P-fimbrial adhesins in *Escherichia coli* enable the coloniza-

tion of host tissues. By mediating attachment to P-blood group 

antigens on uroepithelial cells (6, 12, 16), P fimbriae play a 

critical role in the development of urinary tract infections 

(UTIs). P fimbria production is regulated by a chromosomal 

*pap* operon, containing 11 genes (4). The P-fimbrial-tip adhe-

sin, which is encoded by *papG*, attaches directly to host cells 

(7, 9). The three adhesin classes (*papG*~J96~ [class I], *papG*~AD/IA2~ [class II], and *prsG*~J96~ [class III]) were characterized based on 

their capacities for binding to specific Gal(1–4)Gal-containing 

glycolipids (14). *pf* (pap-related fimbriae) is generalized no-

menclature that includes all gene clusters encoding P fimbriae. 

The *pf* probe contains the most conserved genes and thus 

hybridizes to all of these gene clusters (1). Class I adhesins 

have 45% identity at the amino acid level to class II and 46% 

identity to class III, while class II adhesins have 56% identity to 

class III. Minor *papG* variants have been reported (GenBank 

accession numbers AAF61952 [J. R. Johnson, N. Kaster, T. T. 

O’Bryan, and A. L. Stell, unpublished data], AAF61956 [5], 

AAD13607 [3], and AAA59216 [10]) with homology to the 

three *papG* alleles, ranging in identity from 89 to 96%. We 

present the discovery of a new G allele of P fimbriae with less 

than 65% identity to known adhesin classes.

Three *E. coli* collections were studied: 313 isolates from 

collected from healthy women presenting to the University of 

Michigan Student Health Service for gynecological exams 

between February and March 1996. All *E. coli* isolates were 

cultured and processed as previously described (1, 17).

A total of 815 *E. coli* strains were screened by dot blot hy-

bridization for the presence of *prf*, a cluster of gene sequences 

specific to P-related fimbriae, as described previously (1). Se-

quence homology to *prf* was detected in 332 (41%) of the 

strains tested. The P-fimbrial adhesin class was also deter-

mined by screening those strains positive for *prf* with the three 

class-specific DNA probes using dot blot hybridization (Ta-

ble 1). The adhesin class-specific probes (*papG*~J96~ [class I] 

and *prsG*~J96~ [class III]) were derived from published sequences 

(8, 13, 14) and isolated from control strain J96 or C1212 by PCR 

(1, 2). Strain J96 contains *papG*~J96~ and 

*papG*~AD/IA2~, while control strain C1212 contains *papG*~AD/IA2~. 

We confirmed dot blot results with PCR using unique primers 

for each adhesin class. Identical PCR conditions, except the an-

nealing temperatures, were used for each adhesin class (30 

cycles of 94°C for 60 s and 73°C for 40 s, with an annealing 

time of 35 s). Table 2 lists the annealing temperatures and 

PCR primers. Based on these results, 20 strains positive for 

*prf* and negative for all three adhesin classes were identified 

(Table 1), suggesting the presence of *papG* variants.

Pulsed-field gel electrophoresis analysis showed that all 20 strains differ by three or more bands (15) and therefore do not represent a clonal grouping (data not shown). Because *papF* is conserved among the three known adhesin classes (13), we

| Table 1. P-fimbrial adhesin classes by strain source |
|----------------------------------------|--------|---------------------|---------------------|---------------------|---------------------|---------------------|
| Population | No. (%) of *prf*-positive strains | *papG*~J96~ (class I) | *papG*~AD/IA2~ (class II) | *prsG*~J96~ (class III) | Unknown class |
|------------|---------------------------------|---------------------|---------------------|---------------------|---------------------|
| First UTI (n = 313) | 153 (48.9) | 5 (1.6) | 85 (27.2) | 62 (19.8) | 8 (3.3) |
| Second UTI (n = 51) | 24 (47.1) | 0 (0) | 10 (19.6) | 10 (19.6) | 1 (2.4) |
| Fecal (n = 377) | 128 (34.0) | 0 (0) | 88 (23.3) | 31 (8.2) | 11 (4.1) |
| Periurethral (n = 73) | 27 (37.0) | 0 (0) | 13 (17.8) | 13 (17.8) | 0 (0) |

*Corresponding author. Mailing address: Department of Epidemiology, 109 Observatory St., University of Michigan, Ann Arbor, MI 48109. Phone: (734) 647-2407. Fax: (734) 764-3192. E-mail: cfmarrs@umich.edu.
† Present address: Karmanos Cancer Institute, Detroit, Mich.
assessed whether it could be amplified using PCR in the 20 strains (Fig. 1). A 502-bp fragment was amplified in control strains J96 and C1212 and in 11 of 20 (55%) strains with an unknown papG adhesin class.

In order to determine whether any of these strains contained novel P-fimbrial G alleles, we cloned and sequenced papG from two randomly selected strains (BF1163 and BF31). Southern blot hybridization using a 502-bp papF PCR probe labeled with digoxigenin (Genius System kit; Boehringer Mannheim, Indianapolis, Ind.) detected DNA fragments of 6.5 kb for fecal strain BF31 (Fig. 2), and of 4 and 2.3 kb for UTI strain BF1163 digested with BsaB1 and Psp1406I (data not shown), respectively. Both the 6.5- and the 4-kb DNA fragments were purified following gel electrophoresis and cloned using the pZErO-1 vector (Invitrogen, San Diego, Calif.) with T7 and SP6. Fecal strain BF31 contained a novel papG allele (papG\textsubscript{BF31}), whereas UTI strain BF1163 contained a variant of papG with a deletion. papG\textsubscript{BF31} had amino acid sequence identities of 65% to papG\textsubscript{J96} (class I), 46% to papG\textsubscript{AD/IA2} (class II), and 45% to prsG\textsubscript{J96} (class III) (Fig. 3). We refer to papG\textsubscript{BF31} as the P-fimbrial class IV adhesin gene. BF1163 was most similar (70%) to papG\textsubscript{J96}; however, the open reading frame was truncated at bp 290.

In order to estimate the prevalence of papG\textsubscript{BF31} in other E. coli strains, we screened a sample of strains (n = 308) positive for prf by dot blot hybridization using a 371-bp probe specific to papG\textsubscript{BF31}: papG\textsubscript{BF31} occurred with similar frequency in each collection. The numbers of strains positive for papG\textsubscript{BF31} were as follows: 21 (15%) among the first UTI collection (n = 144), 36 (15%) among the second UTI collection (n = 20), 20 (17%) among the fecal collection (n = 120), and 3 (13%) among the perirethral collection (n = 24). papG\textsubscript{BF31} is positively associated with aer and drb and is negatively associated with prsG\textsubscript{J96}, hly, cnfl, ompT, and sfa (Table 3). Among isolates positive for papG\textsubscript{BF31}, papG\textsubscript{AD} was present in 55%.

PCR was performed on the remaining 19 prf-positive, class I-, II-, and III-negative strains using primers 5'-GACATTC TGTTATGATT-3' and 5'-CAATGAATTAAGTTAG-3' (30 cycles of 95°C for 60 s, 46°C for 40 s and 73°C for 23 s), taken from a unique coding region of papG\textsubscript{BF31}. A 371-bp fragment was amplified in 8 of the 19 (42%) strains, suggesting that other novel G allele variants may exist.

The novel class IV adhesin gene shows 45 to 65% similarity at the amino acid level to the three adhesin classes, thereby representing a unique adhesin class that is found equally among UTI and fecal E. coli strains. Thus, class IV adhesins are not exclusively associated with UTIs, although they could be associated with the pathogenesis of other important diseases.

The large prf probe used in this study hybridized to strains containing novel papG alleles as well as inactive papG variants.
or variants with deletions. Because a PCR fragment specific to the class IV gene was not amplified in 11 of the strains without class I, II, or III adhesins, it is possible that other novel molecular variants of \(papG\) exist. Future work should include hemagglutination assays to determine whether \(papG\) is functional and to identify other novel \(papG\) variants and assess their role in UTIs or other diseases.

**Nucleotide sequence accession number.** The GenBank accession number for the \(papG_{BF31}\) nucleotide sequence is AF304159.

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**TABLE 3. Prevalence of virulence factor genes among the entire collection and among the subset positive for \(papG_{BF31}\).**

| Sample | No. (%) positive for selected virulence factor gene |
|--------|--------------------------------------------------|
| Total collection (\(n = 308\)) | papG\textsubscript{AD}, papG\textsubscript{BF31} | \(papG_{BF31}\), \(papG_{BF31}\)’ | aer\textsuperscript{a} | kpsMT | hly\textsuperscript{b} | cnf1\textsuperscript{b} | ompT\textsuperscript{b} | dtb\textsuperscript{b} | sfa\textsuperscript{a} | fim |
| 183 (59) | 5 (2) | 105 (34) | 142 (46) | 275 (89) | 152 (49) | 105 (34) | 282 (92) | 19 (6) | 112 (36) | 308 (100) |
| 26 (55) | 2 (4) | 1 (2) | 35 (74) | 40 (85) | 10 (21) | 2 (4) | 30 (64) | 11 (22) | 4 (9) | 47 (100) |

\(a\) The virulence factor genes encode the following: aerobactin (aer), group II capsules (kpsMT), \(\alpha\)-hemolysin (hly), cytotoxic necrotizing factor 1 (cnf1), outer membrane protease T (ompT), afimbrial adhesins I to IV and F1845 pili (dtb), S fimbrin (sfa), and type 1 fimbrin (fim). The data are the numbers (percentages) of isolates containing each gene (e.g., among those strains positive for \(papG_{BF31}\), 55% were also positive for \(papG_{AD}\)).

\(b\) The proportion positive for a selected gene among isolates with \(papG_{BF31}\) is significantly (\(P = 0.0001\)) different from the proportion in the total collection.
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