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Escherichia coli FimH adhesins act synergistically with PapGII adhesins for enhancing establishment and maintenance of kidney infection

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Abstract *Background:* FimH adhesin is proposed to enhance *Escherichia coli* kidney infection by acting with PapGII adhesin, but genetic epidemiology study and animal study have not been widely conducted to confirm this hypothesis.
Methods: We compared the prevalence of adhesin gene and their coexistent pattern between upper and lower urinary tract infection (UTI) strains. *fimH* mutant (EC114FM), *papG*II mutant (EC114PM) and *fimH*/*papG*II double mutant (EC114DM) were constructed from a pylonephritogenic strain (EC114). We compared among these strains for the infection ability in bladders and kidneys of female BALB/c mice challenged transurethrally with these bacteria and assessed 1, 3, and 7 days after inoculation.
Results: Strains carrying *fimH*-only genotype were significantly more prevalent in lower UTI ($P < 0.001$). Strains carrying the *fimH*/*papG*II, but not *papG*II-only, were significantly associated with upper UTI ($P = 0.001$). Incidence of kidney infection increased after inoculation with

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EC114 on days 1 and 3, at both low and high dose, as compared with EC114DM; and the effect was greater than the sum of individual effect of EC114PM and EC114FM. Geometric means of quantitative bacterial counts in the kidneys significantly decreased when challenged with EC114FM on days 3 and 7, EC114PM on day 3 and EC114DM on day 1 after inoculation at high dose, as compared with EC114 (all $P < 0.05$).

Conclusions: We confirmed the advantage and synergistic action of FimH and PapGII for *E. coli* kidney infection and concluded that antagonists against FimH and PapGII adhesin may prevent kidney infection and enable its management.

Introduction

When uropathogenic *Escherichia coli* (UPEC) infect the urinary tract, they face numerous dynamic challenges, such as the shear stress of urine flow. For successful colonization in this hydrodynamically challenging environment, UPEC adherence to the epithelium by fimbrial adhesins is essential. Among the various adhesins of *E. coli*, the class II P fimbrial adhesin PapGII is strongly associated with renal infection in humans,[1–3] whereas the type 1 fimbrial adhesin FimH plays a vital role in establishing bladder infection in the mouse model of urinary tract infection (UTI).[4–6] However, the pathogenetic role of FimH in kidney infection remains unclear.

Previous studies have proposed that type 1 fimbriae are essential for colonization of the bladder epithelium, and that P fimbriae are employed when bacteria ascend to establish kidney infection.[7] A live animal model of infection achieved by infusing bacteria into renal proximal tubules demonstrated that P fimbriae facilitated colonization of the tubular epithelium, whereas type 1 fimbriae mediated colonization of the center of tubules.[8] Therefore, *E. coli* carrying both *fimH* and *papG*II adhesin genes may have a higher potential to cause kidney infection than those carrying either gene alone, but no genetic epidemiological study has investigated this yet.

In this study, we compared the prevalence of UPEC carrying a single adhesin gene (denoted as *fimH*$^+$-only, *papG*II$^+$-only, or *foc*$^+$-only) or coexistent adhesin gene (such as *fimH*$^+$*papG*II$^+$ or *foc*$^+$*papG*II$^+$) between cystitis and pyelonephritis patients. We previously demonstrated that a mutation of *papG*II (*papG*II$^-$*fimH*$^+$) in a pyelonephritogenic, *fimH*$^+$*papG*II$^+$ *E. coli* attenuates the virulence of kidney infection caused by the mutant strain.[9] In the present study, we further constructed a *fimH*$^-$(*papG*II$^+$) and a *fimH*$^-$*papG*II$^-$ isogenic mutant from the aforementioned *fimH*$^+$*papG*II$^+$ strain and compared the UTI virulence among these mutant and parent strains in a mouse model of ascending UTI. We analyzed the bacterial counts in the mouse bladder and kidney to assess the function of FimH and/or PapGII adhesin for strains with a *fimH*$^+$*papG*II$^+$ genotype to determine the pathogenic role of cooperative action of FimH and PapGII adhesins in the establishment and maintenance of kidney infection.

Methods

Ethical statement

The Institutional Review Board of National Cheng Kung University Hospital approved the study protocol (No.: A-ER-106-241). All animal study procedures were reviewed and approved by the Institutional Animal Care and Use Committee (No.: 103183) of National Cheng Kung University.

Bacterial isolates and patient selection

For this study, 216 adult patients were recruited from NCKUH who had a definite diagnosis of asymptomatic bacteriuria (ABU; n = 63), lower UTI (n = 57), or upper UTI (n = 96) caused by *E. coli* and for whom a sufficiently detailed medical record and the causative *E. coli* strain were available.

ABU was defined as bacterial colonization of urine without clinical symptoms. The diagnostic criteria for upper UTI were fever of 38.3 °C or greater, flank pain and/or tenderness at the costovertebral angle with or without disturbed voiding. The diagnostic criteria for lower UTI were urinary frequency, dysuria, and/or lower abdominal pain without flank pain or tenderness over the costovertebral angle, and temperature below 38.3 °C. The exclusion criteria were mixed UTI, age less than 18 years and hematogenous infection.

Detection of *E. coli fimH*, *papG*II, and other fimbrial genes

The fimbrial genes of *E. coli* were detected using polymerase chain reaction (PCR). The primer pairs for detection of *fimH*, *papG*I-III, *sfa* (S fimbriae), *foc* (F1C fimbriae) and *afa* (afimbrial adhesins) genes and the amplification procedure are described elsewhere.[2] The PCR results were duplicated and the nucleotide sequence of PCR products was determined using an automatic DNA sequencing system (3100 Genetic Analyzer; ABI Prism, Foster, CA, USA) to confirm that amplification products truly represented the expected sequences.

Construction of mutants, genetic analyses and testing for adhesin phenotypes

We selected *E. coli* EC114, which was isolated from one of our pyelonephritis patients, for construction of the *fimH* mutant. EC114 carries the FimH and PapGII adhesins genotypically and phenotypically but does not harbor most of the other virulence factors (Supplemental Table 1).[9] We used EC114 to construct a *fimH*-deficient (*fimH*$^{-}$*papG*II^{+}) mutant (designated as EC114FM). The previously constructed *papG*II-deficient mutant (EC114PM)[9] was used in this study for construction of a *fimH*-deficient and *papG*II-deficient (*fimH*$^{-}$*papG*II^{-}) double mutant (EC114DM). PCR primers, *fimH* 17f, 5′-ccctgtttgctgtactgctg-3′; *fimH* 883r, 5′-acgccaataatcgattgcac-3′, complementary to the sequence immediate to the 5′ and 3′ ends of the previously sequenced *fimH* (accession number X05672)[10] were used for amplification of *fimH*. The PCR products *fimH* 867 encompassed almost the entire *fimH* gene (867 of 903 bp). A 1.4-kb kanamycin cassette (*Afe*I fragment from plasmid pACYC177; NEB, Beverly, MA, USA) was ligated into the *fimH* 867 to construct an insertion mutation fragment which was then ligated into the pCVD442[11] to create pMW479. The detailed procedure of mating, allelic exchange, and selection of insertion mutants is described previously.[9] In brief, plasmid pMW479 was transformed into EC114 with selection for ampicillin resistance. Colonies were picked and plated onto sodium chloride-free LB agar with and without 5% (w/v) sucrose. Sucrose-resistant colonies were picked and tested for ampicillin sensitivity, indicating the loss of suicide vector sequences. Such colonies were tested for the insertion mutation of the *fimH* through Southern hybridization. Chromosomal DNA of sucrose-resistant and ampicillin sensitive colonies was digested with the restriction enzyme *Hin*CII or *Eco*RV for Southern hybridization. PCR products of the 867-bp DNA fragment from the EC114 *fimH* gene were used as DNA probes. The probes contained the *fimH Xcm*I site where the kanamycin cassette was inserted.

The hemagglutination assay used for the evaluation of PapGII and FimH adhesins is described previously.[12] PapGII adhesin expression is indicated by mannose-resistant hemagglutination to human A1P1 erythrocytes, and FimH adhesin expression is indicated by mannose-sensitive hemagglutination to guinea pig erythrocytes.

Mouse model of ascending UTI

The mouse model of ascending UTI was based on previous studies, with modification.[9,13,14] Ten mice in each group and a total of 240 female BALB/c mice aged 6—8 weeks were used. After disinfection of the peritoneum, a polyethylene catheter (internal diameter: 0.28 mm, outer diameter: 0.61 mm, approximately 25-mm-long, PE10, Intramedic, Becton—Dickinson, Sparks, MD, USA) was inserted through the urethral orifice into the mouse bladder. Subsequently, 50 μL of bacterial suspension, containing 5×10^4 or 5×10^8 CFU of the parental or mutant *E. coli* bacteria was inoculated via the catheter. Mice were sacrificed 1, 3, or 7 days after the bacterial challenge. Upon killing, both the left kidney and bladder were removed and homogenized aseptically for quantitative culture.

Statistics

Proportions were compared using the Pearson chi-square or Fisher's exact tests as appropriate. Quantitative culture results were logarithmically transformed for statistical analyses. Mean numbers of Log10 CFU per bladder or kidney from the culture of tissue homogenates were compared among groups using the ANOVA with post hoc tests. All statistical analyses were performed using SPSS software (SAS Institute Inc., Cary, NC, USA). $P < 0.05$ was considered significant.

Results

Prevalence of fimbrial genes among different sources of *E. coli* strains

Totally 216 *E. coli* strains were analyzed (63 ABU strains, 57 lower UTI strains and 96 upper UTI strains). *fimH* was prevalent in most (95%) UPEC strains and was also found in most (95%) ABU strains (Table 1). By contrast, *papG*II and *foc* were more prevalent in UTI strains than in ABU strains ($P < 0.001$, $P = 0.003$, respectively). The other fimbrial genes (*papG*I, *papG*III, *afa* and *sfa*) were rare or were not associated with UTI strains.

Comparison of the coexistent genotypes of *fimH*, *papG*II, and *foc* genes

Based on the results in Table 1, we selected *fimH*, *papG*II, and *foc* genes for further comparisons of the coexistent genotypes between upper and lower UTI strains (Table 2). UPEC strains carrying only *fimH* were less prevalent in upper UTI ($P < 0.001$). UPEC strains carrying both *fimH* and *papG*II genes (*fimH*$^{+}$*papG*II^{+} genotype) were significantly associated with upper UTI ($P = 0.001$). Among the upper UTI strains, 22 strains (23%) carried *fimH*-only genotype but

Table 1 Comparison of the prevalence of fimbrial genes in *E. coli* strains isolated from different hosts.

Fimbrial genes	ABU (n = 63)	UTI (n = 153)
fimH	60 (95)	146 (95)
*papG*I	0 (0)	0 (0)
*papG*II	9 (14)[a]	84 (55)[a]
*papG*III	8 (13)[b]	6 (4)[b]
foc	0 (0)[c]	20 (13)[c]
afa	12 (19)[d]	2 (1)[d]
sfa	5 (8)	7 (5)

Data are presented as numbers (percentages).
ABU: asymptomatic bacteriuria; UTI: urinary tract infection.
[a]$P < 0.001$; [b]$P = 0.03$; [c]$P = 0.003$; [d]$P < 0.001$.

Table 2 Comparison of the coexisting genotypes of *fimH*, *papG*II, and *foc* genes between *E. coli* strains isolated from upper and lower UTI patients.

Gene category	Upper (n = 96)	Lower (n = 57)
$fimH^+$ only	22 (23)[a]	30 (53)[a]
$papGII^+$ only	1 (1)	0 (0)
foc^+ only	0 (0)	0 (0)
$fimH^+papGII^+foc^-$	56 (58)[b]	18 (32)[b]
$fimH^+papGII^-foc^+$	7 (7)	4 (7)
$fimH^-papGII^+foc^+$	0 (0)	0 (0)

Data are presented as numbers (percentages).
UTI: urinary tract infection.
[a]$P < 0.001$; [b]$P = 0.001$.

only one strain (1%) carried *papG*II-only genotype. The prevalence of the $fimH^+foc^+$ genotype in *papG*II-negative *strains* in upper UTI was not higher than that in lower UTI.

Genetic analysis and phenotype characterization of isogenic mutants

Southern blot analysis revealed that the probe hybridized to a larger *Eco*RV fragment (5.951 kb) from EC114FM (Supplemental Fig. 1A) and EC114DM (Supplemental Fig. 1B) but hybridized to a smaller fragment (4.551 kb) from EC114 and EC114PM (Supplemental Fig. 1B). The result of chromosomal DNA digested with the other restriction enzyme *Hin*CII confirmed this finding (Supplemental Fig. 1A and 1B). These results confirmed that the size of the *fimH* gene had increased because of the insertion of the 1.4 kb kanamycin cassette within the gene.

EC114FM and EC114DM did not agglutinate guinea pig erythrocytes, whereas EC114 and EC114PM did (Supplemental Table 2 and Supplemental Fig. 2). EC114PM and EC114DM did not agglutinate human blood A1P1 erythrocytes, whereas EC114 and EC114FM were positive for mannose-resistant hemagglutination to human A1P1 erythrocytes.

Virulence in the BALB/c mouse model of ascending UTI

Bladder infection

Low dose. When challenged with a low dose (5×10^4 CFU) of wild-type EC114, bladder infection ($\geq 10^3$ CFU in bladder) occurred in 50% and 60% of the mice after 1 and 3 days, respectively (Fig. 1A). By contrast, when the same challenge dose of EC114FM and EC114DM were used, no bladder infection occurred on days 1 and 3 (Fig. 1A). The geometric value of quantitative bacterial count in each mouse bladder was shown in Supplemental Fig. 3a. The geometric means of bacterial counts in the bladders of mice inoculated with EC114FM and EC114DM were significantly lower than those of EC114-inoculated mice on days 1 and 3 after inoculation (all $P < 0.05$, Fig. 1B).

High dose. When challenged with a high dose of wild-type EC114 (5×10^8 CFU), all the mouse bladders were infected by day 1, which remained until day 3, and in 7 mice (70%) on day 7 (Fig. 1C). By contrast, when the same dose of EC114FM was used, bladder infection occurred only in 1 mouse (10%) on day 1 and no bladder infection was found on days 3 and 7 (Fig. 1C). Similar result was found in EC114DM-challenged mice (Fig. 1C). The geometric value of quantitative bacterial count in each mouse bladder was shown in Supplemental Fig. 3b. The geometric means of quantitative bacterial counts in the bladders of mice challenged with EC114FM and EC114DM were significantly lower than those of mice inoculated with EC114 and EC114PM at all the three-time points (all $P < 0.05$, Fig. 1D).

There was no significant temporal or dose-related difference in the percentage of bladder infection or bacterial counts in mouse bladders between EC114 and EC114PM (Fig. 1A–D).

Kidney infection

Low dose. When challenged with a low dose of EC114 (5×10^4 CFU), kidney infection ($\geq 10^3$ CFU in kidney) occurred in 30% of mice after 1 day, and reached 60% by day 3 (Fig. 2A). In contrast to EC114, no kidney infection was found on day 1 and remained through day 3 after challenged with the same dose of EC114FM and EC114DM (Fig. 2A). Similarly, kidney infection occurred only in one mouse on day 1 and no kidney infection occurred on day 3 after challenged with EC114PM. Seven days after low dose of bacterial challenge, both wild-type and mutant strains were essentially cleared from the examined kidneys. The geometric value of quantitative bacterial count in each mouse kidney was shown in Supplemental Fig. 3c. The geometric means of quantitative bacterial counts in the kidneys of mice challenged with EC114 were significantly higher than those challenged with EC114FM and EC114DM on day 1 and day 3 and EC114PM on day 3 after inoculation (all $P < 0.05$, Fig. 2B).

High dose. When challenged with a high dose of bacteria (5×10^8 CFU), kidney infection with EC114 occurred after 1 day in 60% of the mice, reached 70% by day 3, and decreased to 40% after day 7 (Fig. 2C). The percentages of kidney infection by the same challenge dose of EC114FM, EC114PM and EC114DM were less than those of EC114 through day 1 to day 7 after inoculation (Fig. 2C). The geometric value of quantitative bacterial count in each mouse kidney was shown in Supplemental Fig. 3d. The geometric means of quantitative bacterial counts in the kidneys of EC114FM-inoculated mice on days 3 and 7, EC114PM-inoculated mice on day 3 and EC114DM on day 1 were significantly lower than those of EC114-inoculated mice (all $P < 0.05$, Fig. 2D).

Effect of adhesin gene interaction on kidney infection

We evaluated the effect of adhesin genotype $fimH^+$, $papGII^+$ and $fimH^+papGII^+$ on kidney infection by calculating the difference in the incidence of causing kidney infection between strain EC114DM ($fimH^-papGII^-$) and the strain carrying the genotype which would be evaluated (i.e. EC114PM for $fimH^+$, EC114FM for $papGII^+$ and EC114 for $fimH^+papGII^+$).

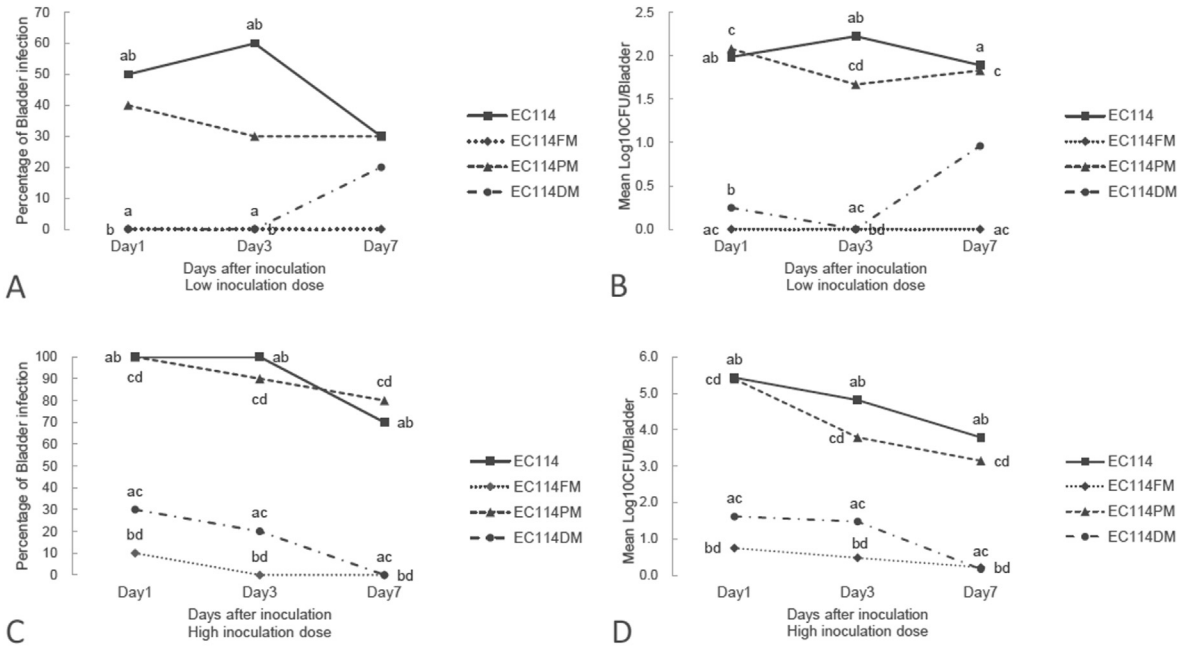

Figure 1. Percentage of bladder infection and geometric means of quantitative bacterial counts in mouse bladder. Each mouse was challenged transurethrally with bacterial suspensions (low dose: 5×10^4 CFU (A and B) or high dose: 5×10^8 CFU(C and D) of wild-type *E. coli* EC114 (■), *fimH* mutant EC114FM (◆), *papG*II mutant EC114PM (▲) or double mutant EC114DM (●). Ten mice in each group and a total of 240 mice were inoculated. (A) and (C) The percentages of bladder infection (defined as $\geq 10^3$ CFU in bladder). (B) and (D) The geometric means of quantitative bacterial counts in mouse bladders. [a]EC114 vs. EC114FM, $P < 0.05$, [b]EC114 vs. EC114DM, $P < 0.05$, [c]EC114PM vs. EC114FM, $P < 0.05$, [d]EC114PM vs. EC114DM, $P < 0.05$.

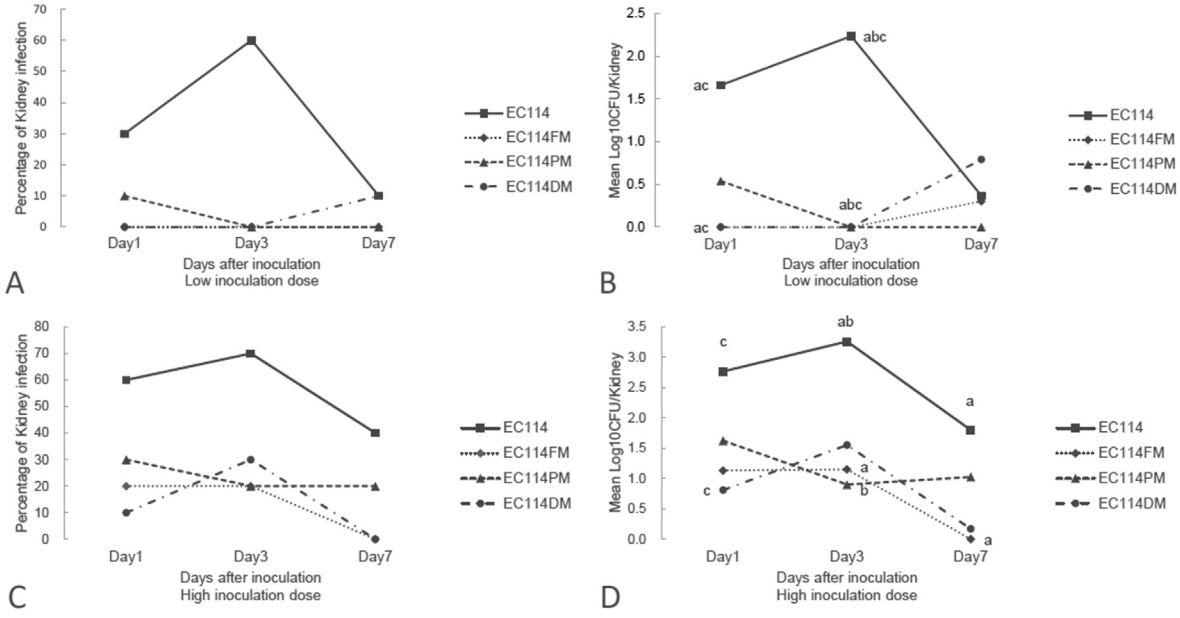

Figure 2. Percentage of kidney infection and geometric means of quantitative bacterial counts in mouse kidney. Each mouse was challenged transurethrally with bacterial suspensions (low dose: 5×10^4 CFU (A and B) or high dose: 5×10^8 CFU(C and D) of wild-type *E. coli* EC114 (■), *fimH* mutant EC114FM (◆), *papG*II mutant EC114PM (▲) or double mutant EC114DM (●). Ten mice in each group and a total of 240 mice were inoculated. (A) and (C) The percentages of kidney infection (defined as $\geq 10^3$ CFU in kidney). (B) and (D) The geometric means of quantitative bacterial counts in mouse kidneys. [a]EC114 vs. EC114FM, $P < 0.05$, [b]EC114 vs. EC114PM, $P < 0.05$, [c]EC114 vs. EC114DM, $P < 0.05$.

The effect on increase in kidney infection by inoculation with *fimH*$^+$*papG*II$^+$ strain (EC114) was greater than the sum of individual effect by inoculation with *fimH*$^+$ strain (EC114PM) and *papG*II$^+$ strain (EC114FM) on days 1 and 3 after low dose inoculation and on days 1—7 after high dose inoculation (Fig. 2A and C and Supplemental Table 3).

Discussion

The role of Type 1 fimbriae in human UTI and development of kidney infection is difficult to reconcile with their occurrence in most of both commensal strains and UPEC.[3,15,16] The same as the previous genetic studies for clinical isolates,[3,15,16] we revealed that *fimH* was found in most of asymptomatic bacteriuria strains and UPEC. However, our genetic epidemiological survey demonstrated that UPEC strains carrying only *fimH* were significantly more prevalent in lower UTI, and the strains carrying the *fimH*$^+$*papG*II$^+$, but not *papG*II$^+$-only or *fimH*$^+$-only, genotype were significantly associated with upper UTI. *E. coli* carrying only *papG*II gene were rarely found among the upper UTI strains. Thus, *E. coli* carrying both *fimH* and *papG*II may have a higher potential to cause kidney infection than those carrying either gene alone. FimH may act with PapG II to enhancing the establishment and maintenance of kidney infection.

The mouse model of ascending UTI in this study revealed the same results as those of previous studies,[4—6,17,18] which showed that type 1 fimbriae receptor-specific adherence, as directed by *fimH*, is critical in the establishment and persistence (i.e. maintained infection until day 7) of bladder infection. More importantly, we also demonstrated that a defect in the expression of FimH adhesin in the *fimH*$^+$*papG*II$^+$ strain could attenuate its ability to cause the establishment of kidney infection, at low bacterial dose, and maintenance of kidney infection even at high dose of bacterial challenge. In contrast, a defect in the expression of PapGII adhesin in the strain could only attenuate its ability in the maintenance of kidney infection and had no effect on its ability in bladder infection. We confirmed the advantage of the *fimH*$^+$*papG*II$^+$ genotype for *E. coli* kidney infection, but we also revealed the temporal and inoculation dose-related difference in kidney infection between *fimH* mutant and *papG*II mutant.

A UPEC CFT073-specific DNA microarray study revealed that the constitutive expression of type 1 fimbriae leads to the downregulation of P fimbrial gene expression,[7] perhaps to enable the sequential occupation of lower and upper urinary tracts. A live rat study revealed the substantial expression of both major fimbrial structural proteins PapA2 and FimA, suggesting that a bacterial population may exhibit heterogeneous expression of adhesins during infection.[8] The authors also demonstrated that P fimbriae mediate binding between bacteria and renal tubule cells, whereas type 1 fimbriae promote interbacterial binding, which results in the formation of biofilm-like communities in the center of lumen.[8] The biofilm-like communities within renal tubules may lead to a focus for persistent kidney infection. The results of our animal study are compatible with the above findings that a defect in the expression of FimH adhesin attenuate the ability of *E. coli* in sequential colonization of lower and upper urinary tract and also affect its ability in the formation of biofilm-like communities which may influence the maintenance of kidney infection.

When UPEC faces filtrate flow in renal tubules, the P and type 1 fimbriae may act in synergy to promote colonization.[8] Synergy is commonly defined as the effect of two or more agents working in combination that is greater than the expected additive effect of said agents.[19—21] In this study, we used *E. coli* EC114 for investigation because it carries the FimH and PapGII adhesins genotypically and phenotypically but does not harbor other fimbrial adhesin. Therefore, we can evaluate the individual effect of adhesin gene on kidney infection by calculating the difference in the incidence of causing kidney infection between strain EC114DM and the strain carrying the adhesin gene which would be evaluated. We demonstrated that the effect by inoculation with *fimH*$^+$*papG*II$^+$ strain was greater than the sum of individual effect by inoculation with *fimH*$^+$ strain and *papG*II$^+$ strain on days 1—7 after both low and high dose challenge, except on day 7 after low dose inoculation. Our mouse model of ascending UTI supports that FimH synergistically act with PapGII for kidney infection.

Regarding the role of P fimbriae in kidney infection, studies using ascending infection models have yielded inconstant results.[9,13,22,23] In the live animal model, P fimbriae may facilitate epithelial attachment and promote bacterial multiplication before infiltration of immune cells,[8] which indicates that P fimbriae may serve as a fitness factor because its expression is not essential but only advantageous for virulence.[8,9] Thus, the result of present study that mutation of PapGII adhesin majorly affected the ability of *E. coli* in maintenance of kidney infection supports the above hypothesis.

Previous studies showed that P or type 1 fimbriae-mediated attachment can activate receptor bearing epithelial cells and enhance host cytokine responses to UPEC.[24,25] Although the wild type strain EC114 displayed enhanced kidney colonization compared to the mutants, a higher proportion of infecting EC114 were cleared from the mouse kidney by 7 days especially after low dose bacterial inoculation. However, the present study revealed a paradoxical increase in EC114DM counts in 10—20% of mouse bladders after 7 days (Fig. 1B) and kidneys after days 3 and 7 (Fig. 2B and D). This result may be related to the defect in innate immune response to the strains lacking P and type 1 fimbrial adhesin which may result in a late infection in the mouse kidney. Nevertheless, this hypothesis needs further study to confirm.

In summary, the results of genetic epidemiological survey taken together with the findings of mouse model of ascending UTI in this study confirm the advantage of the *fimH*$^+$*papG*II$^+$ genotype for kidney infection compared with either *papG*II or *fimH* alone. FimH adhesin plays a role not only in lower UTI, but also in kidney infection by acting synergistically with PapGII adhesin. Thus, antagonists against FimH and PapGII adhesin may prevent kidney infection and enable its management.

Data statement

The datasets of this study are available from the corresponding author on reasonable request.

Declaration of competing interest

All authors declare no conflicts of interest related to this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2020.09.001.