Analysis of C1q polymorphisms suggests association with systemic lupus erythematosus, serum C1q and CH50 levels and disease severity

H A Martens,[1] M W Zuurman,[2] A H M de Lange,[1] I M Nolte,[3] G van der Steege,[4] G J Navis,[2] C G M Kallenberg,[1] M A Seelen,[2] M Bijl[1]

[1] Department of Rheumatology and Clinical Immunology, University Medical Center Groningen and University of Groningen, Groningen, The Netherlands; [2] Department of Nephrology, University Medical Center Groningen and University of Groningen, Groningen, The Netherlands; [3] Department of Epidemiology, University Medical Center Groningen and University of Groningen, Groningen, The Netherlands; [4] Department of Genetics, University Medical Center Groningen and University of Groningen, Groningen, The Netherlands

Correspondence to:
H A Martens, Department of Rheumatology and Clinical Immunology, University Medical Center Groningen, University of Groningen, P.O. Box 30001, 9700 RB Groningen, The Netherlands; h.a.martens@int.umcg.nl

The first two authors contributed equally to this work.

Accepted 9 May 2008
Published Online First 26 May 2008

ABSTRACT

Background: Several findings link systemic lupus erythematosus (SLE) with C1q, the first molecule of the classical complement pathway. Polymorphisms of the *C1qA* gene are associated with low serum C1q levels in patients with cutaneous LE, but C1q polymorphisms have not been studied in patients with systemic lupus.

Objective: To determine whether polymorphisms of the C1q genes are associated with SLE, disease phenotypes, serum C1q and CH50 levels.

Methods: DNA for genetic analysis was obtained from 103 Caucasian patients with SLE and their family members. Five tag single nucleotide polymorphisms (tag SNPs) served as unique markers for underlying SNPs in the genes of the C1q protein. The pedigree disequilibrium test (PDT) was applied to trios to determine association of markers with SLE, SLE phenotypes, low serum C1q and low CH50. Single SNP association and haplotype analysis was also performed.

Results: The PDT revealed a significant association of the tag SNP rs631090 (covering the *C1qB* gene) with SLE ($p = 0.02$). Rs631090 was moderately associated with low serum C1q levels ($p = 0.06$). In addition, the tag SNPs rs292001 and rs294183 were associated with more severe SLE (Systemic Lupus Erythematosus International Collaborating Clinics (SLICC) damage index score>0; $p = 0.007$ and $p = 0.02$, respectively). Haplotype analysis and single SNP association analysis showed no significant associations, but additional analyses revealed that marker rs587585 is associated with low serum C1q and CH50 levels.

Conclusions: C1q polymorphisms are associated with SLE, serum C1q and CH50 levels in a stable founder population of patients with SLE. Although the studied population was small and allele frequencies were low, this is the first study to suggest an association of C1q polymorphisms with SLE.

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterised by the production of autoantibodies against self-antigens. These autoantibodies can affect several organs or organ systems, resulting in a broad spectrum of clinical and immunological manifestations. The exact pathogenesis of SLE is unknown, but genetic predisposition is supposed to play an important role.[1 2]

Several findings link SLE with C1q, the first molecule of the classical complement pathway. First, most individuals with hereditary C1q deficiency develop a clinical syndrome that closely resembles SLE (>90%). Genetic analysis has revealed mutations in the C1q gene in individuals with hereditary C1q deficiency, leading either to the absence of C1q or to the production of a non-functional, low molecular weight C1q.[3 4] Secondly, antibodies directed against C1q (anti-C1q) are present in 20% to 50% of patients with SLE. The presence of anti-C1q is strongly associated with hypocomplementaemia, disease activity and the appearance of renal involvement in patients with SLE.[5 6] Finally, active disease in SLE is often accompanied by low levels of C1q and other complement factors of the classical pathway, reflecting consumption of complement by active inflammation. As C1q plays a critical role in the clearance of immune complexes and apoptotic cells,[7–9] this clearance may be impaired by low C1q levels.

Although various, predominantly immunological, studies link SLE with C1q and C1q deficiency, there is only little data on an association of SLE with polymorphisms of the genes encoding for C1q. The coding region for C1q is localised on chromosome 1p34–36 and consists of three genes, *C1qA*, *C1qB* and *C1qC*. Besides the few cases of lupus with hereditary C1q deficiency, only one other genetic study of the C1q gene has been performed in patients with lupus. In this study, a single nucleotide polymorphism of the *C1qA* gene was associated with decreased levels of serum C1q.[10] However, this study was performed in patients with only a cutaneous form of lupus, and not in patients with systemic lupus.

In this study, we analysed whether C1q polymorphisms are associated with SLE and, therefore, are susceptibility loci for SLE. In addition, we determined whether C1q polymorphisms are accompanied by low levels of C1q or decreased activity of the classical complement pathway (CH50) in a population of SLE cases and first-degree relatives.

METHODS

Subjects

In the period from November 2000 to November 2001, all patients from our SLE cohort were invited to participate in a study on the genetic predisposition of SLE. All patients met the criteria for SLE according to the American College of Rheumatology (ACR).[11] In total, 107 out of 164 patients (65%) gave written informed consent and were included. To avoid influence of ethnicity, 4 patients who were not of Caucasian descent were excluded, yielding 103 patients that could be

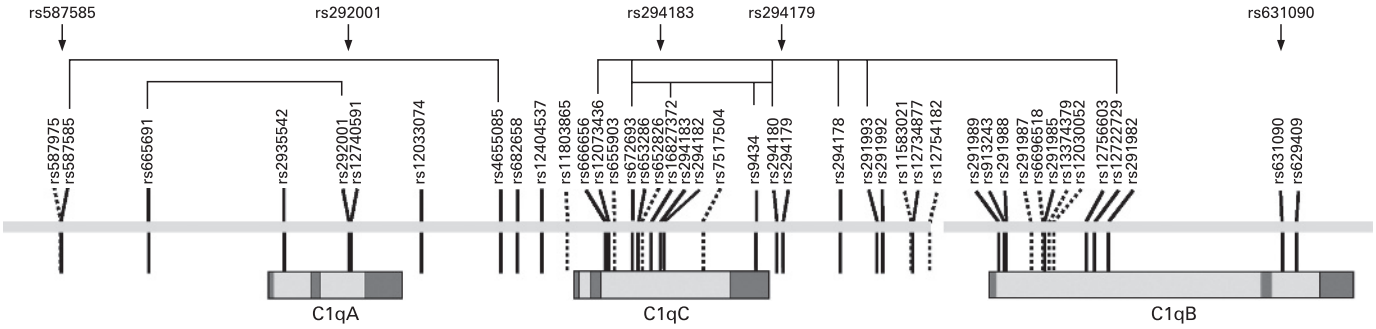

Figure 1 Schematic representation of *C1qA*, *C1qC* and *C1qB* on chromosome 1 and the position of the five tag single nucleotide polymorphisms (SNPs) used in the study population (arrows).

studied for genetic associations with SLE (table 1). The Systemic Lupus International Collaborating Clinics (SLICC)[12] damage index score was obtained for each patient as a measure of chronic damage caused by SLE. We classified disease manifestations using cumulative ACR criteria (table 1).

Participants invited their family members, ideally both parents or spouse and a child, to join the study. None of the family members (n = 195) had SLE. Either the non-transmitted chromosomes of the parents or the chromosomes of the spouse served as controls. These could be either determined directly (n = 60) or determined through other family members (eg, children or sibs of the patient) (n = 13). In the case that neither both parents nor spouse and child were participating (for n = 23 patients), other family members were used for linkage phase determination, but in most cases contributed to only one control haplotype. For seven patients, no family members were willing to participate. The study was approved by the local medical ethics committee.

Table 1 Baseline characteristics of 103 patients with systemic lupus erythematosus (SLE)

Characteristic	Value
Median age (years, range)	43 (23 to 78)
Sex female, n (%)	89 (86%)
Median age of onset (years, range)	31 (8 to 73)
Median duration of disease (months, range)	131 (21 to 516)
Race, n (%):	
Caucasian	103 (100%)
ACR criteria, n (%):	
Malar rash	37 (36)
Discoid rash	31 (30)
Photosensitivity	52 (50)
Oral ulcers	13 (13)
Arthritis	67 (65)
Serositis	39 (38)
Renal disorder	42 (41)
Neurological disorder	7 (7)
Haematological disorder	75 (73)
Immunological disorder	91 (88)
anti-dsDNA antibodies	81 (79)
anti-Sm antibodies	13 (13)
anti-phospholipid antibodies	21 (20)
Anti-nuclear antibody	103 (100)
C1q levels (mean, SD) g/litre	0.17 (0.05)
CH50 levels (mean, SD) %	84% (30)
SLICC score (mean, SD)	0.90 (1.18)
Complement C3 (mean, SD) g/litre	0.97 (0.26)
Complement C4 (mean, SD) g/litre	0.16 (0.09)

ACR, American College of Rheumatology; dsDNA, double-stranded DNA; SLICC, Systemic Lupus International Collaborating Clinics; Sm, Smith.

C1q single nucleotide polymorphism (SNP) selection and genotyping

Five haplotype tagging SNPs from the C1q gene cluster (comprising *C1qA*, *C1qC* and *C1qB*) were selected to be genotyped. Using SNP browser software, V. 3.0 (Applied Biosystems, Forster City, California, USA) the HapMap build 35 database was loaded and subsequently the genotype data of the SNPs from a 30 kb region containing the *C1qC* gene, with flanking sequences including *C1qA* upstream and *C1qB* downstream, for which a TaqMan assay was available, were exported as PED format. Subsequently, the exported data for the HapMap Caucasian sample panel were loaded into Haploview (http://www.broad.mit.edu/mpg/haploview/) using the *Tagger* SNP selection algorithm with default settings, but with forced inclusion of three intragenic SNPs for each of *C1qA*, *C1qC* and *C1qB*, respectively. This resulted in the selection of five SNPs to cover the region: rs587585, rs292001, rs294183, rs294179 and rs631090 (see fig 1 and table 2). The five corresponding TaqMan assays, hCV3176797, hCV3176787, hCV3176770, hCV992987 and hCV992968, respectively, were ordered (Applied Biosystems) and genotyping was performed on an ABI7900HT apparatus.

Laboratory methods

Complement C1q in serum was measured by nephelometry. Activation of the classical complement pathway (CH50) was assessed by measuring haemolysis of antibody-coated sheep erythrocytes. Normal values for C1q are 0.10–0.25 g/litre, for CH50 >65%. Samples were obtained during quiescent disease (Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) ⩽4). C1q and CH50 values were available for 102 and 94 of the 103 patients, respectively.

Statistical analysis

The total number of available family members was 195. For the haplotype analysis, the non-transmitted haplotypes of the parents or the haplotypes of the spouse served as controls, either determined directly or through reconstruction using other family members yielding 146 haplotypes.

The pedigree disequilibrium test (PDT)[13 14] was applied to the trios consisting of the patient and both parents (n = 42) and the families consisting of the patient and unaffected sib(s) (n = 27). This test assesses whether a specific allele is more often transmitted from a parent to the affected child or more often

Table 2 Single nucleotide polymorphisms (SNPs) marked by five tag SNPs in the study population

rs	SNP	Location	MAF (CEU) (%)	MAF (SLE) (%)	CF (CEU) (%)	CF (SLE) (%)
rs587585	A/G	5′ near C1qA	10	13	20	21
rs4655085	A/G	5′ near C1qC	8	NA	16	NA
rs292001	A/G	Intron C1qA	58	42	80	82
rs665691	G/C	5′ near C1qA	44	NA	65	NA
rs294183	G/A	Intron C1qC	36	36	58	60
rs9434	G/T	3′ UTR C1qC	37	NA	61	Na
rs672693	G/A	Intron C1qC	34	NA	57	NA
rs294185	C/T	Intron C1qC	64	NA	87	NA
rs294180	T/G	3′ near C1qC	66	NA	82	NA
rs294179	G/A	3′ near C1qC	56	43	78	79
rs12734877	A/C	5′ near C1qB	40	NA	64	n/a
rs291989	G/A	Intron C1qB	56	NA	78	NA
rs291985	G/T	Intron C1qB	56	NA	78	NA
rs291992	C/T	3′ near C1qC, 5′ near C1qB	56	NA	78	NA
rs653286	C/T	Intron C1qC	53	NA	77	NA
rs294178	C/T	3′ near C1qC, 5′ near C1qB	54	NA	76	NA
rs291988	T/C	Intron C1qB	56	NA	78	NA
rs913243	G/T	Intron C1qB	60	NA	64	NA
rs294182	A/T	Intron C1qC	55	NA	77	NA
rs666656	A/C	Intron C1qC	58	NA	79	NA
rs291982	T/G	Intron C1qB	54	NA	77	NA
rs291993	G/A	3′ near C1qC, 5′ near C1qB	57	NA	78	NA
rs631090	T/C	Intron C1qB	5	6	10	12

Tag SNPs are in bold, SNPs tagged are listed below each tag SNP. HapMap minor allele frequencies (MAF) and carrier frequencies (CF) from 30 mother/father/child trios from the Centre d'Etude du Polymorphisme Humain (CEPH) collection (Utah, USA residents with ancestry from northern and western Europe) are listed (CEU) and from the current patient population (SLE).
NA, not applicable; SLE, systemic lupus erythematosus.

present in an affected sib than in an unaffected sib than expected. Classical association analysis was performed by comparing the genotype frequencies of each tag SNP between cases and controls using a χ^2 test or Fisher exact test, as appropriate. Haplotype frequencies were estimated using the expectation–maximisation (EM) algorithm and compared between cases and controls by means of a likelihood ratio test (in-house software).

In addition, patients were analysed for serum C1q, CH50 levels and SLICC score. The PDT was applied separately to subgroups of patients with values above and below the median. Haplotype analyses were performed comparing patients with values above the median to those with values below. In a quartile analysis of serum C1q levels and CH50, haplotype groups were compared using a χ^2 test. The two major haplotypes were named 1.1 and 1.2. The minor haplotype A (MHA) was defined as the haplotypes containing the minor allele of the tag SNP rs586585. Minor haplotype B (MHB) represented all other minor haplotypes. Correlation between C1q and CH50 levels was determined according to the Pearson correlation test.

Table 3 Linkage disequilibrium

r2	Marker				
Marker	**rs587585**	**rs292001**	**rs294183**	**rs294179**	**rs631090**
rs587585		0.06	0.00	0.03	0.12
rs292001	0.06		0.32	0.50	0.00
rs294183	0.00	0.32		0.69	0.09
rs294179	0.03	0.50	0.69		0.07
rs631090	0.12	0.00	0.09	0.09	

RESULTS

Before association of C1q gene polymorphisms was SLE analysed, linkage disequilibrium was determined (table 3). This showed a weak disequilibrium between the markers rs292001, rs294183 and rs294179.

To determine whether polymorphisms of the C1q genes are associated with SLE, we performed a PDT. An association between SLE and the tag SNP rs631090 could be established (number transmitted 7, non-transmitted 1; $p = 0.02$) (table 4). To see whether polymorphisms have functional consequences (eg, leading to lower or less functional C1q), we analysed the association of C1q polymorphisms with serum C1q and CH50 levels. Although such an association of tag SNP rs631090 with low serum C1q levels is suggested, a significant relation could not be established ($p = 0.06$). To determine whether polymorphisms are involved in severity of disease, association of C1q polymorphisms with the SLICC score was analysed. Transmission of rs631090 was not associated with the SLICC score, but an association of the tag SNPs rs292001 and rs294183 with SLICC score was found ($p = 0.007$ and $p = 0.02$, respectively). There was no association between the other tag SNPs with either SLE, C1q, CH50 levels.

When single SNP association analysis was performed, none of the tag SNPs were associated with SLE, serum C1q, CH50 levels or SLICC score (table 5). However, a tendency towards an association of tag SNP rs587585 with low serum C1q (minor allele frequencies C1q below median: 16.4%, above median: 8.7%; $p = 0.11$) and low CH50 levels (allele frequencies CH50 below median: 17%, above median: 8.7%; $p = 0.09$) with the tag SNP rs587585 was suggested.

To further investigate the influence of this C1q polymorphism on the levels of serum C1q and CH50, an additional quartile analysis was performed. In this analysis serum C1q levels and

Table 4 Results of the pedigree disequilibrium test (PDT) for the minor alleles of each single nucleotide polymorphism (SNP) for systemic lupus erythematosus (SLE) and various other clinical phenotypes.

	rs587585					rs292001					rs294183					rs294179					rs631090				
Phenotype	**T**	**NT**	**AF**	**UN**	**p Value**	**T**	**NT**	**AF**	**UN**	**p Value**	**T**	**NT**	**AF**	**UN**	**p Value**	**T**	**NT**	**AF**	**UN**	**p**	**T**	**NT**	**AF**	**UN**	**p Value**
SLE	13	8	7	5	0.18	33	32	23	18	0.24	32	25	17	14	*0.07*	34	35	23	19	0.39	7	1	3	0	**0.02**
Low CH50	10	7	8	7	0.47	23	22	20	20	0.81	21	16	12	12	0.27	24	23	18	19	0.82	5	1	3	1	*0.10*
High CH50	3	1	2	0	*0.05*	10	10	10	6	0.12	11	9	9	9	0.31	10	12	11	9	0.55	2	0	1	0	0.83
Low C1q	10	6	9	7	0.20	21	20	20	18	0.62	18	15	12	12	0.46	21	20	18	17	0.65	4	0	2	1	*0.06*
High C1q	3	2	1	0	0.41	12	12	10	8	0.26	14	10	9	9	0.15	13	15	11	11	0.81	3	1	2	0	0.18
SLICC = 0	8	5	4	2	0.20	14	21	16	18	0.24	18	16	11	16	0.93	17	22	17	20	0.45	5	1	2	1	*0.10*
SLICC>0	5	3	6	5	0.41	19	11	14	8	**0.007**	14	9	10	5	**0.02**	17	13	12	8	*0.09*	2	0	2	0	*0.08*

Low: below or equal to median; high: above median; ctrl: control (ie, non-transmitted alleles or alleles of the spouse). Bold type indicates significance.
AF, affected; NT, non-transmitted; SLICC, Systemic Lupus International Collaborating Clinics; T, transmitted; UN, unaffected (discordant sibs).

the CH50 activity of different haplotype groups were compared. The MHA (containing the rs587585 minor allele) was more associated with C1q levels and CH50 in the lower quartile then were the major haplotypes 1.1 and 2.1, although not reaching significance ($p = 0.051$ and $p = 0.077$, respectively; fig 2A). When the MHA was compared with all the other haplotypes, there were significantly more patients with C1q and CH50 values in the lower quartile in the MHA group ($p = 0.049$, fig 2B). This difference confirms the trends already found for rs587585 in the single SNP association analysis.

No difference in haplotype frequencies was observed between SLE subjects and controls (table 6). To investigate the association of different SLE phenotypes (disease manifestations according to ACR criteria) with polymorphisms of the C1q region, haplotype analysis was performed, but no significant association was found (data not shown). Of note, a stepwise increase of percentage of patients positive for ACR skin criteria (1, 2 and 3) was observed after stratification by rs292001 genotypes: 65% (11/17) AA, 70% (35/50) AG and 81% (29/36) GG. However, this also did not reach statistical significance.

DISCUSSION

This study shows that polymorphisms of the C1q gene are associated with SLE in a sample of 103 Caucasian trios from the northern part of The Netherlands. In addition, another polymorphism in the same region is associated with low serum C1q and CH50 levels.

A significant association of SLE with the tag SNP rs631090, which covers part of the *C1qB* gene, was found in the PDT analysis. Our results suggest an association with low serum C1q levels for this tag SNP. Patients with an absolute C1q deficiency were not present in our cohort. This might be expected, as hereditary C1q deficiency is a very rare condition and is the cause of SLE only in a few cases. The presence of anti-C1q antibodies might also contribute to lower C1q and CH50 levels, but these antibodies were not measured in our population. A possible explanation for the association between polymorphisms in the C1q region and low CH50 levels is that patients having these polymorphisms might be the producers of less or less functional C1q.

Several studies suggest that lower or less functional C1q might contribute to the development of autoimmunity. In C1q deficient mice, the development of autoimmunity is related to impaired clearance of apoptotic cells.[15] An in vitro study demonstrated reduced uptake of apoptotic cells by macrophages in the presence of sera of patients with SLE with low complement levels.[16] In addition, low C1q and CH50 levels may also reflect complement consumption by disease activity. However, all samples in this study were obtained during quiescent disease (SLEDAI $\leqslant 4$). Furthermore, the C1q and CH50 levels (see table 1) are fully comparable with those found in an other study where patients with SLE with low disease activity were analysed.[17] In addition, the median complement C3 and complement C4 levels were within the normal range. Thus, although the influence of complement consumption on

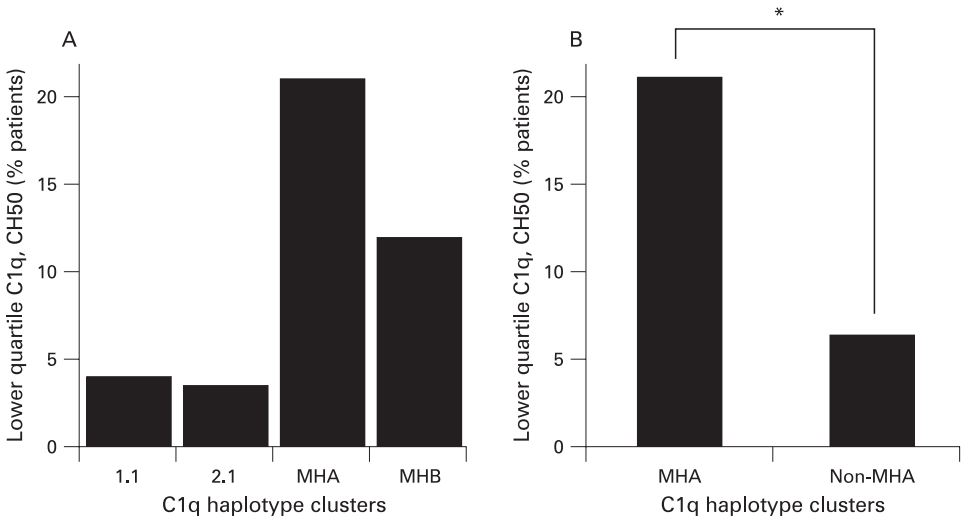

Figure 2 A. Quartile analysis for lower quartile serum C1q combined with lower quartile CH50 comparing the two major haplotypes (named 1.1 and 2.1) with minor haplotype A (MHA) and minor haplotype B (MHB). MHA vs 1.1, $p = 0.051$; MHA vs 2.1, $p = 0.077$. B. Quartile analysis comparing MHA with all other haplotypes, $p = 0.049$.

Table 5 Single single nucleotide polymorphism (SNP) association

Phenotype	rs587585			rs292001			rs294183			rs294179			rs631090		
	Control	Case	p Value	Control	Case	p Value	Control	Case	p Value	Control	Case	p Value	Control	Case	p Value
SLE	13.1	13.1	1.00	36.1	41.5	0.30	31.7	36.2	0.37	40.2	42.9	0.61	3.2	6.4	0.16
	Low	High	p Value	Low	High	p Value	Low	High	p Value	Low	High	p Value	Low	High	p Value
CH50	17	8.7	*0.09*	43.5	36.4	0.33	37.8	34.8	0.68	47.7	37.5	0.17	7.8	5.4	0.52
C1Q	16.4	8.7	0.11	38.7	43.3	0.51	34.3	37.8	0.61	42.2	43.3	0.87	6.6	6.5	0.98
SLICC	0	>0	p Value	0	>0	p Value	0	>0	p Value	0	>0	p Value	0	>0	p Value
	11.5	14.7	0.50	41.4	41.7	0.96	40.4	31.6	0.20	46.2	39.1	0.32	8.8	4	0.16

Allele frequencies are given for SLE cases and controls and for subgroups of SLE cases. Low: below or equal to median; high: above median; ctrl: control (ie, non-transmitted alleles or alleles of the spouse). Bold type indicates significance; italic type indicates values that suggest a trend but do not reach significance.
SLE, systemic lupus erythematosus; SLICC, Systemic Lupus International Collaborating Clinics.

C1q and CH50 levels cannot be excluded, this effect is probably negligible.

Our results from the single tag SNP analysis were not as strong as the results from the PDT analysis. A trend for low serum C1q and CH50 levels was found in association with tag SNP rs587585, which covers the *C1qA* promoter region. The association of tag SNP rs587585 with lower serum C1q and CH50 levels was further analysed by comparing C1q levels and CH50 for different haplotype groups. Lower C1q levels and CH50 were found for the minor haplotype A group when compared with the other groups. This finding confirms the association of the rs587585 minor allele with low serum C1q and CH50, found in the single-SNP analysis. The finding that polymorphisms of the *C1qA* promoter region are associated with low C1q and CH50 levels is supported by a study by Miura-Shimura *et al*.[18] In this study, a polymorphism of the *C1qA* upstream region was associated with low C1q and the development of nephritis in New Zealand Black mice.

None of the other tag SNPs in the PDT and single tag SNP analyses were associated with either SLE, serum C1q or CH50 levels. However, an association of rs292001 and rs294183 with a SLICC score >0 was found. In the haplotype analysis, C1q polymorphisms were not associated with SLE. The differences in results between PDT, the single tag SNP analysis and the haplotypes analysis can be explained by different kinds of information obtained from the data. The PDT focuses specifically on inheritance information whereas tag SNP analysis only compares allele frequencies between patients and controls. The haplotype analysis analyses the evolutionary history of mutations.

Because SLE is a very heterogeneous disease with a large variety of phenotypes, we also performed a haplotype analysis for the different SLE phenotypes according to the (cumulative) ACR criteria. This analysis revealed no significant association between polymorphisms of the C1q region and the different phenotypes, probably because groups with certain disease manifestations were either very small (eg, neurological manifestations) or contained almost all patients, as in the case of anti-nuclear antibodies and antibodies to anti-double-stranded DNA (dsDNA).

The finding that low C1q levels are associated with a polymorphism in the *C1qA* region,[10] was not confirmed in this study. In our study, the marker rs587585 covered part of the *C1qA* promoter region and not the *C1qA* gene itself. We did not find significant results for our marker rs292001, covering part of the *C1qA* gene itself. This difference in findings can be explained by the fact that the study by Racila *et al* contained a relatively small number of patients. Furthermore, these patients had exclusively subacute cutaneous LE. Our study is the first that analysed the involvement of polymorphisms of the C1q region in systemic LE.

Even though the number of patients in our cohort is relatively low and allele frequencies were low, some conclusions might be drawn. Our study had 80% power to discover associations for alleles. Allele frequencies of 5% and 40% have a relative risk for susceptibility for SLE of 3.8 and 2.2, respectively; for difference in subgroups of SLE cases a relative risk of 4.1 and 2.3, respectively.[19] These are moderate to high risks, but the crude odds ratios in this study (~11 for rs631090 for SLE susceptibility, ~6 for rs587585 for high CH50 level, and ~3.0 and ~2.4

Table 6 Haplotype frequencies in systemic lupus erythematosus (SLE) cases and controls.

Haplotype	Cases (%)	Controls (%)	OR*	χ^2 p value*	Part of haplotype group
1-1-1-1-1	49.4	52.3	0.89	0.59	1.1
1-1-1-2-1	1.0	2.2	0.45	0.36	MHB
1-1-2-2-1	3.3	5.1	0.64	0.40	MHB
1-1-2-2-2	0.8	0.0	0	0.28	MHB
1-2-1-1-1	2.4	2.9	0.83	0.77	MHB
1-2-1-2-1	2.2	2.4	0.92	0.90	MHB
1-2-2-2-1	25.2	22.7	1.15	0.59	1.2
1-2-2-2-2	2.6	0.1	25	0.06	MHB
2-1-2-1-1	0.6	0.9	0.67	0.74	MHA
2-1-2-2-1	0.6	0.8	0.75	0.82	MHA
2-1-2-2-2	2.7	2.2	1.23	0.77	MHA
2-2-1-1-1	4.5	2.6	1.75	0.35	MHA
2-2-1-2-1	4.2	5.9	0.70	0.47	MHA
2-2-2-2-2	0.5	0.0	0	0.39	MHA

*Comparing the specific haplotypes with all others. p Value = 0.67 (for haplotypes >3%); controls, non-transmitted alleles or alleles of the spouse; 1 = major allele; 2 = minor allele (left to right: rs587585, rs292001, rs294183, rs294179, rs631090).
MHA, minor haplotype A; MHB, minor haplotype B; OR, odds ratio; 1.1, major haplotype 1.1; 1.2, major haplotype 1.2.

for rs292001 and rs294183, respectively, for positive SLICC scores) are higher. Considering the size of our study population, we expect that the effects of C1q polymorphisms will be more pronounced in a larger population. Nevertheless, the results need to be confirmed in future study, preferably in al larger population of patients with SLE. Furthermore, this study was performed in a 100% Caucasian, stable founder population, in contrast to a lot of other genetic studies concerning SLE which have been performed in multiethnic populations.

In conclusion, this is the first study that suggests an association of C1q polymorphisms with SLE, serum C1q levels, CH50 and disease severity. Further studies should confirm this association and reveal the functional genetic variation that underlies our observations.

Competing interests: None declared.

Ethics approval: The study was approved by the local medical ethics committee.

REFERENCES

1. **Nath SK,** Kilpatrick J, Harley JB. Genetics of human systemic lupus erythematosus: the emerging picture. *Curr Opin Immunol* 2004;**16**:794–800.
2. **Tsao BP.** The genetics of human systemic lupus erythematosus. *Trends Immunol* 2003;**24**:595–602.
3. **Petry F,** Le DT, Kirschfink M, Loos M. Non-sense and missense mutations in the structural genes of complement component C1q A and C chains are linked with two different types of complete selective C1q deficiencies. *J Immunol* 1995;**155**:4734–8.
4. **Marquart HV,** Schejbel L, Sjoholm A, Martensson U, Nielsen S, Koch A, *et al*. C1q deficiency in an Inuit family: identification of a new class of C1q disease-causing mutations. *Clin Immunol* 2007;**124**:33–40.
5. **Flierman R,** Daha MR. Pathogenic role of anti-C1q autoantibodies in the development of lupus nephritis-a hypothesis. *Mol Immunol* 2007;**44**:133–8.
6. **Trendelenburg M.** Antibodies against C1q in patients with systemic lupus erythematosus. *Springer Semin Immunopathol* 2005;**27**:276–85.
7. **Botto M,** Walport MJ. C1q, autoimmunity and apoptosis. *Immunobiology* 2002;**205**:395–406.
8. **Walport MJ,** Davies KA, Botto M. C1q and systemic lupus erythematosus. *Immunobiology* 1998;**199**:265–85.
9. **Walport MJ.** Complement. Second of two parts. *N Engl J Med* 2001;**344**:1140–4.
10. **Racila DM,** Sontheimer CJ, Sheffield A, Wisnieski JJ, Racila E, Sontheimer RD. Homozygous single nucleotide polymorphism of the complement C1QA gene is associated with decreased levels of C1q in patients with subacute cutaneous lupus erythematosus. *Lupus* 2003;**12**:124–32.
11. **Tan EM,** Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, *et al*. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982;**25**:1271–7.
12. **Gladman D,** Ginzler E, Goldsmith C, Fortin P, Liang M, Urowitz M, *et al*. The development and initial validation of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index for systemic lupus erythematosus. *Arthritis Rheum* 1996;**39**:363–9.
13. **Martin ER,** Monks SA, Warren LL, Kaplan NL. A test for linkage and association in general pedigrees: the pedigree disequilibrium test. *Am J Hum Genet* 2000;**67**:146–54.
14. **Martin ER,** Bass MP, Kaplan NL. Correcting for a potential bias in the pedigree disequilibrium test. *Am J Hum Genet* 2001;**68**:1065–7.
15. **Botto M,** Dell'Agnola C, Bygrave AE, Thompson EM, Cook HT, Petry F, *et al*. Homozygous C1q deficiency causes glomerulonephritis associated with multiple apoptotic bodies. *Nat Genet* 1998;**19**:56–9.
16. **Bijl M,** Reefman E, Horst G, Limburg PC, Kallenberg CG. Reduced uptake of apoptotic cells by macrophages in systemic lupus erythematosus: correlates with decreased serum levels of complement. *Ann Rheum Dis* 2006;**65**:57–63.
17. **Grevink ME,** Horst G, Limburg PC, Kallenberg CG, Bijl M. Levels of complement in sera from inactive SLE patients, although decreased, do not influence in vitro uptake of apoptotic cells. *J Autoimmun* 2005;**24**:329–36.
18. **Miura-Shimura Y,** Nakamura K, Ohtsuji M, Tomita H, Jiang Y, Abe M, *et al*. C1q regulatory region polymorphism down-regulating murine c1q protein levels with linkage to lupus nephritis. *J Immunol* 2002;**169**:1334–9.
19. **Gauderman WJ.** Sample size requirements for association studies of gene-gene interaction. *Am J Epidemiol* 2002;**155**:478–84.

Analysis of C1q polymorphisms suggests association with systemic lupus erythematosus, serum C1q and CH50 levels and disease severity

H A Martens, M W Zuurman, A H M de Lange, et al.

Ann Rheum Dis 2009 68: 715-720 originally published online May 26, 2008
doi: 10.1136/ard.2007.085688