Introduction

Chemokines belong to the family of cytokines whose primary function is the recruitment of leukocytes to inflammatory sites. Chemokine receptor 2 (CCR2) is expressed on monocytes, macrophages, activated T cells and activated endothelial cells and is a receptor for monocyte chemoattractant protein-1 (MCP-1), which specially mediates monocyte chemotaxis. CCR2 enhance directly T helper type immune responses. The regulated on activation normal T-cell expressed and secreted protein (RANTES) and macrophage inflammatory protein-1 (MIP-1) chemokine is a ligand for the chemokine receptor 5 (CCR5) which is expressed on monocytes, activated T cells and natural killer cells. Recently, genetic variants of CCR2 and CCR5 are defined. A point mutation in the CCR2 gene leads to a single, conservative amino acid change, which substitutes isoleucine for valine at position 64 (CCR2 64I). Genotypes of CCR2 are classified as CCR2 GG (+/+), CCR2 GA (+/64I) and CCR2 AA (64I/64I). A genetic variant of CCR5 exists consisting of a 32-nucleotide deletion (delta 32). Genotypes of CCR5 are classified as CCR5 GG (+/+), CCR5 GA (+/delta 32) and CCR5 AA (delta 32/delta 32). A genetic variant of CCR5 exists consisting of a 32-nucleotide deletion (delta 32). Genotypes of CCR5 are classified as CCR5 GG (+/+), CCR5 GA (+/delta 32) and CCR5 AA (delta 32/delta 32).
are classified as CCR5 +/+, CCR5 +/delta 32 and CCR5 delta 32/delta 32. CCR2 64I and CCR5 delta 32 are known to effect chemokine receptor function and or expression in primary cells. The role of CCR2 and CCR5 polymorphisms are shown in patients with HIV infection, sarcoidosis, asthma, rheumatoid arthritis and renal transplant survival. The common finding of these diseases is that they are all characterized by the recruitment of mononuclear cells.

Focal segmental glomerulosclerosis (FSGS) is a common cause of nephrotic syndrome in children and in adults. The clinical course and prognosis of FSGS is heterogeneous. Approximately 30-40% of patients reach renal failure 10 years after diagnosis. The initial podocyte injury of FSGS might result from infections, toxic agents, immunological factors or genetic mutations. Besides the factors listed above, an individual's genetic may be important in disease frequency and clinical course. The progression of the disease may be related with inflammatory or oxidative components and mechanisms against inflammatory response may have a role in protection and tapering down the rate of progression in nephrotic syndrome.

As CCR2 and CCR5 play important roles in the recruitment of monocytes and T cells in inflammation, we hypothesized that genetically determined differences in the immune response might influence the risk of developing FSGS. No adequate knowledge exists as to whether the CCR2 64I and CCR5 delta 32 variants affect the development of FSGS in pediatric patients. Aim of our study was to compare the frequency of CCR2 64I and CCR5 delta 32 in Turkish children with FSGS and healthy controls.

Materials and Methods

This study was carried out at Marmara University Medical Faculty between July 2000 and July 2005 prospectively. We studied CCR2 64I and CCR5 delta32 gene polymorphisms in 25 children with steroid resistant biopsy proven primary FSGS and 40 healthy controls. Cases and controls were from similar ethnic origin. The study was approved by the ethics committee of our medical faculty and written informed consent was taken from all patient families before the study.

Blood specimen from all subjects were collected into tubes containing EDTA and DNA was isolated by the method of Miller et al and Polymerase Chain Reaction (PCR) for CCR2 V64I, CCR5 delta 32 genotypes were performed by using a previously performed method. Chemokine receptor gene polymorphisms were typed by visualization under ultraviolet light and photographed with a polaroid camera and the chemokine restriction fragment length polymorphism (RFLP) alleles for each genotype were identified in each sample.

To determine the CCR2 V64I genotype, BsaB1 was used to digest the PCR product by a previously described method. Either an uncut 173 bp or cut 149 and 24 bp amlicons were produced after the PCR products and digestion by using enzyme BsaB1. The PCR product for CCR5 delta32 genotype was either a 233bp wild type amplicon or a 201 bp deleted product.

Definitions

Nephrotic syndrome is defined by the International Study of Kidney Disease in Children (ISKDC) as edema, serum albumin levels below 2.5 g/dl, and urinary protein excretion greater than 40 mg/m2 per hour. The histopathological diagnosis of FSGS was made according to histopathological diagnosis criteria as described by ISKDC for children with nephrotic syndrome.

Blood samples were obtained at the initiation of study.

Patient selection and clinical investigation

FSGS patients

25 steroid resistant and biopsy proven primary FSGS patients were included in this group. FSGS patients were grouped according to the severity of the disease. Patients who remained in complete or partial remission with preserved renal functions were defined as “FSGS with stable renal function” (n:17). “FSGS with declining renal function” group consisted of patients with declining renal function. The definition of declining renal function was made as doubling of serum creatinine from the onset of nephrotic syndrome, to the time of the initiation of study, or diminished GFR and high serum creatinine levels for age group and gender despite all therapeutic interventions (n:8). This group included patients receiving renal replacement therapy.

Control subjects

The control group consisted of 40 healthy children with no history of hypertension, renal, cardiac, hepatic disease or family history of renal disease.
Statistical analysis was performed using SPSS software package, version 10.0. Laboratory data were expressed as mean ± SD when assessing group results (n>20), or median and range when assessing subgroup results (n<20). Mann Whitney U test was used for comparison.

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**

**FSGS patients**

There were 25 patients in FSGS group of whom 18 were boys and 7 were girls. The mean age of the patients was 9.6 ± 5.2 years and the mean follow up period was 3.8 ± 2.6 years.

The clinical characteristics and the laboratory results of FSGS patients are shown on Table 1. These patients had high serum total cholesterol and low density lipoprotein (LDL) cholesterol levels, normal serum high density lipoprotein (HDL) cholesterol levels and low serum albumin levels.

For the entire group of children with FSGS, the frequencies of the CCR2 64I polymorphisms as GG, GA and AA genotypes were 23 (92%), 2 (8%), and 0 respectively. The data is shown on table 2. None of the patients showed CCR5 delta 32 mutation (Table 2).

**Control subjects**

There were 40 subjects, 25 boys and 15 girls in the control group with mean age of 11 ± 3.8 years. All had normal serum creatinine, serum albumin, total cholesterol, LDL cholesterol and HDL cholesterol levels (Table 1).

Distribution of CCR2 64I polymorphisms (GG, GA, AA) were 29 (72.5%), 10 (25%), and 1 (2.5%) respectively (Table 2). None of the control subjects had CCR5 delta 32 polymorphism.

**Comparison of the study parameters between FSGS patients and controls**

The mean age and gender distribution of the FSGS patients and controls were similar. Children with FSGS had significantly higher total cholesterol, LDL cholesterol levels and lower serum albumin levels than those

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**Table 1**
Clinical characteristics and laboratory data of FSGS patients and controls

<table>
<thead>
<tr>
<th></th>
<th>FSGS</th>
<th>Control</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>M/F</td>
<td>18 / 7</td>
<td>25 / 15</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years)</td>
<td>9.6 ± 5.2</td>
<td>11 ± 3.8</td>
<td>NS</td>
</tr>
<tr>
<td>Age at onset (years)</td>
<td>5.8 ± 4.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follow up (years)</td>
<td>3.8 ± 2.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum total cholesterol (mg/dl)</td>
<td>319 ± 109.4</td>
<td>124 ± 44</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Serum LDL cholesterol (mg/dl)</td>
<td>264 ± 66</td>
<td>72 ± 12</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Serum HDL cholesterol (mg/dl)</td>
<td>51.8 ± 14.2</td>
<td>66 ± 14</td>
<td>NS</td>
</tr>
<tr>
<td>Serum albumin (g/dl)</td>
<td>1.6 ± 0.4</td>
<td>4.4 ± 0.6</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Baseline serum creatinine (mg/dl)</td>
<td>0.8 ± 1.1</td>
<td>0.4 ± 0.1</td>
<td>NS</td>
</tr>
</tbody>
</table>

FSGS: focal segmental glomerulosclerosis, M/F: male/female, LDL: low density lipoprotein, HDL: high density lipoprotein, NS: not significant

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**Table 2**
The distribution of CCR2 64I and CCR5 delta 32 genotypes of nephrotic children and control subjects

<table>
<thead>
<tr>
<th>Patient groups</th>
<th>CCR2 64I n (%)</th>
<th>CCR5 delta 32 n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSGS (n: 25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSGS stable renal function</td>
<td>23 (92%)</td>
<td>-</td>
</tr>
<tr>
<td>FSGS declining renal function</td>
<td>15 (88%)</td>
<td>2 (12%)</td>
</tr>
<tr>
<td>Control (n: 40)</td>
<td>29 (72.5%)</td>
<td>10 (25%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patient groups</th>
<th>+/-</th>
<th>+/delta32</th>
<th>delta 32/delta 32</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSGS (n: 25)</td>
<td>25 (100%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FSGS stable renal function</td>
<td>17 (100%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FSGS declining renal function</td>
<td>8 (100%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Control (n: 40)</td>
<td>40 (100.5%)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

FSGS vs Control: p >0.05
of control subjects. HDL cholesterol and baseline serum creatinine levels of FSGS patients and controls were in normal limits (Table 1).

Statistical difference between FSGS patients and control subjects with respect to the distribution of CCR2 64I polymorphism was not significant.

Comparison of the study parameters between subgroups of FSGS

There were no differences between subgroups of FSGS (stable renal function versus declining renal function) in terms of age, age at onset, serum total cholesterol, LDL cholesterol, HDL cholesterol, albumin and baseline serum creatinine levels (Table 3).

CCR2 64I polymorphism and the course of renal disease

The distribution of the CCR2 64I genotype in patients with declining renal function was GG in 8 subjects (100%), and the distribution of CCR2 64I genotype in children who had preserved renal function were GG in 15 (88%), AG in 2 (12%). The distribution of CCR2 64I genotype of FSGS subgroups – stable renal function and declining renal function- were shown on Table 2. We could not make any statistical comparisons because of the small sample size of each allelic subgroup.

Discussion

The present preliminary study demonstrates the distribution of CCR2 64I and CCR5 delta 32 polymorphisms in Turkish children with FSGS and in control subjects for the first time. FSGS is a common cause of nephrotic syndrome. Most children with FSGS do not respond to any form of therapy and one third of patients develop end stage renal disease. Recent studies focus on risk factors in relation with FSGS development and progression.

CC-chemokine receptors are predominantly expressed on the surface of leukocytes but are also expressed on endothelial cells, macrophages which are involved in the interstitial inflammation of chronic proteinuric glomerulopathies. Because oxidative and inflammatory processes in the form of glomerulosclerosis is important in the progression of renal diseases, we investigated the risk of developing FSGS and progression in Turkish children in respect to CCR2 and CCR5 polymorphisms. Both of the studied polymorphisms involve inflammatory components, however we did not observe any difference in the distribution of CCR2 and CCR5 polymorphisms in FSGS patients in respect to control subjects. The similar distribution of genotype frequencies in patients and controls suggest that these
polymorphisms did not contribute to the risk of FSGS development.

We also evaluated the distribution of polymorphisms in the subgroups of FSGS that are grouped according to the severity of the disease. Patients with preserved renal function had GG genotype in 15 (88%) and A g genotype in 2 (12%) and patients with declining renal function had GG genotype in all subjects (n: 8, 100%). We could not make any statistical comparisons because of the small sample size of each allelic group. None of the patients displayed CCR5 delta 32 polymorphism.

Studies concerning the role of CCR2 and CCR5 in renal diseases are very few and focused on the progression of diseases. CCR2 ligand, MCP-1, which is an important chemoattractant for monocytes has been shown to be markedly elevated in FSGS in agreement with moderate glomerular cell infiltration. Studies of tissue samples from patients with Ig A nephropathy revealed an increased expression of the CCR5 ligands MIP-1 alpha and RANTES and demonstrated a correlation between the intensity of renal infiltration of CCR5-positive leukocytes and disease progression. Similarly, increased expression of RANTES has been found in FSGS patients. Fischeder et al reported that homozygous for CCR5 delta 32 had significantly longer renal transplant survival than CCR5 heterozygotes and CCR5 +/- individuals. The presence of CCR5 delta 32 mutation seems favorable in renal diseases and no data exists as to whether CCR2 64I polymorphism predicts the development of any renal disease. None of the patients in our study group and control subjects showed CCR5 delta 32 genotype. It has been reported that CCR5 delta 32 genotype is relatively rare about 4% to 16% in Europeans and 0% in 85 Turkish renal transplant patients. Because of the small sample size of our study group we may lack to observe this mutation. The effect of CCR2 and CCR5 polymorphisms in FSGS course was also analysed in our study. We divided our FSGS patients into two subgroups according to the severity of the disease, but we were unable to make any statistical comparisons because of the limited number of patients in each allelic group.

In conclusion, our preliminary results suggest that CCR2 64I and CCR5 delta 32 polymorphisms were not different in patients and controls, indicating no significant influence on FSGS initiation. Future studies with larger number of patients are needed to confirm the CCR2 and CCR5 receptor polymorphism associations with FSGS development and course.

References


