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A polymorphism in the type one complement receptor (CR1) involves an additional cysteine within the C3b/C4b binding domain that inhibits ligand binding

Daniel J. Birmingham^{*,1}, Fawzi Irshaid², Katherine F. Gavit¹, Haikady N Nagaraja³, C. Yung Yu⁴, Brad H. Rovin¹, and Lee A Hebert¹

*1*Division of Nephrology, Department of Internal Medicine, The Davis Heart and Lung Research Institute, The Ohio State University, Columbus, Ohio.

*3*The Department of Statistics, The Ohio State University, Columbus, Ohio.

*4*The Children's Research Institute, The Ohio State University, Columbus, Ohio.

*2*Al al-Bayt University, Mafrqa, Jordan

Abstract

The type one complement receptor (CR1) contains a variable number of binding domains for C3b and C4b, formed through a nearly identical set of repeating units known as short consensus repeats (SCRs). Each SCR contains 4 cysteines that, by forming two disulfide bonds, impart a conformation critical for function. In this study, we identified a CR1 single nucleotide polymorphism (1597C>T) that results in an additional cysteine (483R>C) in SCR 8 of the N-terminal C3b/C4b binding domain, and occurring sporadically in corresponding SCRs of other repeated C3b/C4b binding domains. The normal carrier frequency for 483-C was 6.3% in 175 African Americans, and 2.4% in 153 Caucasians. In expression constructs containing one C3b/C4b binding domain, the 483-C residue reduced binding to C3b, C3bi, and C4b by over 80% (each $p < 0.0001$), versus the wildtype construct. Full-length CR1 from 483-C carriers also exhibited reduced binding to C3b and C4b, although the effect was influenced by the total number of binding domains present. Race-matched comparisons between SLE patients (86 African Americans, 228 Caucasians) and the normal cohort showed that 483-C carrier status alone is not a risk factor for SLE or lupus nephritis. The physiological role of this polymorphism remains to be determined.

Keywords

CR1; polymorphism; complement; SLE

1. Introduction

The type one complement receptor (CR1, CD35) is an integral membrane glycoprotein that binds the cleaved products of the third (C3b, C3bi) and fourth (C4b) component of the complement system (Krych-Goldberg and Atkinson, 2001). While CR1 is expressed on most

* Address correspondence to Daniel J. Birmingham, Ph.D., Division of Nephrology, Ohio State University, N210 Means Hall, 1654 Upham Dr. Columbus, OH 43210, Telephone: 614-688-3842, Fax: 614-293-3073, E-mail: Dan.Birmingham@osumc.edu

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circulating cells, in humans, approximately 90% of the total circulating pool is found on erythrocytes, with an average level of 400-500 receptors per erythrocyte. This weighted distribution has physiological consequences, as complement-opsonized immune complexes (IC), containing C3b and C4b, are bound by erythrocytes in the circulation through a process known as immune adherence (Birmingham and Hebert, 2001). Through the process of immune adherence, erythrocyte CR1 (E-CR1) promotes the safe removal of IC from the circulation.

The gene for CR1 is located in a cluster on chromosome 1 at q32. Members encoded at this cluster, termed the regulators of complement activation (RCA), all share a repeating unit, known as the short consensus repeat (SCR), also known as the complement control protein repeat (CCP) (Hourcade, Holers and Atkinson, 1989). These SCRs, ranging from approximately 60 to 70 amino acids, contain a number of consensus residues, the most invariant being four cysteines that form two disulfide bonds that impart a double looped structure considered critical for function.

The extracellular domain of CR1 is composed entirely of SCRs, and a pattern exists in some regions of CR1 where every 8th SCR shares greater than >98% identity (Hourcade et al., 1988; Klickstein et al., 1987). This intramolecular homology results in repeating ligand binding domains. The most N-terminal domain involves SCR 1, 2, and 3 and binds predominantly C4b, while SCRs 8, 9, and 10 form a binding domain for both C3b and C4b, a domain which is repeated in SCRs 15, 16, and 17 (Kalli et al., 1991; Klickstein et al., 1988; Krych, Hourcade and Atkinson, 1991).

The CR1 gene is expressed from a single locus as four allotypes ranging from 190,000 M_r to 280,000 M_r (Dykman et al., 1983; Dykman et al., 1985; Dykman, Hatch and Atkinson, 1984). The two most common size allotypes are the 220,000 M_r A allotype (~80% allele frequency) and the 250,000 M_r B allotype (~15% allele frequency). The difference in size is due to the presence of 30 total SCRs in the A allotype, and 37 total SCRs in the B allotype (Wong et al., 1989). The additional 7 SCRs in the B allotype impart a third C3b/C4b binding domain between the first and second. Thus three C3b binding domains occur in the B allotype, at SCRs 8-10, SCRs 15-17, and SCRs 22-24. Although it isn't clear whether all of the binding domains on a single CR1 molecule participate in the binding of complement opsonized IC, the B allotype appears to bind C3b dimers with higher avidity than the A allotype (Wong and Farrell, 1991).

We have postulated that E-CR1, by mediating immune adherence, represents a critical component for the appropriate clearance of IC from the circulation, and as such contributes to the prevention of IC diseases such as SLE (Birmingham and Hebert, 2001; Hebert et al., 1991; Hebert and Cosio, 1987). The involvement of CR1 in SLE has long been recognized through numerous studies showing that detectable E-CR1 levels are abnormally low in SLE (Walport and Lachmann, 1988). We have recently shown that fluctuations in detectable E-CR1 levels occur rapidly and routinely in SLE patients with active disease and are likely the consequence of normal E-CR1 function, and that loss of this function is associated with kidney damage during disease flare (Birmingham et al., 2006). Together, these data imply that defects in E-CR1 that affect function or expression would be risk factors for SLE or its nephritis. The purpose of the present study was to determine if functional genetic variability exists in the region encoding the C3b/C4b binding domain, and if so, whether it represents risk for SLE or its nephritis.

2. METHODS

2.1 Subjects

Whole blood was collected in EDTA from normal healthy volunteers and SLE patients following informed consent, and with adherence to the Declaration of Helsinki, as determined

by the Ohio State University human subjects Institutional Review Board. The normal volunteers included 175 African Americans and 153 Caucasians from central Ohio. The SLE patients (86 African Americans and 228 Caucasians) were recruited from the Division of Nephrology and Division of Rheumatology at The Ohio State University, and met at least 4 of 11 of the revised 1982 ACR criteria for the diagnosis of SLE.

2.2 Identification of CR1 polymorphisms

RNA was isolated from 4 ml of blood using RNeasy (Qiagen, Crawfordsville, IN). DNA was isolated from 1 ml of blood using Puregene (Gentra Systems, Minneapolis, MN), as previously described (Birmingham et al., 2003).

Initial identification of the CR1 single nucleotide polymorphism (SNP) reported herein within SCR 8 was done through cDNA analysis of the sequence encoding N-terminal C3b/C4b binding domain in a panel of Caucasians and African Americans. Specifically, from individuals of the AA size genotype, CR1 cDNA segment encoding SCRs 7 through 13 were amplified using the sense primer GGAAAACCTCTGGAAGTCTT and the antisense primer GAACACTGCTATTCCAAAGG, as previously described (Birmingham et al., 1994). The PCR products were directly sequenced, and the polymorphism were identified by visual inspection.

2.3 Identification of SNP carriers

The 1597C>T polymorphism was characterized in the normals and SLE cohort using genomic DNA and the SnapShot multiplex methodology (Applied Biosystems, Foster City CA), following the manufacturers protocol. Briefly, a 225 bp region of the CR1 gene containing the exon for SCR 8 was amplified using a sense primer from the 5' end of SCR 8 (GTCACCGTCAAGCCCCAGATC) and an antisense primer from intronic sequence downstream from the SCR 8 exon (GAAGCTTTTGCAGACATGGG). These primers also match 100% with the comparable region of SCR 15 of the A and B size alleles, and with SCR 22 of the B size allele (UCSC Genome Bioinformatics Website). Following amplification, the PCR products were treated with shrimp alkaline phosphatase and *Exo* I to digest and remove the primers and dNTPs. Primer-extension reactions were then performed in the presence of dye-labeled ddNTPs using the PCR products as templates along with a 23 nucleotide antisense sequence (TGGGCTCACGTAGTACTCAGGAC) that bordered on the 3' end with the 1597 residue. Following primer extension and incorporation of dye-labeled ddNTP, the reactions were treated with calf intestinal phosphatase and electrophoresed on an ABI PRISM 3700 DNA analyzer. Identification of the incorporated ddNTP was made visually from the resulting chromatograph.

Due to 100% homology between the 225 bp sequence surrounding SCR 8 and SCR 15 for the A size allele, and SCR 8, SCR 15, and SCR 22 for B size allele, determinations of the exact SCR and the total number of variant alleles in a given individual were not possible using genomic DNA. Thus, those individuals identified as having at least one variant were reported as "carriers". To gain insight as to different ways the 483-C variant residue occurred, we amplified cDNA from those normal carriers for whom RNA and data on CR1 size genotypes (see below) were available. Specifically amplification of CR1 cDNA using primers from SCR 7 (sense) and SCR 13 (antisense), as described above, yielded sequence that included SCR 8 from the A size allele, and both SCR 8 and SCR 15 from the B size allele (Fig 2). Amplification using primers from SCR 13 (sense: GACTTCATGGGCCAACTTCT) and SCR 19 (antisense: GTGAAAAGTTGTCCTGATGG) yielded sequence encoding the C-terminal C3b/C4b binding domain, including sequence for SCR 15 for the A allele and SCR 22 for the B allele.

2.4 Functional effects of the CR1 polymorphism

To determine the effect of the 483R>C polymorphism on CR1 ligand binding domains, single binding domain constructs were used. These constructs were prepared originally from a naturally occurring alternative splice product of chimpanzee CR1 (Birmingham et al., 1994) in which the chimp sequence was inserted into the expression vector pBK-CMV (Stratagene, San Diego, CA), and then exchanged for human sequence, as we've previously described (Birmingham et al., 2003). The specific construct from that study that was used in the current study contained sequence SCRs 8-11, SCRs 5-6, and SCRs 28-30, followed by the CR1 transmembrane and cytoplasmic domain. This construct was used either expressing 100% wildtype sequence (termed WT), or expressing the 483-C variant. The variant was introduced into the wildtype construct by replacing the wildtype SCR 8-11 sequence with the same region amplified from cDNA of 483-C carriers, as we've previously described (Birmingham et al., 2003). Clones containing the variant residue were sequenced in entirety to ensure 100% sequence fidelity.

The two constructs were transiently expressed in HEK 293 cells using Lipofectamine (Invitrogen), and then tested for relative ligand binding activity, as previously described (Birmingham et al., 2003). The pBK-CMV plasmid alone was also used to transfect HEK 293 as a negative control. In brief, solubilized construct proteins were incubated in ELISA plate wells previously coated with C3b, C3bi or C4b (Advanced Research Technologies, San Diego, CA). To ensure equal concentrations, wells were also coated with antibody to the C-terminus of CR1 (Santa Cruz, Biotechnology, Inc., Santa Cruz, CA). Bound protein was detected with a biotinylated non-blocking anti-CR1 mAb (E11, Ancell Corp., Bayport, MN), and the data were read as OD₄₉₀ following the addition of streptavidin conjugated with horseradish peroxidase (Zymed/Invitrogen, Carlsbad, CA). The data were normalized within each plate by subtracting the OD values exhibited by the membranes isolated from cells transfected with pBK-CMV alone (negative controls), and then dividing by the OD values for the anti-CR1 coated wells. Three replicate assays (plates) were performed, and samples were assessed in duplicate within each assay.

To determine the effects of these polymorphisms on ligand binding by full-length CR1, erythrocyte CR1 (E-CR1) from normal healthy 483-C carriers for which fresh erythrocyte samples were available (4 AA size genotypes, 4 AB size genotypes) were tested for differences in ligand binding activity compared to a panel of wildtype E-CR1 matched for CR1 size genotype (5 AA size genotypes, 4 AB size genotypes). After isolating and solubilizing erythrocyte membranes, solubilized E-CR1 preparations were adjusted by dilution in 0.01M Tris, 0.1 M NaCl, 1% Igepal (Sigma Chemical Company, St. Louis, MO) to achieve approximate equal concentrations, and tested for ligand binding to C3b and C4b as described above, with the following modification. To ensure comparable E-CR1 concentrations, wells were coated with E11, and bound E-CR1 was detected using chicken anti-CR1 antibody (Accurate), followed by HRP-labeled rabbit anti-chicken IgG (Invitrogen). All assay measurements for E-CR1 for individuals of the AA size genotype were done together in the same assay, as were all comparisons for individuals of the AB CR1 genotypes. Duplicate assays were performed for each E-CR1 sample, and samples were assessed in duplicate within each assay.

2.5 CR1 allelic size determination

The CR1 size genotypes were determined as phenotypes in some of the E-CR1 samples by western blot analysis under non-reducing conditions as we have previously described (Birmingham et al., 1996).

2.6 Statistical analyses

For the assays involving the CR1 constructs, differences in binding to C3b, C3bi, and C4b between the wildtype and 483-C variant constructs were determined by least square estimation following appropriate linear modeling. For the assays involving E-CR1, mean relative binding values were determined for binding to C3b or C4b for normal wildtype and 483-C carrier groups for each CR1 size genotype (AA, AB). Differences in mean binding between wildtype and 483-C carrier groups within the same CR1 size genotype were determined for each ligand (C3b or C4b) by unpaired t-tests. Differences in carrier frequency between populations were assessed by Fisher's exact test.

3. Results

3.1 Identification of the 1597C>T SNP

Visual of assessment of direct sequencing data of the CR1 cDNA SCR 7-13 PCR products from a group of Caucasians and African Americans revealed a polymorphism in SCR 8 at 1597C>T (relative to the published cDNA sequence (Hourcade et al., 1988)) (Fig 1). This polymorphism changes an arginine at amino acid 483 (relative to the N-terminal end of the mature protein) to a cysteine. This polymorphism will subsequently be referred to by the amino acid designation (483R>C).

3.2 Frequency of the 483-C variant in the normal population

To characterize the frequency of the 483-C variant in normal healthy individuals, a genotype assay was developed to interrogate genomic DNA samples. This assay, based on DNA amplification using primers from the start of the SCR 8 exon (sense) and from the intron proximal to the 3' end of the SCR 8 exon (antisense), was run on 175 normal African Americans and 153 normal Caucasians. Eleven carriers were identified in the African Americans cohort (6.3% carrier frequency) and 4 carriers were identified in the Caucasian cohorts (2.6 % carrier frequency).

The high degree of homology between this region and other repeated regions in the CR1 gene predicts that the method to amplify SCR 8 from genomic DNA would also amplify SCR 15, and for the B allele also SCR 22. Thus, this genotype assay could only identify carriers of the 483-C variant, and not the exact genotype (e.g. heterozygous vs. homozygous, which SCR). To provide more information concerning the total number and SCR position of the 483-C variant in carriers, cDNA was synthesized and sequenced from leukocyte RNA available from 9 of the healthy normal carriers who were also typed for the CR1 allelic size polymorphism. As described in Methods (and shown in Fig 2), this approach allowed determination of the exact nature of the 483-C variant (which SCR) for the A size allele. However, for the B size allele, which contains an additional C3b/C4b binding domain, this approach would only identify if the 483-C variant was carried in one of the two N-terminal ligand binding domains, while providing definitive information for the C-terminal binding domain.

The results of sequencing these cDNA PCR products are shown in Table 1. All four of the carriers of the AA size genotype were heterozygous for the 483-C variant in SCR 8 of the N-terminal domains. One of the four carriers was heterozygous for this variant in SCR 15 of the C-terminal ligand binding domain. For AB size genotypes, the 483-C variant appeared in one of the N-terminal domains (SCR 8, or SCR 15, or both) in all five carriers. Two of these carriers also had the 483-C variant in the C-terminal ligand binding domain (SCR 22, one heterozygous, one homozygous).

3.3 Ligand binding effects of the 483-C variants

To determine if the 483-C variant could affect the ligand binding domain, single-binding domain constructs were used that contained SCRs 8-11 of the N-terminal C3b/C4b binding domain, followed by SCRs 5 and 6, SCRs 28-30, and the CR1 transmembrane and cytoplasmic domains (Fig 3A). After expressing these constructs in HEK293 cells as membrane receptors, the membrane were solubilized, adjusted to equal concentrations, and compared in ELISAs for binding differences to C3b and C4b. As can be seen in Fig 3B, the construct containing the 483-C variant residue bound over 80% less to C3b, C3bi, and C4b (all $p < 0.0001$), relative to the wildtype construct.

The data shown in Fig 3 shows the effect of the 483R>C polymorphism on a single C3b/C4b binding domain. To determine how this polymorphism influences the overall binding activity of intact CR1 containing multiple binding domains, solubilized E-CR1 from 8 of the normal healthy carriers shown Table 1 were tested for ligand binding activity, compared to a panel of freshly isolated wildtype E-CR1 that were matched for the CR1 size genotype. For homozygous AA size genotypes, E-CR1 isolated from all four 483-C carriers bound on average, significantly less to C3b ($p < 0.0001$) and C4b ($p = 0.0001$), relative to wildtype E-CR1 (Fig 4A). For the three carriers with one 483-C variant, E-CR1 bound on average 36% less to C3b ($p < 0.0001$) and 43% less to C4b ($p = 0.0003$). Erythrocyte CR1 from the fourth carrier, also expressing the 483-C variant in one of the C-terminal C3b/C4b binding domains (SCR 15), appeared to bind less than the other three single carriers to both C3b and C4b.

For heterozygous AB size genotypes, E-CR1 isolated from the 483-C carriers also exhibited reduced ligand binding compared to wildtype controls, but did so to a lesser extent (Fig 4B). Specifically, E-CR1 from the 483-C carriers exhibited on average 14% less binding to C3b ($p = 0.0185$) and 28% less binding to C4b ($p = 0.0133$). For the two carriers with the 483-C residue occurring only in the N-terminal C3b/C4b binding domains, E-CR1 exhibited 8% less binding to C3b, and 27% less binding to C4b. One carrier with an additional 483-C variant residue in one of the C-terminal C3b/C4b binding domains appeared bind even less to C3b and C4b. However, this enhanced binding defect was not seen in E-CR1 from the carrier with homozygous expression of the 483-C residue in the C-terminal domains.

3.4 Frequency of the 483-C variant in SLE patients

To determine if the 483-C variant plays a role as a risk factor for SLE, a cohort of African American ($n = 86$, 65 with nephritis) and Caucasian ($n = 228$, 107 with nephritis) SLE patients were interrogated for this polymorphism. Five African American (5.8% carrier frequency) and one Caucasian (0.4% carrier frequency) were identified as carriers. These frequencies were not significantly different from the frequencies of the race-matched normal controls, nor was there an association with lupus nephritis.

4. Discussion

The safe clearance of immune complexes (IC) from the circulation of humans requires appropriate interaction between IC and a number of soluble and membrane bound proteins that interact with IC, such as complement proteins and Fc receptors (Hebert, 1991). Genetic variation affecting the expression levels or functions of these proteins could theoretically lead to variation in the efficiency of proper IC clearance, and thus to risk for IC diseases such as SLE. Evidence for this includes genetic deficiencies in certain complement components (reviewed in (Manderson, Botto and Walport, 2004)) and variation in FcγRIIa binding capacity due to a functional SNP (Karassa, Trikalinos and Ioannidis, 2002), both of which are recognized as risk factors for SLE.

In the current study, genetic variation affecting function was sought for CR1, another protein involved in the handling of IC. This search was conducted by sequencing the encoding region for the N-terminal C3b/C4b binding domain (SCRs 8-10) in a panel of Caucasians and African Americans. The results identified a SNP at 1597C>T that causes a 483R>C amino acid change in SCR 8. This was not a rare SNP, as it occurred in 6.3% of the normal African Americans and 2.4% of the normal Caucasians that we studied. Using a CR1 protein construct containing the N-terminal C3b/C4b binding domain revealed that this was a functional polymorphism, with the 483-C substitution causing greater than 80% reduction in binding of the construct to C4b, C3b, and C3bi.

The strong effect of the 483-C substitution on the CR1 binding domain is not unexpected. This polymorphism involves two different types of amino acids, replacing a positively charged arginine with an uncharged cysteine. Positively charged amino acids are known to enhance CR1 ligand binding activity (Krych et al., 1994; Krych, Hauhart and Atkinson, 1998), and thus this substitution would likely weaken the CR1/ligand interaction. Moreover, work by Smith et al showed that replacing this arginine with a negatively charged glutamic acid in the comparable position in SCR 15 (residue 933) substantially reduce binding to both C3b and C4b (Smith et al., 2002). Perhaps more importantly than a change in charge, however, is that this substitution results in an SCR with a total of 5 cysteines, one more than that found in all RCA SCRs studied to date (Hourcade, Holers and Atkinson, 1989; Perkins et al., 1988). We suggest this fifth cysteine could alter the position of the two disulfide bonds that normally occur within SCRs, thereby changing the conformation of this SCR that is critical for the function of the binding domain.

Using genomic DNA identifies carriers of the 483-C variant, but does not allow one to distinguish which SCR contains the variant residue, nor whether a carrier is heterozygous or homozygous at that SCR. Analysis of cDNA was more informative, and revealed that all four carriers that were of the AA size genotype were heterozygous for the 483-C variant in the SCR 8, with one carrier also being heterozygous in SCR 15 of the C-terminal domain (Table 1). The five carriers of the AB size genotype also all contained the 483-C variant in the N-terminal domains, though it couldn't be determined if the variant occurred in SCR 8 of the A allele, or SCR 8 or SCR 15 of the B allele (or what the total number of variant residues were). Interestingly, four of the five AB size carriers also had the 483-C variant residue in SCR 22 of the C-terminal C3b/C4b binding domain. Comparing this to the AA size genotype carriers, where only one of four carried the 483-C variant in the C-terminal binding domain, suggests that this variant residue occurs in the C-terminal binding domain more frequently in CR1 encoded by the B allele than by the A allele.

To understand the effect of the 483-C variant on full-length CR1, binding assays were also performed using the carriers characterized above (Fig 4). These assays, using CR1 solubilized from erythrocytes, reflected the over-all binding activity of E-CR1 encoded from both alleles from individuals who were homozygous for the A size allele (AA genotypes), or who had both the A and B allele (AB genotypes). Analysis of the AA size genotypes (Fig 4A) reveals that the 483-C variant, when present in the N-terminal C3b/C4b binding domain of the A size allotype, appeared to have a similar effect on binding as that observed for the single binding domain construct (Fig 3B). This is inferred from the data for the three AA size genotypes carrying one variant residue, where the average decrease in binding was 36% for C3b and 43% for C4b. Because half of the CR1 in these solubilized fractions would exhibit normal binding (i.e. half were wildtype), doubling the percent binding inhibition exhibited by the entire E-CR1 fraction would approximate the extent of inhibition on the CR1 allotype carrying the 483-C residue, i.e. 72% less binding to C3b and 86% less binding to C4b. This suggests that the presence of the second, C-terminal C3b/C4b binding domain in the A size allotype does not provide much compensation for the defect in the N-terminal C3b/C4b binding domain. The

presence of the 483-C variant in the C-terminal C3b/C4b binding domain does appear to have some effect, but whether this is a combined effect (occurring in the same allotype as the N-terminal 483-C variant) or an independent effect (occurring alone) cannot be determined with the present data.

In contrast, the binding data from the AB size genotype group suggests that the presence of the CR1 B allele, encoding a third C3b/C4b binding domain, does provide some compensation for the overall defect caused by the 483-C variant residue (Fig 4B). This is perhaps best reflected by comparing the two carriers with variant residues only in the N-terminal domains with the wildtype controls, where there was only an 8% reduction in binding to C3b, and a 27% reduction in C4b. The results, however, are confounded somewhat by the inability to determine which allele (A or B) expresses the 483-C variant, and by the inability to determine the total number of 483-C variant residues in the N-terminal C3b/C4b binding domains (SCR 15 for the A allotype, SCR 8 and 15 for the B allotype). These determinations would require cloning CR1 cDNA from these carriers across all of the C3b/C4b binding domains (~3.8 kb), an effort that was outside the scope of this study.

The rationale for this study was in part to determine if genetically-based variation in CR1 function was a risk factor for SLE or its nephritis. Interrogating genomic samples from a cohort of SLE patients for the 483R>C polymorphism indicated that being a carrier of the 483-C variant alone was not a risk factor. Other variables could coexist that would aggravate or mitigate the physiological effect of the 483-C variant, including genetic variation or acquired changes in Fc receptors and complement proteins, and variations of E-CR1 expression, function, and number of binding domains as discussed above. It is intriguing to note that, while five of the nine normal 483-C carriers reported in this study also carried a B allele, only one of five SLE patients who carried the 483-C variant also carried a B allele, and that one patient expressed an average of only 39 CR1/E, which is less than 10% of the average level (data not shown). A larger study population will be required to determine if the 483-C variant represents an SLE risk factor only when occurring with other influencing variables.

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      H   C   Q   A   P   D   H   F   L   F   A   K   L   K   T   -   465
CAC TGT CAA GCC CCA GAT CAT TTT CTG TTT GCC AAG TTG AAA ACC - 1545

      O   T   N   A   S   D   F   P   I   G   T   S   L   K   Y   -   480
CAA ACC AAT GCA TCT GAC TTT CCC ATT GGG ACA TCT TTA AAG TAC - 1590

      E   C   C   P   E   Y   Y   G   R   P   F   S   I   T   C   -   495
GAA TGC CGT CCT GAG TAC TAC GGG AGG CCA TTC TCT ATC ACA TGT - 1635
      C
      R
      TGT

      L   D   N   L   V   W   S   S   P   K   D   V   C   K   R   -   510
CTA GAT AAC CTG GTC TGG TCA AGT CCC AAA GAT GTC TGT AAA CGT - 1680

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Figure 1.

Sequence of CR1 SCR 8, showing the 1597C>T SNP position that results in a change of an arginine to a cysteine.

Figure 2.

Schematic representation of the amplicons in the two major CR1 size allotypes. The A size allele encodes a receptor containing 30 SCRs (shown as circles numbered 1 through 30 from the N-terminus) and two C3b/C4b binding domains. The B size allele encodes a receptor containing 37 SCRs and three C3b/C4b binding domains. The brackets identify the regions of high homology, where each SCRs separated by 7 are greater than 98% homologous (eg. SCR3, SCR 10, SCR 17). Solid lines show the encoding sequences that are amplified from each allele using primers from SCR 7 and 13, and primers from SCR 13 and 19.

Figure 3.

The effect of the 483-C variant residue in a single C3b/C4b binding domain construct. A. The single binding domain construct used in the binding assay, containing SCRs 8-11 (with either the wildtype 483-R residue or the variant 483-C residue), SCRs 5-6, SCRs 28-30, and the transmembrane and cytoplasmic regions. B. Relative binding of the wildtype construct (WT) and the construct containing the 483-C variant residue to wells coated with C3b, C3bi, and C4b. ** $p < 0.0001$ for differences between WT and the 483-C construct for each complement ligand.

Figure 4.

The effect of the 483-C residue on the C3b or C4b binding by E-CR1 isolated from wildtype or normal 483-C carriers of the AA size CR1 genotype (panel A) or the AB size genotype (Panel B). In panel A, carriers were identified who were heterozygous for the 483-C residue in the N-terminal binding domain alone (1/0) or with heterozygous expression of 483-C residue in the C-terminal binding domain (1/1). In panel B, carriers were identified who were heterozygous for the 483-C residue in the N-terminal binding domains alone (1/0) or with heterozygous (1/1) or homozygous (1/2) expression of 483-C residue in the C-terminal binding domain. P values refer to differences in mean relative binding between WT and 483-C carriers for each complement ligand.

Table 1

Distribution and number of CR1 483-C variant residues in normal 483-C carriers of the AA or AB CR1 size genotype.

Sample	CR1 size genotype	N-term ¹	C-term ²
Carrier 1	AA	1	0
Carrier 2	AA	1	1
Carrier 3	AA	1	0
Carrier 4	AA	1	0
Carrier 5	AB	1	2
Carrier 6	AB	1	0
Carrier 7	AB	1	1
Carrier 8	AB	1	1
Carrier 9	AB	1	0

¹ For AA size genotypes, a “1” refers to 483-C residue expression in SCR 8 of one of the two A alleles (heterozygous expression). For the AB size genotypes, a “1” refers to expression of at least one 483-C residue in SCR 8, SCR 15, or both, but not homozygous expression in both.

² For both AA and AB size genotypes, the number refers to the number of alleles with the 483-C residue in the C-terminal C3b/C4b binding domain (SCR 15 for the A allele, SCR 22 for the B allele).