Lack of Association of C-C Chemokine Receptor 5 Δ32 Deletion Status with Rheumatoid Arthritis, Systemic Lupus Erythematosus, Lupus Nephritis, and Disease Severity

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ABSTRACT. ***Objective***. C-C chemokine receptor 5 (CCR5) plays an important role in inflammation. A 32 base-pair (Δ32) deletion in the CCR5 gene leads to a nonfunctional receptor. This deletion has been reported to have a protective effect on the development and progression of several autoimmune diseases. We investigated whether the Δ32 deletion is associated with disease susceptibility in a population of patients with rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and lupus nephritis (LN); and whether it is associated with disease severity.

Methods. DNA samples from 405 RA patients, 97 SLE patients, 113 LN patients, and 431 healthy controls were genotyped for the CCR5 Δ32 deletion. Differences in genotype frequencies were tested between patients and controls. Association of genotypes with disease severity was analyzed.

Results. Genotype frequencies of each group were in Hardy-Weinberg equilibrium. The genotype frequencies of patients did not differ significantly from controls (CCR5/Δ32, Δ32/Δ32: RA 18.3% and 1.2%, respectively; SLE 17.5% and 2.1%; LN 13.3% and 1.8%; controls 20.0% and 2.8%). However, there was a trend for lower Δ32 deletion allele frequency in LN patients compared to controls ($p = 0.08$). There was no significant association between the CCR5 status and disease severity in RA, SLE, or LN.

Conclusion. Although an association with LN cannot be excluded, the CCR5 Δ32 deletion does not seem to be a disease susceptibility genotype for RA, SLE, or LN. No significant effect of the Δ32 deletion on disease severity was demonstrated. (First Release August 1 2010; J Rheumatol 2010;37:2226–31; doi:10.3899/jrheum.091468)

Key Indexing Terms:
C-C CHEMOKINE RECEPTOR 5 Δ32 BASE-PAIR DELETION
SYSTEMIC LUPUS ERYTHEMATOSUS RHEUMATOID ARTHRITIS LUPUS NEPHRITIS

Chemokines, a family of proteins that play an important role in inflammation, attract inflammatory cells such as leukocytes, lymphocytes, and macrophages by interacting with chemokine receptors that are expressed on the surface of these cells. Chemokines are important in homeostatic as well as in inflammatory conditions. In several autoimmune diseases, an enhanced level of chemokines and increased expression of chemokine receptors have been found. One of these receptors, C-C chemokine receptor 5 (CCR5), has several ligands, including CCL5/RANTES (regulated on activation, normal T cell expressed and secreted). CCR5 is highly expressed on T lymphocytes and is important for recruitment of T cells[1,2]. A deletion of 32 base pairs (Δ32 bp deletion) in the gene encoding for CCR5 leads to the pro-

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duction of a nonfunctional receptor. The clinical importance of this deletion was first demonstrated in human immunodeficiency virus (HIV) infection, where CCR5 acts as an important coreceptor. In HIV, homozygosity for the Δ32 bp deletion leads to almost 100% resistance against the HIV virus[3]. Subsequently, the Δ32 bp deletion was also reported to have a protective effect on susceptibility for several autoimmune diseases such as rheumatoid arthritis (RA)[4], on progression of (inflammatory) kidney diseases, and on solid organ allograft rejection[5].

The Δ32 bp deletion has been reported to have a protective effect against the development of RA[4]. In established RA, the Δ32 bp deletion seems to influence disease severity[6,7]. However, results have been conflicting[8,9]. In patients with systemic lupus erythematosus (SLE), there are few data on the role of the Δ32 bp deletion. Although in 2 studies no direct correlation of the Δ32 bp status with SLE was found[10,11], another study reported an increased risk for SLE in subjects with the Δ32 bp deletion[12]. In a mouse model of SLE, expression of chemokines and the CCR5 receptor was highly associated with the development and progression of renal disease (lupus nephritis, LN)[13,14]. Also in human LN, increased expression of CCR5-positive cells was found[5]. To date, except for the study by Mamtani, *et al*[12], no study has focused on the role of the CCR5 Δ32 bp deletion in LN.

Thus CCR5 may play a role in several autoimmune diseases, including RA and SLE. We evaluated the association of the CCR5 Δ32 bp status with susceptibility for RA and SLE. In addition to studying a large, well defined, and stable founder population of RA and SLE patients, we analyzed a large, well defined and homogeneous population of patients with LN. Further, we determined whether the Δ32 bp deletion is associated with disease severity in RA, SLE, and LN.

MATERIALS AND METHODS

Patient selection. RA. In 2002, all RA patients who fulfilled the American College of Rheumatology (ACR) criteria for RA[15] treated at the University Medical Center Groningen (UMCG) were asked to participate in a study on genetic predisposition of RA. In total, 405 patients were included. In a subgroup of 107 patients, prospective information on disease severity was available. This included cumulative C-reactive protein (CRP) levels over a period of 24 months, Disease Activity Score (DAS) according to Van der Heijde with 3 variables (number of swollen joints, erythrocyte sedimentation rate, Ritchie articular index)[16] at disease onset, Ritchie articular index[17], and progression of erosions after 24 months of followup (measured by the modified Sharp-van der Heijde score[18]). Also rheumatoid factor and anti-cyclic citrullinated peptide (anti-CCP) levels were available.

SLE. In the period November 2000 to November 2001 we contacted all patients with SLE treated at UMCG to invite them to participate in a study on the genetic predisposition of SLE. All patients met ACR criteria for SLE[19]. In total, 106 patients were eligible. Nine of these patients, however, were already included in the lupus nephritis studies (see below), so 97 SLE patients were included for further analysis.

Lupus nephritis. From September 1995 until November 2006, patients with biopsy-proven proliferative LN (World Health Organization ISN/RPS class III or IV) were included in the first and second Dutch LN studies. Patients included in this study were treated according to a protocol (first study: azathioprine combined with methylprednisolone and prednisone versus cyclophosphamide with prednisone[20]; second study: prednisone with cyclophosphamide followed by mycophenolate mofetil). Blood samples were available for genetic analysis from 113 of these patients. In LN, proteinuria and creatinine clearance results (measured by the Cockcroft-Gault formula) at the time of inclusion, after 5 months, and after 30 months of therapy were used as markers for disease severity. If the followup was shorter than 30 months, the last available followup data were included. Treatment failure was defined as a renal relapse requiring escalation of therapy or renal failure requiring dialysis or renal transplant. In addition, complement C3 and C4 levels and anti-dsDNA antibody levels at baseline were available.

Controls. Partners of patients included in the study of the RA and SLE patients of the UMCG cohort were included as controls. Controls had no RA or SLE. Additional healthy controls were included from a study of risk for development of colorectal cancer as described[21]. These controls also had no RA or SLE.

All patients and controls gave written informed consent to participate in this study. The study was approved by the local medical ethics committee.

Genotyping. DNA samples were genotyped for the CCR5-del32 variation by the 5'-nuclease assay (TaqMan). Primer and probe sequences were essentially as described[22], using TET and FAM as reporter dyes, and TAMRA as quencher. 5'-CAG GAA TCA TCT TTA CCA GAT CTC AA was used as forward primer, 5'-CGA GTA GCA GAT GAC CAT GAC AA as reverse primer, TET-AGT CAG TAT CAA TTC TGG AAG AAT TTC CAG ACA TTA A-TAMRA as 'ins' probe, and FAM-CAG CTC TCA TTT TCC ATA CAT TAA AGA TAG TCA TCT TG-TAMRA as 'del' probe. The assay was performed as a standard TaqMan assay in 10 μl volumes with primer concentrations at 700 nM and probe concentrations 100 nM. Results were analyzed on an ABI-7900HT system and with SDS software (Applied Biosystems, Foster City, CA, USA).

Statistical analysis. The genotype frequencies of controls were tested for Hardy-Weinberg equilibrium using the chi-square test on the observed versus the expected frequencies according to the Hardy-Weinberg formula. Differences in genotype frequencies were tested between patients and controls using the Pearson chi-square test. After combining the Ins/Del and the Del/Del groups, Fisher's exact test was used to investigate significant differences.

In a subgroup of 107 RA patients, the association of genotypes with disease severity was analyzed. For these analyses we dichotomized the allele groups into individuals homozygous for the insertion (ins/ins) and individuals homozygous or heterozygous for the deletion (del/del or ins/del). We tested for differences in these 2 groups with regard to cumulative CRP levels, radiologic damage, and Ritchie articular index using the t-test for parametric variables and the Mann-Whitney U test for nonparametric variables. For differences in disease measures in LN patients, Mann-Whitney or Student t-test was used as appropriate.

RESULTS

In total, 405 patients with RA, 97 patients with SLE, 113 patients with LN, and 431 healthy controls were included in our study. For additional characteristics of the SLE and RA patients, see Table 1.

As a measure for quality of the markers, the genotype frequencies were checked for Hardy-Weinberg equilibrium. The genotype frequencies of the controls were in Hardy-Weinberg equilibrium.

In order to determine whether the CCR5 Δ32 bp deletion is associated with either RA or SLE, we analyzed the genotype frequencies of the Δ32 bp deletion in these groups

Table 1. Baseline characteristics of patients with SLE and RA.

Characteristic		
SLE, n = 97		
Median age, yrs (range)	44	(23–78)
Female, n (%)	83	(86)
ACR criteria, n (%)		
Malar rash	36	(37)
Discoid rash	30	(31)
Photosensitivity	50	(52)
Oral ulcers	13	(13)
Arthritis	61	(63)
Serositis	38	(40)
Renal disorder	38	(40)
Neurologic disorder	7	(7)
Hematologic disorder	68	(70)
Immunologic disorder	79	(81)
Anti-dsDNA	75	(77)
Anti-Sm	13	(13)
Antiphospholipid antibodies	16	(16)
Antinuclear antibody	97	(100)
RA, n = 405		
Median age, yrs (range)	61	(25–86)
Female, n (%)	295	(73)
Rheumatoid factor-positive (%)	379	(94)
Erosive disease (%)	379	(94)

(Table 2). The genotype frequencies did not differ between RA patients and controls (p = 0.2), or between SLE patients and controls (p = 0.8). In addition, we analyzed the number of carriers of the Δ32 bp deletion in RA and SLE patients, but found no differences in numbers of carriers compared with controls (p = 0.3 and 0.5, respectively; Table 3). In addition, we found no difference in allele frequencies between either patient group and controls (data not shown).

To analyze whether the Δ32 bp deletion is involved in disease severity in RA patients, we performed a subgroup analysis in 107 patients. In this group, disease severity markers were available: cumulative CRP, Disease Activity Score, Ritchie index at disease onset, and radiographic damage after 24 months of followup. We determined the differences of several disease severity markers between RA patients who were carriers of the Δ32 bp deletion (n = 18) and those who were not (n = 89, Table 4). For all disease severity markers, there were no differences between carriers of the Δ32 bp deletion and noncarriers. In addition, there were no differences in prevalence of and level of rheumatoid factor and anti-CCP levels between the 2 groups.

Table 3. CCR5 Δ32 bp deletion carriers.

Patient Group	CCR5d32 Ins/Ins	Del	Total	p†
Rheumatoid arthritis	326 (80.5)	79 (19.5)	405 (100.0)	0.3
Systemic lupus erythematosus	78 (80.4)	19 (18.9)	97 (100.0)	0.5
Lupus nephritis*	96 (85.0)	17 (15.0)	113 (100.0)	0.08
Controls	333 (77.3)	98 (22.7)	431 (100.0)	
Total	833 (79.6)	213 (20.4)	1046 (100.0)	

* Lupus nephritis versus SLE was not significant (p = 0.7). CCR5d32: Δ32 bp deletion; Ins: Δ32 bp insertion; Del: Δ32 bp deletion; † p value differences between patient groups and controls, Fisher's exact test.

We also analyzed whether the CCR5 Δ32 bp deletion was involved in the degree of disease severity in SLE patients. For this purpose, we determined the CCR5 Δ32 bp deletion allele frequencies in the original group of 106 SLE patients who developed biopsy-proven proliferative LN during the course of their disease (n = 26). Compared to SLE patients who developed no LN, patients with LN had the same number of CCR5 Δ32 bp deletion alleles (p = 1.0).

To determine whether the CCR5 Δ32 bp deletion was associated with disease susceptibility in LN, we also analyzed the Δ32 bp deletion in a group of 113 patients with biopsy-proven, proliferative LN. The genotype frequencies of LN did not differ from controls (p = 0.4), or from the 97 SLE patients from the UMCG cohort. The number of Δ32 bp deletion carriers and the Δ32 bp deletion allele frequencies also did not differ significantly between LN patients and controls or between LN patients and SLE patients. However, there was a trend for difference in the number of CCR5 Δ32 bp carriers between LN patients and controls (p = 0.08). In the LN group, there were fewer patients that carried the CCR5 Δ32 bp deletion allele (15.0% in LN vs 22.7% in controls).

As CCR5 might play a role in the progression of inflammatory kidney diseases, we analyzed the association of the CCR5 Δ32 bp deletion with disease severity and outcome of proliferative LN (Table 5). In patients with and without the

Table 2. CCR5 Δ32 bp deletion genotype frequencies and Hardy-Weinberg equilibrium. Values are number (%).

	CCR5d32 Ins/Ins	Ins/Del	Del/Del	Total	p_{HW}	$p_{chi\text{-}square}$
Rheumatoid arthritis	326 (80.5)	74 (18.3)	5 (1.2)	405 (100.0)		0.2
Systemic lupus erythematosus	78 (80.4)	17 (17.5)	2 (2.1)	97 (100.0)		0.8
Lupus nephritis	96 (85.0)	15 (13.3)	2 (1.8)	113 (100.0)		0.4
Controls	333 (77.3)	86 (20.0)	12 (2.8)	431 (100.0)	0.1	
Total	833 (79.6)	192 (18.4)	21 (2.0)	1046 (100.0)		

CCR5d32: Δ32 bp deletion; Ins: Δ32 bp insertion; Del: Δ32 bp deletion; p_{HW}: p value Hardy-Weinberg equilibrium; $p_{chi\text{-}square}$: p value genotype differences between patients and controls.

Table 4. Prospective data on disease severity in 107 patients with RA..

Patient Group	CCR5d32 Ins/Ins, n = 89	CCR5d32 Del, n = 18	p
CRP cumulative, t = 24	1099 [587–2253]	1525 [1073–1938]	0.18
Erythrocyte sedimentation rate	38 [18–61]	36 [26–49]	0.93
RF level	142 [65–375]	132 [50–335]	0.86
RF positivity, n (%)	84 (79)	18 (17)	0.45
Anti-CCP level	283 [105–644]	230 [54–802]	0.83
Anti-CCP positivity, n (%)	77 (72)	16 (15)	0.38
Disease Activity Score	6.27 ± 1.36	6.53 ± 1.02	0.37
Ritchie index	7 [4–13]	11 [6–16]	0.20
XTT, t24 – t0	18 [7–30]	16 [9–33]	0.75

Ins: Δ32 bp insertion; Del: Δ32 bp deletion; CRP cumulative t = 24: cumulative CRP after 24 mo followup; XTT, t24 – t0: difference in radiologic damage between start of followup and after 24 mo. p: nonparametric variables, displayed as median [25th percentile–75th percentile], tested by Mann-Whitney U-test; categorical variables, displayed as n (%), by chi-square test; parametric variables, displayed as mean ± standard deviation, tested by Student t-test.

Table 5. Followup data in patients with lupus nephritis in relation to CCR5 Δ32 bp status.

CCR5 d32	Ins/Ins	Del	p
Urinary protein excretion g/24h			
T0	3.4 (1.4–5.9), n = 86	3.1 (2.2–5.8), n = 16	0.6
T1	0.8 (0.2–1.5), n = 69	0.6 (0.2–1.1), n = 16	0.6
T2	0.3 (0.1–0.7), n = 66	0.1 (0.0–0.2), n = 13	0.3
T3	0.3 (0.1–0.7)*, n = 55	0.2 (0.0–0.9)**, n = 10	0.7
Creatinine clearance ml/min			
T0	65.10 ± 23.84, n = 91	63.30 ± 31.11, n = 16	0.8
T1	75.92 ± 26.11, n = 90	68.48 ± 24.22, n = 16	0.3
T2	77.97 ± 27.69, n = 82	78.73 ± 17.87, n = 16	0.9
T3	74.69 ± 23.36†, n = 55	78.67 ± 14.97††, n = 10	0.6

* $p < 0.001$ vs T0; ** $p = 0.005$ vs T0; † $p < 0.001$ vs T0; †† $p = 0.06$ vs T0. T0: time of inclusion; T1: after 5 months of treatment; T2: after 24 months; T3: after 2.6 years of treatment or latest available followup data if followup < 2.6 years. n = number of patients for whom data were available.

CCR5 Δ32 bp deletion, proteinuria decreased significantly over time. Renal function as measured by creatinine clearance improved in all patients. These changes in proteinuria and creatinine clearance did not differ between the patients with the CCR5 Δ32 bp deletion and those without. Renal relapse and renal failure (n = 10) also were not associated with the CCR5 Δ32 bp deletion status in a Cox regression model ($p = 0.61$). In addition to the followup data, we also analyzed the difference in complement levels and anti-dsDNA levels at baseline in our LN patients. Levels of complement C3, complement C4, and anti-dsDNA were not different for patients with the CCR5 Δ32 bp deletion versus those without ($p = 0.5$, $p = 0.9$, and $p = 0.1$, respectively).

DISCUSSION

In a group of 405 RA, 97 SLE, and 113 LN patients, we could not find an association with CCR5 Δ32 bp deletion status and disease susceptibility. In addition, the CCR5 Δ32 bp deletion did not seem to be associated with the severity of these diseases.

CCR5 plays an important role in inflammation and inflammatory diseases such as RA[23]. In patients with RA, an enrichment of CCR5-positive monocytes in the synovial fluid has been demonstrated[7]. In synovial tissue of RA patients, increased expression of the CCR5 ligand RANTES has been found in lining layer cells, macrophages, and fibroblasts[24]. Thus, increased expression of both the CCR5 receptor and its ligand has been demonstrated in patients with RA. Whether this expression is associated with the Δ32 bp deletion status is less clear. In our study, a negative association of the Δ32 bp deletion with the development of RA could not be demonstrated. The fact that we did not find a significant association of the Δ32 bp deletion with RA makes an important effect of the CCR5 Δ32 bp deletion unlikely. In the literature, results regarding the association of CCR5 Δ32 bp deletion with RA have been conflicting. Some studies could find no protective effect of the Δ32 bp deletion for RA[7,8]. In contrast, a metaanalysis of 5 studies comprising 1790 RA patients did suggest a protective effect of the Δ32 bp deletion on the development of RA (pooled

odds ratio 0.65, 95% CI 0.55–0.77)[4]. Thus, the functional effect of the CCR5 Δ32 bp deletion seems rather small. However, the fact that we did not find an effect of the CCR5 Δ32 bp deletion does not exclude that other CCR5 variants (such as the HHC and HHE haplotype) may play a role in RA. In addition, other chemokines and receptors may also play an important role in RA. A large variety of chemokines and their receptors have been associated with RA[23]. In addition, RA is a heterogenic and polygenic disease. Therefore, other genetic factors may contribute more to susceptibility for RA. The HLA locus, particularly variants within the HLA-DRB1 genes, has proven to have the highest attributable risk in RA[25]. Also, the PTPN22 gene is more strongly associated with RA in populations of European descent, suggesting that the CCR5 Δ32 bp deletion may only have a low attributable risk in RA.

In the analysis of a subgroup of 107 RA patients, we were not able to demonstrate an (protective) effect of the Δ32 bp deletion on the severity of RA. In the literature, as for association with development of RA, results with regard to association of Δ32 bp deletion with disease severity are conflicting. Some studies suggest a protective effect of the Δ32 bp deletion on disease severity[6,26,27], while others could not confirm this[11,28].

Data on the severity of RA were available in a subgroup of our patients who originated from a prospective cohort study in which RA patients were included consecutively. Although this still represents over 25% of the total group, a possible effect of the Δ32 bp deletion on the severity of RA cannot be fully excluded. In addition, the time of followup of 2 years might be too short to detect any differences.

Activation of the chemokine system is also important in SLE. In SLE patients, increased levels of chemokines during active disease have been found[29]. Compared with healthy controls, SLE patients have significantly higher levels of the CCR5 receptor ligand RANTES[30,31]. In our study, there were no differences in CCR5 Δ32 bp deletion status between patients and controls, so neither a protective nor a susceptibility effect of the Δ32 bp deletion on SLE could be established. Only a few studies have been performed into the association of SLE with the CCR5 Δ32 bp deletion. Although a protective effect of the Δ32 bp deletion has been suggested for HIV and in some studies for RA, the available data on SLE suggest an increased risk of SLE for the Δ32 bp deletion[11,12]. In the study by Mamtani, *et al*, the genotypes containing the Δ32 bp deletion were associated with increased risk for SLE[12]. Gomez-Reino, *et al* found no difference in Δ32 bp deletion status between SLE patients and controls[11]. Aguilar, *et al*[10] found a slight contribution of the Δ32 bp deletion to the production of anti-dsDNA autoantibodies. In our study, there was no association of the CCR5 Δ32 bp deletion with anti-dsDNA levels.

To date, no genetic studies have been performed on CCR5 Δ32 bp deletion status in patients with LN. Our study is the first to analyze the Δ32 bp deletion in a homogenous, well defined population of patients with biopsy-proven proliferative LN. We also analyzed the CCR5 Δ32 bp deletion in a well defined population of RA and SLE patients and assessed disease severity in these patients.

In the RA and the SLE patients, there were no significant differences in genotypes or in Δ32 bp deletion allele frequencies compared to LN patients and controls. However, a small contribution of CCR5 Δ32 bp status to susceptibility for LN cannot be excluded, as there was a trend for fewer Δ32 bp del carriers in the LN patients compared to control patients. This could mean that the CCR5 Δ32 bp deletion might be a protective phenotype for the development of LN. However, since LN patients also had SLE, it cannot be clearly differentiated whether this difference in CCR5 Δ32 bp deletion would refer more to SLE or to LN.

CCR5 Δ32 bp deletion status was not associated with outcome in LN. Proteinuria decreased and creatinine clearance increased in all patients, irrespective of CCR5 status. Renal failure or renal relapse was not associated with CCR5 status. There was no association of CCR5 status with complement levels and anti-dsDNA antibody levels in LN patients. Although CCR5 plays an important role in inflammatory kidney diseases including LN[5], as well as in a mouse model of SLE[13,14], we could not relate this to the CCR5 Δ32 bp deletion status in our patients.

The number of patients in our study was relatively low. However, the well characterized and homogeneous cohort strengthens the power of the study, as other genetic studies in complex diseases are often hampered by phenotypic heterogeneity. Because linkage analysis studies in SLE have proven that stratification by clinical symptoms increases power to detect genetic association[32,33], we think that firm conclusions can be drawn from our study.

Another drawback of our study is the relatively short duration of followup; effects of CCR5 Δ32 bp deletion in the long term might have been missed.

Although the relevance of the CCR5 Δ32 bp deletion in RA and SLE has not fully been elucidated, CCR5 might still be a therapeutic target in these diseases. The availability of CCR5 receptor antagonists opens possibilities for trials with these drugs in both RA and SLE. A phase II clinical trial in RA patients comparing treatment with methotrexate combined with a CCR5-blocking agent (maraviroc) versus methotrexate with placebo is under way (NCT00427934). The first results are expected to be available soon.

In a well defined, stable founder population of RA, SLE, and LN patients, we could not demonstrate a significant association of these diseases with the CCR5 Δ32 bp deletion status. Therefore, the Δ32 bp deletion does not seem to be involved in susceptibility for these diseases, although a small (protective) effect of the CCR5 Δ32 bp deletion on the development of LN cannot be excluded. In addition, there was no association of the Δ32 bp deletion with the severity of RA, SLE, or LN.

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