The Influence of an Elastase-Sensitive Complement C5 Variant on Lupus Nephritis and Its Flare

Chris R. Toy1, Huijuan Song1, Haikady N. Nagaraja2, Julia Scott1, Jessica Greco1, Xiaolan Zhang1, Chack-Yung Yu3, James A. Tumlin4, Brad H. Rovin1, Lee A. Hebert1 and Daniel J. Birmingham1

1Department of Internal Medicine, Davos Heart and Lung Research Institute, Ohio State University Medical Center, Columbus, Ohio, USA; 2Division of Biostatistics, Ohio State University College of Public Health, Columbus, Ohio, USA; 3The Abigail Wexner Research Institute at Nationwide Children’s Hospital, Columbus, Ohio, and Department of Pediatrics, Ohio State University, Columbus, Ohio, USA; and 4NephroNet Clinical Research Consortium, Atlanta, Georgia, USA

Introduction: A C5 polymorphism (rs17611, 2404G>A) exists where the G allele associates with enhanced C5a-like production by neutrophil elastase. This cohort study investigated the influence of this polymorphism as a risk factor for lupus nephritis (LN), and on C5a and membrane attack complex (MAC) levels in LN during flare.

Methods: A cohort of lupus patients (n = 155) was genotyped for the 2404G>A polymorphism. A longitudinal LN subset (n = 66) was tested for plasma and urine levels of C5a and MAC 4 and/or 2 months before and at nonrenal or LN flare.

Results: The 2404G allele and 2404-GG genotype were associated with LN in black, but not white, lupus patients. In the longitudinal cohort, neither urine nor plasma C5a levels changed at nonrenal or LN flare regardless of 2404G>A genotype or race. Urine (but not plasma) C5a levels increased at LN flare independent of race, more so in 2404-GG patients where 8 of 30 LN flares exhibited very high C5a levels. Higher proteinuria and serum creatinine levels also occurred in these eight flares. Urine (but not plasma) MAC levels also increased at LN flare in 2404-GG patients and correlated with urine C5a levels.

Conclusions: The C5 2404-G allele/GG genotype is a potential risk factor for LN uniquely in black lupus patients. The GG genotype is associated with sharp increases in urine C5a and MAC levels in a subset of LN flares that correspond to higher LN disease indices. The lack of corresponding changes in plasma suggests these increases reflect intrarenal complement activation.

Kidney Int Rep (2021) 6, 2105–2113; https://doi.org/10.1016/j.ekir.2021.05.029
KEYWORDS: complement; C5a; lupus nephritis
© 2021 International Society of Nephrology. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

See Commentary on Page 2031

The role of complement in systemic lupus erythematosus is complex. The reasons for this are many and include (1) the various roles of complement in immunomodulation, immune complex processing, and tissue damage; (2) the different effector molecules involved in these roles; and (3) the different complement activation pathways that can protect or damage tissue.1,2 These complexities have complicated the use of complement changes as clinical tools in the management of lupus and LN.

Genetic variations in the complement genes also affect the utility of complement in monitoring LN disease activity and understanding LN pathogenesis. We have previously shown an example of this in the effect that a common factor H polymorphism has on the relationship between C3 activation and onset of LN flare.3 Factor H is a complement regulatory protein that controls C3 activation, and the risk for LN flare as C3 was activated was shown to be the highest in LN patients who were homozygous for the defective factor H variant.

Another example of genetic variation in complement has been reported in other diseases for a common nonsynonymous polymorphism occurring in the gene for C5. This polymorphism (rs17611) is a nucleotide substitution (2404G>A) that causes a valine to isoleucine change at amino acid 802. The original report of
this showed that the polymorphism influenced circulating C5 levels. Subsequent studies have reported that this polymorphism is associated with ischemic stroke, periodontitis, and pneumococcal meningitis outcomes. A recent study revealed the likely mechanism behind these associations, specifically that the 802V C5 form was more susceptible to cleavage by leukocyte elastase. This cleavage produced a C5a-like molecule slightly larger than recombinant C5a derived from C5 convertase cleavage of C5, but with comparable biological function as measured by Ca++ flux from C5aR1 expressing U937 cells. However, the cleavage did not lead to higher levels of soluble MAC.

Based on these associations, we hypothesized that the 2404G>A polymorphism might be relevant for LN pathogenesis and its flare. The present study was designed to address this by exploring whether this polymorphism represented a risk factor for LN onset, and by measuring, in a longitudinal 2404G>A genotyped cohort of LN patients, plasma and urine levels of C5a and MAC during periods leading to flare.

**METHODS**

**Patient Cohort**

The patients in this study were from the Ohio SLE Study (OSS), a large cross-sectional and longitudinal study of both nonrenal lupus patient and LN patients recruited between 2001 and 2005 under institutional review board–approved informed consent and in accordance with the Declaration of Helsinki. The entire longitudinal cohort (N = 104), which was followed at regular bimonthly intervals (with blood and urine collections at each visit) for as long as 7 years (median: 46 months), was genotyped for 2404 G>A polymorphism. In addition, the black patients from the cross-sectional cohort of the OSS (N = 51) were included to strengthen the analysis in this patient group. Table 1 shows the baseline demographic and clinical data for the cohorts.

Table 1 also shows, by race and disease type (nonrenal or LN), the number of longitudinal patients experiencing at least one flare (nonrenal or LN flare), the number of flares, and the number of flares that were tested for urine and ethylenediamine tetraacetic acid–plasma levels of C5a and MAC. This testing was limited to longitudinal LN patients and included all moderate to severe flares visits (both nonrenal and LN flares) in which urine samples were available (37 of 40 LN patients with flare). Plasma samples from the same dates were also tested. Also included in the testing were all available samples from 2 and/or 4 months before each flare. The three visit months (4 and 2 months before flare and at flare) were together termed a flare interval.

Flares were identified in the OSS cohort by prespecified criteria and were classified as mild, moderate,
or severe, as we have previously described.\textsuperscript{3,9–12} Briefly, a nonrenal flare was declared if a patient developed or had increased severity in one or more symptoms or signs of nonrenal systemic lupus erythematosus in the absence of increased renal manifestations that were attributable to systemic lupus erythematosus and were of sufficient severity that the managing OSS study physician increased therapy. An LN flare was declared based on threshold changes in urinalysis or in proteinuria or serum creatinine. For the present study, only moderate to severe flares were included.

**Genotyping the C5 2404 G>A Polymorphism**
The C5 2404G>A polymorphism was genotyped by amplifying leukocyte DNA using TGCAATGTC-TAAAACATGCAAT as the forward primer, and AAAAGAGGAGGTGAAAGGAGAAA as the reverse primer. Each amplicon was visualized in 1% agarose under ethidium bromide staining, and sequenced using a 3730 DNA Analyzer (Applied Biosystems, Inc., Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) through the Ohio State University Genomics Shared Resource. The genotype for each patient was determined through visual inspection of the sequence chromatographs.

**Measurements of Free C5a and Soluble MAC**
Sandwich enzyme-linked immunosorbent assays (ELISAs) were developed for free C5a and soluble MAC for measurements in urine and plasma. For C5a, wells were coated with mouse anti-C5a antibody (clone 295003, R&D Systems, Minneapolis, Minnesota, USA) at 2 ug/ml. Patient samples of urine or ethylenediamine tetraacetic acid–plasma, typically diluted 1/2 or 1/40, respectively, in 6% bovine serum albumin in 0.01 M phosphate buffer with 0.15 M NaCl (phosphate-buffered saline), and 0.015 M ethylenediamine tetraacetic acid, were added and incubated for 1 hour at room temperature. Bound C5a was detected using biotinylated mouse anti-C5a (R&D, clone 295009), followed by the addition of streptavidin horseradish peroxidase (R&D), and developed with 3,3’,5,5’-tetramethylbenzidine. A standard curve of purified C5a (Complement Technology, Tyler, Texas, USA) was used in each assay plate, and all samples were normalized by urine creatinine (uCr) content. This ELISA had a lower limit of detection of 100 pg/ml urine and an interassay coefficient of variation of 3.9%. The ELISA was specific for free C5a, as shown by measuring the amount of C5a in plasma samples from five normal healthy individuals, where less than 1% of the amount of C5a in intact plasma C5 was detected. This ELISA was also compared to a commercial C5a ELISA (Quidel Corporation, San Diego, California, USA), commonly reported in studies measuring C5a, for 27 patient urine and plasma samples. The correlation between the two assays was 0.93 for the urine and 0.84 for plasma ($P < 0.0001$ for both).

The same general ELISA protocol described above was used to measure soluble MAC in urine and plasma, except the coating antibody was a monoclonal specific for a neoepitope in the MAC (Quidel), the detection antibody was a biotinylated rabbit polyclonal raised against soluble MAC (Complement Technology), and the plasma samples were diluted 1/10. A standard curve of purified soluble MAC (Complement Technology) was used in each assay plate. This ELISA had a lower limit of detection of 12.5 ng/ml urine and an interassay coefficient of variation of 7.9%. As with C5a, this MAC ELISA was compared to a standard commercial MAC assay (Quidel) for the same 27 samples. The correlation between the two assays was 0.83 for the urine and 0.79 for plasma ($P < 0.0001$ for both).

**Western Blot Analysis of Urine C5a**
Urine samples containing four of eight highest C5a levels at LN flare, all with the 2404-GG genotype, were analyzed by western blot analysis under nonreduced conditions. The four urine samples were diluted to 8 ng C5a/ml in 1× lithium dodecyl sulfate buffer (Thermo Fisher), and 10 ul of each, along with purified C5a (3 ng/ml) for comparison (Complement Technology), were electrophoresed through NuPAGE 4% to 12% Bis-Tris Protein Gels (Thermo Fisher), electroblotted to nitrocellulose, probed with either biotinylated goat anti–C5a (R&D, 0.5 ug/ml), or biotinylated goat immunoglobulin G (R&D, 1 ug/ml) as a negative control, followed by streptavidin horseradish peroxidase, and visualized by autoradiography using SuperSignal West Femto chemiluminescence (Thermo Fisher).

**Statistical Analyses**
The Fisher exact test was used to assess for differences among 2404G>A genotypes, and for differences in allele frequencies, between black and white patients, and between nonrenal and LN OSS patients.

For the longitudinal data, regression models using analysis of variance methodology applicable to repeated measures data (SAS/JMP, Cary, North Carolina, USA) were used to determine if analyte levels changed temporally in relation to LN flare for all analytes except for the urine MAC data. For these models, the flare interval month (-4, -2, or 0 [at flare]) was set as the fixed effect, the flare interval was set as random effects, and the analyte levels were used as the response, with an alpha level for significance set at 0.05. Only flare intervals with at least one preflare...
value were included in the regression models, and we assume that missing data were generated by the “missing completely at random” mechanism. Neither the effects of age nor race were found to be significant. Because of sample size restrictions, the data for 2404-GA and 2404-AA patients were combined and analyzed separately from the 2404-GG patients. For each analysis, two models were run, one using untransformed analyte levels, and a second using data that was transformed by square root (to account for values of 0). The final model that was selected was the one that provided the best fit, as reflected by (1) higher R², a measure of model fit; and (2) distribution of residuals being closer to the normal distribution. For final models with \( P < 0.05 \), Tukey’s honest significant difference test was run to determine where these differences occurred (-4 vs. -2, -4 vs. 0, and -2 vs. 0).

The urine MAC data contained too many values below the detectable limit (and assigned a value of “0”) to meet the regression model assumption of random variability in the observations. As an alternative approach, these data were analyzed by paired \( t \) tests or the Wilcoxon test, as appropriate, using a Bonferroni correction of 0.05/3 (thus, \( P < 0.0167 \)) for the cutoff value for significance for the 3 comparisons (-4 vs. -2, -4 vs. 0, and -2 vs. 0). A linear regression model was used to determine if urine C5a levels correlated with urine MAC levels.

Differences in clinical variables between high and low urine C5a expressers were determined by unpaired \( t \) tests of natural log-transformed data.

**RESULTS**

**Influence of C5 2404 G>A Genotype on LN**

The G allele has been reported to be higher in blacks than in whites in the general population (0.91 vs. 0.56 allele frequency, Grand Opportunity Exome Sequencing Project: https://esp.gs.washington.edu/drupal/). Because of this, the genotype and allele frequencies in the lupus patients in this study were analyzed for blacks separately from whites. Table 2 shows both genotype and allele frequencies determined in 90 black lupus patients (29 nonrenal patients, 61 LN patients) and 65 white lupus patients (24 nonrenal patients, 41 LN patients). Overall, the GG genotype and G allele frequencies were higher in the black lupus cohort compared to the white cohort, as expected (\( P < 0.001 \) for both frequencies). The black LN patients also had higher GG genotype and G allele frequencies compared to black nonrenal patients (\( P = 0.008 \) and \( P = 0.004 \), respectively). These differences were not observed with the white patient cohort.

**Influence of the C5 2404 G>A Genotype on Urine and Plasma C5a and MAC Levels at Flare**

Of the 40 LN patients from the longitudinal cohort who were included in this study, 23 patients were genotyped as 2404-GG, 14 patients were genotyped as 2404-GA, and 3 patients were genotyped as 2404-AA. Because of the small sample size for the 2404-AA cohort, their data were pooled with the data from the 2404-GA LN cohort (from 13 tested nonrenal flares and 23 tested LN flares), and statistical analyses were performed for this group separate from the 2404-GG patients (with 13 nonrenal flares tested and 30 LN flares tested) to assess the influence of this polymorphism on C5a levels.

Urine C5a increased in both genotype groups at the time of LN flare (Figure 1a). However, the degree of increase was greater in the 2404-GG cohort, with 8 of 30 LN flares having urine C5a levels greater than 10 ng/mg uCr, ranging from 12.2 to 88.7 ng/mg uCr. These eight LN flares were from six different LN patients (3 black and 3 white). Parallel measurements in plasma did not reflect the urine changes, instead showing no change in C5a levels during LN flare intervals (Figure 1b). For nonrenal flares, neither urine nor plasma C5a levels changed during flare intervals (Figure 1c and d).

To assess if increases in urine C5a levels led to corresponding changes in MAC levels, urine and plasma samples from LN flare intervals were also tested for MAC levels. Urine MAC levels did not change

**Table 2. Genotype and Allele Frequencies of the C5 2404G>A Polymorphism for Black and White Nonrenal Lupus and Lupus Nephritis Cohorts**

| Race | Type | 2404 Genotype | 2404 Allele |
|------|------|---------------|-------------|
|      |      | GG | GA | AA | %GG | %GA | %AA | \( P^a \) | G | A | %G | %A | \( P^b \) |
| B    | All  | 72 | 17 | 1  | 80a | 19 | 1   | 0.008 | 161 | 19 | 89 | 11 | 0.004 |
| B    | NR   | 18 | 10 | 1  | 62  | 34 | 3   | 0.008 | 46  | 12 | 79 | 21 | 0.004 |
| B    | LN   | 54 | 7  | 0  | 89  | 11 | 0   | 0.008 | 115 | 7  | 94 | 6  | 0.004 |
| W    | All  | 27 | 29 | 9  | 42a | 45 | 14  | 0.605 | 83  | 47 | 64 | 36 | 0.450 |
| W    | NR   | 12 | 9  | 3  | 50  | 38 | 13  | 0.605 | 33  | 15 | 69 | 31 | 0.450 |
| W    | LN   | 15 | 20 | 6  | 37  | 49 | 15  | 0.605 | 50  | 32 | 61 | 39 | 0.450 |

\( B \), black; LN, lupus nephritis; NR, nonrenal; W, white.

\( a \)The GG genotype and G allele frequencies are significantly higher in the overall black lupus cohort than the white lupus cohort (\( P < 0.001 \) for both comparisons, by Fisher exact test).

\( b \)\( P \) values are for NR vs. LN comparisons.
significantly in the 2404-GA/AA cohort during LN flare intervals, although there was an apparent trend for increasing levels at flare (Figure 2a). In contrast, urine MAC levels increased significantly in the 2404-GG cohort at LN flare and correlated with urine C5a levels ($r = 0.742, P < 0.001$), with seven of the eight samples with the highest urine C5a levels also having the highest urine MAC levels (Figure 2a, filled circles). As with C5a, plasma levels of MAC did not change in either genotype cohort during LN flare intervals (Figure 2b).

Table 3 summarizes the $P$ values from the various models for changes over time of urine and plasma C5a and MAC levels in the C5 2404-GG and 2404-GA/AA cohorts during the LN flare intervals (Figures 1 and 2).

**High Urine C5a Levels and LN Disease Activity**

To determine if there was a relationship between the high urine C5a levels observed in 8 of 30 2404-GG urine samples at LN flare (Figure 1a) and the degree of LN disease activity, the urine protein/creatinine ratios and serum creatinine levels were compared between the two groups. The levels of both of these renal disease indicators were higher in the eight high–C5a level urine samples compared to the low–uC5a samples (Figure 3a and b).

**Western Blot Analysis of Urine C5a**

Urine samples from four of eight at-flare, high–C5a urine samples from 2404-GG LN patients (Figure 1a) were analyzed by western blot under nonreducing conditions to identify the size of the urine C5a detected by ELISA. As can be seen in Figure 4, all four urine samples (lanes 9 through 12) contained a urine C5a fragment of the same relative molecular weight as purified urine C5a (lane 7). There was also a larger band of approximately 65 kDa that was uniquely identified by
the anti-C5a antibody in two of four LN urine samples (compared to control lanes 3 through 6).

**DISCUSSION**

The present study addressed whether a polymorphism in the C5 gene (2404G>A, amino acid V802A) that influences C5 cleavage by neutrophil elastase plays a role in LN and its flare. The data presented here suggest that it does in two ways. First, in a cohort of lupus patients, both the 2404G allele and the 2404-GG genotype frequencies were significantly higher in black LN patients, compared to black nonrenal lupus patients, identifying the G allele as a potential risk factor for LN onset. These differences were not observed in the white lupus cohort. Second, in approximately 25% of LN flares from patients with the 2404-GG genotype, the onset of LN flare resulted in sharp increases in urine C5a, and a corresponding increase in MAC levels, independent of race, and corresponding increases in LN disease measurements. Although these associations do not prove causality, they do provide an important link between a common functional C5 polymorphism and LN and its severity during active disease.

It is unclear why the apparent role of the 2404G allele and 2404-GG genotype as a risk factor for LN onset is limited to black lupus patients. The OSS cohort used in this study is limited in size, and the findings will need to be confirmed in an independent cohort. Nevertheless, based on the current findings, these data may simply suggest that complement activation products (C5a and/or MAC) have a stronger contribution in blacks to the development of LN during the early stages of lupus onset. Or this may reflect that black lupus patients are generally more sensitive to LN manifestations, and C5a and/or MAC may have a

---

**Figure 2.** Levels of membrane attack complex (MAC) in the urine (a) and plasma (b) during lupus nephritis (LN) flare intervals. Individual levels are shown as open circles, and least squares means are shown as lines. The top seven urine values at LN flare (solid circles) correspond to seven of eight urine samples with the highest C5 levels (Figure 1a). uCr, urine creatinine.

---

**Table 3.** Summary of P Values From the Various Models for Changes Over Time for Urine and Plasma C5a and MAC Levels in the C5 2404-GG and 2404-GA/AA Cohorts

| 2404 G>A Genotype | Response Variable | Model/Method | P Value | Time Effect | Month-4 vs. -2 | Month-4 vs. -0 | Month-2 vs. 0 |
|-------------------|------------------|--------------|---------|-------------|---------------|---------------|---------------|
| GG                | SqRt uC5a        | LMM          | <0.001  | 0.684d      | <0.001c       | <0.001c       |               |
| GG                | pC5a             | LMM          | 0.858   |             |               |               |               |
| GAAA              | SqRt uC5a        | LMM          | 0.008   | 0.973d      | 0.038d        | 0.011d        |               |
| GAAA              | pC5a             | LMM          | 0.924   |             |               |               |               |
| GG                | uMAC             | Wilcoxon test | NA      | 0.500d      | 0.056d        | <0.001d       |               |
| GG                | SqRt pMAC        | LMM          | 0.168   |             |               |               |               |
| GAAA              | uMAC             | Paired t or Wilcoxon test | NA | 0.125d | 0.023d | 0.074d |               |
| GAAA              | SqRt pMAC        | LMM          | 0.053   |             |               |               |               |

LMM, linear mixed model; MAC, membrane attack complex; p, plasma; SqRt, square root; u, urine.

*As shown in Figures 1 and 2.

*Square-root transformed data.

*P values based on post hoc Tukey’s honest significant difference test (under 0.05 is significant).

*P values from Wilcoxon test.

*P values from paired t test. P value for uMAC significance was set at 0.0167 to account for the three comparisons.
greater impact in pushing kidney damage into bona fide LN. Finally, there may be other complement polymorphisms associated with the black population that accentuate the pro-LN effects of C5a or MAC.

The mechanism by which this C5 polymorphism affects urine C5a levels in the LN patients at LN flare, and does so in only a subset of the flares, is also unclear. The report describing the neutrophil elastase sensitivity of the 802V variant showed no enhanced C5 cleavage by C5-convertase. Neutrophils are involved in active LN, and neutrophil elastase may be driving the high levels of urine C5a in some of the 2404-GG patients. However, the size of C5a in the LN patient urines shown herein is identical to C5a derived from C5 convertase. There is also a corresponding increase in urine MAC. Elastase cleavage of C5 results in a slightly larger form of C5a, without a corresponding increase in MAC formation. Together this suggests that some other proteolytic mechanism affected by the 2404 G>A polymorphism is behind this seemingly non-complement driven C5 cleavage.

Elastase is a serine protease, and other serine proteases have been shown to cleave C5, resulting in

Figure 3. Urine protein/creatinine ratios (uPCR) (a) and serum creatinine (sCr) levels (b) in C5 2404-GG patients at lupus nephritis (LN) flare where urine C5a (uC5a) levels were greater than 10 ng/mg urine creatinine (uCr) (>10) or less than 10 ng/mg uCr (<10). Individual levels are shown as open circles, and median levels are shown as lines.

Figure 4. Western blot analysis of urine C5a. The blots of urine samples from four of eight highest C5a levels from 2404-GG patients at lupus nephritis flare (lanes 3 through 6 and 9 through 12) were probed with either nonspecific goat immunoglobulin G (lanes 1 through 6) or goat anti-C5a (lanes 7 through 12). Lanes 1 and 7 contained purified C5a, and lanes 2 and 8 were blank. The numbers on the far left are the molecular weight marker positions. The dotted line arrow identifies approximately 65-kDa fragments containing C5a, and the solid arrow identifies C5a.
functional C5a-like products. These include both thrombin and plasmin, proteases that become activated during thrombotic events and fibrinolysis. The occurrence of thromboses in association with lupus has long been recognized. Thus, the high levels of urine C5a occurring in the subset of the LN cohort at flare shown in this study may be driven by thrombin and/or plasmin. Thrombin produces a 35-kDa C5a-like fragment that eventually is reduced to a size identical to complement-driven C5a; C5 cleavage by thrombin or plasmin leads to MAC formation. Other possible serine proteases that may be affected by the elastase-sensitive C5 polymorphism include urokinase-type plasminogen activator, plasminogen, and prostatin, all of which are elevated in the urine of albuminuric kidney transplant recipients. Importantly, it is not known if cleavage of C5 by thrombin, plasmin, or any other serine protease is affected by this elastase-sensitive polymorphism.

The corresponding increase in urine MAC levels in the high urine C5a samples suggests that the resulting C5b fragment is capable of combining with C6-C9 to form MAC. Alternatively, high urine C5a levels may drive increased tissue damage at flare that leads to more complement activation and subsequent MAC formation. There is evidence for such a pathogenic consequence, with the high urine C5a levels being associated with more proteinuria and higher serum creatinine levels (lower glomerular filtration rate).

These findings also have implications as to the site of complement activation. Plasma levels of C5a did not change during the LN flare interval, but interpreting this is difficult, as C5a is approximately 10 to 11 kDa and positively charged, and any C5a formed in the circulation may be freely filtered into the urine. However, plasma MAC levels also did not change and, at a molecular weight of more than 1000 kDa, any MAC formed in the circulation would likely not be readily filtered even during proteinuric states, indicating that the increases in urine C5a and MAC indeed represent intrarenal complement activation.

The results of this study do not show casualty between the 2404G>A C5 polymorphism and LN onset (in blacks) or high levels of C5a at LN flare. These associations may be due to other variations in genes proximal to the C5 gene that linked to the 2404G>A polymorphism. One such candidate is the gene for tumor necrosis factor receptor associated factor 1 (TRAF1), which is an important in mediating tumor necrosis factor alpha signaling. In fact, studies have identified a TRAF1/C5 locus (rs10818488) being associated with lupus in some, but not all, studies, as recently reported in a meta-analysis. Importantly, this TRAF/C5 haplotype block does not contain the 2404G>A polymorphism, and so does not account for the associations shown in the current study. Nevertheless, until causality is shown, other proximal genetic variation that may be causal should be considered.

In summary, the present study implicates the C5 2404G allele that is associated with enhanced elastase-sensitive C5a-like production as an important contributor in the pathogenesis of LN, both as a risk factor for LN onset in black lupus patients, and as a driver of high urine C5a levels and MAC levels at the time of LN flare that appear to be local and are associated with more severe LN flare. That this occurred in only approximately 25% of the LN flares in patients with the 2404-GG genotype indicates that whatever protease is cleaving this C5 variant, its involvement is sporadic. Identifying this protease along with determining the 2404G>A genotype of LN patients may be an important screening tool for determining in whom complement-based therapies, and the target of those therapies, is warranted.

**DISCLOSURE**

JAT is a consultant for Alexion Pharmaceuticals.

BHR is a consultant for Alexion Pharmaceuticals, Aurinia, Callidatis, Chemocentryx, Retrophin, Novartis, Morphosys, EMD Serono, Bristol Myers Squibb, Janssen, Astra Zeneca, and Omeros. The remaining authors have nothing to disclose.

**ACKNOWLEDGMENTS**

The study was funded by NIH R01 AR073311 (CYY, DJB).

**SUPPLEMENTARY MATERIAL**

Supplementary File (PDF)

**STROBE Statement.**

**REFERENCES**

1. Pickering MC, Botto M, Taylor PR, et al. Systemic lupus erythematosus, complement deficiency, and apoptosis. *Adv Immunol*. 2000;76:227–324.

2. Birmingham DJ, Hebert LA. The complement system in lupus nephritis. *Semin Nephrol*. 2015;35:444–454.

3. Birmingham DJ, Irshaid F, Nagaraja HN, et al. The complex nature of serum C3 and C4 as biomarkers of lupus renal flare. *Lupus*. 2010;19:1272–1280.

4. Hillebrandt S, Wasmuth HE, Weiskirchen R, et al. Complement factor 5 is a quantitative trait gene that modifies liver fibrogenesis in mice and humans. *Nat Genet*. 2005;37:835–843.

5. Greisenegger S, Zehetmayer S, Bauer P, et al. Polymorphisms in inflammatory genes and the risk of ischemic stroke and transient ischemic attack: results of a multilocus genotyping assay. *Clin Chem*. 2009;55:134–138.

6. Chai L, Song YQ, Zee KY, et al. Single nucleotide polymorphisms of complement component 5 and periodontitis. *J Periodontal Res*. 2010;45:301–308.
7. Woehrl B, Brouwer MC, Murr C, et al. Complement component 5 contributes to poor disease outcome in humans and mice with pneumococcal meningitis. *J Clin Invest*. 2011;121:3943–3953.

8. Giles JL, Choy E, van den Berg C, et al. Functional analysis of a complement polymorphism (rs17611) associated with rheumatoid arthritis. *J Immunol*. 2015;194:3029–3034.

9. Birmingham DJ, Gavit KF, McCarty SM, et al. Consumption of erythrocyte CR1 (CD35) is associated with protection against systemic lupus erythematosus renal flare. *Clin Exp Immunol*. 2006;143:274–280.

10. Birmingham DJ, Nagaraja HN, Rovin BH, et al. Fluctuation in self-perceived stress and increased risk of flare in patients with lupus nephritis carrying the serotonin receptor 1A -1019 G allele. *Arthritis Rheum*. 2006;54:3291–3299.

11. Birmingham DJ, Hebert LA, Song H, et al. Evidence that abnormally large seasonal declines in vitamin D status may trigger SLE flare in non-African Americans. *Lupus*. 2012;21:855–864.

12. Birmingham DJ, Bitter JE, Ndukwe EG, et al. Relationship of circulating anti-C3b and anti-C1q IgG to lupus nephritis and its flare. *Clin J Am Soc Nephrol*. 2016;11:47–53.

13. Gonzalez LA, Toloza SM, Alarcon GS. Impact of race and ethnicity in the course and outcome of systemic lupus erythematosus. *Rheum Dis Clin North Am.* 2014;40:433–454. vii–viii.

14. Nishi H, Mayadas TN. Neutrophils in lupus nephritis. *Curr Opin Rheumatol*. 2019;31:193–200.

15. Wetzel RA, Kolb WP. Expression of C5a-like biological activities by the fifth component of human complement (C5) upon limited digestion with noncomplement enzymes without release of polypeptide fragments. *J Exp Med*. 1983;157:2029–2048.

16. Amara U, Flierl MA, Rittirsch D, et al. Molecular intercommunication between the complement and coagulation systems. *J Immunol*. 2010;185:5628–5636.

17. Krisinger MJ, Goebeler V, Lu Z, et al. Thrombin generates previously unidentified C5 products that support the terminal complement activation pathway. *Blood*. 2012;120:1717–1725.

18. Castiblanco-Valencia MM, Fraga TR, Pagotto AH, et al. Plasmin cleaves fibrinogen and the human complement proteins C3b and C5 in the presence of Leptospira interrogans proteins: a new role of LigA and LigB in invasion and complement immune evasion. *Immunobiology*. 2016;221:679–689.

19. Petri M. Thrombosis and systemic lupus erythematosus: the Hopkins Lupus Cohort perspective. *Scand J Rheumatol*. 1996;25:191–193.

20. Foley JH, Walton BL, Aleman MM, et al. Complement activation in arterial and venous thrombosis is mediated by plasmin. *EBioMedicine*. 2016;5:175–182.

21. Hinrichs GR, Michelsen JS, Zachar R, et al. Albuminuria in kidney transplant recipients is associated with increased urinary serine proteases and activation of the epithelial sodium channel. *Am J Physiol Renal Physiol*. 2018;315:F151–F160.

22. Xu K, Peng H, Zhou M, et al. Association study of TRAF1/C5 polymorphism (rs10818488) with susceptibility to rheumatoid arthritis and systemic lupus erythematosus: a meta-analysis. *Gene*. 2013;517:46–54.

23. Kurreeman FA, Padyukov L, Marques RB, et al. A candidate gene approach identifies the TRAF1/C5 region as a risk factor for rheumatoid arthritis. *PLoS Med*. 2007;4:e278.