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Studies on Clinical Characteristics, Urovirulence Factor and Host Susceptibility Gene in Pediatric Acute Lobar Nephronia

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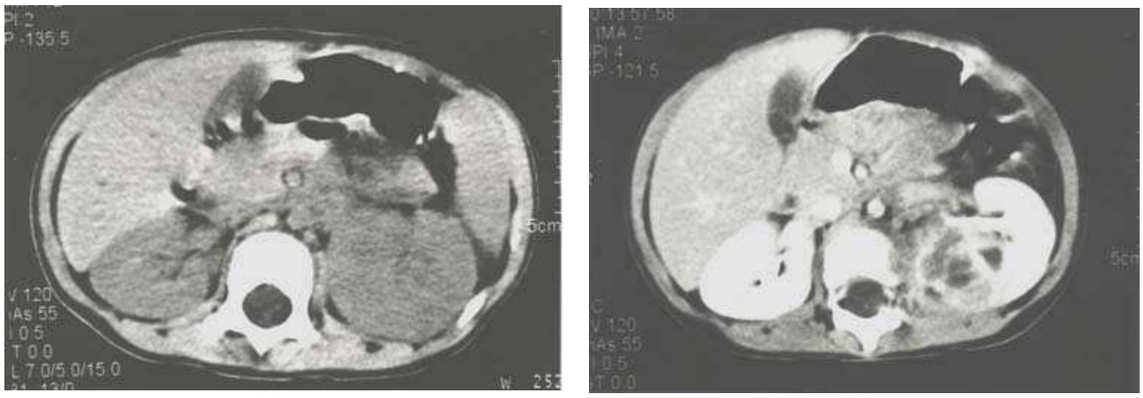
1. Introduction

Urinary tract infections (UTIs) have been described as one of the most common serious bacterial diseases affecting infants and young children. Approximately 3% of prepubertal girls and 1% of prepubertal boys are diagnosed with UTIs (Riccabona 2003; Ma and Shortliffe 2004). The clinical severity of acute renal bacterial infection spans continuously from an uncomplicated lower urinary tract infection (i.e. cystitis) to frank abscess formation (Soulen et al., 1989). Among these UTIs, renal parenchymal infections, including uncomplicated acute pyelonephritis (APN), acute lobar nephronia (ALN), and intrarenal abscess, are considered to be more serious forms of UTI.

Acute lobar nephronia (ALN), also known as acute focal bacterial nephritis, is an acute localized bacterial renal infection presenting as an inflammatory mass without liquefaction (Rosenfield *et al*., 1979; Zaontz *et al*., 1985; Kline *et al*., 1988; Klar *et al*., 1996, Uehling *et al*., 2000). The typical clinical presentations include fever, flank pain, leukocytosis, pyuria and bacteriuria, similar to presentations of patients with renal abscess or acute pyelonephritis (Zaontz *et al*., 1985; Soulen *et al*., 1989). It has previously been indicated as a complicated form of acute renal infection, representing the progression of the inflammatory process of APN (Nosher *et al*., 1988). ALN may also represent a relatively early stage of the development of renal abscess (Shimizu *et al*., 2005). The management of these renal parenchymal infections differs widely. Most patients with renal abscess require intensive medical therapy with or without surgical intervention, whereas treatment of those with ALN, like uncomplicated APN, entails only intravenous and oral antibiotics (Zaontz *et al*., 1985; Rathore *et al*., 1991; Klar *et al*., 1996). Hence it is important to differentiate these renal parenchymal infections. In this Chapter, we would like to review the diagnosis scheme, treatment modality, bacterial urovirulence factors, host susceptibility gene and the renal scar outcome of ALN.

2. Effective ultrasonographic predictor for the diagnosis of acute lobar nephronia

Sonographically, ALN generally presents as severe nephromegaly or a poorly defined, irregularly margined focal mass with hyper-, iso- or hypoechogenicity, depending on the temporal sequence of the lesions and the resolution of the disease (Rathore *et al*., 1991; Boam and Miser, 1995). Although renal ultrasonography (US) has been considered as the best and most-effective screening method, various false positive and false negative findings have been reported previously (Rosenfield *et al*., 1979; Soulen *et al*., 1989). Computed tomography (CT), instead, has been currently recognized as the most-sensitive and -specific imaging modality for diagnosing ALN (Kline *et al*., 1988; Soulen *et al*., 1989; Rathore *et al*., 1991; Klar *et al*., 1996; Uehling *et al*., 2000). CT images of the ALN-infected areas typically appear as wedge-shaped, poorly defined regions of decreased nephrogenic density after contrast medium administration (Figure 2.1) (Kline *et al*., 1988; Soulen *et al*., 1989; Rathore *et al*., 1991; Cheng *et al*., 2004), and mass-like hypodense lesions in the more-severe form (Lee *et al*., 1980). CT, however, is costly and requires the sedation of a young patient.



(a) (b)

Fig. 2.1. The characteristic non-enhanced (a) and contrast-enhanced (b) CT scans for a 2-year-old patient with acute lobar nephronia, who presented with severe left nephromegaly while without a focal mass sonographically. Note no attenuation area seen in the kidney before enhancement (Cheng *et al*., 2004).

A new systemic radiologic imaging evaluation scheme, the combination of renal US and CT scanning, was proposed to assist ALN diagnosis in pediatric patients. Enlarged kidneys and/or focal masses were utilized to be the sonographic preselection features for subsequent CT evaluation. CT scan is used when the patient shows (1) a markedly enlarged kidney or focal mass on the initial US scan; or (2) poor response to the initial 72 hours antibiotic treatment while his/her kidneys appear borderline nephromegaly sonographically (Cheng *et al*., 2004).

From our results (Cheng *et al*., 2004), severe nephromegaly (i.e. renal length greater than mean +3 SD for age) on at least one side of the kidneys is very sensitive for the diagnosis of ALN. A higher sensitivity was achieved when US focal mass findings were included with severe nephromegaly as the diagnosing criteria. In terms of the kidneys, focal masses on US scans correlated much better with the final ALN diagnosis than other US characteristics evaluated. The use of focal mass findings as an effective predictor for ALN was limited since

its sensitivity was only 25% despite a specificity of 100%. Severe nephromegaly was a very useful sonographic diagnostic criterion for kidneys affected with ALN with a sensitivity of 90.0%. Sensitivity increased to 95% when severe nephromegaly together with focal mass was used as the sonographic predictor.
In summary, pediatric ALN was effectively predicted using sonographic findings of severe nephromegaly and/or focal mass prior to CT scanning.

3. Effective duration of antimicrobial therapy for the treatment of acute lobar nephronia

Treatment for patients with ALN generally requires intravenous and oral antibiotic medication as does treatment for uncomplicated APN (Zaontz *et al*., 1985; Rathore *et al*., 1991; Klar *et al*., 1996). Surgical intervention is rarely needed for ALN patients, except for those with concomitant urological abnormalities which may increase the risks of occurrence of acute bacterial infection (Uehling *et al*., 2000). Although it has been suggested that the treatment duration for ALN needs to be at least the same as that for uncomplicated APN, recommendations for the duration of antibiotic treatment still remains somewhat inconclusive, and to the best of our knowledge, for neither condition, has a rigorous therapeutic efficacy comparison of relevant medication been performed (Rathore *et al*., 1991).
We have performed a study sought to determine the appropriate duration of effective antibiotic therapy for the management of pediatric ALN patients (Cheng *et al*., 2006). Patients who first presented as febrile UTI and who later were diagnosed with positive CT findings of ALN were entered into this study for receiving either a two-week or a three-week intravenous and oral antibiotic therapeutic program. The demographic data and clinical results of these patients were compared. In addition, the identification of any clinical or laboratory factors that are likely associated with treatment failure was also attempted.
These two treatment groups had similar demographic data and clinical results (Table 3.1). Most of the patients had been febrile for around three-to-four days, ranging from one day to two weeks or so, prior to admission. Once patients had been admitted, all responded well to the initial antibiotic treatment regimen and the fever generally subsided within about a week. CT scans indicated that 18 patients had left ALN, 12 right ALN, and 11 bilateral ALN in the two-week treatment group. Corresponding figures for the three-week treatment group were 16, 12 and 11 patients respectively. The distribution of these ALN diagnoses was quite similar between the two treatment groups.
Among the 80 patients participating in this study, *Escherichia coli* was the most-common pathogen cultured from the patient urine samples (59/61) such a finding being consistent with the results of previous studies (Kline *et al*., 1988; Nosher *et al*., 1988; Rathore *et al*., 1991; Boam and Miser, 1995; Uehling *et al*., 2000). Interestingly, the proportion (percentage) of *Escherichia coli* cultured in cases of ALN appears to be much greater than the corresponding figure reported for first-time UTIs (Hoberman and Wald, 1999).
Sixty-nine ALN patients, 40 from the two-week treatment group and 29 from the three-week treatment group, underwent VCUG evaluation. Sixteen patients in the two-week treatment group (40%) and eleven in the three-week treatment group (38%) had vesicoureteral reflux (VUR). Among the patients with VUR, a grade-III or greater was noted in eight and nine patients respectively, treated by the two-week and the three-week antibiotic courses. For patients who underwent VCUG, no difference as regards the presence of VUR in either frequency or severity was found between the two treatment groups.

	Two-week treatment group (n=41)	Three-week treatment group (n=39)	P
Age, years	3.72 ± 4.14	4.16 ± 4.22	NS
Range	4 mo – 16 yr	4 mo - 14yr 8mo	
Girls	24 (58.5%)	23 (59.0%)	NS
Fever duration prior to admission, days	3.90 ± 3.06	3.97 ± 2.72	NS
Range, days	1 – 14	1-13	
Fever continuation following antibiotic treatment, days	2.73 ± 1.28	3.43 ± 2.05	NS
Range, days	1 – 7	1 – 8	
White blood cell count, (cells/μL)	19,107 ± 8,772	19,600 ± 10,212	NS
Leukocytosis (>15,000 WBC/μL)	28 (68.3%)	25 (64.1%)	NS
C-reactive protein, (mg/L; normal <5)	137.7 ± 98.1	119.3 ± 74.0	NS
Urine culture			
Escherichia coli	31 (75.6%)	28 (71.8%)	NS
E. coli > 10^5cfu/mL	25 (61.0%)	25 (64.1%)	NS
Klebsiella pneumoniae	1 (2.4%)	---	---
Pseudomonas aeruginosa	---	1 (2.6%)	---
No isolatable organism	9 (22.0%)	10 (25.6%)	NS
Blood culture			
Escherichia coli	1 (2.4%)	2 (5.1%)	---
Staphylococcus aureus	---	1 (2.6%)	---
No isolatable organism	40 (97.6%)	36 (92.3%)	NS
Treatment failure	7 (17.1%)	0 (0%)	0.01

NS indicates not significant

Table 3.1. Clinical and laboratory data for 80 children with ALN enrolled for different treatment protocol (Cheng *et al*., 2006).

None of our patients revealed any evidence of underlying diseases such as diabetes mellitus, immunodeficiency, nor did any feature structural abnormality of the urinary-tract system such as neurogenic bladder, or upper or lower urinary-tract obstruction apart from VUR. Reflux was noted in about 40% of the ALN children in this study, a figure quite comparable to that in several previous studies (Kline *et al*., 1988; Klar *et al*., 1996; Uehling *et al*., 2000). This frequency of VUR among patients with ALN is close to that in children with UTI (Ilyas *et al*., 2002), thus, VUR may not be a necessary prerequisite for the development of ALN.

Overall, seven treatment failures were noted in this study (8.8%; 95% CI, 2.6%-14.9%), all of which had been managed by a two-week antibiotic course (17.1%; 95% CI, 5.6%-28.6%). Statistical significance was noted in regard to treatment success rate between these two groups (p=0.01). Among these patients with treatment failure, one demonstrated persistent infection during the treatment course, and six others were considered to be relapse by revealing a positive *Escherichia coli* urine culture with the same antibiotic sensitivity profile as had been the case previously.

Table 3.2 lists the clinical characteristics of these seven patients as compared with those successfully treated with the two-week antibiotic course. Proportionally more girls may be noted in the failures group than the non-failures group, but the difference was not statistically significant (p=0.21). The patients failing the two-week antibiotic treatment presented with a more-pronounced fever duration prior to admission (6.00 ± 5.54 vs. 3.47 ± 2.16 days; p=0.04), and they were more likely to be *Escherichia coli* infection positive [$>10^5$ cfu/mL; 100% (7/7) vs. 52.9% (18/34); p=0.03]. The distribution of ALN foci, VCUG characteristics, and other clinical results revealed no difference between the failures and non-failures groups.
For the treatment-failure patients, the antibiotic treatment course was extended/restarted for an additional ten days. Subsequent urine culture and clinical-symptom evaluation at the follow-up exams revealed eventual successful treatment.

	Failures with two-week treatment protocol (n=7)	Non-failures with two-week treatment protocol (n=34)
Age, years	4.07 ± 4.31	3.65 ± 4.16
Range	4 mo – 9 yr	4 mo – 16 yr
Girls	6 (85.7%)	18 (52.9%)
Fever duration prior to admission, days†	6.00 ± 5.54	3.47 ± 2.16
Range, days	1 – 14	1-10
Fever continuation following antibiotic treatment, days	2.14 ± 1.21	2.85 ± 1.28
Range, days	1 – 4	1-7
White blood cell count, (cells/μL)	22,257 ± 8,656	18,459 ± 8,781
Leukocytosis (>15,000 WBC/μL)	6 (85.7%)	22 (64.7%)
C-reactive protein, (mg/L; normal <5)	107.3 ± 113.3	143.4 ± 95.9
Escherichia coli in urine culture	7 (100%)	24 (70.6%)
E. coli > 10^5cfu/mL‡	7 (100%)	18 (52.9%)

†: p=0.04; ‡: p=0.03

Table 3.2. Comparison of demographic data, clinical characteristics and laboratory results between the ALN patients with treatment failure and those with treatment success for the two-week antibiotic therapy protocol (Cheng *et al.*, 2006).

From our results, all ALN patients with the three-week antibiotic course were successfully treated, whereas seven treatment failures (17.1% of treated patients) were noted in the two-week treatment group. This observation suggests that the two-week antibiotic treatment, usually scheduled for APN, may not be appropriate for the treatment of ALN. Patients who failed the two-week treatment modality were more likely to have prolonged fever prior to admission and to reveal positive *Escherichia coli* infection ($>10^5$ cfu/mL). The longer febrile history prior to admission may suggest that these patients may be prone to develop a more severe disease state than their counterparts, and that, by necessity, a longer antibiotic treatment course will be needed for such individuals. Indeed, these treatment failures were

all successfully dealt with by an additional ten-day antimicrobial therapy regimen. Whether host factors, or the virulence of *Escherichia coli*, relates to ALN and plays a role leading to treatment failure remains an issue that should be clarified.

4. A high incidence of renal scarring is associated with child with acute lobar nephronia

The pathogenesis of renal scarring after a febrile UTI remains unclear. Some risk factors making children with a UTI more vulnerable to renal damage include young age at the time of infection (Winberg *et al*. 1982), delayed treatment (Miller and Phillips 1981; Winberg *et al*. 1982), the presence of vesicoureteral reflux (VUR) (Biggi *et al*. 2001; Chroustova *et al*. 2006; Faust *et al*. 2009), and although mentioned infrequently, the extent of kidney lesions (Biggi *et al*. 2001). ALN is a severe disease entity, with extensive renal parenchymal involvement (Cheng *et al*. 2009). Thus, we performed a prospective study to evaluate renal scar formation after ALN as compared with APN. In this prospective study (Cheng *et al*. 2010a), we also examined nearly all the previously proposed risk factors for renal scarring.

DMSA scintigraphy is a sensitive diagnostic method for renal scarring but does not always distinguish between new and old lesions, or differentiate renal dysplasia from acquired post-infection scars. It is possible that we might have overestimated the occurrence of renal scars that were related to the index infection. Thus, the exclusion of children with history of prior UTI or the development of recurrent UTI before DMSA scintigraphy in current study was designed to keep any overestimation of renal scarring to a minimum.

In this investigation, a total of 218 children with a first documented febrile UTI (109 APN, 109 ALN) who fulfilled our patient selection criteria and completed the final DMSA scintigraphy were analyzed. Patient characteristics were comparable between the two ALN treatment groups (Table 4.1). The frequency of renal scarring at scintigraphy was similar between the 2-week and 3-week successful treatment groups. The demographic and clinical data for the APN (all had received 10-day treatment) and ALN patients are shown in Table 4.2. Acute lobar nephronia was a more severe disease than APN, as judged by higher inflammatory indices and longer fever duration after and/or before treatment. The incidence of renal scarring was much higher in ALN than in APN patients.

Regression analysis of the 218 patients with a first febrile UTI showed that renal scarring was more likely to occur in children with higher inflammatory indices (white blood count: 19802±7652 vs. 15478± 6853; and C-reactive protein: 124.6±89.8 vs. 68.4±69.6; $P < 0.001$), longer duration of fever after ($P < 0.001$) and/or before treatment ($P = 0.001$), and the presence of VUR ($P = 0.044$). However no relationship was found between renal scarring and age at diagnosis or gender.

Higher inflammatory indices and longer fever duration after and/or before treatment were strongly correlated with ALN (Table 4.2), these factors, henceforth, were determined not to be independent predictor variables in a multiple logistic regression analysis on renal scarring. ALN was shown to be the only independent risk factor for renal scarring ($P < 0.001$; Table 4.3)

The duration and route of administration of antibiotics have been shown not to influence the risk for renal scarring in patients with APN (Hoberman and Wald *et al*. 1999; Bouissou *et al*. 2008). Our previous prospective study (Cheng *et al*. 2006) suggested that 3 weeks of antibiotic therapy was the treatment of choice for all radiographically documented ALN patients; a longer duration of antibiotic use resulted in the successful treatment for ALN but did not reduce the risk for renal scarring. In most reported studies, including ours, the

outcome in terms of renal scarring seems to be unrelated to the mode and duration of antibiotic treatment.

Parameter	2-wk Treatment ALN Group	3-wk Treatment ALN Group	*P*
Patient number	54	55	
Male/Female	25/29	24/31	NS
Age (years)	2.62 ± 3.08 Median: 1.00 Range: (0.25, 15.00)	3.17 ± 3.01 Median: 1.33 Range: (0.07, 9.42)	NS
WBC count, cells/μl	21144 ± 7205	22014 ± 9608	NS
C-reactive protein, mg/L	156.1 ± 94.4	150.0 ± 85.1	NS
Vesicoureteral reflux	48.1% (25/52)	35.9% (19/53)	NS
E. coli percentage in urine culture[a]	95.1% (39/41)	85.7% (36/42)	NS
Fever duration before treatment, days	3.54 ± 2.23	4.07 ± 3.37	NS
Fever duration after treatment, days	3.19 ± 1.76	4.02 ± 2.81	NS
Time from ALN to DMSA renal scan, years	1.27 ± 1.02	1.40 ± 1.18	NS
Renal scar formation	88.9% (48/54)	89.1% (49/55)	NS

WBC, white blood cell; DMSA, dimercaptosuccinic acid; NS, not significant. [a] excluding urine cultures showing no growth.

Table 4.1. Demographic and clinical data for 109 patients with ALN (Cheng *et al*. 2010a).

Parameter	APN	ALN	*P*
Patient number	109	109	
Male/Female	49/60	49/60	NS
Age (years)	2.80 ± 3.59 Median: 1.00 Range: (0.16, 15.00)	2.90 ± 3.04 Median: 1.16 Range: (0.07, 15.00)	NS
WBC count, cells/μl	14729 ± 4656	21583 ± 8475	< 0.001
C-reactive protein, mg/L	53.4 ± 46.5	153.0 ± 89.5	< 0.001
Vesicoureteral reflux	34.6% (36/104)	41.9% (44/105)	NS
E. coli percentage in urine culture[a]	78.8% (82/104)	90.4% (75/83)	0.033
Fever duration before treatment, days	1.90 ± 1.62	3.81 ± 2.86	< 0.001
Fever duration after treatment, days	1.02 ± 0.75	3.61 ± 2.37	< 0.001
Time from APN or ALN to DMSA renal scan, years	1.21 ± 1.06	1.34 ± 1.10	NS
Renal scar formation	34.9% (38/109)	89.0% (97/109)	< 0.001

[a] excluding urine cultures showing no growth.

Table 4.2. Demographic and clinical data for patients with APN and patients with ALN (Cheng *et al*. 2010a).

Variable	aOR	95% CI		*P*
		Lower	Upper	
Disease				
APN	1.00	--	--	--
ALN	13.56	6.53	28.19	< 0.001
Gender				
Male	1.00	--	--	--
Female	0.95	0.47	1.93	NS
Age				
< 1 year	1.00	--	--	--
1-5 years	0.85	0.39	1.86	NS
> 5 years	0.91	0.35	2.33	NS
Vesicoureteral reflux (VUR)				
No VUR	1.00	--	--	--
VUR	1.83	0.90	3.74	0.096

Table 4.3. Multiple logistic regression analysis for scar formation (Cheng *et al*. 2010a).

The severity of APN as evaluated by the extent of renal lesions on acute DMSA scanning has been suggested to be a predictor for renal scarring (Biggi *et al*. 2001; Chiou *et al*. 2001). Our data on higher renal scar odds ratio in ALN, a more severe form of acute renal infection than APN, as well as in higher inflammatory indices and longer fever duration after and/or before treatment strongly support this suggestion.

The role of VUR in the development of renal scars remains controversial (Hoberman and Wald *et al*. 1999; Gordon *et al*. 2003; Hoberman *et al*. 2003; Moorthy *et al*. 2005). Some recent prospective studies (Hoberman *et al*. 2003; Chroustova *et al*. 2006; Polito *et al*. 2006; Bouissou *et al*. 2008) and a cross-sectional meta-analysis (Faust *et al*. 2009) have shown a significant association between the presence of VUR and the risk for renal scarring. However, the presence of VUR was a weak predictor of renal scarring in the present study. The additional effect of VUR above that achieved by including only the presence of ALN (nephromegaly and/or severity of infection) in the multiple logistic regression model was not statistically significant for predicting renal scarring.

In conclusion, our results showed a new finding that ALN is associated with a very high incidence of renal scarring, in comparison to APN, irrespective of the duration of antibiotic treatment.

5. Comparison of urovirulence factors and genotypes for bacteria causing acute lobar nephronia and acute pyelonephritis

Escherichia coli is the most common cause of various UTIs, including cystitis, prostatitis and pyelonephritis (Johnson and Kuskowski *et al*. 2005). Our early studies showed that *E. coli* was the most common pathogen cultured from the patients with ALN (Cheng *et al*. 2004, 2006), having a higher percentage of pathogens than the first-time UTIs (Hoberman and Wald 1999). This finding has led us to this investigation of the pathogenetic association of the bacterial virulence factors as well as the genotypes of the *E. coli* isolates in pediatric ALN.

Henceforth, we have sought to determine the role of *E. coli* urovirulence factors in the development of ALN as compared to APN in pediatric patients who have no underlying

diseases or urinary anatomical anomalies except vesicoureteral reflux (VUR) (Cheng *et al*., 2007). Through our previous published systematic diagnostic scheme (Cheng *et al*. 2004, 2006), patients who first presented as febrile UTIs and later were diagnosed with positive CT findings of ALN or positive technetium 99m-dimercaptosuccinic acid scintigraphic (^{99m}Tc-DMSA) findings of APN were enrolled into this study.

Patients were included for study only if *E. coli* was the sole isolate recovered from their urine specimens. Single colonies of the *E. coli* were randomly selected from the initial culture plate and stored in 20% glycerol at –70°C until used. Urovirulent factors examined included genes associated with various fimbrial and nonfimbrial adhesins (*pap*G I, *pap*G II, *pap*G III, *fimH*, *sfa*, *foc*, *afa*), aerobactin receptor (*iutA*), hemolysin (*hlyA*), and cytotoxic necrotizing factor I (*cnf1*) (Tseng *et al*. 2001, 2002; Johnson 2003; Johnson and Russo 2005). The difference in the prevalence of various *E. coli* urovirulent factors for the pediatric patients with ALN or APN was statistically analyzed. In addition, genotyping of these *E. coli* isolates was also performed to examine the possible clonal differences.

A total of 88 patients who fulfilled enrollment criteria were included for study. Among these, 46 patients were diagnosed with ALN and 42 cases with APN. Seventy-two patients, 42 from the ALN group and 30 from the APN group, underwent VCUG evaluation. Seventeen (40.5%) patients in the ALN group and 12 (40%) in the APN group had VUR. Among the patients with VUR, grade-IV reflux or greater was noted in 3 patients each with APN and ALN. Among patients who underwent VCUG, no difference in the presence of VUR in either frequency or severity was found between the two disease categories.

Among the 88 *E. coli* clinical isolates, *pap*G adhesin genes (including classes I to III) were detected in 44 of the ALN isolates and 32 of the APN ones (95.7% vs. 76.2%, $p<0.05$). The class II allele was more commonly noted in the group of ALN (95.7% vs. 73.9%, $p<0.05$; Table 5.1) (Cheng *et al*., 2007). In contrast, no significant difference was found for the class III allele between the two groups. None of the isolates had the class I allele. In addition, *pap*G II allele was noted in all ALN patients with normal VCUGs (25/25) while only in 16 of the 18 APN patients with normal VCUGs. The genetic determinant for type 1 fimbriae, *fimH*, was the most common virulence factor (95.5%) found among the isolates; however, no statistically significant difference between the two groups was noted. Similarly, the remaining genetic determinants for other virulence factors did not reveal any significant difference between the two groups. Multivariate logistic regression analysis revealed that *pap*G II allele was significantly associated with ALN ($p<0.005$; odds ratio, 17.16, 95% CI: 2.76-106.70). This association was independent of the presence of VUR.

To cause bacterial infections of the upper urinary tract, the microorganisms need to reach the kidney through ascending or hematogenous routes. Bacterial adherence to the uroepithelial cells by fimbrial or nonfimbrial adhesins is considered to be an important factor in the development of upper urinary tract infection via the ascending route (Tseng *et al*. 2001). Among these adhesins, *pap*G variants, which are located at the tip of P-fimbriae and bind preferentially to different Gal (α 1-4) Gal-containing glycolipids in the human epithelium of proximal and distal tubules and in collecting ducts, have been implicated to be associated with the severity of renal infection (Källenius *et al*. 1981; Johnson 1991; Wang *et al*. 2002; Johnson and Russo 2005). Previous studies have shown that the *pap*G II allele is associated with acute pyelonephritis (APN) (Johanson *et al*. 1993; Otto *et al*. 1993; Jantunen *et al*. 2000), while the *pap*G III allele predominates in less severe genitourinary infections, such as acute cystitis and prostatitis (Johanson *et al*. 1993; Johnson *et al*. 1998; Ruiz *et al*. 2002).

Virulence factor	ALN group (n=46)	APN group (n=42)	P
*pap*G (P-fimbriae)			
Class I	0 (0%)	0 (0%)	---
Class II	44 (95.7%)	31 (73.8%)	0.01
Class III	5 (10.9%)	4 (9.5%)	NS
fimH (type 1 fimbriae)	44 (95.7%)	40 (95.2%)	NS
sfa (S-fimbriae)	7 (15.2%)	4 (9.5%)	NS
foc (F1C-fimbriae)	5 (10.9%)	8 (19.1%)	NS
afa (afimbrial adhesins)	3 (6.5%)	4 (9.5%)	NS
iutA (aerobactin receptor)	35 (76.1%)	32 (76.2%)	NS
hlyA (hemolysin)	21 (45.7%)	23 (54.8%)	NS
Cnf1 (cytotoxic necrotizing factor 1)	8 (17.4%)	14 (33.3%)	NS

NS indicates not significant

Table 5.1. Comparison of virulence factors of *Escherichia coli* isolated from patients with ALN and APN (Cheng *et al*., 2007).

Results in this study (Cheng *et al.* 2007) also indicated that *pap*G II was significantly more prevalent in pediatric patients with ALN than those with APN. This finding provides further evidence that the *pap*G II allele might play a more important pathogenic role than other adhesins in the development of severe renal infectious diseases. In addition, this finding may offer an insight for the future development of vaccine against such severe renal parenchymal inflammatory diseases.

The *fimH* gene sequence which encodes the type I fimbriae was present uniformly in most of the isolates from either ALN or APN patients. This is in accordance with the fact that type I fimbriae is present in nearly all *E. coli* isolates from patients with various UTIs, ranging from cystitis, prostatitis to APN (Johnson and Stell 2000; Johnson and Russo 2005). This study further extends the proposed mechanism that *fimH* gene (i.e. type I fimbriae) was generally required for renal bacterial infection disease to be occurred, no matter what degree of severity it is. In contrast, other fimbrial and nonfimbrial adhesins (i.e. *sfa*, *foc*, and *afa* genes) were rarely detected among our isolates and their pathogenic roles in ALN and APN are likely of less importance, a finding similar to those reported previously in the less severe renal infection categories (Siitonen *et al*. 1993; Blanco *et al*. 1997; Mitsumori *et al*. 1998).

Host compromise can decrease the requirements for bacterial virulence in causing severe urinary tract infections, and, henceforth, change the distribution of *pap*G pathogenetic determinants among the clinical isolates studied (Jantunen *et al*. 2000; Tseng *et al*. 2001, 2002; Johnson and Russo 2005). In this study, the lack of influence of VUR, the only host compromising factor revealed, on the determination of urovirulence factors could be due to the similar distribution and severity of VUR between these two groups. Such similarities in VUR severity distribution and occurrence frequency were also reported in our earlier studies and many others (Kline *et al*. 1988; Sargent and Stringer 1995; Uehling *et al*. 2000; Cheng *et al*. 2004, 2006). In addition, this frequency of VUR among patients with ALN or APN (~ 40%) is close to that in children with UTI (Ilyas *et al*. 2002). Thus, VUR may not be a necessary prerequisite (i.e. significant predisposing host factor) for the development of ALN.

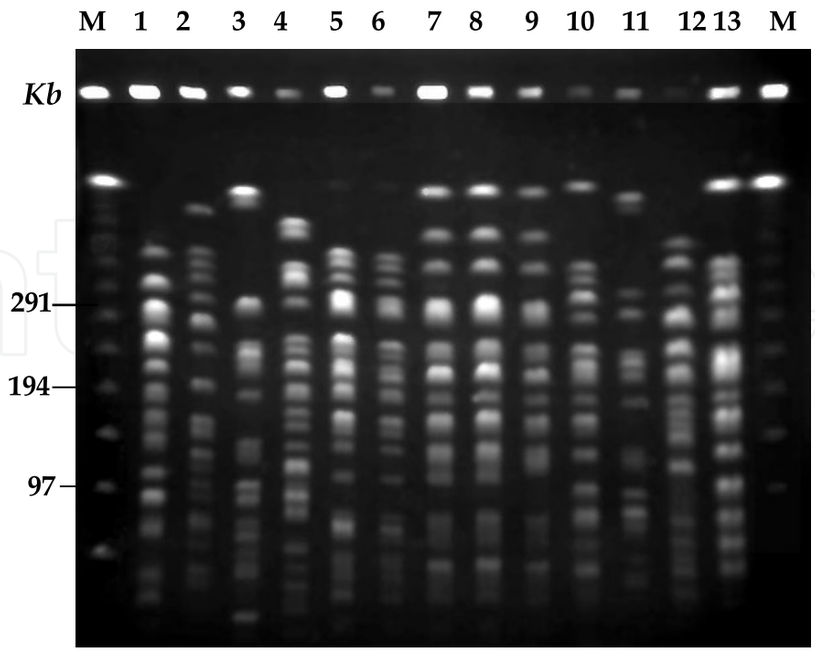


Fig. 5.1. PFGE pattern of representative *Xba*I-digested genomic DNA from *Escherichia coli* isolates. Lane M, lambda DNA concatemer standard; lanes 1-5, 7, and 8, clinical isolates in the APN group; lanes 6, 9-13, clinical isolates in the ALN patients. (Cheng *et al.*, 2007).

A total of 85 genotypes were found among the 88 *E. coli* isolates. Some representative banding patterns are shown in Figure 5.1 (Cheng *et al.*, 2007). These isolates from both ALN and APN patients demonstrated a variety of genotypes. A total of 85 genotypes contained multiple isolates, and 2 genotypes contained isolates from both ALN and APN groups, suggesting that isolates of the same clone could be associated with either entity.
In summary, PFGE analysis indicated that no major genotype was associated with the disease category among these 88 clinical *E. coli* isolates. As to the urovirulence factors examined, the *pap*G class II gene was the most strongly associated pathogenic determinant for the pediatric ALN patients who have no underlying diseases except VUR. Furthermore, even without VUR, patients can still develop clinical symptoms and radiographic characteristics associated with ALN in the presence of *pap*G II gene.

6. Comparison of bacterial urovirulence genotypes among patients with urosepsis, acute pyelonephritis, and acute lobar nephronia

Complex pathogen–host interactions determine the patient's susceptibility to bacterial infections (Rushton 1997; Ma and Shortliffe 2004). Various virulence factors have been identified that enhance *E. coli* uropathogenicity, including the facilitation of colonization and invasion of the host, avoidance or disruption of host defense mechanisms, injury to host tissue, and/or stimulation of a noxious host inflammatory responses (Rushton 1997; Johnson and Stell 2000). Furthermore, some virulence factors are more prevalent in specific urinary tract infectious diseases, thus offering insights into future vaccine development (Jantunen *et al*. 2000; Ruiz *et al*. 2002; Tseng *et al*. 2002).
We sought to further elucidate the roles of *E. coli* virulence factors in the development of urosepsis and two other severe renal parenchymal infectious diseases, APN and ALN, in

pediatric patients who show no host-compromising factors, except for vesicoureteral reflux (VUR) (Cheng *et al.,* 2010b). Twenty-five virulence factors were analyzed, including genes associated with fimbrial and nonfimbrial adhesins (*pap*AH, *pap*C, *pap*EF, *pap*G I, *pap*G II, *pap*G III, *sfaS*, *focG*, *afa*, *bmaE*, *gafD*, *nfaE*, *fimH*), toxins (*hlyA*, *cnf1*, *cdtB*), siderophores (*fyuA*, *iutA*), capsule synthesis (*kpsMT* II, *kpsMT* III), invasion of brain endothelium (*ibeA*), serum-resistance (*traT*), markers for virulence-associated *E. coli* serogroup O4 (*rfc*) and colcin V plasmids (*cvaC*), and the coding region of PAI from the uropathogenic strain CFT073 (PAI) (Johnson and Stell 2000; Jantunen *et al*. 2000; Tseng *et al*. 2002; Johnson and Kuskowski *et al*. 2005; Cheng *et al*. 2007). Moreover, the prevalence rates of these 25 urovirulence genes for patients with the three invasive UTIs (i.e. APN, ALN, and urosepsis) will also be compared with those for patients diagnosed as cystitis.

The inclusion criteria and diagnostic scheme for patients with documented episodes of ALN or APN were the same as those stated in our earlier publication (Cheng *et al*. 2007). Urosepsis was defined as a patient with bacteremia arising from a urinary tract source (Johnson *et al*. 1988). Cystitis was defined as afebrile pediatric patients with just only localizing symptoms such as dysuria, frequency, urgency, cloudy urine or lower abdominal discomfort. Exclusion criteria included any evidence of underlying diseases such as diabetes or immunodeficiency, or any structural anomalies such as neurogenic bladder, posterior urethral valve, urinary diversion, bladder diverticulum, ureterocele, and urinary tract obstruction apart from VUR.

Among the 123 *E. coli* isolates from APN, ALN and urosepsis, the overall prevalence rate of various virulence factors ranged from 2% (*nfaE*, nonfimbrial adhesin-1) to 97% (*fimH*, Type I fimbriae). In addition, all but one APN clinical isolate presented at least one adhesin. Of the 25 virulence factors examined, 17 showed a statistically significant distribution among these three invasive UTI categories (Table 6.1). ALN isolates differed significantly from other invasive UTI isolates (i.e. APN and urosepsis) due to their lower prevalence of *cdtB* and a medium prevalence of *cvaC*. Moreover, ALN isolates showed a higher prevalence of *pap*AH, *pap*C, *pap*EF, and *pap*G II, compared with APN isolates, and a lower prevalence of *pap*G I, *focG*, *afa*, *bmaE*, *hlyA*, *cnf1*, *iutA*, *kpsMT* III, *rfc*, and *traT* compared with urosepsis isolates. APN isolates were significantly different from other two types of invasive isolates due to a lower prevalence of *cvaC*. Additionally, APN isolates had a lower prevalence of *pap*G I, *focG*, *afa*, *bmaE*, *iutA*, *kpsMT* III, *rfc*, *traT,* and PAI compared with urosepsis isolates. Finally, urosepsis isolates significantly differed from all other two types of invasive UTI isolates due to a higher prevalence of *pap*G I, *focG*, *afa*, *bmaE*, *iutA*, *kpsMT* III, *rfc*, *cvaC,* and *traT* (Table 6.1).

In contrast, among the 24 clinical isolates from cystitis, eight virulence genes were not noted, and in which, six were bacterial adhesins (i.e. *pap*G I, *sfaS*, *afa*, *bmaE*, *gafD*, *nfaE*). However, the highest prevalence rate (100%) was noted in *fimH* adhesin, the virulence gene sequence encoding type I fimbriae. As compared to the combination of three invasive UTI diseases (i.e. APN, ALN and urosepsis), the cystitis isolates had a lower prevalence of *pap*AH, *pap*C, *pap*EF, *pap*G II, *sfaS*, *afa*, *bmaE*, *hlyA*, *cdtB*, *fyuA* and *ibeA* (Table 6.1).

In this investigation, none of the patients presented with any evidence of underlying disease or structural anomalies except VUR, and a similar distribution of severity and frequency of occurrence of VUR was noted among the three different invasive UTI disease groups; APN, ALN, and, urosepsis. Hence, distinct syndrome-specific differences in distribution for certain virulence factors, but conservation across syndromes for others, is likely related to differences in bacterial urovirulence and uropathogenicity among these three invasive bacterial urinary infectious diseases.

Virulence factor	Cystitis group (n = 24)	APN group (n = 45)	ALN group (n = 48)	Urosepsis group (n = 30)	P[b]			
					Cystitis vs. (APN+ ALN+ Urosepsis)	APN vs. ALN	ALN vs. Urosepsis	APN vs. Urosepsis
Adhesin								
*pap*AH (P-fimbriae)	8 (33%)	32 (71%)	43 (90%)	24 (80%)	<0.0001	0.0242	---	---
*pap*C (P-fimbriae)	6 (25%)	32 (71%)	45 (94%)	25 (83%)	<0.0001	0.0038	---	---
*pap*EF (P-fimbriae)	6 (25%)	34 (76%)	44 (92%)	26 (87%)	<0.0001	0.0348	---	---
*pap*G (P-fimbriae)								
Class I	0 (0%)	0 (0%)	0 (0%)	13 (43%)	---	---	<0.0001	<0.0001
Class II	4 (17%)	33 (73%)	44 (92%)	26 (87%)	<0.0001	0.0192	---	---
Class III	4 (17%)	4 (9%)	6 (13%)	6 (20%)	---	---	---	---
sfaS (S-fimbriae)	0 (0%)	6 (13%)	7 (15%)	9 (30%)	0.0251	---	---	---
focG (F1C-fimbriae)	6 (25%)	8 (18%)	5 (10%)	20 (67%)	---	---	<0.0001	<0.0001
afa (afimbrial adhesin)	0 (0%)	4 (9%)	4 (8%)	27 (90%)	0.0028	---	<0.0001	<0.0001
bmaE (M blood group antigen-specific M fimbriae)	0 (0%)	1 (2%)	1 (2%)	16 (53%)	0.0446	---	<0.0001	<0.0001
gafD (glucosaminyl-specific G fimbriae)	0 (0%)	2 (4%)	7 (15%)	5 (17%)	---	---	---	---
nfaE (nonfimbrial adhesion-1)	0 (0%)	2 (4%)	0 (0%)	0 (0%)	---	---	---	---
fimH (type 1 fimbriae)	24 (100%)	43 (96%)	46 (96%)	30 (100%)	---	---	---	---
Toxin								
hlyA (hemolysin)	4 (17%)	24 (53%)	22 (46%)	22 (73%)	0.0006	---	0.0172	---
Cnf1 (cytotoxic necrotizing factor 1)	4 (17%)	14 (31%)	9 (19%)	16 (53%)	---	---	0.0015	---
cdtB (cytolethal distending toxin)	0 (0%)	21 (47%)	4 (8%)	17 (57%)	0.0009	<0.0001	<0.0001	---

Virulence factor	Cystitis group (n = 24)	APN group (n = 45)	ALN group (n = 48)	Urosepsis group (n = 30)	P[b]			
					Cystitis vs. (APN+ ALN+ Urosepsis)	APN vs. ALN	ALN vs. Urosepsis	APN vs. Urosepsis
Siderophore								
fyuA (yersiniabactin receptor)	14 (58%)	41 (91%)	48 (100%)	29 (97%)	<0.0001	---	---	---
iutA (aerobactin receptor)	16 (67%)	35 (78%)	36 (75%)	30 (100%)	---	---	0.0024	0.0047
Miscellaneous								
kpsMT II (capsule synthesis, group II)	16 (67%)	38 (84%)	41 (87%)	24 (80%)	---	---	---	---
kpsMT III (capsule synthesis, group III)	0 (0%)	3 (7%)	3 (6%)	9 (30%)	---	---	0.0081	0.0101
rfc (marker for virulence-associated *E. coli* serogroup O4)	2 (8%)	6 (13%)	9 (19%)	16 (53%)	---	---	0.0015	0.0002
ibeA (invasion of brain endothelium gene)	14 (58%)	34 (77%)	41 (85%)	28 (93%)	0.0090	---	---	---
cvaC (marker for ColV, colcin V, plasmids)	4 (17%)	4 (9%)	13 (27%)	24 (80%)	---	0.0233	<0.0001	<0.0001
traT (serum-resistance associated gene)	16 (67%)	30 (67%)	39 (81%)	30(100%)	---	---	0.0108	0.0004
PAI (coding region of PAI from uropathogenic strain CFT073)	16 (67%)	31 (69%)	39 (81%)	28 (93%)	---	---	---	0.0114

[a] Data are presented as the number (%) of indicated urovirulence factors.

[b] The *P* values, as determined by χ^2 analysis or 2-sided Fisher's exact tests, as appropriate, are shown only when $P < 0.05$.

Table 6.1. Comparison between virulence factors among 147 *Escherichia coli* isolates from patients with cystitis, acute pyelonephritis (APN), acute lobar nephronia (ALN), or urosepsis. (Cheng *et al*., 2010b).

The *fimH*, the gene sequence that encodes type I fimbriae, was found in nearly all strains (97%) from these three invasive UTI diseases and did not vary statistically among these three syndromes. This finding further extends the proposed mechanism, namely, that the *fimH* gene is generally required for renal bacterial infectious disease to occur, regardless of level (Johnson and Stell 2000; Tseng *et al*. 2002; Johnson and Russo 2005; Moreno *et al*. 2005; Cheng *et al*. 2007).

An aggregate virulence factor score for each isolate was calculated as the number of unique virulence factors detected, with adjustment for multiple detection of *pap* (P-fimbriae) and *sfa/foc* (S/F1C fimbriae) (Johnson and Kuskowski *et al*. 2005). The median scores were 6.5 (range: 1-12), 9 (range: 2–12), 9 (range: 6–13), and 14 (range: 9–17) for cystitis, APN, ALN, and urosepsis isolates, respectively. A Kruskal-Wallis nonparametric one way ANOVA analysis indicated the aggregate virulence factor score was significantly different among these four disease groups ($p < 0.0001$). *Post hoc* analyses using Dunn method (2-sided) between any two disease categories indicated that urosepsis isolates presented significantly higher aggregate virulence scores than isolates from any other three diseases (urosepsis vs. cystitis, urosepsis vs. APN and urosepsis vs. ALN; $p < 0.0001$). Isolates from cystitis, rather, showed significantly lower aggregate virulence scores than those from any other three invasive UTI diseases (cystitis vs. APN and cystitis vs. ALN; $p < 0.01$; cystitis vs. urosepsis; $p < 0.0001$). However, no significant difference was noted between the APN and ALN isolates ($p = 0.88$).

In summary, for the three invasive urinary infectious diseases, distinct syndrome-specific differences in distribution for certain virulence factors, but conservation across syndromes for others is noted. This likely resulted from the differences in bacterial urovirulence and uropathogenicity. Our findings also suggested that urosepsis isolates carry more virulence factors and are therefore likely more urovirulent compared with cystitis, APN and ALN isolates.

7. Genetic polymorphisms and susceptibility for pediatric patients with parenchymal renal infections

Despite of aforementioned efforts on correlating urovirulence factors of uropathogenic *E. coli* with the disease severity, the intra-individual differences in clinical presentations are still noted among UTI patients. This underlies the importance of host factors, such as mechanistic dysfunctions like vesicoureteral reflux (VUR) and genetic variations, in patient's susceptibility to the bacterial invasion and infection (Artifoni *et al*. 2007; Lundstedt and Leijonhufvud *et al*. 2007; Lundstedt and McCarthy *et al*. 2007; Hawn, Scholes and Li *et al*. 2009; Sivick and Mobley 2010).

The inflammatory response caused by the attachment/invasion of uropathogenic *E. coli* into the urinary tract is determined by different molecular interactions between the bacteria and epithelial cells (Artifoni *et al*. 2007; Sivick and Mobley 2010). The initial recognition for bacterial attachment/invasion occurs through the coordination efforts of various toll-like receptors and different Pathogen-Associated Molecular Patterns (PAMPs) such as bacterial flagellin and lipopolysaccharide (Hawn, Scholes and Li *et al*. 2009). Following that, potent chemoattractants secreted by the infected epithelial cells will attract inflammatory cells, and the chemokine receptors will then direct recruited inflammatory cells' interactions with mucosal barrier. Subsequent steps in the inflammatory process will determine the balance

between the health and the disease severity (Godaly *et al*. 2001; Artifoni *et al*. 2007). Neutrophil-dependent innate host defense system is considered to be an important antimicrobial process to maintain the sterility of the urinary tract. It starts from signal transmission by cooperative efforts of toll-like receptor 4 (TLR-4) and P fimbriae of uropathogenic *E. coli*, followed by the secretion of main chemoattractant for neutrophils, IL-8. The IL-8 mediates its effects on neutrophil chemotaxis, transepithelial infiltration into the urinary tract, activation and phagocytosis and killing of bacteria through the receptors CXCR1 and CXCR2 (Godaly *et al*. 2001; Lundstedt and Leijonhufvud *et al*. 2007; Lundstedt and McCarthy *et al*. 2007).

Henceforth, we sought to determine the correlations in the polymorphisms for genes regulating the initial recognition of bacterial invasion (i.e. TLR-4, toll-like receptor 4) and subsequent neutrophil infiltration and activation for bacteria clearance (i.e. IL-8, interleukin-8; and CXCR1, CXCR2; receptors for interleukin-8) among the pediatric UTI patients with different clinical severity; namely, acute pyelonephritis (APN) and the clinically more severe UTI disease, acute lobar nephronia (ALN) (Cheng *et al*., 2011a). In addition, since VUR is a well-known risk factor for severe parenchymal infectious disease as APN (Orellana *et al*. 2004; Artifoni *et al*. 2007), a subgroup of APN and ALN patients without VUR will also be examined to exclude the possible effects caused by VUR.

Statistical analyses using log-additive model has revealed that only IL-8 (rs4073) showed significant difference in genotype frequency between the control group and APN, ALN or combined cases (Table 7.1) for APN vs. control; ALN vs. control and combined vs. control, respectively). In addition, the genotype AA in IL-8 (rs4073) was associated with the severe upper UTIs (i.e. APN and ALN) in comparison to the TT and TA genotypes (Table 7.1) for APN vs. control; ALN vs. control and combined vs. control, respectively). The allele frequency analyses have shown that the minor allele, "A", in IL-8 (rs4073) is more prevalent in the severe upper UTI groups than in the control for APN vs. control; ALN vs. control and combined vs. control, respectively (Table 7.2) (Cheng *et al.* 2011a).

Since vesicoureteral reflux (VUR) has been suggested as the significant host risk factor for upper UTIs (Orellana *et al*. 2004; Artifoni *et al*. 2007), we subsequently evaluated the results of genetic analysis in the subgroup of APN and ALN patients with no VUR. In the no-VUR subgroup of APN and ALN patients, only ALN and APN+ALN group presented a statistically significant difference in IL-8 (rs4073) genotype frequency using log-additive model (OR (95% CI): 1.47 (1.03, 2.10); 1.50 (1.09, 2.06) for ALN vs. control; and combined vs. control, respectively). In comparison to the TT and TA genotypes in IL-8 (rs4073) SNP, a significant higher AA genotype frequency was noted in the no-VUR subgroup of ALN and APN+ALN cases (recessive model, OR (95% CI): 2.31 (1.15, 4.65); 2.15 (1.13, 4.09) for ALN vs. control; and combined vs. control, respectively). These two no-VUR subgroups (i.e. ALN and APN+ALN) also presented a significant higher minor allele (i.e. "A" in IL-8 (rs4073)) frequency than in the control (OR (95%CI): 1.43 (1.02, 2.01); 1.45 (1.07, 1.96) for ALN vs. control and combined vs. control, respectively).

This investigation (Cheng *et al.* 2011a) has indicated that APN and ALN patients have distinctive higher AA genotype frequency and A allele occurrence in IL-8 (rs 4073) as compared to the controls. In contrast, no differences in TLR-4 (rs10759932), CXCR1 (rs16858808) and CXCR2 (rs4674258) were noted among the APN, ALN and control. The polymorphism for IL-8 (rs4073) occurs at -251A>T position in the 5' promoter region of IL-8.

SNP	Group	Genotype (%)			Log-additive Model		Dominant Model (01, 11 vs 00)		Recessive Model (11 vs 00, 01)	
		00	01	11						
		CC	CT	TT	OR (95% CI)	P^a	OR (95% CI)	P^a	OR (95% CI)	P^a
CXCR1 (rs16858808)	Control	214 (96.4)	8 (3.6)	0 (0)						
	APN	108 (95.6)	5 (4.4)	0 (0)	1.24 (0.40, 3.88)					
	ALN	156 (94.0)	9 (5.4)	1 (0.6)	1.79 (0.73, 4.37)	0.32	1.71 (0.66, 4.44)	0.27		0.43
	Combined	264 (94.6)	14 (5.0)	1 (0.4)	1.57 (0.68, 3.63)	0.58	1.52 (0.63, 3.65)	0.34		
		CC	CT	TT	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
CXCR2 (rs4674258)	Control	101 (45.7)	94 (42.5)	26 (11.8)						
	APN	50 (44.2)	50 (44.2)	13 (11.6)	1.03 (0.73, 1.43)	0.88	1.06 (0.67, 1.67)	0.80	0.98 (0.48, 1.98)	0.94
	ALN	80 (48.2)	72 (43.4)	14 (8.4)	0.88 (0.64, 1.19)	0.39	0.90 (0.60, 1.35)	0.63	0.69 (0.35, 1.37)	0.28
	Combined	130 (46.6)	122 (43.7)	27 (9.7)	0.93 (0.72, 1.22)	0.62	0.96 (0.68, 1.37)	0.84	0.80 (0.45, 1.42)	0.45
		TT	TA	AA	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
IL-8 (rs4073)	Control	94 (42.9)	107 (48.9)	18 (8.2)						
	APN	40 (35.4)	54 (47.8)	19 (16.8)	1.45 (1.03, 2.06)	**0.03**	1.37 (0.86, 2.19)	0.18	2.26 (1.13, 4.50)	**0.02**
	ALN	57 (34.3)	81 (48.8)	28 (16.9)	1.49 (1.09, 2.02)	**0.01**	1.44 (0.95, 2.18)	0.09	2.27 (1.21, 4.26)	**0.01**
	Combined	97 (34.8)	135 (48.4)	47 (16.8)	1.46 (1.12, 1.91)	**0.01**	1.41 (0.98, 2.03)	0.06	2.26 (1.27, 4.02)	**0.01**

SNP	Group	Genotype (%)			Log-additive Model		Dominant Model (01, 11 vs 00)		Recessive Model (11 vs 00, 01)	
		00	01	11						
		CC	CT	TT	OR (95% CI)	P^a	OR (95% CI)	P^a	OR (95% CI)	P^a
		TT	TC	CC	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
TLR-4 (rs10759932)	Control	118 (53.6)	86 (39.1)	16 (7.3)						
	APN	65 (58.6)	39 (35.1)	7 (6.3)	0.86 (0.59, 1.24)	0.42	0.82 (0.52, 1.30)	0.40	0.86 (0.34, 2.15)	0.74
	ALN	87 (52.7)	68 (41.2)	10 (6.1)	0.99 (0.72, 1.37)	0.96	1.04 (0.69, 1.55)	0.86	0.82 (0.36, 1.86)	0.64
	Combined	152 (55.1)	107 (38.8)	17 (6.1)	0.94 (0.70, 1.25)	0.65	0.94 (0.66, 1.35)	0.75	0.84 (0.41, 1.70)	0.62

[a] The P values are shown in bold when $P < 0.05$.

[b] Combined: APN+ALN

Table 7.1. Genotypic analysis of single nucleotide polymorphisms (SNPs) (Cheng et al., 2011a).

Hence, current finding is in parallel to an earlier report in which A allele in -251A>T is significantly associated with the presence of dimercapto-succinic acid scan documented APN (Artifoni *et al*. 2007). This has been attributed to the association of A allele with an increase in IL-8 production (Hull *et al*. 2001; Artifoni *et al*. 2007). In addition, AA genotype has been linked to the increased level of fecal IL-8 and the occurrence of enteroaggregative *E. coli*-associated diarrhea (Jiang *et al*. 2003). Therefore, the IL-8 (rs4073) SNP for APN and ALN patients could be related to the up-regulated IL-8 expression that has subsequently resulted in severe clinical inflammatory responses noted clinically. Furthermore, after elimination of VUR, the well-known risk factor for severe UTIs, from analyses, only ALN patients presented SNP in IL-8 (rs4073) while APN did not. Since the inflammatory responses in ALN patients are more sever than in APN ones (e.g. higher CRP value and longer fever duration after antibiotic treatment), this finding further supports the role of polymorphism in IL-8 (rs4073, -251A>T) in IL-8 up-regulation.

In summary, the SNP in the inflammatory chemokine IL-8, a higher frequency in AA genotype and A allele, is involved in the susceptibility and clinical responses in pediatric APN and ALN cases. In addition, after removing VUR, the significant risk factor for parenchymal infection, from statistical analysis, the IL-8 SNP is only noted in the no-VUR subgroup of clinically more severe ALN patients. This suggests IL-8 (rs4073) SNP is correlated to the clinical severity of parenchymal infection, likely due to the up-regulated IL-8 expression by the AA genotype and A allele.

SNP	Minor allele frequency[a] (%)				APN vs. Control		ALN vs. Control		Combined vs. Control	
	Control	APN	ALN	Combined[b]	OR (95% CI)	*P*[c]	OR (95% CI)	*P*[c]	OR (95% CI)	*P*[c]
CXCR1 (rs16858808)	1.80	2.21	3.31	2.87	1.23 (0.40, 3.81)	0.72	1.87 (0.74, 4.70)	0.18	1.61 (0.68, 3.79)	0.28
CXCR2 (rs4674258)	33.03	33.63	30.12	31.54	1.03 (0.73, 1.44)	0.88	0.87 (0.64, 1.19)	0.39	0.93 (0.72, 1.22)	0.62
IL-8 (rs4073)	32.65	40.71	41.27	41.04	1.42 (1.02, 1.97)	**0.04**	1.45 (1.08, 1.95)	**0.01**	1.44 (1.11, 1.86)	**0.01**
TLR-4 (rs10759932)	26.82	23.87	26.67	25.54	0.86 (0.59, 1.24)	0.41	0.99 (0.72, 1.37)	0.96	0.94 (0.70, 1.24)	0.65

[a] Minor allele: CXCR1, T; CXCR2, T; IL-8, A; TLR-4, C.

[b] Combined: APN+ALN

[c] The *P* values are shown in bold when $P < 0.05$.

Table 7.2. Allele frequency analysis of single nucleotide polymorphisms (SNPs) by logistic regression model. (Cheng et al., 2011a).

8. Conclusion

A new imaging scheme that combines US and CT has been developed for effective ALN diagnosis. In this scheme, patients suspected of suffering from UTI [i.e. who had pyuria (> 5

WBCs/high-power field), fever without focus or any symptoms/signs related to UTI, such as knocking pain, dysuria and frequency] underwent renal US during the 1st-2nd day following their admission to hospital. The CT assessment followed immediately when the initial US findings met either one of these two criteria, evidence of: (1) unilateral or bilateral nephromegaly; and (2) a focal renal mass. For children who presented with borderline nephromegaly ultrasonographically, CT was performed when the patient remained febrile for 72 hours subsequent to antibiotic-treatment commencement. ALN diagnosis was made on the basis of positive CT findings.

Further, with this scheme, we have identified that a three-week antimicrobial therapy protocol, rather than the two-week scheme commonly used for APN treatment, should constitute the treatment of choice for all radiographically documented ALN patients. As for the likelihood in scar formation following the severe parenchymal infections, we have confirmed that pediatric patients with ALN could have higher possibility for scar formation than with APN.

Through the urovirulence factor analyses, we have noted that the *pap*G class II gene (gene associated with P-fimbriae of uropathogenic *E. coli*) was the most strongly associated pathogenic determinant for the pediatric ALN patients who have no underlying diseases except VUR. But PFGE analysis indicated that no major genotype was associated with the disease category. Thus the major pathogenic determinants may not be unique to any specific genetic lineage. In addition, using the MDCK epithelial cells model, we have confirmed that the ability to adhere to and produce cytotoxicity against uroepithelial cells appears a prerequisite factor for *E. coli* to cause more severe bacterial kidney infection, such as ALN (Cheng *et al.*, 2011b). Much more, we have confirmed that *E. coli* isolates from urosepsis patients carried more virulence factors as compared to those from patients with cystitis, APN and ALN. This implicated that the number of urovirulent genes found in pathogenic isolates may be correlated with the clinical severity noted in pediatric UTIs.

For the host factors that could influence the patient's susceptibility to the sever parenchymal infections, we have identified SNP in the inflammatory chemokine IL-8, a higher frequency in AA genotype and A allele, is involved in the susceptibility and clinical responses in pediatric APN and ALN cases. Further, among the patients without VUR, this IL-8 SNP is only noted in the ALN patients while not in the APN cases. This finding implicates that this IL-8 SNP could lead to a higher IL-8 secretion level after bacterial infection, and, subsequently, more severe inflammatory responses found in the ALN patients.

Despite of abovementioned findings in the pediatric patients with ALN, we have not explored the host genetic factors for the ALN patient proned to have recurrent UTIs. These patients might have some genetic polymorphisms that increase patient's susceptibility to UTIs. A detailed longitudinal clinical follow-up plan would be needed to further elucidate the likely polymorphisms in these recurrent-UTI-prone ALN patients. In addition, building a proper animal model for ALN study will be attempted in the future study. The IL-8 expression level among the patients diagnosed as cystitis, APN and ALN will also be compared to each other. Since the ALN patients are also likely to have renal scarring according to our previous findings, genetic polymorphisms likely to reduce the renal scars will also be evaluated in the future studies.

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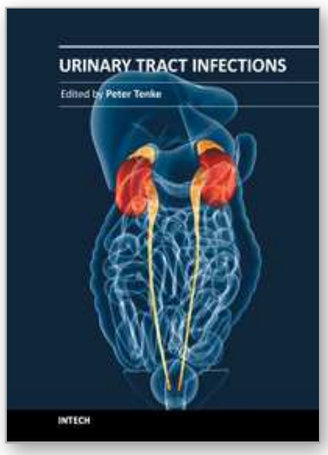

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Urinary tract infections (UTIs) are among the most common bacterial infections worldwide, and they are also the leading cause of hospital-acquired infections. Therefore, the appropriate management of UTIs is a major medical and financial issue. This book covers different clinical manifestations of UTI, with special emphasis on some hard-to-treat diseases, and special conditions in respect of treatment; antibiotic resistance and the available alternative strategies for the prevention and treatment of UTIs and it deals with urinary tract infections in children. The aim of this book is to give a summary about the different aspects of the diagnosis, management and prevention of urinary tract infections for all medical disciplines.

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