

Assessing association of common variation in the C1Q gene cluster with systemic lupus erythematosus

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Summary

Recent studies have tested genetic variation at the *CIQA*, *CIQB* and *CIQC* (complement component 1, q subcomponent, A chain, complement component 1, q subcomponent, B chain and complement component 1, q subcomponent, c chain) loci in relation to systemic lupus erythematosus (SLE) risk. Evidence for a significant effect of *CIQ* locus gene polymorphisms on SLE predisposition remains unclear. We aimed to identify associations between common *CIQ* polymorphisms and SLE risk and serum C1q, C3 and C4 levels. We performed family-based association tests in 295 nuclear families with one affected proband. Tag-single nucleotide polymorphisms (SNPs) ranging from 35.4 kb upstream of the *CIQA* gene to 28 kb downstream of the *CIQB* gene were selected to represent the entire *CIQ* gene locus. We performed transmission disequilibrium tests for affectation status and continuous traits, including C1q, C3 and C4 levels using family-based association tests (FBAT). There was no evidence for a significant role of *CIQ* locus gene polymorphisms in SLE risk predisposition. The strongest association was observed with a variant in the 3'UTR region of the *CIQB* gene (rs294223, $P = 0.06$). We found nominally significant associations with a second variant (rs7549888) in the 3'UTR region of the *CIQB* gene and C1q ($P = 0.01$), C3 ($P = 0.004$) and C4 levels ($P = 0.01$). In a large family-based association study of *CIQ* gene cluster polymorphisms no evidence for a genetic role of *CIQ* locus SNP in SLE risk predisposition was obtained in patients of European ancestry. This is in contrast to other cohorts, in which single variants associated with C1Q, C3 and C4 levels and nephritis have been studied and shown associations.

Keywords: *CIQA*, *CIQB*, *CIQC*, complement concentrations, SLE

Accepted for publication 31 March 2010

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Introduction

The complement proteins are known to be involved in the pathogenesis of systemic lupus erythematosus (SLE). Initial observations confirmed that during active disease, complement proteins, and in particular C3, are deposited at the site of inflammation in various organs, including the spleen, heart and kidney [1]. This, in turn, leads to reduced serum levels of complement as it is rapidly consumed and bound with immune complexes in such patients. These early studies suggested that inadvertent activation of complement during autoimmunity was proinflammatory, and played a similar role as it does in host defence against infectious disease [2]. However, studies on patients with homozygous deficiencies for complement proteins have revealed that complement

deficiencies promote rather than inhibit the development of SLE [3–14]. This has led to the 'paradoxical hypothesis' being proposed by Walport and colleagues [15] that complement activation, although primarily a proinflammatory immune mechanism, is actually anti-inflammatory during the development of certain types of autoimmunity. This may be due to the anti-inflammatory functions of C1q, namely its ability to help solubilize immune complexes and aid in the clearance of apoptotic debris [16], which is defective in SLE patients [17].

Despite homozygous complement gene deficiencies being rare, the absences of complement genes are very powerful markers of disease susceptibility. A hierarchical grade of susceptibility exists in which 93%, 75%, ~10%, 13% and >5% of individuals will develop lupus-like illness if they are deficient

in C1q, C4, C2, C3 or C5–9, respectively [18]. The rarity of these genetic deficiencies has led to further sequence analysis of the A, B and C chains of C1q to identify single nucleotide polymorphisms (SNP) that may account for defective C1q function, C1q antigenicity or decreased concentration in the serum of SLE sufferers [19–22]. However, these studies have been small, or have examined only a small number of variants. Here we extend analysis from previous studies to study associations of common haplotype tagging SNPs across the *C1Q* locus and SLE affection status, C1Q, C3 and C4 levels. We used HapMap Build 35 information to select tag-SNPs across an 87.8 kb region in a sample of 295 trios.

Methods

Patients and samples

Details of samples used in this analysis are given in Table 1. The cohort investigated here included 295 nuclear families (including unaffected parents and a single affected proband). All the participants included in this analysis are of European ancestry [23] and had provided informed consent. The patient recruitment and samples were approved by the London Multi-Centre Research Ethics Committee (MREC) and the study protocol approved further by Exeter MREC 06/Q2102/56. Details of patient recruitment have been reported previously [24]. Briefly, patients were recruited via attendance at rheumatology and/or renal clinics at the Hammersmith Hospital or responded to advertisements in the media and then their disease status checked by medical questionnaire of their symptoms and through their clinical records by collaborating physicians in the United Kingdom. Each patient gave written permission to obtain medical record information. The SLE status of each patient was determined according to the American College of Rheumatology (ACR) 1982 and 199 revised criteria for SLE [25,26]. As far as possible, the initial onset date for the initial diagnosis of SLE was recorded in 118 of 295 subjects. C1q, C3 and C4 were measured in a subset of 166 parents and 84 affected SLE

probands by commercial enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's instructions.

Tag SNP selection

A total of 23 tagging polymorphisms across the *C1QA*, *C1QB* and *C1QC* loci were selected to capture all common SNPs in the CEU samples of the Hapmap project [minor allele frequency (MAF) >5%] at $r^2 > 0.8$. These SNPs extended from 35.4 kb upstream of the *C1QA* gene to 28 kb downstream of the *C1QB* gene (Fig. 1). Tag SNPs were selected using the tagger SNPs selection algorithm available in Haploview (<http://www.broad.mit.edu/mpg/haploview/>).

Genotyping and quality control

The polymorphisms were all typed by MALDI-TOF (matrix-assisted laser desorption ionization time-of-flight) mass spectrometry (Sequenom, Hamburg, Germany), using the standard conditions described for the iPLEX kit. All polymerase chain reactions (PCRs) were carried out on DNA Engine Tetrads (MJ Research Incorporated, Waltham, MA, USA) in either a 96- or 384-well format. The details of all amplification and extension primers are available from the authors. Of the 23 variants selected to capture the majority of common variation (MAF >5%) across the C1q locus, based on HapMap Phase II Build 35 data, 18 SNPs were acceptable for analysis following quality control (QC). This included removing SNPs with recorded Mendelian error rates >1%, SNPs with Hardy–Weinberg equilibrium (HWE) *P*-values below 0.01 significance level in the unaffected parent samples and SNPs with call rates <85% in the trios.

Statistics

We used the transmission disequilibrium test (TDT), as implemented in the FBAT program, to assess the evidence that alleles were transmitted differentially from parents to SLE-affected probands. We used the quantitative-trait

Table 1. Characteristics of subjects and clinical parameters presented as mean (standard deviation).

Number of participants [†]	295 nuclear participants, 997 individuals	Normal range of complement proteins [29]
Age of probands	38.50 (9.53)	
Age of parents	66.20 (9.99)	
Age at diagnosis (<i>n</i> = 118)	23.30 (9.61)	
C1q levels probands (<i>n</i> = 84)	192.10 mg/l (50.21)	50–250 mg/l
C1q levels parents (<i>n</i> = 166)	214.08 mg/l (52.46)	
Complement C3 probands (<i>n</i> = 84)	944.26 mg/l (322.83)	750–1650 mg/l
Complement C3 parents (<i>n</i> = 166)	1178.94 mg/l (357.82)	
Complement C4 probands (<i>n</i> = 84)	163.48 mg/l (96.10)	140–540 mg/l
Complement C4 parents (<i>n</i> = 166)	238.15 mg/l (83.39)	

[†]All patients were Caucasian and residents of the United Kingdom. All subjects were recruited while attending the rheumatology and/or renal clinics of the Hammersmith Hospital, London or referred from collaborating physicians in the United Kingdom.

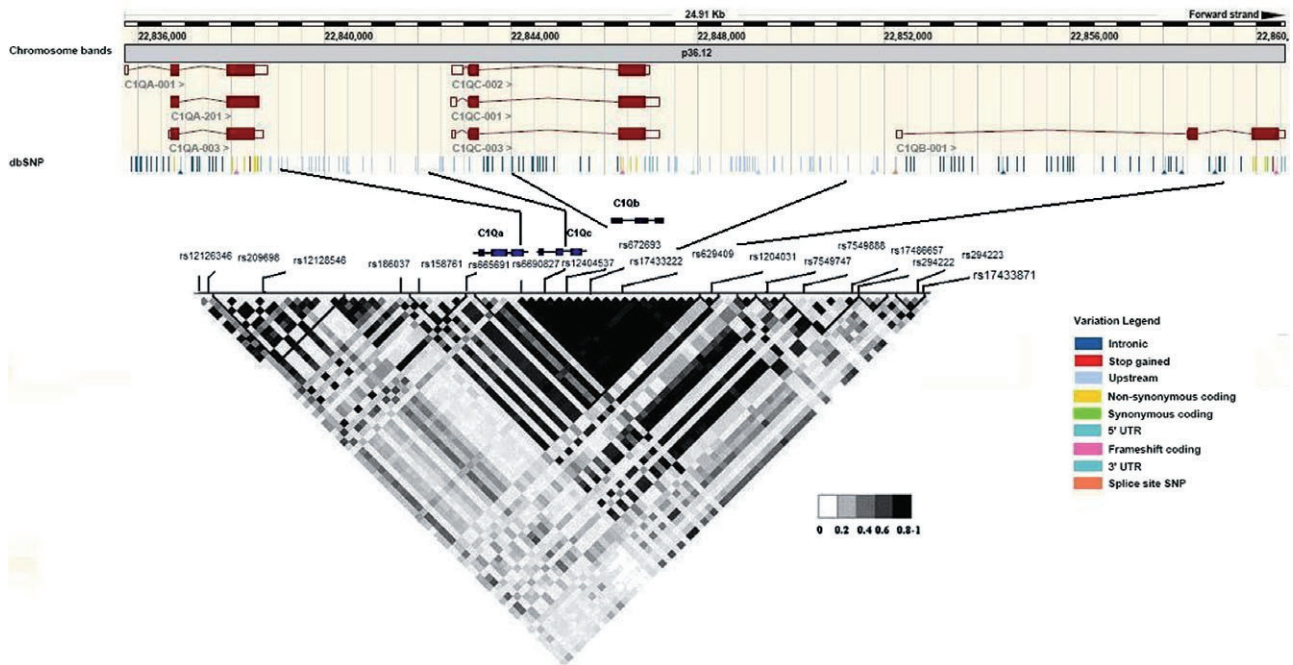


Fig. 1. Positions of the 18 common gene variants studied are marked along the linkage disequilibrium plot of the *CIQ* locus. Positions of these variants along the actual genetic co-ordinates according to the dbSNP database are marked in the upper half of the diagram.

disequilibrium test (QTD) test to study associations with C1q, C3 and C4 levels as continuous traits. The strength of association in FBAT is specified by the significance of the P -value and the standardized FBAT ' U -statistic', represented as Z . ' U ' is based on a linear combination of genotypes and traits [27] and is calculated as the difference between expectation of the product of offspring genotypes and traits and the product of coded traits values and offspring genotypes. Power calculations were performed using <http://hydra.usc.edu/GxE/>. This study had 88% power to detect a genetic effect size of odds ratio (OR) = 1.5, at $\alpha = 0.05$ for a SNP with MAF = 0.25. For a more modest effect size equivalent to an OR of 1.2 we had 28% power to detect associations at $\alpha = 0.05$.

Results

Of the 23 variants included after genetic coverage analysis, 18 variants passed quality control analysis and were included in association tests (Fig. 1). The selected variants across the *CIQA*, *CIQC* and *CIQB* genes captured 30% and 75% of variation based on HapMap Phase II Build 36 data at $r^2 > 0.8$ and $r^2 > 0.5$, respectively. Extending this region to 20 kb either side of the *CIQ* locus allowed us to capture 38% and 73% of variation at $r^2 > 0.8$ and $r^2 > 0.5$, respectively.

No evidence that C1Q SNPs are associated with SLE

We performed TDT analysis in 292 nuclear families to test for association of SNPs residing in the *CIQ* locus and SLE

affection status. Of the 18 variants included in single marker association tests, none of these were associated with affection status in TDT analysis (Table 2). The lowest observed P -value did not reach nominal significance (rs294223, $P = 0.06$, $Z = -1.88$). Similarly, no evidence for association was found when transmission of haplotypes formed by allelic combinations of SNPs ranging from 5' transcription start site of the *CIQA* gene to the 3' transcription stop site of the *CIQB* gene were studied (Table 3). The two most common haplotypes were observed at frequencies of 53% and 16%, and these were marked by the common and rare alleles of five SNPs which covered the *CIQ* locus, respectively (Table 3).

C1Q SNPs and associations with C1Q, C3 or C4 levels

We tested the 18 variants analysed for association with disease status for further associations with C1q, C3 and C4 levels, which were measured in a subset of the probands ($n = 84$) and parents ($n = 116$) (Table 4). These tests therefore included both SLE patients and their predominantly unaffected parents, but it is important to note that genetic associations cannot be confounded by, or secondary to, disease processes and that the use of both unaffected and affected SLE patients is unlikely to result in false positive associations between C1Q variants and circulating protein levels. The minor allele of a variant (rs7549888) in the 3'UTR region of the *CIQB* locus was associated with lower C1q levels ($P = 0.01$) and higher C3 ($P = 0.004$) and C4 levels ($P = 0.01$). The variant associated most strongly with SLE status in this study was in moderate linkage disequilibrium

Table 2. Association of informative single nucleotide polymorphisms (SNPs) with systemic lupus erythematosus disease status.

SNP ID	Major allele	Minor allele	Location	P-value	Z-statistic for minor alleles	MAF parents	MAF probands
rs12126436	G	A	22672978	0.27	1.10	0.12	0.14
rs209698	T	C	22674119	0.17	1.35	0.33	0.35
rs12128546	T	G	22682400	0.94	-0.07	0.21	0.21
rs186037	T	C	22697692	0.86	-0.17	0.13	0.13
rs158761	G	T	22699678	0.36	-0.91	0.41	0.40
rs665691	G	C	22705660	0.94	-0.06	0.44	0.44
rs6690827	G	A	22712802	0.89	0.14	0.27	0.28
rs12404537	C	T	22714674	0.66	-0.43	0.19	0.18
rs672693	G	A	22716752	0.72	-0.35	0.33	0.32
rs294180	G	T	22720100	0.33	-0.96	0.38	0.36
rs17433222	G	A	22723952	0.69	-0.39	0.26	0.25
rs12040131	C	G	22734004	0.17	-1.35	0.21	0.19
rs7549747	T	C	22741877	0.96	-0.06	0.43	0.44
rs7549888	G	T	22749325	0.74	0.32	0.36	0.36
rs17486657	C	T	22752481	0.37	-0.90	0.29	0.29
rs294222	G	G	22752866	1.00	0.00	0.22	0.23
rs294223	A	C	22761312	0.13	-1.45	0.25	0.23
rs17433871	T	G	22761409	0.77	-0.38	0.25	0.25

MAF: minor allele frequency.

Table 3. Haplotype structure resulting from single nucleotide polymorphisms (SNPs) across the C1q locus and 20 kb either side; association analysis with affectation status.

Haplotype	rs6690827	rs12404537	rs672693	rs294180	rs17433222	Haplotype frequency	Haplotype specific association P-value
1	1	1	1	1	1	0.53	0.16
2	2	2	2	2	2	0.16	0.80
3	1	1	2	2	1	0.09	0.06

Table 4. Association of single nucleotide polymorphisms (SNPs) 20 kb either side of C1Q locus with C1q, C3 and C4 levels.

SNP ID	Alleles	Location	P-value for association with C1q levels	Z-statistic for minor allele	P-value for association with C3 levels	Z-statistic for minor allele	P-value for association with C4 levels	Z-statistic for minor allele
rs12126436	G/A	22672978	0.22	1.20	0.07	-1.83	0.16	1.38
rs209698	T/C	22674119	0.08	1.72	0.18	1.33	0.08	1.71
rs12128546	T/G	22682400	0.59	-0.52	0.47	-0.71	0.42	-0.79
rs186037	T/C	22697692	0.73	0.34	0.32	0.98	0.18	1.35
rs158761	G/T	22699678	0.66	0.43	0.62	0.49	0.53	0.62
rs665691	G/C	22705660	0.96	0.05	0.92	-0.09	0.81	-0.24
rs6690827	G/A	22712802	0.61	-0.51	0.81	-0.23	0.70	-0.38
rs12404537	C/T	22714674	0.02	-2.30	0.01	-2.01	0.01	-2.50
rs672693	G/A	22716752	0.83	-0.20	0.94	0.08	0.84	0.19
rs294180	G/T	22720100	0.61	-0.50	0.55	-0.60	0.79	-0.26
rs17433222	G/A	22723952	0.04	-2.28	0.01	-2.35	0.01	-2.50
rs12040131	C/G	22734004	0.16	-1.85	0.07	-1.80	0.05	-1.91
rs7549747	T/C	22741877	0.69	0.39	0.65	0.45	0.47	0.72
rs7549888	G/T	22749325	0.01	-2.33	0.004	2.84	0.01	2.40
rs17486657	C/T	22752481	0.33	-0.97	0.17	-1.38	0.34	-0.94
rs294222	G/C	22752866	0.71	0.36	0.44	0.77	0.29	1.04
rs294223	A/G	22761312	0.29	-1.05	0.37	-0.90	0.38	-0.94
rs17433871	T/C	22761409	0.18	-1.35	0.14	-1.48	0.41	-0.82

with rs7549888 ($r^2 = 0.52$), although this variant itself was not associated with disease status ($P = 0.81$, $Z = -0.14$).

In a recent study of 103 SLE cases and 195 family members weak associations of two tag-SNPs at the *C1Q* locus and C1q levels were described. The strongest association in this study was observed with the variant rs587585 ($P = 0.051$) [20]. We tagged this SNP with a proxy at $r^2 = 0.43$. No association of this proxy variant (rs186037) with C1q levels was observed in our study ($P = 0.32$). The association between the rs7549888 variant and C1q levels is the strongest association identified between a *C1Q* locus SNP and serum C1q levels, although this association does not survive a conservative Bonferroni correction for 18 SNPs tested ($P = 0.14$). Further studies are required to confirm this association.

Previous studies have analysed associations of two further candidate SNPs in the exonic regions [19,21,22] (*C1QA*-GLY70 (rs172378) (G/A) and *C1QC*-Pro14 (rs15940) (T/C)) of the *C1QA* and the *C1QC* genes and SLE affectation status. Neither of these polymorphisms have been genotyped in Caucasian participants of the HapMap project. We identified a tag-SNP (rs292001) for the *C1QA*-GLY70 polymorphism from the Seattle SNPs project at $r^2 > 0.8$. This SNP is in strong linkage disequilibrium (LD) with a SNP (rs665691, $r^2 = 0.90$) that we genotyped for association analysis. This SNP was not associated with C1q levels ($P = 0.92$) and SLE status ($P = 0.67$).

Discussion

The C1Q locus is an important candidate locus for association analysis with SLE. Here we report association analysis of 18 SNPs spread across the C1q encoding locus and the regulatory regions of these genes. We report a novel association of a variant in the 5'UTR region of the C1Q locus and C1q levels, but replication studies are required to increase the evidence for these associations. In our association screen of selected variants across 295 nuclear families, we failed to identify any strong associations between *C1Q* locus variants and SLE status. Our results do not provide any evidence that common variants at the *C1Q* locus confer high risk of SLE ($OR \geq 1.5$), at least in our European population, although we may have missed weaker associations ($OR \leq 1.2$) in our study, given the limited power and lack of complete coverage. Identification of *C1Q* variants associated with SLE would improve understanding of the role of *C1Q* locus in SLE predisposition. In addition, a recent study by Namjou *et al.* identified specific SNPs in the C1q gene (rs665691 and rs172378) that correlated with kidney nephritis, but this was specific to African American patients [28]. The SNP rs172378 is also known to be associated with lower levels of C1q in European ancestry patients with subacute cutaneous lupus [22]. In our study, we measured C1q titres in our European ancestry families with SLE and there was no difference in C1q titres compared with normal levels of C1q. These different associations with a single SNP of the *C1QA*

gene appear to be important in different subsets of lupus patients. This might go some way in explaining the complex results and lack of correlation between C1q SNPs and association with SLE in mixed cohorts of patients. It would appear that the lupus cohort plays an influential role between complement genetics and phenotypic expression of disease activity.

In conclusion, we have performed a comprehensive analysis of common genetic variation at the C1q protein encoding loci. We found no evidence to suggest a causal role of common C1Q locus polymorphisms in SLE risk predisposition in a cohort of European ancestry.

Acknowledgements

This work was supported by research grants from the Arthritis Research Campaign, grants E0521, E0543, 17231 and 16537, and Lupus UK and the support of the Peninsula NIHR Clinical Research facility. We thank Mrs Susan Westoby for secretarial assistance.

Disclosure

The authors have no financial conflicts of interest.

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