bb, ≤1.6 μg/ml; C5, 13–26.3 mg/dl; and sMAC, ≤250 ng/ml.

[b]24-hour urine protein (in milligrams).

Gilead. M.J. Wick reports serving on the board of Informed DNA. M.A.V. Willrich reports serving on the Clinical Chemistry and Laboratory Medicine (published by De Gruyter) editorial board, as chair of the Clinical Diagnostic Immunology Division of the American Association for Clinical Chemistry, and as vice-chair of the Diagnostic Immunology and Flow Cytometry Committee of the College of American Pathologists; having consultancy agreements with Sebia Inc.; and receiving research funding from Sebia Inc., Siemens Healthineers, and The Binding Site. All remaining authors have nothing to disclose.

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Author Contributions

L. Alrahmani was responsible for investigation and project administration; L. Alrahmani and M.A. Cousin were responsible for data curation; L. Alrahmani, M.A. Cousin, V.D. Garovic, M.L. Gonzalez Suarez, A.M. Moyer, K. Narang, W.M. White, M.J. Wick, and M.A.V. Willrich reviewed and edited the manuscript; L. Alrahmani, M.A. Cousin, V.D. Garovic, M.J. Wick, and M.A.V. Willrich wrote the original draft; L. Alrahmani, M.A. Cousin, M.L. Gonzalez Suarez, A.M. Moyer, and K. Narang were responsible for methodology; L. Alrahmani, V.D. Garovic, M.L. Gonzalez Suarez, A.M. Moyer, K. Narang, and W.M. White conceptualized the study; M.A. Cousin, V.D. Garovic, L.J. Tostrud, W.M. White, M.J. Wick, and M.A.V. Willrich were responsible for resources; M.A. Cousin, A.M. Moyer, L.J. Tostrud, and M.A.V. Willrich were responsible for formal analysis; V.D. Garovic and M.A.V. Willrich were responsible for validation and provided supervision; and M.A.V. Willrich was responsible for software.

Supplemental Material

This article contains the following supplemental material online at http://kidney360.asnjournals.org/lookup/suppl/doi:10.34067/KID.0000992021/-/DCSupplemental.

Supplementary Table 1. Genetic variants in complement alternative pathway genes.

Supplementary Table 2. Complement serology functional results.

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