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RAPID COMMUNICATION

Heat Shock Protein 72 (*HSPA1B*) Gene Polymorphism and Toll-Like Receptor (TLR) 4 Mutation Are Associated with Increased Risk of Urinary Tract Infection in Children

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ABSTRACT: Innate immunity and urinary tract response play a central role in the development of urinary tract infection (UTI). Heat shock protein (HSP) 72 and Toll-like receptor (TLR) 4 are among the key elements of innate defence mechanisms. This study assesses the role of *HSPA1B* A(1267)G and *TLR4* A(896)G polymorphisms using allele-specific polymerase chain reaction in 103 patients treated with recurrent UTI. Allelic prevalence was compared with reference values of 235 healthy controls. Clinical data were also statistically evaluated. *TLR4* (896)AG genotype and *TLR4* (896)G allele had also higher prevalence in UTI patients *versus* controls ($p = 0.031$ and 0.041, respectively). Our data indicates a relationship between the carrier status of *HSPA1B* (1267)G and *TLR4* (896)G alleles and the development of recurrent UTI in childhood independently of other renal abnormalities, while raising further questions about the clinical and therapeutic relevance of these polymorphisms in everyday pediatric nephrology. **(*Pediatr Res* 61: 371–374, 2007)**

UTI is the second most common bacterial infection in the pediatric population, affecting approximately 1% of boys and 3% of girls before their 11th birthday. UTI involving the kidney may cause chronic pyelonephritis and renal scarring leading to hypertension and chronic renal failure (1). Anatomic abnormalities, such as primary and secondary VUR are often associated with UTI, however, the majority of the patients have structurally and functionally normal renal tracts. The factors causing recurrent infection are not always obvious, as bladder dysfunction, bacterial virulence, and host reaction also play important role in the pathogenesis (2). Treatment options are limited and long-term prophylactic antimicrobial treatment is often required.

There is an increasing amount of data available about the relevance of host response, particularly of uroepithelial cells in the recognition and local immune response to bacterial invasion, which in turn is an important determinant of the clinical outcome. Inadequate bacterial clearance leads to recurrent infections or to sustained inflammatory response with subsequent scarring (3).

The early response against invading pathogens is provided by innate immunity, in which TLR, especially TLR4 provide, a significant role. TLR4 detects lipopolysaccharides of the cell walls of Gram-negative bacteria such as *Escherichia coli*; therefore, TLR4 function could be of importance in the innate defence against UTI (4).

Numerous other factors and mechanisms contribute the activation of innate immunity including the role of HSP, which is receiving increasing attention lately. Members of the HSP70 cytosolic group are either constitutively expressed (HSC70) or can be induced by a broad range of stress factors (HSP72). They are not only limiting tubular injury, restoring renal function, and accelerating renal recovery but also take part in the activation of the innate immune system (5). As endogenous stimulus for the TLR4 signal pathway, they induce the components of innate immunity and contribute to improved bacterial killing and facilitated recovery after bacterial challenge (6).

Finally, the individual's response to the infection is also variable. The predisposition is inheritable, indicating the importance of host's genetic factors. Recently various genetic polymorphisms have been reported to be associated with increased risk of infections. Agnese *et al.* (7) described the A-G substitution at nucleotide 896 at the start of the codon of *TLR4* A(896)G, which results in an aspartic acid–to-glycine substitution (Asp299Gly) and is associated with an increased risk of Gram-negative infections.

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Abbreviations: HSP, heat shock protein; **TLR,** toll-like receptor; **UTI,** urinary tract infection; **VUR,** vesicoureteral reflux

Previously an A(1267)G polymorphism has been described in the *HSPA1B* gene of HSP72 protein (8). However, as this polymorphism does not lead to any amino acid change in the HSP72 protein, it is rather likely to be a marker for other polymorphic sites that lead to the change in biologic function, explaining the relationship with various infection-related diseases (9–11).

Regarding the central role of HSP72 and TLR4 in innate response to bacterial infection, here we hypothesized that the *HSPA1B* A(1267)G polymorphism and *TLR4* Asp299Gly mutation might be important contributing factors of recurrent UTI and predictive of scarring and other infection outcomes.

PATIENTS AND METHODS

Patients. After obtaining informed parental consent, blood samples were collected from 103 patients (81 girls/22 boys, age: 7.3 ± 4.5 y) with recurrent UTI. A recurrent UTI was defined as a UTI episode within 6 mo after successful treatment of pyelonephritis or cystitis (a negative urine culture, absence of pyuria, and absence of fever) (12). Data on gender and previous medical history were systematically reviewed. Laboratory tests and diagnostic imaging, including abdominal ultrasonography and voiding cystoureterography were also performed on all patients. VUR was diagnosed and classified as grade I–V according to the International Reflux Classification (13). Following a 6-mo UTI-free period, a nuclear scan with technecium-99m-dimercaptosuccinic acid was carried out. It was considered abnormal according to the recent guidelines if focal or generalized uptake defects were detected (14).

Healthy subjects. The prevalence of *HSPA1B* A(1267)G and *TLR4* A(896)G genotypes was determined in a random, unrelated population of 235 healthy controls. All subjects were of Hungarian ethnic origin. Ethical committees of the Institutional Review Board of Semmelweis University approved the present study.

Samples and genotyping. Whole blood was collected and genomic DNA was extracted using the standard phenol-chloroform method. *HSPA1B* A(1267)G and *TLR4* A(896)G genotypes were detected as previously described (11,15).

Statistical analysis. The data were analyzed on STATISTICA.6 software (StatSoft Inc., Tulsa, OK). The statistical significance was calculated by χ^2 test followed by Fisher's exact test if it was needed. For linkage and distribution calculations, Arlequin software (available at http://anthropologie.unige.ch/arlequin/) was used. Significance was set at $p < 0.05$.

RESULTS

Clinical data. Urinary microbiological analyses have detected the urinary pathogen as follows: *E. coli:* 49 patients (47%); *Enterococcus faecalis:* 13 patients (12%); *Proteus:* 13 patients (12%); *Klebsiella:* 7 patients (7%); *Pseudomonas* spp.: 7 patients (7%), *Enterobacter* spp.: 6 patients (6%), *Staphylococcus aureus:* 3 patients (3%); others: 5 patients (5%).

VUR was present in 50 patients (38 girls/12 boys), among which 28 cases were unilateral (left 13, right 15), and 22 were had bilateral reflux. High-grade VUR (IV–V) was detected in 19 cases, while 31 patients had only mild (I-III) VUR. Of those patients who did not have VUR, one had hypospadiasm, one had pyelon duplex, one had unilateral renal agenesia, and two suffered from bladder diverticulum.

Renal scarring was found in 40 patients (30 girls/10 boys). It was more common in patients suffering from high-grade VUR than in others, independent of unilateral or bilateral reflux. The prevalence of renal scarring was similar in patients with low grade (I–III) VUR and those who had recurrent UTI infections without any VUR (10 cases).

Genotype distribution and allele frequencies of *HSPA1B* A(1267)G and *TLR4* A(896)G polymorphisms are shown in Table 1 and Table 2, respectively. Genotype distributions of the tested polymorphism fulfilled the Hardy-Weinberg criteria in the control population.

HSPA1B (1267)G allele occurred more frequently in UTI patients than in healthy subjects [$p = 0.0001$ *versus* healthy controls (OR: 1.92, 95% CI: 1.38–2.68)], whereas (1267)GG

Table 1. *Genotypic frequencies at the* HSPA1B *A(1267)G and* TLR4 *A(896)G polymorphisms in patients suffering UTI compared with healthy reference populations*

		HSPA1B A(1267)G			*TLR4* A(896)G		
Group	No.	AA	AG	GG	AA	AG	GG
Healthy controls	235	93 (40%)	119 (50%)	23 (10%)	218 (92%)	17 (8%)	0 (0%)
UTI patients total	103	12 (11%)	77 (75%)	14* (14%)	88 (85%)	15† (15%)	0 (0%)
Patients with renal scarring	40	2 (5%)	29 (72%)	9** (23%)	35 (87.5%)	5 (12.5%)	0 (0%)
Patients without renal scarring	63	10 (16%)	48 (76%)	5 (8%)	53 (81%)	10 (19%)	0 (0%)
Patients with VUR	50	9 (18%)	39 (72%)	5 (10%)	46 (92%)	4 (8%)	0 (0%)
Patients without VUR	53	3 (5%)	38 (80%)	9 (15%)	42 (79%)	11‡ (21%)	0 (0%)

** $p = 0.036$ *vs* patients without renal scarring [odds ratio (OR): 3.37, 95% confidence interval (CI): 1.04–10.93]; † $p = 0.034$ *vs* healthy controls (OR: 2.19, 95% CI: 1.05–4.57); ‡ $p = 0.067$ *vs* patients with VUR (OR: 0.33, 95% CI: 0.10–1.12).

Table 2. *Allelic frequencies at the* HSPA1B *A(1267)G and* TLR4 *A(896)G polymorphisms in patients suffering UTI compared with healthy reference populations*

		HSPA1B A(1267)G		*TLR4* A(896)G	
Group	No.	A	G	A	G
Healthy controls	235	305 (65%)	165 (35%)	453 (96%)	17 (4%)
UTI patients total	103	101 (49%)	105* (51%)	191 (92%)	15† (8%)
Patients with renal scarring	40	33 (41%)	47 (59%)	75 (93%)	5 (7%)
Patients without renal scarring	63	68 (54%)	58 (46%)	116 (92%)	10 (8%)
Patients with VUR	50	51 (51%)	49 (49%)	98 (98%)	2 (2%)
Patients without VUR	53	44 (41%)	62 (59%)	93 (87%)	11‡ (13%)

* $p = 0.0001$ *vs* healthy controls (OR: 1.92, CI: 1.38–2.68); † $p = 0.041$ *vs* healthy controls (OR: 2.71, 95% CI: 1.33–4.12); ‡ $p = 0.051$ *vs* patients with VUR (OR: 0.35, 95% CI: 0.10–1.1).

Table 3. *Risk of UTI by combined* HSP1AB *A(1267)G and* TLR4 *A(896)G polymorphism in patients* versus *healthy controls*

HSPA1B A(1267)G and *TLR4* A(896)G N	Controls (%) 235	UTI patients (%) 103
(1267)AA + (896)AA	41 (17%)	10 (10%)
(1267)AA + (896)AG	6 (2%)	2 (2%)
(1267)AA + (896)GG	0 (0%)	0 (0%)
(1267)AG + (896)AA	151 (64.5%)	66 (65%)
(1267)AG + (896)AG	13 (5%)	11 (10%)
(1267)AG + (896)GG	0 (0%)	0 (0%)
(1267)GG + (896)AA	22 (9.5%)	11 (10%)
(1267)GG + (896)AG	2 (1%)	3 (3%)
(1267)GG + (896)GG	0 (0%)	0 (0%)

genotype was associated with a higher risk of renal scarring [$p = 0.036$ *versus* patients without renal scarring (OR:3.37, 95% CI:1.04–10.93)]. The relationship between *HSPA1B* (1267) GG genotype and scarring remained significant when it was adjusted for the presence of anatomical abnormalities (*e.g.* high-grade VUR), as a risk factor.

TLR4 (896)AG genotype and *TLR4* (896)G alleles also had higher prevalence among UTI patients than in controls [(896)AG: $p = 0.034$ *versus* healthy controls (OR: 2.19, 95% CI: 1.05–4.57); (896)G: $p = 0.041$ (OR: 2.71, 95% CI: 1.33–4.12), respectively].

TLR4 (896)AG genotype and (896)G allele tended to occur more frequently in patients with recurrent UTI without VUR than in patients with vesicoureteral abnormalities [(896)AG: $p = 0.067$ (OR: 0.33, 95% CI: 0.10–1.12); (896)G: ‡$p = 0.051$ *versus* patients with VUR (OR: 0.35, 95% CI: 0.10–1.1), respectively].

We also assessed the risk of UTI by various combination of *HSPA1B* A(1267)G and *TLR4* A(896)G genotypes (Table 3). Although the greatest risk was provided in those subjects, who carried the G alleles at both of the polymorphic sites, the association was statistically not significant ($p = 0.09$).

DISCUSSION

Taking into account both epidemiologic studies and genomic analyses, there is no doubt that genetic variations influence the frequency and the course of infectious diseases. In the present study we analyzed the prevalence of *HSPA1B* A(1267)G and *TLR4* A(896)G genetic polymorphisms in children with recurrent UTI. Our data indicate a relationship between the carrier status of *HSPA1B* (1267)G and *TLR4* (896)G alleles with the development of recurrent UTI in childhood independently of other renal abnormalities that predispose to the disease. Moreover, we showed that the combination of *HSPA1B* (1267)AG and *TLR4* (896)AG genotype further increases the risk for UTI in these population.

Accumulating evidence indicates that recurrent UTI is caused mainly by the host reaction to bacterial invasion and innate immunity. HSP are crucial for the maintenance of cell integrity during pathophysiological conditions, such as infection or inflammation. Functioning mainly as molecular chaperones, HSP70s are also involved in diverse biologic activities, including protein restoration and cytoprotection.

Various studies have already investigated the relevance of *HSPA1B* A(1267)G in several infection-related diseases (11,16), however, the possible significance of the *HSPA1B* gene polymorphism is still far from clear. This polymorphism has also been shown to associate with lower HSP72 mRNA expression (17). Previously, we demonstrated that *HSPA1B* (1267)GG genotype frequency is increased in prematurity, which is also influenced by infections (10). Recently, in renal transplant recipients we showed an association between the carriage of *HSPA1B* (1267)G and *TLR4* (896)AG allele and chronic allograft nephropathy and acute rejection, which underlines further the importance of these polymorphisms in innate immunity (18).

Here we found that children carrying *HSPA1B* (1267)G allele suffer more often from UTI. Furthermore, we showed that renal scarring is also more prevalent in patients with (1267)G allele. Based on these data one can speculate hat the protein and tissue restoring function of HSP72 and thereby renal recovery might be impaired in these patients, which could be an additional risk factor of UTI and might be more predisposing to renal fibrotic changes.

HSP70s also play a central role in innate immunity as endogenous stimuli for the TLR4 signal receptor pathway. The recently characterized *TLR4* A(896)G polymorphism is associated with functional changes, as demonstrated by decreased bronchial responsiveness to LPS (19).

The data reported here further supports the clinical importance of TLR4 mutations in bacterial infection. It provides the first evidence that human *TLR4* Asp(299)Gly mutation is associated with increased risk of UTI in children with and without anatomic abnormalities. Our data are in line with previous studies, which revealed similar occurrence of *TLR4* (896)AG genotype in septic shock (20) and in Gram-negative infection (7). We have also found that the prevalence of *TLR4* (896)AG, as well as *TLR4* (896)G alleles, is increased among UTI patients without VUR compared with patients suffering from VUR. This supports the assumption that in UTI patients without any predisposing anatomical abnormalities, genetic factors such as TLR4 polymorphism might have an increased importance in the development of the disease.

In summary, our results indicate that the risk of recurrent UTI is associated with certain *HSP1AB* and *TLR4* genotypes and raise further questions about the clinical and therapeutic relevance of these polymorphisms in everyday pediatric nephrology.

Clinical studies are needed to demonstrate if those UTI patients who carry these genetic variants should need a different approach of treatment (for example continuous prophylaxis *versus* treatment of actual infections only) to prevent subsequent irreversible renal damage.

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