CCR2 Polymorphism in Chronic Renal Failure Patients Requiring Long-Term Hemodialysis

Ilhan Sezgin¹, Binnur Koksal¹, Gokhan Bagci¹, Hande Kucuk Kurtulgan¹ and Ozturk Ozdemir²

Abstract

Objective A number of chemokines and chemokine receptors are produced by intrinsic renal cells as well as by infiltrating cells during renal inflammation. The CCR2 chemokine receptor mediates leukocyte chemotraction in the initiation and amplification phase of renal inflammation. The polymorphism, CCR2-V64I, changes valine 64 of CCR2 to isoleucine. We aimed to determine the frequency of CCR2-V64I polymorphism in patients with chronic renal failure requiring long-term hemodialysis.

Methods and Patients The PCR-based restriction fragment length polymorphism (PCR-RFLP) technique was used to assess the gene frequencies of CCR2-641 in CRF patients (n=210) and healthy controls (n=139) in the current study.

Results The frequencies of the CCR2 genotype were 0.68 for V/V, 0.28 for V/I, and 0.4 for I/I in the CRF patients and 0.81 for V/V, 0.18 for V/I and 0.1 for I/I in healthy controls. The distribution of the CCR2-V64I mutant genotype was significantly different between subjects with CRF and healthy control subjects (X²=7.197 and p=0.027).

Conclusion We found that the CCR2-V64I polymorphism was significantly high in CRF patients. In addition to the contribution to disease pathogenesis, it was recently found that chemokines have therapeutic importance in chronic renal failure. The frequency of CCR2-V64I and other chemokine and chemokine receptor polymorphisms in renal pathologies must be further investigated in larger study populations and in different renal diseases.

Key words: CCR2 gene, chronic renal failure, chemokines

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Introduction

Chemokines are proinflammatory cytokines that function in leukocyte chemotraction (1). Chemokines can be divided into four families based on their amino acid sequence in relation to their cysteine moieties/differences in structure: CC, CXC, CX3C and XC (2). CCR2 is a cognate receptor of MCP1, a member of the CC family of chemokines (also termed monocyte chemoactive and activating factor, or CCL2), which is mainly expressed on monocytes (3).

CCR2 protein has 374 amino acids and the polymorphism, CCR2-V64I (also named CCR2G190A) is a transition mutation that changes valine 64 of CCR2 to isoleucine. Studies indicate that CCR2 V64I mutation does not affect the CCR2 expression level (4). The amino-terminal domain of CCR2 is necessary for binding of MCP1. CCR2 mutations have been associated with insulin-dependent diabetes mellitus (5), a reduced risk for severe coronary artery disease (6), and a delay in the progression to AIDS in human immunodeficiency virus (HIV)-infected individuals (7).

Locally secreted chemokines mediate leukocyte recruitment during the initiation and amplification phase of renal inflammation. In the initiation phase, renal cells release chemokines and induce leukocyte infiltration to the place of injury. In the amplification phase the infiltrating leukocytes

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contribute to renal damage by releasing inflammatory and profibrotic factors (8). Termination of the chemokine signal is critical for the resolution of the inflammatory process (9). In the case of chronic disease process, even if the initial injury to the kidney subsides, chemokine-mediated leukocyte recruitment can be maintained or exacerbated by other mechanisms such as infection, activation of rennin-angiotensin system, hypoxia, and proteinuria (10).

Chemokines also have been implicated in acute cardiac and renal allograft rejection. It was hypothesized that for acute allograft rejection monocytes and T-effector cells are directed into the transplant and produce a characteristic tubular or vascular infiltrate. The complex process of extravasation and influx of leukocyte subsets into the site of tissue injury appears to be mediated by specifically expression of the CC-chemokine MCP-1 together with the corresponding chemokine receptor CCR2 can be detected in mononuclear cells infiltrating the kidney graft (11, 12). The association of human chemokine receptor genetic variants, CCR5-Delta32, CCR5-59029-A/G, CCR2-V64I, CX3CR1-V249I, and CX3CR1-T280M, with outcome of renal transplant recipients was examined by Abdi et al and significant reductions were found in the risk of acute renal transplant rejection in recipients who possessed the CCR2-64I allele (13). Kang et al found a significant increase for the risk of late acute rejection in recipients who were homozygous for the MCP-1-2518G polymorphism and no difference in the incidence of rejection among recipients stratified by the CCR2-V64I genotype (14).

We aimed to determine the frequency and possible role of inherited CCR2 gene mutation in patients with chronic renal failure.

Materials and Methods

Study population

Two hundred and ten Turkish patients (average age of 57.4±14.3) who suffered from chronic renal impairment and required hemodialysis for approximately 5.0±4.4 years in the four different dialysis centres from Sivas city were enrolled in the current study. Mean hemodialysis duration was 5.4±4.9 hours per week. One hundred thirty-nine age-matched healthy individuals with no history of Type II diabetes, hypertension, renal, cardiac, or family history of renal disease were selected as healthy controls.

CCR2 V64I genotyping

Peripheral blood samples obtained from both the subjects in the CRF and control groups were collected in tubes containing 1 mL of EDTA and stored at -20°C. Total genomic DNA was obtained from a 100 μL peripheral blood sample with an Invitek kit extraction technique (Invisorb spin blood; Invitek, Berlin, Germany). Genotype was determined by polymerase chain reaction/restriction fragment length polymorphism (PCR-RFLP) analysis.

The PCR mixture in a 25-μL final volume consisted of 12.5 μL PCR master mix (Fermentas, St. Leon-Rot, Germany), 9.5 μL ddH2O, 1 μL of each primer, and 1 μL DNA. The G to A mutation at position 190 of CCR2 gene was determined by PCR-RFLP. The following primers were used for amplification: forward 5’-CAT TGC AAT CCC AAA GAC CCA CTC-3´ and reverse 5´-TTG GTT TTG TGG GCA ACA TGA TGG-3´. Initial denaturation at 94°C for 5 minutes was followed by 33 cycles of denaturation at 94°C for 30 seconds, annealing at 56°C for 30 seconds and extension at 72°C for 30 seconds. Final extension step was at 72°C for 5 minutes.

PCR product (5 μl) was digested for 2 hours at 65°C with 2.5 U of BsaBI restriction endonuclease (Fermentas). Digestion products were analyzed by electrophoresis on 3% agarose gel in TBE buffer and visualized using ethidium bromide staining. Samples with a single 173 bp band were identified as having GG genotype, samples with two bands, 149 bp and 24 bp as AA genotype and those with three bands, 173 bp, 149 bp and 24 bp as GA heterozygotes.

Statistical analysis

Statistical analysis was performed using SPSS software package, version 15.0. Differences in the distribution of chemokine polymorphisms between cases and controls were tested using chi square test. Results were considered significant when the p value was less than 0.05.

Results

Hypertension, Type II Diabetes, atherosclerosis and haematological diseases were found in 52.38%, 27.62%, 11.42% and 2.9% of the patients respectively. 11.2% of patients have parental consanguinity and 2.5% of patients have FMF history in their families. There was no renal disease in 82.6% of patients before chronic renal failure (Table 1).

For the entire group of patients with CRF, the frequencies of the CCR2 V64I polymorphisms as GG, GA and AA genotypes were 68.1% (n=143), 28% (n=58), and 4.3%(n=9) respectively. Distribution of CCR2 V64I polymorphisms (GG, GA, AA) in healthy controls were 80.6% (n=112), 18% (n=25), and 1.4% (n=2), respectively (Table 2). The distribution of the CCR2-V64I mutant genotype was significantly different between subjects with CRF and healthy control subjects (X2= X2=7.197 and p=0.027). Distribution of CCR2 genotypes in three different disease sub-groups of CRF patients were also evaluated and no significant association was found in these diseases and CCR2 genotypes (Table 3).

Discussion

Clarifying the chemokine network that functions in leukocyte recruitment may represent a promising therapeutic option for progressive renal disorders (8). The importance of chemokine and chemokine receptors in renal diseases was
investigated by blocking these ligands and receptors with neutralizing antibodies and chemokine receptor antagonists and targeted disruption of genes encoding chemokine receptor genes in animal models (8). The strategy of blocking MCP-1/CCR2 interaction found to be effective in preventing macrophage-induced tissue damage. Neutralizing antibodies against CCL2/MCP-1 blocked glomerular infiltration of macrophages in the rat anti-thymocyte antibody-induced glomerulonephritis model (15). Treatment anti-CCL2/MCP-1 antibodies reduced proteinuria and glomerular macrophage influx in rat nephrotic serum nephritis (16). Consistently, a preliminary report demonstrated that blockade of CCR2 with a series of antagonists ameliorated disease, including glomerular injury, in the rat model (17). Kitagawa et al found that the therapeutic strategy of blocking CCR2 may prove beneficial for progressive fibrosis via the decrease in infiltration and activation of macrophages in the diseased kidneys (3). Chemokines and their receptors have many other functions in addition to leukocyte migration to sites of tissue injury. These includes homeostatic functions in leukocyte development, cell trafficking during immune surveillance, hematopoiesis and, angiogenesis (10). In a knockout mice study, mice lacking CCR2 for CCL2/MCP-1, the proteinuria and glomerular pathology of nephrotic serum nephritis, were worse despite reduced glomerular macrophage infiltration. This study indicates that the absence of CCR2 may influence other immune mechanisms in addition to local cell infiltration (18). In a preliminary study Biyikli et al (2006), found no significant difference in CCR2 and CCR5 polymorphisms between 25 children with biopsy proven focal segmental glomerulosclerosis (FSGS) and 40 healthy age-matched controls (19). In the current study we find that the frequency of CCR2-V64I mutant genotype was significantly higher than in healthy controls. This higher mutation frequency may be related to the heaviness of the CRF and especially in the CRF with chronic disease. CCR2 is only one of the chemokine receptors that is expressed by inflammatory cells after renal injury. Characteristic chemokines and chemokine receptors participate in particular pathologic changes at specific time points. Chemokine cascades in ischemia-reperfusion injury pathologic change of tubular necrosis after ischemic acute kidney injury are mainly mediated by the action of CCR2 on macrophages. Regeneration of tubular epithelial cells in the late phase of the injury was mediated by IP-10, a CXC class chemokine, producing macrophages. Chronic interstitial fibrosis was mediated by the action of CX3CR1 on macrophages and platelets (20).

Monocyte/macrophage infiltration has been detected in the glomeruli of rats with streptozotocin-induced diabetes and in renal biopsy specimens from patients with diabetic nephropathy, suggesting that the secretion of chemokines is enhanced in diabetes and that monocyte recruitment to renal tissues and differentiation to macrophages may be associated with the development or progression of diabetic nephropathy (21-23). Although Prasad et al and Nakajima et al found an association between CCR5 polymorphisms and diabetic

### Table 1. Demographic Findings of Study and Control Group

<table>
<thead>
<tr>
<th>Demographic Findings</th>
<th>Patients % (n)</th>
<th>Controls % (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>male</td>
<td>51.90 (109)</td>
<td>50</td>
</tr>
<tr>
<td>female</td>
<td>48.10 (101)</td>
<td>95</td>
</tr>
<tr>
<td>Average age</td>
<td>57.4±14.3</td>
<td>57.24±9.71</td>
</tr>
<tr>
<td>Parental consanguinity</td>
<td>9.52 (20)</td>
<td>unknown</td>
</tr>
<tr>
<td>HT</td>
<td>52.38 (110)</td>
<td>0</td>
</tr>
<tr>
<td>DM</td>
<td>27.62 (58)</td>
<td>0</td>
</tr>
<tr>
<td>Atherosclerosis</td>
<td>11.42 (24)</td>
<td>0</td>
</tr>
</tbody>
</table>

HT: Hypertension, DM: Diabetes Mellitus, FMF: Familial Mediterranean Fever

### Table 2. Comparison of CCR2 G190A Genotype and Allele Frequency between Study Group and Healthy Controls

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Case (n=210) %</th>
<th>Control (n=139) %</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>(143) 68.1</td>
<td>(112) 80.6</td>
<td></td>
</tr>
<tr>
<td>GA</td>
<td>(58) 27.6</td>
<td>(25) 18</td>
<td>X2=7.107</td>
</tr>
<tr>
<td>AA</td>
<td>(9) 4.3</td>
<td>(2) 1.4</td>
<td>p=0.027</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Allele</th>
<th>Case (n=210) %</th>
<th>Control (n=139) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>(344) 81.90</td>
<td>(249) 89.57</td>
</tr>
<tr>
<td>A</td>
<td>(76) 18.10</td>
<td>(29) 10.43</td>
</tr>
</tbody>
</table>
nephropathy they did not find any association between CCR2 genotypes and diabetic nephropathy (23, 24). Muntinghe et al have investigated the interaction between CCR5 genotype and levels of high-sensitivity C-reactive protein (hsCRP) and found that the CCR5A32 polymorphism attenuates the adverse effects of inflammation on overall and cardiovascular mortality in ESRD (25). Joo et al have genotyped single nucleotide polymorphism (SNPs) in the MCP-1G-2518A, CCR2G46295A, RANTES C-28G and G-403A in 177 diabetic end-stage renal disease (ESRD) patients and 184 patients without renal involvement (controls) in order to investigate the effects of these SNPs on DNA in Korean patients with type 2 DM. They found no associations of MCP-1, CCR2 and RANTES promoter SNPs with diabetic ESRD in the Korean population (26). We have evaluated the relationships between CCR2 genotypes and disease sub-groups which cause CRF in patients. No significant associations were found between CCR2 genotypes and DM, HT and atherosclerosis (Table 3). These results have shown that in the current study group the presence of DM, HT and atherosclerosis did not affect the results. In order to reveal the role of chemokines in renal pathogenesis further investigations should be carried out using other chemokines and chemokine receptors in different renal diseases.

The authors state that they have no Conflict of Interest (COI).

**References**

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### Table 3. Genotypic Distribution of Etiologic Reasons Causing Renal Damage

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Patients With HT (%)</th>
<th>Patients Without HT (%)</th>
<th>p</th>
<th>Patients With DM (%)</th>
<th>Patients Without DM (%)</th>
<th>p</th>
<th>Patients With Atherosclerosis</th>
<th>Patients Without Atherosclerosis</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>75(68.18)</td>
<td>68(68)</td>
<td></td>
<td>41(70.69)</td>
<td>102(67.1)</td>
<td></td>
<td>14(58.33)</td>
<td>129(69.35)</td>
<td></td>
</tr>
<tr>
<td>GA</td>
<td>32(29.09)</td>
<td>26(26)</td>
<td>$X^2=1.491$</td>
<td>14(24.14)</td>
<td>44(28.95)</td>
<td>$X^2=0.578$</td>
<td>10(41.67)</td>
<td>48(25.81)</td>
<td>$X^2=3.347$</td>
</tr>
<tr>
<td>AA</td>
<td>3(2.72)</td>
<td>6(6)</td>
<td>p=0.4</td>
<td>3(5.17)</td>
<td>6(3.95)</td>
<td>p=0.749</td>
<td>0(0)</td>
<td>9(4.84)</td>
<td>p=0.176</td>
</tr>
<tr>
<td>TOTAL</td>
<td>110(100)</td>
<td>100(100)</td>
<td></td>
<td>58(100)</td>
<td>152(100)</td>
<td></td>
<td>24(100)</td>
<td>186(100)</td>
<td></td>
</tr>
</tbody>
</table>

**Allel**

- **A**: 182 (%82.73) 162 (%81) 96 (%82.76) 248 (81.58) 38 (79.17) 306 (82.26)
- **G**: 38 (%17.27) 38 (%19) 20 (%17.24) 56 (18.42) 10 (20.83) 66 (17.24)


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