CONCISE COMMUNICATION

Predominance of Class II papG Allele of Escherichia coli in Pyelonephritis in Infants with Normal Urinary Tract Anatomy

M. E. Jantunen,1,2 A. Siitonen,2 O. Koskimies,1 S. Wikström,1 UM. Kärkkäinen,3 E. Salo,1 and H. Saxén1

1Hospital for Children and Adolescents, University of Helsinki, and 2Laboratory of Enteric Pathogens, National Public Health Institute, Helsinki, and 3Department of Clinical Microbiology, Kuopio University Hospital, Kuopio, Finland

P-fimbrial genotypes of Escherichia coli strains and their possible association with urinary tract abnormalities were studied in infants with pyelonephritis. A total of 153 urinary E. coli strains were analyzed by polymerase chain reaction for class I, II, and III alleles of the pyelonephritis-associated adhesin gene papG. Strains with any class II papG alleles were found significantly more often in infants with normal anatomy and function or in infants with clinically insignificant abnormalities than they were in infants with significant abnormalities (90 of 119 vs. 14 of 34 infants; P < .001). On the other hand, strains without any papG alleles were found significantly more often in infants with major urinary tract abnormalities (11 of 34 vs. 17 of 119 infants; P = .016). Our genotypic findings indicate that, especially in infants with a normal urinary tract, infection is caused by more-virulent E. coli than is present in infants without a normal urinary tract. This virulence could be due to expression of pyelonephritogenic P fimbriae by an infecting E. coli strain.

Urinary Escherichia coli isolates from children and adults with nonobstructive pyelonephritis (PN) have been shown to have a predominantly P-fimbriated phenotype [1, 2]. Specific PapG adhesins on the tips of P fimbriae—that is, adhesins that are coded by papG genes—are major virulence factors of these uropathogenic E. coli, and they promote bacterial binding to uroepithelial cells. PapG adhesins can be divided into 3 classes (I, II, and III), each of which recognizes a specific receptor on uroepithelial cells [3].

Novel single-tube polymerase chain reaction (PCR)-based methods have provided the opportunity to study conveniently the prevalence of P-fimbrial adhesin genotypes in E. coli isolates [4, 5]. The class II papG adhesin gene has been shown to be predominant in those E. coli strains that cause PN [6, 7] and the class III papG adhesin gene in those that cause cystitis [7]. Although several studies have shown that E. coli-expressing P fimbriae are found frequently in urinary tract infections (UTIs) in both adults and children, no genotypic analysis of isolates from infants with urinary tract abnormalities has been published.

Patients and Methods

Study population. An open, prospective, multicenter follow-up study was done in 5 hospitals in Finland between April 1995 and August 1999. The study population comprised 214 children. The inclusion criteria included a positive urine culture (growth of ≥105 bacteria/mL in 2 sterile-bag specimens or any growth in suprapubic bladder aspirate) and 2 of 3 of the following criteria: pyuria (leukocyte count >10/mm3), fever ≥38.0°C, or a C-reactive protein (CRP) level of >25 mg/L. In addition, eligible children were 1–24 months of age, had received no oral or intravenous antibiotic treatment within the preceding 2 weeks, had no previously known congenital anomalies of the urinary tract, and had no central nervous system-associated anomalies. Thirty-four of the recruited children were later excluded from the analysis for the following reasons: negative urine culture (n = 7); age of <1 month or >24 months (n = 9); or receipt of oral or intravenous antibiotic treatment within 2 weeks prior to hospitalization (n = 3). Fifteen children were excluded because they failed to meet more than 2 of the above-mentioned inclusion criteria (pyuria, fever, and CRP).

Radiological imaging studies. Renal ultrasound was performed on all children. In addition, children underwent either a voiding cystourethrography (VCU; 178 of 180 patients) or a radioisotope-voiding cystourethrography (2 of 180 patients) to determine possible vesicoureteral reflex (VUR). When indicated, additional imaging studies, such as intravenous urogram (IVU; 41 of 180 patients), renal scanning with technetium-labeled 2,3-dimercapto-
succinic acid (36 of 180 patients), radioisotopic renography using technetium-labeled diethylenetriaminepentaacetic acid (11 of 180 patients), or a urodynamic study (17 of 180 patients), were performed.

All VUCs and IVUs initially were performed and the results analyzed by the local radiologist at each center and later by an experienced radiologist (in a blinded manner) at the Hospital for Children and Adolescents (University of Helsinki). For unclear cases, another pediatric radiologist was consulted. Abnormal radiological findings also were evaluated by a pediatric urologist (S.W.), who interpreted the urodynamic studies.

Urinary tract findings were divided into 4 categories: normal anatomy, bladder dysfunction, unilateral or bilateral VUR, and obstructive anomalies. Children with VUR were further divided into 2 groups on the basis of the more severely affected side: either the group with nondilating VUR (grades I and II) or the group with dilating VUR (grades III–V) [8]. Finally, the children were classified into 2 groups according to the clinical significance of the findings. Group A comprised children with normal anatomy or minor, “borderline” urinary tract abnormalities. Group B included children with significant urinary tract abnormalities that caused changes in structure and function.

Processing of urinary samples. All urine samples were cultured before the initiation of therapy. Positive cultures were sent to the National Public Health Institute (Helsinki), where they were identified using standard methods [9] and were stored in skim milk at −70°C for further analysis.

PCR assay. Bacteria were cultured on cystine-lactose electrolyte-deficient (Oxoid, Basingstone, UK) agar plates. A loopful of an overnight pure culture of *E. coli* was suspended in Tris-EDTA buffer and was boiled for 10 min. One microliter of this lysate was used for allele-specific single-tube PCR detection of *papG*, as described elsewhere [5].

Statistical methods. Pearson’s χ² test and Fisher’s exact test were used. A *P* value of <.05 was considered significant.

Results

The study group comprised 107 girls and 73 boys, with median ages of 7.1 months (range, 1.0–23.8) and 3.4 months (range, 1.0–20.8), respectively. Radiological examinations revealed normal anatomy of the urinary tract in 102 (57%) of the 180 patients, VUR grades I and II in 30 (17%) of the 180 patients, and VUR grades III–V in 40 (22%) of the 180 patients. The following obstructive anomalies were found in 6 (3%) of the 180 patients: ureteropelvic junction (UPJ) obstruction (n = 3); posterior urethral valves (n = 1); UPJ obstruction with bilateral VUR grade III (n = 1); and UPJ obstruction with ipsilateral VUR grade II (n = 1). Detrusor overactivity was the only abnormal finding in 2 (1%) of the 180 patients.

*E. coli* was found in 171 (95%) of the urinary samples. Of the 171 *E. coli* strains, 153 (89%) *papG* alleles were available for PCR, and only these were included in the statistical analysis. The remaining 18 strains were lost. Class II *papG* alleles were significantly more common in isolates from group A (90 [76%] of 119 patients) than in isolates from group B (14 [41%] of 34 patients; *P* < .001), whereas isolates with no genes coding for P fimbriae were significantly more common in group B (11 [32%] of 34 patients) than in group A (17 [14%] of 119 patients; *P* = .016) (table 1). There also was a significant difference between groups A and B in terms of the frequency of class III *papG* alleles only (10 [8%] of 119 patients vs. 9 [26%] of 34 patients; *P* = .015).

Discussion

The present study, which used genotyping of P-fimbrial adhesins of *E. coli*, showed for the first time that class II *papG* alleles were associated strongly with normal urinary tract anatomy and with clinically insignificant urinary tract abnormalities in infants with PN. This finding supports the earlier phenotype reports on the association of P fimbriae with nonobstructive PN [1] in children, which suggests that efficient P fimbriae-mediated adhesion is necessary for vigorous colonization of the urinary tract in these infants.

Our finding that *E. coli* strains lacking P-fimbrial genes were found more frequently in infants with clinically significant urinary tract abnormalities is in agreement with earlier studies of adult and pediatric phenotypes [10, 11]. This similarity indicates that *E. coli* strains with no *papG* adhesin genes (and, thus, those without corresponding gene products) are less virulent and are able to cause infections only in a certain group of infants, as has been suggested to be the case in adults [12]. In our study, 17 (14%) of 119 samples of *E. coli* isolated from infants with normal anatomy lacked *papG* alleles. This could be explained by the fact that VUR is not a steady-state phenomenon [13]. Thus, the radiographic imaging performed 4–6 weeks after the initial infection does not necessarily describe the situation during the acute phase of the infection. On the other hand, one could speculate about whether the adhesin of the pathogen is an ultimate requirement for UTI in a host with a normal urinary tract.

Our finding of a high frequency of class III *papG* alleles only among infants with complicating factors (such as significant
urinary tract abnormalities) is of interest. In earlier studies this allele was shown to be associated with cystitis in children [7] and with urosepsis in compromised adults [14]. We are currently investigating the possible association between class II papG and class III papG alleles and urosepsis.

At present, invasive, laborious, and costly routine diagnostic imaging studies are performed on all children who are experiencing their first UTI. However, to date no study has shown the effectiveness of these interventions in reducing renal sequelae [15]. Therefore, new approaches are needed. Genotypic studies of bacterial isolates (such as our study) in infants and also in older children could be helpful in determining which children are at risk for urinary tract abnormalities. Thus, the detection of the absence of papG gene(s) could be useful for the clinician: radiological resources and antimicrobial prevention could be focused on children who display a higher likelihood of experiencing anatomical abnormalities (i.e., on those children whose UTIs are caused by papG-negative strains). Although, according to our study, papG-positive strains seem to have an 82% negative predictive value for the presence of significant urinary tract abnormalities, this finding does not justify the elimination of reflex imaging studies. On the other hand, the recognition of the association between the presence or absence of papG genes and radiographic urinary tract findings is of great importance when adhesin-based vaccine trials against UTI are being designed and when children are being recruited for these trials.

Acknowledgments

We thank Anna Föhr and Eino Marttinen for their evaluation of the radiographs; Liisa Immonen, Tarja Heiskanen, Aino Kyyhkyinen, and Ritva Taipalinen for excellent technical assistance; and Virva Jäntti for statistical advice. We also thank the staff members in the participating hospitals: Aurora Hospital (Helsinki); Hospital for Children and Adolescents, University of Helsinki (Helsinki); Jorvi Hospital (Espoo, Finland); Päijät-Häme Central Hospital (Lahti, Finland); and the Department of Pediatrics at Tampere University Hospital (Tampere, Finland).

References