The Genetics of Urinary Tract Infections and the Innate Defense of the Kidney and Urinary tract

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Abstract

Keywords

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The urinary tract is a sterile organ system. Urinary tract infections (UTIs) are common and often serious infections. Research has focused on uropathogen, environment, and host factors leading to UTI pathogenesis. A growing body of evidence exists implicating genetic factors that can contribute to UTI risks. In this review, we highlight genetic variations in aspects of the innate immune system critical to the host response to uropathogens. This overview includes genetic variations in pattern recognition receptor molecules, chemokines/cytokines, and neutrophil activation. We also comprehensively cover murine knockout models of UTI, genetic variations involved in renal scarring as a result of ascending UTIs, and asymptomatic bacteriuria.

Introduction

The urinary tract, under normal conditions, is considered a sterile organ system. Urinary tract infections (UTIs) represent a common and, in certain populations, serious infection. The presence of bacteria in the urine and urinary tract can represent asymptomatic bacteriuria (ABU), cystitis, or acute pyelonephritis (APN). The organism most commonly associated with UTI is uropathogenic *Escherichia coli* (UPEC). Several host factors have been identified that contribute to the defense and clearance of invading pathogens including environmental conditions within the urinary tract, host blood type, anatomical variation, and the host innate immune response. This review will briefly address environmental blood type and structural aspect to UTI risk. Primarily, we will highlight genetic innate immune factors, which when perturbed allow for bacterial invasion (cystitis/UTI), coloni-

zation (ABU), or ascension to the kidney (APN) from the lower urinary tract.

Inheritance of Urinary Tract Infection Susceptibility

Inheritance of Acute Cystitis Susceptibility

Several studies have associated personal and family histories of UTI with an increased risk of acute cystitis.^{1,2} One study showed that patients with a history of UTI have increased frequency of UTI-prone family members compared with controls.³ This study was recently confirmed, and these data were extended to also include APN.⁴ The risk of developing a UTI increased with the number of prone family members, especially females.

Inheritance of Acute Pyelonephritis Susceptibility

The inheritance of APN morbidity was studied in three-generation pedigrees of families.⁵ An increased frequency

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of APN was detected in family members of APN-prone children, regardless of gender, compared with controls without UTI. However, no consistent inheritance pattern was observed, indicating that both dominant and recessive genetic determinants might be involved. The frequency of cystitis was similar in both groups, however, emphasizing that APN and cystitis are controlled by different genetic mechanisms.

Environmental Factors Contributing to Urinary Tract Defense

Although UTI and APN have a genetic component, environmental factors clearly play a role. If UPEC or other uropathogens invade the lower urinary tract, several host and environment factors contribute to the pathogen's ability to cause disease.⁶ For example, the composition of urine can

influence bacterial growth and UTI susceptibility. Optimal pH and osmolality as well as the presence of iron and glucose can affect bacterial growth in urine.⁷ Obstruction or altered urinary tract emptying can lead to increased UTI risk, presumably from decreased clearance from the urinary tract. But before pathogens can invade, they must also evade host innate immune defenses and attach to uroepithelial surfaces.

Host Blood Group and Uroepithelial Attachment

Epithelial receptor expression is also influenced by the P and ABO blood groups as well as secretor state.⁴ The receptors for P fimbriae on uroepithelial cells are antigens in the P blood group system.^{8,9} The globoseries of glycosphingolipids vary in expression and molecular identity with the host erythrocyte

Fig. 1 Schematic of TLR4 (toll-like receptor 4) signaling: MyD88 (myeloid differentiation primary response 88)-dependent and MyD88-independent pathways mediate TLR4 signaling. Genetic variations in key proteins or inflammatory mediators within TLR4 signaling pathways can result in increased asymptomatic bacteriuria (white stars), cystitis (striped stars), or pyelonephritis (black stars) risk. Pathway crosstalk and other involved pathway proteins are present but omitted from the figure for illustration purposes. ABO, asymptomatic bacteriuria; APN, acute pyelonephritis; CCL, chemokine C-C motif ligand; CCL20, chemokine (C-C motif) ligand 20; CXCL8, chemokine (C-X-C motif) ligand 8; CXCR, C-X-C chemokine receptor; ICAM, intercellular adhesion molecule; IFNB1, interferon β 1, fibroblast; IRF, interferon regulatory factor; IRF3, interferon regulatory factor 3; LPS, lipopolysaccharide; NF- κ B, nuclear factor of kappa light polypeptide gene enhancer in B-cells 1; PRR, pattern recognition receptors; PTX3, pentraxin 3; TGF β 1, transforming growth factor β 1; TICAM, toll-like receptor adaptor molecule 1; TIRAP, toll-IL 1 receptor (TIR) domain containing adaptor protein; TLR, toll-like receptor; TNF, tumor necrosis factor; UMOD, uromodulin; UPEC, uropathogenic *Escherichia coli*; UTIs, urinary tract infections; VEGF, vascular endothelial growth factor; VUR, vesicoureteral reflux.

blood group. In addition, in peripheral tissues the glycolipid repertoire is affected by additional variables, including secreted anti-adhesive molecules, as known as host secretor status.¹⁰

Due to P blood group-dependent variation in receptor expression,^{11–13} individuals of blood group P1 have an ~17-fold increased risk for APN. These individuals also have increased carriage of P-fimbriated *E coli* in their intestinal flora and subsequent increased risk of recurrent APN.^{11–13} In contrast, individuals of blood group P lack the Gb3 glucosyl-transferase and fail to express functional receptors for P fimbriae.^{11–13} In the ABO blood groups, individuals expressing globo-A epitope are preferentially infected by *E coli* expressing the prsG adhesin, which uses group A as a receptor.¹⁴ By influencing the receptor repertoire of uroepithelial cells, the host blood group has an impact on both uropathogen attachment and innate immune activation.

Vesicoureteral Reflux

Vesicoureteral reflux (VUR) is commonly seen as a risk factor for APN in children. VUR likely contributes to renal scarring by facilitating the ascent of bacteria into the renal pelvis^{15,16} and its prevalence is increased up to 62% in children with febrile UTI.^{17–19} However, after corrective surgery, individuals with VUR seem to remain susceptible to UTI.²⁰ This observation suggests that VUR itself may not be a primary determinant for UTI susceptibility but just a conduit for bacteria to reach the upper urinary tract more readily when they invade.¹⁰

The Innate Immune Defense of the Urinary Tract

The host innate immune response serves as the primary factor for the defense of the kidney and urinary tract. Unlike the adaptive immune system, the human innate immune system is poised to provide continuous, generalized, and potent defense to a broad spectrum of pathogens in almost every organ system. The innate immune system also serves as the activator of the adaptive immune response to prevent subsequent similar infections. The innate immune system of the kidney and urinary tract includes peptides with antimicrobial properties, cytokines and chemokines, pattern recognition receptors (PRRs), and phagocytes.²¹ Antimicrobial peptides are small, cationic molecules made by epithelia and innate immune cells that have potent antimicrobial activity. The host secretes antimicrobial peptides into the urine stream to directly kill or prevent uroepithelial attachment. Furthermore, host iron-scavenging molecules limit free iron in the environment, a necessary component for growth. If a pathogen attaches to a uroepithelial cell, PRRs recognize pathogen-associated molecular patterns. The most studied PRR, toll-like receptor 4 (TLR4), recognizes gram-negative bacteria's lipopolysaccharide (LPS). If a PRR is activated, several intracellular signaling pathways are activated, which leads to transcription of proinflammatory molecules including chemokines and cytokines (►Fig. 1). These molecules then activate and target phagocytes to the site of infection.

Several animal models exist that demonstrate that alterations in these processes lead to UTI susceptibility. Furthermore, an increasing number of human genetic alterations in the innate immune system have been identified and associated with bacterial infections and colonization of the urinary tract. This review will highlight important studies in both transgenic murine models and genetic studies of human disease.

Murine Models of Urinary Tract Infection and Asymptomatic Bacteriuria

Experimental models of UTI have increased our knowledge of the importance of innate immunity in UTI susceptibility. Further, these models support the hypothesis that single genes have effect on both acute and chronic disease.^{22–24} Several murine genetic models have been used to better understand the susceptibility of UTIs. A majority of the genes important for susceptibility belong to the distal signaling pathways of TLR4, where single gene knockouts are enough to develop severe infection and pathology.^{22,25–27}

Antimicrobial Peptides and Proteins with Antibacterial Properties

In the murine urinary tract, only one cathelicidin is expressed.²⁸ Cathelin-related antimicrobial peptide $-/-$ mice deficient for the cathelin-related antimicrobial peptide develop kidney pathology, septicemia with increased mortality^{28,29} and have increased bacterial counts compared with wild-type mice in response to UPEC infections.³⁰ No genetic polymorphisms in the human ortholog cathelicidin (CAMP) gene have been identified. Beta defensins have been identified and described in the urinary tract. Beta-defensin 1 knockout mice had a high incidence of spontaneous *Staphylococcus* bacteriuria.³¹ When challenged with UPEC, beta-defensin 1 knockout mice did not have increased bacterial burdens in the bladders or kidneys.³² The role for this antimicrobial peptide in the human kidney and urinary tract is unknown.

Additional genes have been implicated in murine cystitis models, although their role in the pathogenesis is less understood. Tamm-Horsfall protein, or uromodulin (UMOD), is expressed in the kidneys and released into the urine via proteolysis. UMOD acts as a receptor for UPEC's type 1 fimbriae,^{33,34} indicating a role for UMOD in host defense. UMOD $-/-$ mice, however, showed higher numbers of bacterial number in bladders compared with wild-type mice. No difference was observed in renal bacterial burdens.³⁵ UMOD-mRNA expression was reduced in *Ptgs2* $-/-$, cyclo-oxygenase-2 deficient mice, which showed increased susceptibility to type-1 fimbriated *E coli*.³⁶

TLR4 Variants and Bacterial Response

Murine Models of Asymptomatic Bacteriuria

Patients with ABU can carry the same bacterial strain in their bladders for several months or years without developing symptoms of UTI.³⁷ Murine genetic models that interfere

with *Tlr4* signaling pathways often create an ABU-like state. Mice without functional *Tlr4*, when infected with UPEC strains, have no or little early innate immune response, including mucosal cytokine response, activation of chemokine receptors, and neutrophil recruitment. The antibacterial effector functions are suppressed, leading to bacterial persistence in the host without signs of inflammation. Also, these mice have a higher tendency to develop intracellular bacterial communities.^{38–40} A similar phenotype occurs with *Trif* $-/-$ and *Myd88* (myeloid differentiation primary response 88) $-/-$ mice where no pathologic/inflammatory changes are observed, and the cytokine responses are absent or low.^{26,41} Thus, interference at the proximal level of the murine *Tlr4* signaling cascade protects the host by abrogating the innate immune response to UPEC infection. Further, prevention of innate immune activation favors long-term bacteriuria without inflammation. Whether these models truly reflect similar biology to human ABU is a hot topic for scientific debate.

TLR4 Structure

DNA sequencing of *TLR4* in children with ABU and healthy controls demonstrates significant genetic variations associated with the condition.⁴² An association of the *Asp299Gly* and *Thr399Ile* mutations in patients with recurrent UTI but without VUR has been observed.⁴³ An increased prevalence of the *Asp299Gly* mutation was confirmed in Chinese patients with acute cystitis and urethritis.⁴⁴ Association studies implicated that the *TLR4 Asp299Gly* variant protected from recurrent cystitis in adults but this was not seen in children.⁴⁵ However, no in vivo effect of the *Asp299Gly* variant was detected in LPS challenge studies on healthy volunteers with or without the single nucleotide polymorphism (SNP).^{46,47} Cytokine production in response to LPS was actually stronger in individuals with the *Asp299Gly* mutation than those without.⁴⁸ Additionally, other mechanisms of *TLR4* regulation or downstream signaling molecules contribute to UTI susceptibility.

TLR4 Expression and Signaling

TLR4 expression is lower in children with ABU than in age-matched controls or APN patients.⁴⁹ Elevated levels of the *TICAM1* TRIF (toll-like receptor adaptor molecule 1) adaptor protein and reduced level of the *TLR4* inhibitor single immunoglobulin interleukin (IL)-1-related receptor were also observed in this patient group. But *MyD88* and *TICAM2* (TRAM) expression levels did not differ between ABU patients and the other patient groups. *TICAM1* (TRIF) sequences from ABU-prone children have been compared with those from healthy controls. Several polymorphic sites were identified, but no association with UTI was found.^{43,44} Furthermore, no association between *TIRAP* (toll-IL 1 receptor [TIR] domain containing adaptor protein) polymorphisms, +539 C/T and +558 C/T and UTI susceptibility was found in a cross-sectional analysis of women with ABU.⁵⁰ The results suggest that low *TLR4* expression and signaling might protect the host against symptomatic UTI and promote the development of ABU.^{43,44} Yin et al⁴⁴ found decreased *TLR4* expression in adult patients with history of recurrent UTIs compared with controls. *TLR4* +896 AG genotype and *TLR4* +896 G

allele also had higher prevalence among UTI patients than in controls.

TLR4 Promoter Polymorphisms

The *TLR4* promoter is highly polymorphic, and *TLR4* promoter genotypes with reduced function are common in children with ABU.⁵¹ In reporter assays, promoter-sequence variants from ABU patients showed reduced *TLR4* expression. In vivo studies, patients with the ABU-associated genotypes showed reduced innate immune responses to therapeutic inoculation of the urinary tract with *E coli*.⁵¹ Genetic variation in the *TLR4* promoter may be an essential mechanism to influence *TLR4* expression and susceptibility to pathogens with *TLR4*-activating virulence ligands.

Genes Critical to Neutrophil Recruitment and Activation

Interferon Regulatory Factor 3 (IRF3) Expression and Promoter Polymorphisms

After UPEC activation of *TLR4*, intracellular signaling leads to increased expression of *IRF3*. *IRF3* is a transcription factor that, together with *IRF-7*, controls the expression of genes involved in the innate immune response, including, IL-6, CXCL2 chemokine ([C-X-C motif] ligand 2), tumor necrosis factor (TNF) and *IFNB1* (interferon, β 1, fibroblast).^{52,53} In murine models, this molecule was a critical component for UPEC clearance. *Irf3* $-/-$ mice are highly susceptible to APN, with development of urosepsis and severe kidney pathology during UPEC infection. Recruitment of neutrophils to the site of infection is intact. However, their antibacterial functions are impaired, resulting in increased bacterial burden in the tissues. Not surprisingly, a similar phenotype was observed in *Ifnb1* $-/-$ mice, suggesting that *IFNB1* and downstream effects of *IRF3* have essential antibacterial effector functions.²⁵

Polymorphisms affecting transcription factor expression have been associated with susceptibility in APN-prone patients. *IRF3* promoter polymorphisms were studied in an epidemiologically defined population of APN- or ABU-prone children and in a follow-up adult population ~30 years after their first febrile UTI episode. -925 A/G and -776 C/T polymorphisms were strongly associated with human APN susceptibility, occurring in ~70% of APN-prone patients compared with the ABU group.²⁵ The in vitro transcriptional activity from the promoter sequence associated with APN (A-C) was shown to be ~50% lower than the ABU promoter (A-T). Together, these results demonstrate the importance of the downstream factors of the *TLR-4* signaling cascade in UTI susceptibility.

CXCL8 and Receptors, CXCR1 and CXCR2, Expression and Genetic Polymorphisms

The *CXCL8* chemokine, also known as IL-8, and its receptors *CXCR1/2* are essential for recruitment of neutrophils following infection in the urinary tract. In addition to the *IRF3* increased expression discussed above, *TLR4* activation leads to additional chemokine responses, including increases in

CXCL8 levels in both urine and blood. Concurrently, the expression of the chemokine receptor *CXCR1* increases. This receptor is critical for recruiting neutrophils to the site of infection.^{54,55} Although mice do not express CXCL8, they express analogous chemokines that bind to the receptor *CXCR2*. Mice lacking this receptor are unable to recruit neutrophils to the infection site during UTI. The *Cxcr2* $-/-$ mice develop severe APN with urosepsis and renal scarring.²²⁻²⁴

APN-prone children were shown to have reduced *CXCR1* expression levels compared with pediatric controls and reduced *CXCR1* expression was detected in family members of the APN-prone children.²² Five SNPs were found in the *CXCR1* gene. Four were predicted to affect *CXCR1* expression and one was shown to reduce the efficiency of transcription.⁵⁶ *CXCR2* levels were also reduced in premenopausal women with recurrent UTI compared with controls.⁵⁷ In association studies, a *CXCR1* polymorphism +2608 G/C as well as CXCL8 polymorphisms -251 A/T and +2767 A/G were not APN-associated.⁵⁸ On the other hand, the -251 TT genotype was more frequently associated with children with negative dimercaptosuccinic acid scan-confirmed APN. Another study found no difference in *CXCR1* expression levels in adult patients with history of recurrent UTIs compared with controls.⁴⁴ An additional study confirmed a CXCL8 SNP that correlated to APN susceptibility and severity in children.⁵⁹ Findings from a cross-sectional analysis revealed increased frequency of three *CXCR1* polymorphisms in women with ABU caused by gram-positive organisms.⁵⁰ The *CXCR1* +827 G/C polymorphism was associated with increased CXCL8 levels.⁵⁰ This highlights multiple factors at play that lead to ABU and APN susceptibility. Thus, reduced *CXCR1* expression is a risk factor for APN, and variations in the *CXCR2* receptor and the CXCL8 chemokine lead to UTI susceptibility.

Genetic Polymorphisms Important in Renal Scarring

Several studies showed that *VEGFA* (vascular endothelial growth factor A) and *TGFB1* (transforming growth factor, β 1) polymorphisms predisposed to progressive renal disease,⁶⁰⁻⁶² and the TNF variant -238 G/A had increased frequency in UTI-prone patients with early rheumatoid arthritis.⁶³ Both the *TGFB1* -509 T allele and the *VEGFA* -406 CC genotype were associated with a risk of renal scarring after UTI but not to isolated VUR.⁶⁴ Interestingly, the *VEGFA* -460 C allele was shown to increase the basal promoter activity.⁶⁵ Another study also associated *TGFB1* promoter variants -800 G/A and -509 C/T polymorphisms with UTI and VUR in children.⁶⁰ The *TGFB1* +869 CC and -509 CC genotypes were more common in patients with renal scarring.⁶⁶ Those findings were supported in a European cohort⁶⁷ but not in a group of Asian patients with renal scarring. The *VEGFA* -460 CC promoter variant was increased in patients with VUR regardless of UTI,⁶⁶ which indicates variation in risk of scarring between ethnic groups. *HSPA1B* (heat shock protein 1B) +1267 G allele occurred more frequently in UTI patients

than in healthy subjects, whereas +1267 GG genotype was associated with a higher risk of renal scarring.⁴³

Polymorphisms in Other TLRs and Other Pattern Recognition Molecules

Besides *TLR4*, other murine pattern recognition molecules have been implicated in UTI susceptibility. *Tlr11* is strongly expressed in the murine kidney. With UPEC challenge, the kidneys of *Tlr11* $-/-$ mice have severe infection and defective neutrophil infiltration compared with wild-type mice.⁶⁸ Murine *Tlr5* is expressed in both bladders and kidneys and is mobilized to the cell surface in response to infection. *Tlr5* $-/-$ mice show a gradual increase in bacterial numbers in the bladder, with superficial bacterial micro-abscesses in parallel with submucosal edema compared with wild-type mice.⁶⁹

TLR2 has been implicated in the bacterial response in the kidney and urinary tract as well. *TLR2* recognizes several molecules, but primarily recognizes lipoteichoic acid from gram-positive bacteria. The *TLR2* +2258 G/A polymorphism caused impaired innate immune signaling in transfected human embryonic kidney cells. This SNP in *TLR2* was also associated with ABU.⁵⁰ Markedly reduced cytokine release was observed in whole blood from individuals heterozygous for this SNP after stimulation with *Borrelia burgdorferi* (the causative organism of Lyme disease) lysate.⁷⁰ Additionally, patients carrying the *TLR2* Arg753Gln allele had higher risk of UTI with gram-positive pathogens.⁷¹

The body of evidence for *TLR5*'s role in urinary tract defense is growing. *TLR5* recognizes the motile bacterial flagellin protein. A stop codon polymorphism in the ligand-binding domain of *TLR5* (Leu392X) has been shown to abrogate flagellin-induced signaling and has been reported to increase the risk of recurrent UTIs but not pyelonephritis.⁴⁵ Interestingly, this study also implicated *TLR1* variant (+1805 G/T) with protection from APN.

Finally, the PRR molecule, pentraxin 3 (*PTX3*), is induced by UPEC in a *TLR4*- and *MyD88*-dependent manner.⁷² *Ptx3*-deficient mice (*Ptx3* $-/-$) had higher bladder bacterial burdens at day 5 following UPEC challenge and higher kidney burdens at days 1 and 5. Furthermore, SNPs were identified in this gene in patients with APN and UTI that correlated to UTI susceptibility.

Other Genes Associated with the Bacterial Response

CCL5 (chemokine [C-C motif] ligand 5) (*RANTES*) polymorphisms have been investigated, and the *CCL5* -403 G allele was significantly associated with UTI risk in a pediatric population.⁷³ Polymorphisms in *ICAM1* (intercellular adhesion molecule 1), a cell adhesion molecule, were also investigated. Notably, the *ICAM1* exon 4 (G/A) polymorphism was less common in patients who developed renal scars following infection than in controls.⁷⁴ Genetic variants affecting coding region of lymphotoxin α have been associated with recurrent UTI but not APN.⁶³

Conclusion

The innate defense of the kidney and urinary tract is a complex and multifactorial process. Several components work in concert to prevent and immediately clear invading pathogens. As we review in the manuscript, different variations in the same gene can lead to significantly different phenotypes ranging from ABU to APN and renal scarring. While several significant mutations have been identified in known critical components, much is still to be discovered. Antimicrobial peptides and epithelial cell functions have not been well studied. For example, mice with intercalated cell-targeted dysfunctions have increased risk of UTI with bacterial challenge.^{75,76} Well-phenotyped cohorts of these conditions and genome-wide studies are needed to identify the entire milieu of the innate defense that may be targeted for novel therapeutics in preventing or treating UTIs.

Conflict of Interest

The authors report no conflict of interest.

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