Abstract
Microorganisms adhere to uroepitelium and trigger adaptive immune response with synthesis of chemokine's and cytokines. Chemokine and chemokine receptors lead to recruitment of inflammatory cells results in inflammatory response: healing or scaring. FcR initiates effector functions: degranulation, cytokine, superoxide production, and arrangement of antibody synthesis, antibody dependent cellular cytotoxicity, and phagocytosis. Aim was to determine FcγR polymorphism role in urinary tract infection (UTI).

131 UTI and 151 healthy subjects without any urinary tract abnormality were participated. Polymorphisms were determined by amplification refractory mutation system PCR.

FcγRIIa R/R genotype and FcγRIIa R allele are found significantly higher in study group than control. FcγRIIa genotype distribution and allele frequency is not significantly different between UTI and control group. FcγRIIb-NA2/NA2 genotype and NA2 allele are found statistically higher when compared to control FcgRIIa-131-R allele is found to be related; lower UTI (p=0.015), gram negative bacterial infection risk (p=0.012) FcgRIIb-NA2 allele is found to be related; upper UTI (p=0.001) gram negative bacterial infection (p=0.001), renal scar development risk (p=0.001). FcγRIa-R131R and FcγRIIb-NA2/NA2 gene polymorphisms may increase risk and susceptibility to UTI in children.

Keywords: FcγR gene polymorphisms, UTI, children

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INTRODUCTION
Urinary tract infection (UTI) is one of the most common infections in childhood. The pathogenesis of UTIs is dependent primarily on two factors, host defense and pathogen related virulence (1). Bacteria adhere to the uroepithelium and trigger an adaptive immune response with the synthesis of chemokine’s and cytokines. Chemokine and chemokine receptors lead to the recruitment of inflammatory cells, resulting in an inflammatory response, followed by healing or scarring (2).

IgG is the predominant subclass of antibodies produced by immune cells in response to bacterial polysaccharide antigens. Fc receptor region (FcR) of immune globulins plays a crucial role in phagocytosis. FcR initiates effector functions, such as degranulation, cytokine production, and arrangement of antibody synthesis, antibody dependent cellular cytotoxicity, superoxide production, and phagocytosis. FcR are divided in three classes: FcγRI (CD 64), FcγRII (CD 32), and FcγRIII (CD16) (3-7). These receptors differ in cell distribution and affinity for IgG subclasses and display heterogeneity between individuals according to genetic structure and ethnicity. The polymorphic nature, also known as diverse nature, of the FcγR, is a risk factor in certain infectious and autoimmune diseases (8).

FcγRIIa is the only FcR class that interacts with the human IgG2 molecule located on chr.1q23. It carries either an arginine (FcγRIIa-R131) or histidine (FcγRIIa-H131) at the amino acid position 131 due to a G to A point mutation in exon 4 of FcγRIIa. FcγRIIa has an important role in the pathogenesis of autoimmune diseases. It bears a phenylalanine (FcγRIIa-158F) or valine (FcγRIIa-158V) at amino acid position 158 due to G to T point mutation at FcγRIIIa 559 of FcγRIIIa. FcγRIIIb, most commonly found in neutrophils, increases the capability of phagocytosis especially against gram-negative microorganisms. It bears the neutrophil antigen (NA) polymorphism caused by four amino acid substitutions, with wild type named NA1, and a polymorphic variant named NA2 (9, 10).

Our aim in this case-control study is to determine the role of FcγR polymorphisms in urinary tract infections, to investigate the genotypic diversity of FcγR, and to demonstrate phenotype-genotype relationship in UTI.

MATERIALS
One-hundred thirty-one patients presented with a UTI but did not have a vesicoureteral reflux or any other congenital anomaly (107 females and 24 males with the mean age of 7.21±4.19 years and age disturbance range was 1 month -17 years) and 151 healthy Turkish subjects (73 females and 78 males with a mean age of 9±4.2 years), without any laboratory (complete blood cell count and acute phase reactants), abnormal physical examination finding, or past history of UTI or urinary tract abnormality, based upon renal ultrasonography, participated in our study.

Infections were defined by a positive urine culture. The indirect laboratory criteria for localization of UTI were fever, erythrocyte sedimentation rate, C-reactive protein, leukocytosis and urine osmolality. A patient with three or more of these criteria was clinically diagnosed to have an upper UTI. Upper/lower UTI ratio was 70/61. Reinfection was defined as the recurrence of infection with a different pathogen, while relapse was defined as the recurrence of the infection with the same pathogen.

Patients with recurrent UTI and pyelonephritis were investigated with 99mTc-DMSA for renal damage, the renal Scar (+)/ (-) ratio was 30/101. Renal scarring was classified with the Goldraich scarring grade from Type 1 to Type 4 (11).

Type 1 less than 2 renal scars
Type 2 more than 2 renal scars with normal parenchymal area in between
Type 3 generalized parenchymal damage
Type 4 atrophic kidneys (with <5% function)

The University Ethical Research Committee approved the study. Parents of subjects and controls provided written informed consent prior to the study.

Both patients and controls were investigated for FcγR polymorphisms. Polymorphisms were analyzed by allele-specific PCR and a direct DNA sequencing based method from genomic DNA.

METHODS
DNA purification
Genomic DNA from patients and healthy controls was extracted from peripheral blood leukocytes with QIAamp DNA Blood Mini Kits (Qiagen GmbH, Hilden, Germany) according to the manufacturer’s instructions.

FcγRIIa genotyping
FcγRIIa genotyping (figure1) was performed by using the method defined by Flesch BK et al (12). We used a 25-μL PCR mixture containing 2.5 μl of genomic DNA, 2.5 μl of 10× PCR buffer (Applied Bio systems, Foster City, CA, USA), 2 mM MgCl2, 200 μmol/L of each dNTP (Promega, Madison, USA), and 0.5 U Ampli Tag DNA Polymerase (Applied Bio systems).

In addition, 0.5 μmol/L of H131-specific sense primer (5'- ATCC-CAGAAATTCTCAGA-3') from the second extracellular domain or 0.5 μmol/L R 131-specific sense primer (5'-ATCCGAGAATTCTCCG-3') was used. Qiagen Operon Co synthesized all primer oligonucleotides, 0.5μmol/L common anti-sense primer from an area downstream of the intron (5'-CAATTTGCTGCTATGGC-3'). The resulting fragment was 253 bp in length. As an internal PCR control, we...
used 0.125 μmol/I human growth hormone (HGH)-1 forward primer (5′-CAGTGCCTCCACCACTCCCTTA-3′) and 0.125 μmol/I HGH-II reverse primer (5′-ATCCACTCACGGATTTCTGTTGTTTC-3′), which resulted in a 439-bp fragment.

**Figure 1**: FcγRIIa genotyping
A thermal cycler (Gene Amp 9700, Applied Bio systems) was used to perform a hot-start PCR as follows; 5 min at 95 °C, 10 cycles of 1 min at 95 °C, 2 min at 57 °C, and 1 min at 72 °C; thereafter, to enhance the sensitivity, we used 22 cycles of 1 min at 95 °C, 2 min at 54 °C, and 1 min at 72 °C and a final extension step for 5 min at 72 °C. The PCR amplification products were separated on a 2% agarose gel and visualized by ethidium bromide staining. To validate accuracy and reproducibility of the results obtained using the above-described techniques, we randomly ran 20% of samples from both groups (study and control groups) for FcγRIIa genotypes by using a direct DNA sequencing technique with ABI PRISM 310 Genetic Analyzer System (Applied Bio systems).

**Polymerase chain reaction for FcγRIIIa (PCR):**
In the case of FcγRIIIa genotyping (figure 2), primer (KIM-G (V): 5′-TCT CTG AAG ACA CAT TTC TAC TCC CTA C-3′ and KIM-1 (F): 5′-TCT CAT AAG ACA CAT TTC TAC TCC CTA A-3′) primer A013 (5′-ATA TTT ACA GAA TGG CAC AGG-3′)

**Control primers** Ctrl-1and Ctrl-2, as explained in FcγRIIa, DNA’s fragments of 270-bp multiplied. This process was made in a 25 μl reaction solution using 175 ng of genomic DNA. The reaction solution containing: 2.5 μl PCR Gold Buffer, 62.5 nmol MgCl2, 1.25 nmol, from each of the four dNTP, 11.9 pmol KIM-G (V), 66.4 pmol KIM-G (F), 15.4 pmol A013, 1.1 pmol from each of the two control primers and 0.5 U Ampli Tag Gold (Applied Bio systems).

PCR was denatured at 94° in 10 minutes. 32 cycles of 30 seconds at 95°C, thereafter 32 cycles for 20 seconds at 57°C and for 25 seconds at 72°C anda final extension step for 7 minutes at 72°C.

**FcRIIIB genotyping**
In the case of FcγRIIIB-NA1/NA2 genotyping (figure 3), PCR was modified from Bux et al (13). The NA1-specific primer (5V-CAGTGGTTTTCAATGTGAA-3V) gave a 141-bp fragment, and the NA2-specific primer (5V-CATGCTACACGGTGCTT-3V) amplified a 219-bp fragment. The reverse primer (5V-ATGGACTTCTACTCGAC-3V) did not discriminate between the two allotypes. Because there was a substantial difference in length between the NA1-specific and NA2-specific reaction products, both alleles could be detected in the same reaction. The 25-μl reaction mixture used contained 100 ng of genomic DNA, 3.7 μl of 10x PCR buffer (Applied Bio systems), 25 nM MgCl2, 1 nmol/l of each dNTP (Promega), 4 pmol of each of the control primers, 0.012 nmol of the NA1 and NA2 primers, 0.025 nmol of the reverse primer, and 0.5 U AmpliTaq DNA Polymerase (Applied Bio systems). After denaturation for 3 min at 95°C, 30 PCR cycles consisting of 95°C for 1 min, 57°C for 2 min, and 72°C for 1 min were run. After a final extension step of 1 min at 72°C, samples were resolved in 2% agarose gels stained with ethidium bromide.

**Figure 3**: FcγRIIIb receptor genotyping

**Statistics**
Statistical analysis of the data was performed with SPSS package v.11.0 program. The allele distribution FcγR polymorphism was tested with the Hardy Weinberg equilibrium equation in patient and control
groups. The chi-square test and Fisher’s exact test were used for comparison of the groups for genotypic distribution and allelic frequencies. The data was expressed as mean ± standard deviation (SD) and p value less than 0.05 and an odds ratio with 95% confidence not including 1 was considered as statistically significant.

RESULTS
Genotypic distributions of the case-control study did not deviate significantly from the Hardy-Weinberg equilibrium expectations in each group (P>0.05). The most common pathogen was E. coli (n=90) and Gram (-) infections were seen in 108 patients. With respect to the localization of UTI, the upper and lower UTI ratio was similar to each other. Complicated UTI, renal scarring and relapse were found to be more frequent in upper UTI whereas re-infection was more common in the lower UTI group (Table 1).

FcγRIIa Genotype Distribution and Allele Frequency
In study group, FcγRIIa R/R genotype and FcγRIIa R allele were found significantly higher than in the control group (Table 2).

<table>
<thead>
<tr>
<th>FcγRIIa Genotype Distribution and Allele Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>The chi-square and Fisher’s exact tests were used for comparison of the groups for genotypic distribution and allelic frequencies</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>H131</th>
<th>H131</th>
<th>R131</th>
<th>H</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>78</td>
<td>59</td>
<td>14</td>
<td>215(7%</td>
<td>87</td>
</tr>
<tr>
<td>(n=151)</td>
<td></td>
<td></td>
<td></td>
<td>2%</td>
<td>28</td>
</tr>
<tr>
<td>UTI(n=131)</td>
<td>51</td>
<td>59</td>
<td>21</td>
<td>161(6%</td>
<td>101(39</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1%</td>
<td>13</td>
</tr>
<tr>
<td>p</td>
<td>ns</td>
<td>ns</td>
<td>0.01</td>
<td>ns</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Table 2: FcγRIIa Genotype Distributions and Allele Frequency
The chi-square test and Fisher’s exact tests are used for comparison of the groups for genotypic distribution and allelic frequencies.

FcγRIIIa Genotype Distribution and Allele Frequency
FcγRIIIa genotype distribution and allele frequency is not significantly different between the UTI and control group, which is due to the fact that the basic function of this receptor is related to autoimmune diseases (Table 3).

<table>
<thead>
<tr>
<th>Group</th>
<th>F158F</th>
<th>F158V</th>
<th>V158F</th>
<th>F</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>53</td>
<td>67</td>
<td>31</td>
<td>173(57%</td>
<td>129(43</td>
</tr>
<tr>
<td>(n=151)</td>
<td></td>
<td></td>
<td></td>
<td>3%</td>
<td>3</td>
</tr>
<tr>
<td>UTI(n=131)</td>
<td>33</td>
<td>84</td>
<td>14</td>
<td>150(57%</td>
<td>112(43</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3%</td>
<td>3</td>
</tr>
<tr>
<td>P</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

Table 3: FcγRIIIa Genotype Distributions and Allele Frequency
The chi-square test and Fisher’s exact tests are used for comparison of the groups for genotypic distribution and allelic frequencies.

Relations of FcγRIIa-131-R Allele Polymorphisms with Phenotypic Features
FcγRIIa-131-R allele was found to be related with lower urinary tract infections (OR =1.12, 95% CI 0.86-1.46, p=0.015) and gram-negative bacterial infection risk (OR= 1.20, 95% CI 0.79-2.01) and renal scar development risk(OR=1.95% CI 0.76-3.17, p=0.001) (Table 5).

<table>
<thead>
<tr>
<th>Allele (n=131)</th>
<th>Upper UTI</th>
<th>Lower UTI</th>
<th>E.Coli</th>
<th>Gram (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R (+)</td>
<td>41</td>
<td>40</td>
<td>61</td>
<td>67</td>
</tr>
<tr>
<td>R (-)</td>
<td>29</td>
<td>21</td>
<td>31</td>
<td>41</td>
</tr>
</tbody>
</table>

Table 5: Relations of FcγRIIa-131-R Allele Polymorphisms with Phenotypic Features

Relations of FcγRIIIb-N Allele Polymorphisms with Phenotypic Features
The FcγRIIIb-NA2 allele was found to be related with upper UTI (OR =1.11, 95% CI 0.4-2, p=0.001), gram-negative bacterial infection (OR= 1.2, 95% CI 0.85-1.4, p=0.001) and renal scar development risk(OR=1.95% CI 0.76-3.17, p=0.001) (Table 6).

<table>
<thead>
<tr>
<th>Allele (n=131)</th>
<th>Upper UTI</th>
<th>Lower UTI</th>
<th>Renal Scar</th>
<th>E.Coli</th>
<th>Gr (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA2+</td>
<td>49</td>
<td>44</td>
<td>22</td>
<td>64</td>
<td>80</td>
</tr>
<tr>
<td>NA2-</td>
<td>28</td>
<td>7</td>
<td>3</td>
<td>11</td>
<td>11</td>
</tr>
</tbody>
</table>

Table 6: Relations of FcγRIIIb-N Allele Polymorphisms with Phenotypic Features

DISCUSSION
Urinary tract infection is a common problem, which differs in clinical severity from asymptomatic bacteriuria to pyelonephritis. It may be recurrent due to anatomic anomalies, environmental factors and immune-system defects. Determining the risk factors...
will prevent long-term sequel of UTI, such as hypertension, renal scarring and renal failure. Genetic factors which may lead to a predisposed UTI susceptibility is still unknown.

In recent years, genetic studies have suggested that receptors for the Fc fragments of immunoglobulin G (IgG) (FcγRs) may be implicated in the susceptibility or the progression of some autoimmune and infectious diseases. Receptors for IgG play an important role in immune complex clearance and immune response (14-16).

There is limited study in the literature regarding the FcγRIIIa genotype and its relationship to infectious disease. Researchers examined FcγRIIIa-158FF, FcγRIIIa-158VV, FcγRIIIa-158VF polymorphisms and FcγRIIa and FcγRIIIb polymorphisms coexistence in 462 adults from Thailand with a Plasmodium falciparum malaria infection. Nevertheless, they did not find any relationship between the severity of malaria and polymorphisms (17).

In this study, we found no significant relationship with FcγRIIIa polymorphism in patients with a UTI and the control group (p=0.63), we suspect this is because the main function of the receptor is related to autoimmune disease processes. FcγRIIIa has a role in clearance of circulating immune complexes, but has no correlation with UTI tendency.

A predisposition to certain bacterial infections has been observed with a histidine to arginine substitution at the amino acid position 131, affecting FcγRIIa and receptor binding of IgG₂ (18,19). The leucocytes carrying the FcγRIIa-131RR genotype have lower phagocytic ability for immunoglobulin G₂ opsonized particles than the leucocytes carrying the FcγRIIa-131HH genotype (20,21). In American patients with bacterial pneumonias, we found the FcγRIIa-131RR genotype to have an increased frequency (22), and increased host sensitivity due to FcγRIIa-IgG₂ association was not observed. IgG₂ is the most important immunoglobulin subgroup in patients, as it provides protection against capsulated pathogens, such as Neisseria meningitidis, Haemophilus influenza and Streptococcus pneumonia. Patients carrying the FcγRIIa-131HH genotype showed increased phagocytic activity against IgG₂ opsonized bacteria when compared to FcγRIIa-131RR homozygote genotype carrying patients. In one study investigating the severity of Neisseria meningitis infection with the FcγRIIa gene polymorphism, it was reported that hosts carrying only FcγRIIa-131RR polymorphism had a more severe disease and severe complications (23). Fang Fang Yuan et al. reported that Australian patients the FcγRIIa-131RR polymorphism had more occurrences of Streptococcus pneumonia sepsis, as compared to the healthy control group; in addition, the FcγRIIa-131HH polymorphism was very rare as compared to the FcγRIIa-131RR genotype in these patients (24).

In our study, we found that the FcγRIIa-131RR genotype and FcγRIIa-131R allele frequency was significantly higher in the patient group as compared to the control group (p<0.05). Patients with the FcγRIIa-131R allele have a significantly higher risk of a lower UTI and gram-negative bacterial infections than others. Although the occurrence of a complicated UTI, upper UTI, renal scarring and recurrence and reinfection rates were not different than patients without the FcγRIIa-131R allele.

FcγRII regulates the stimulation of neutrophils degranulation and phagocytic functions. A neutrophil functional deficiency due to an FcγRIIa polymorphism may be one factor causing patients to have a predisposition to UTIs.

FcγRIIb is the most common Fcγ receptor found in neutrophils and shows neutrophil antigen (NA) polymorphism. These polymorphic glycoproteins called NA1 and NA2 (100). FcγRIIb-NA1/NA1 induces IgG₁ and IgG₃ opsonized particles to undergo phagocytosis. Neutrophils with the FcγRIIb-NA1/NA1 genotype have a stronger phagocytic ability than neutrophils with the FcγRIIb-NA2/NA2 alleles (25).

In our study, the FcγRIIb-NA2/NA2 polymorphism and NA2 allele were found to be statistically significantly higher in the patient group than in the control group. In patients with the FcγRIIb-NA2 allele, they had a significantly more upper UTIs and lower UTIs, infection recurrence, renal scar development risk, more UTIs caused by a gram negative pathogen, and a higher potential to develop a complicated UTI than without NA2.

FcγRIIb, which is the most common receptor found on neutrophils, with an ability to enhance the phagocytic capability against gram negative bacteria, has been shown to have a higher polymorphism ratio in children with UTIs. We thought that 82.7% of microorganisms, which were most commonly identified from urine cultures, have been found gram (-) bacteria therefore this polymorphism could be an important risk factor in development of UTI.

The studies investigating FcγRIIa, FcγRIIa and FcγRIIb genotypes conducted in different healthy ethnic groups showed that genotypic diversity exists between different ethnic groups. The FcγRIIa-131RR genotype was found to be lowest in in Japan with a ratio of 3.8%. This was similar for our study, with a ratio of 9.2% in Turkish children for FcγRIIa-131RR. The higher ratios, such as 50-60% for FcγRIIa-131HH genotype, can be explained by existence of an innate
resistance against capsulated bacteria. Finding of lower FcγRIla-131RR genotype ratio can decrease tendency to infections in these ethnic groups. FcγRIla and FcγRIIIB polymorphisms have an important role in the development of a genetic tendency toward bacterial infections. At the same time, the severity of a bacterial infection and the frequency of a bacterial infection, are also directly affected.

UTI has a very important place among pediatric bacterial infections; to the best of our knowledge there is no other study conducted for investigation of a relationship between pediatric UTIs and the FcγR receptor in the literature.

FcγRIla and FcγRIIIB polymorphisms were thought to be risk factors for patients with a definite UTI. We have found the more common FcγRIIIB polymorphism in our patients; therefore it likely results in a deficiency of the neutrophil phagocytic capacity for gram-negative pathogens. More gram-negative bacterial growth, as seen more often in urine cultures, may be related with this polymorphism. People with the FcγRIIIB-NA2/NA2 polymorphism have less effective gram-negative bacterial phagocytosis capacity. In particular, people carrying the FcγRIIIB-NA2/NA2 polymorphism or FcγRIla-131RR and FcγRIIIB-NA2/NA2 genotype coexistence, have more severe meningococcal infections and complications (20,26).

As a result, investigation of FcγR polymorphisms is useful when there is a diagnosis of an upper UTI or complicated UTI in patients without factors predisposing them to a UTI, such as an underlying congenital urinary tract abnormality, VUR etc. We must be more careful when clinically following and providing prophylaxis for patients carrying the FcγRIla-131RR or FcγRIIIB-NA2/NA2 genotypes, as the ratio of serious complications, such as renal scar development, a prime cause of hypertension and renal failure, could be decreased.

In conclusion FcγRIla and FcγRIIIB polymorphisms are thought to be risk factors for a predisposition to a UTI. The FcγRIla-131-R allele was found to be related with lower urinary tract infections and gram-negative bacterial infection risk. The FcγRIIIB-NA2 allele was also found to be related with an increased occurrence of upper UTIs, gram-negative bacterial infection and renal scar development risk.

REFERENCES