

The Phase-Variable Allele of the Pilus Glycosylation Gene *pglA* Is Not Strongly Associated with Strains of *Neisseria gonorrhoeae* Isolated from Patients with Disseminated Gonococcal Infection^{∇†}

P. M. Power,¹ S. C. Ku,¹ K. Rutter,¹ M. J. Warren,¹ E. A. Limnios,²
J. W. Tapsall,² and M. P. Jennings^{1*}

School of Molecular and Microbial Sciences, The University of Queensland, St. Lucia, Queensland 4072, Australia,¹
and Neisseria Reference Laboratory, Microbiology Department, The Prince of Wales Hospital,
Barker St., Randwick, New South Wales 2031, Australia²

Received 19 September 2006/Returned for modification 2 November 2006/Accepted 30 January 2007

The *Neisseria gonorrhoeae pglA* gene has two alleles, one of which is phase variable. A previous study reported that all disseminated gonococcal infection (DGI) isolates contained the phase-variable allele and proposed a causal link. In the present study of 81 strains no absolute correlation between DGI and the phase-variable *pglA* allele was observed.

Neisseria gonorrhoeae infections result in a spectrum of disease from asymptomatic carriage and mucosal gonococcal infection (MI) to disseminated gonococcal infection (DGI). Pili are a major virulence factor of *N. gonorrhoeae*, facilitating adhesion to the epithelial surface. Pili are posttranslationally modified by glycosylation, and a number of genes involved in the glycosylation of pili have been identified (5, 8, 9, 16, 17). *pglA* was the first of these genes to be identified (8) and is responsible for the transfer of the first galactose to the trideoxyhexose sugar in the pilin-linked Gal(β1-4) Gal(α1-3) 2,4-diacetamido-2,4,6-trideoxyhexose in *N. meningitidis* (see Fig. 1B). Subsequent work by Banerjee et al. (5) reported on the equivalent gene in *N. gonorrhoeae* (*pgtA* [96% identical at the nucleotide level]) (8). Hence, *pgtA* is referred to as *pglA*.

Jennings et al. (8) and Banerjee et al. (5) have reported that the expression of *pglA* is phase variable. Phase variation is the reversible high-frequency on-off switching of the expression of a gene and is commonly associated with virulence factors in a number of host-adapted human pathogens (reviewed in reference 11). The *N. gonorrhoeae pglA* gene has two distinct alleles: one containing a homopolymeric tract that mediates phase variation (random on/off switching of expression) and another lacking the tract. The phase variation of *pglA* is mediated by a tract of G residues within the *pglA* coding sequence (see Fig. 1A). Variation in the number of G residues can result in a frameshift mutation and a nonfunctional *pglA* gene product. An analysis of the *pglA* gene in a number of strains conducted by Jennings et al. (8) revealed a wide variation in the number of G residues in the homopolymeric tract in pathogenic *Neisseria*.

Banerjee et al. (5) analyzed 52 strains of *N. gonorrhoeae* for

the presence of this homopolymeric tract. All of the 24 strains from patients with DGI contained the *pglA* allele with the repeat tract. In contrast, only 8 of the 28 MI strains contained the *pglA* allele with the homopolymeric tract. This was an exciting observation since it identified the first example of a virulence factor where the potential for phase-variable expression was invariably associated with progression to a distinct pathology, and this has been subsequently cited in the literature (4, 7, 18). The reasons why some cases of gonorrhoea progress from uncomplicated mucosal disease to DGI is not well characterized. Several prior studies (6, 12, 13) of the differences between MI and DGI strains have found no single factor that was predictive of whether an *N. gonorrhoeae* strain was a potential DGI strain (such as arginine, hypoxanthine, and uracil auxotrophy and the gonococcal pathogenicity island). Banerjee et al. (5) suggested that the phase variation of *pglA* is likely to be involved in the conversion of MI to DGI because of its absolute correlation of the phase-variable *pglA* allele with DGI.

In the present study, the observations made by Banerjee et al. (5) were tested in a larger, more diverse strain collection and are extended to examine the relationship between other phase-variable genes involved in the posttranslational modification of pili and the distinct pathologies of MI and DGI. The strain collection was derived from isolates referred from public and private sectors in two geographically distinct Australian jurisdictions, New South Wales and the Northern Territory, between 1988 and 2002. The sampling methods used were those of the Australian Gonococcal Surveillance Programme as detailed in annual reports (2, 3). Some cases were identified as having presented in Australia after being contracted overseas (i.e., in the Middle East, United Kingdom, Thailand, and Fiji). Strains were classified as being from DGI if they were isolated from blood or joint cultures or as MI if they were isolated from anogenital sites or the eye. The details of the strains are recorded in Table S1 in the supplemental material and include data on the isolation site and antibiotic resistances. In addition, an effort was made to exclude strains that were presumptively “clonal.” The criteria for exclusion included

* Corresponding author. Mailing address: School of Molecular and Microbial Sciences, The University of Queensland, Brisbane, QLD 4072, Australia. Phone: 61 7 3365 4879. Fax: 61 7 3365 4620. E-mail: jennings@uq.edu.au.

† Supplemental material for this article may be found at <http://iai.asm.org/>.

∇ Published ahead of print on 12 February 2007.

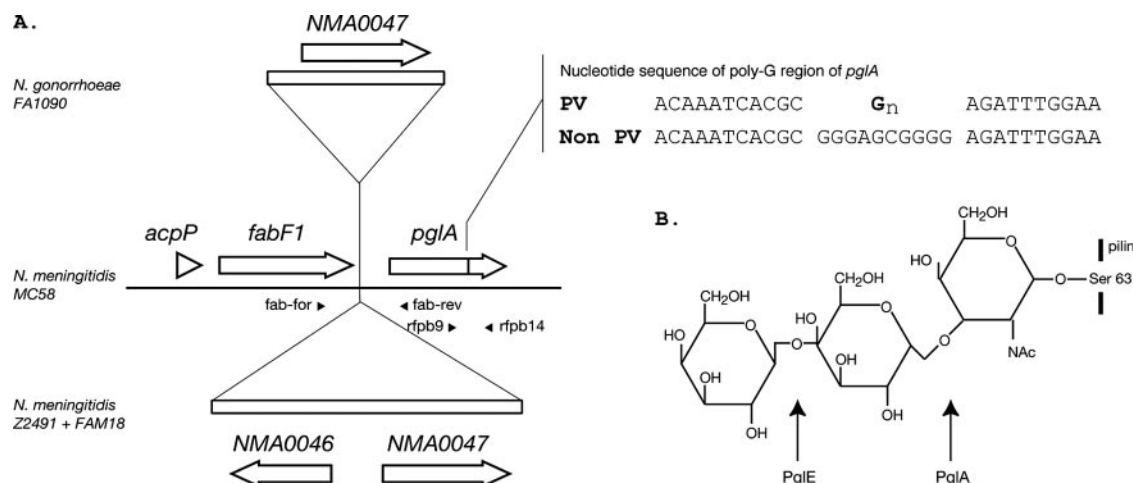


FIG. 1. Genetic map of *pglA* region in *Neisseria* and structure of posttranslational modification of *N. gonorrhoeae* pilin. (A) Genetic map of *pglA* region in various *Neisseria* strains (as indicated on left). Open reading frames are represented by unfilled arrows and are named according to the *N. meningitidis* strain Z2491 genome annotation. The key region defining the phase-variable and non-phase-variable alleles of *pglA* is indicated in the top right of the figure. G_N indicates that a different number of G residues may be observed in this position in different strains (range, 11 to 17). The positions of the primers are indicated by small black triangles, and their names are adjacent. (B) The predicted structure of the pilin-linked glycan from *N. gonorrhoeae* with both PglE and PglA expressed. Arrows indicate the linkages created by PglE and PglA.

geographic, temporal, and antibiotic resistance clustering. The repeat region of the *pglA* gene in each strain was sequenced as previously described (as described in reference 8). Phase-variable and non-phase-variable alleles of *pglA* were defined as shown in Fig. 1A. Similar to the study of Banerjee et al., non-phase-variable alleles were defined as having the sequence 5'-GGGAGCGGG-3' in place of the homopolymeric tract.

We observed no absolute correlation between the phase-variable *pglA* allele and DGI; only 37% of DGI strains examined in the present study had the PV tract in *pglA* (Table 1). Among the MI strains, 18% had a phase-variable allele. We conclude that there is no absolute correlation between the presence of the phase-variable allele and either pathology.

Two characterized *pglA*-linked polymorphisms were described by Power and Jennings (15) (see Fig. 1A). There are up to two additional genes inserted between *pglA* and *fabF* (8), referred to by their *N. meningitidis* Z2491 annotation: NMA0047, a member of the PFAM sugar transporter family (10) (PFAM PF00083), and NMA0046, a member of a family of bile acid/Na⁺ symporter family (PFAM PF01758). The primers Fab-for (5'-ATGAGTCAGAGAAGAGTAG-3') and Fab-rev (5'-TCAGCCTTTGAAGCGTTT-3') were used to PCR amplify the region in which the polymorphisms had been identified. The size of the products was used to determine which allele was present. The results revealed that NMA0046 was present in all *N. gonorrhoeae* strains studied. The alternative genetic arrangements found in *N. meningitidis* MC58 (19)

(no genes) and in *N. meningitidis* Z2491 (14) (two genes) were not found in any *N. gonorrhoeae* strains.

Two other phase-variable genes associated with pilin-linked posttranslational modifications were also examined to look for a correlation with MI or DGI. *pglE* is responsible for the addition of the terminal galactose in the *N. meningitidis* pilin-linked trisaccharide (17). *pptA*, is involved in the addition of ChoP to pilin in *N. meningitidis* and a similar role has been suggested for *pptA* of *N. gonorrhoeae* (1, 20). The potential for phase variation, as defined by the presence or absence or polymorphism in the size of the repeat tracts of *pglA* or *pptA*, did not correlate with either pathology (see Table S1 in the supplemental material).

In the present study we demonstrate that there is no absolute correlation between the potential of the pilin glycosylation gene *pglA* to phase vary and DGI. We assume that the correlation reported by Banerjee et al. (5) was due to the use of a clonal strain collection and indicates the importance of accessing a large, diverse, and well-characterized bacterial strain collection in undertaking such studies.

This study was supported by program grant 284214 from the National Health and Medical Research Council of Australia.

We acknowledge the Australian National Neisseria Network, who provided access to the strain collection used in this study.

REFERENCES

- Aas, F. E., W. Egge-Jacobsen, H. C. Winther-Larsen, C. Lovold, P. G. Hitchen, A. Dell, and M. Koomey. 2006. *Neisseria gonorrhoeae* type IV pili undergo multisite, hierarchical modifications with phosphoethanolamine and phosphocholine requiring an enzyme structurally related to lipopolysaccharide phosphoethanolamine transferases. *J. Biol. Chem.* **281**:27712–27723.
- Australian Gonococcal Surveillance Programme. 2005. Annual report of the Australian Gonococcal Surveillance Programme. *Commun. Dis. Intell.* **29**: 137–142.
- Australian Gonococcal Surveillance Programme. 1984. Penicillin sensitivity of gonococci in Australia: development of the Australian Gonococcal Surveillance Programme. *Br. J. Vener. Dis.* **60**:226–230.
- Banerjee, A., and S. K. Ghosh. 2003. The role of pilin glycan in neisserial pathogenesis. *Mol. Cell Biochem.* **253**:179–190.

TABLE 1. Strain survey of *pglA* allele from DGI and MI strains

Strain	No. (%) of strains with <i>pglA</i> allele type present:	
	Phase-variable	Non phase-variable
DGI	15 (37)	26 (63)
MI	7 (18)	33 (82)

5. Banerjee, A., R. Wang, S. L. Supernavage, S. K. Ghosh, J. Parker, N. F. Ganesh, P. G. Wang, S. Gulati, and P. A. Rice. 2002. Implications of phase variation of a gene (*pgtA*) encoding a pilin galactosyl transferase in gonococcal pathogenesis. *J. Exp. Med.* **196**:147–162.
6. Dillard, J. P., and H. S. Seifert. 2001. A variable genetic island specific for *Neisseria gonorrhoeae* is involved in providing DNA for natural transformation and is found more often in disseminated infection isolates. *Mol. Microbiol.* **41**:263–277.
7. Ghosh, S. K., J. Zhao, M. C. Philogene, A. Alzaharani, S. Rane, and A. Banerjee. 2004. Pathogenic consequences of *Neisseria gonorrhoeae* pilin glycan variation. *Microbes Infect.* **6**:693–701.
8. Jennings, M. P., M. Virji, D. Evans, V. Foster, Y. N. Srikhanta, L. Steeghs, P. van der Ley, and E. R. Moxon. 1998. Identification of a novel gene involved in pilin glycosylation in *Neisseria meningitidis*. *Mol. Microbiol.* **29**:975–984.
9. Kahler, C. M., L. E. Martin, Y. L. Tzeng, Y. K. Miller, K. Sharkey, D. S. Stephens, and J. K. Davies. 2001. Polymorphisms in pilin glycosylation locus of *Neisseria meningitidis* expressing class II pili. *Infect. Immun.* **69**:3597–3604.
10. Lee, E. H., and W. M. Shafer. 1999. The *farAB*-encoded efflux pump mediates resistance of gonococci to long-chained antibacterial fatty acids. *Mol. Microbiol.* **33**:839–845.
11. Moxon, E. R., R. E. Lenski, and P. B. Rainey. 1998. Adaptive evolution of highly mutable loci in pathogenic bacteria. *Perspect. Biol. Med.* **42**:154–155.
12. Noble, R. C., R. R. Reyes, M. C. Parekh, and J. V. Haley. 1984. Incidence of disseminated gonococcal infection correlated with the presence of AHU auxotype of *Neisseria gonorrhoeae* in a community. *Sex Transm. Dis.* **11**:68–71.
13. O'Brien, J. P., D. L. Goldenberg, and P. A. Rice. 1983. Disseminated gonococcal infection: a prospective analysis of 49 patients and a review of pathophysiology and immune mechanisms. *Medicine* **62**:395–406.
14. Parkhill, J., M. Achtman, K. D. James, S. D. Bentley, C. Churcher, S. R. Klee, G. Morelli, D. Basham, D. Brown, T. Chillingworth, R. M. Davies, P. Davis, K. Devlin, T. Feltwell, N. Hamlin, S. Holroyd, K. Jagels, S. Leather, S. Moule, K. Mungall, M. A. Quail, M. A. Rajandream, K. M. Rutherford, M. Simmonds, J. Skelton, S. Whitehead, B. G. Spratt, and B. G. Barrell. 2000. Complete DNA sequence of a serogroup A strain of *Neisseria meningitidis* Z2491. *Nature* **404**:502–506.
15. Power, P. M., and M. P. Jennings. 2003. The genetics of glycosylation in gram-negative bacteria. *FEMS Microbiol. Lett.* **218**:211–222.
16. Power, P. M., L. F. Roddam, M. Dieckelmann, Y. N. Srikhanta, Y. C. Tan, A. W. Berrington, and M. P. Jennings. 2000. Genetic characterization of pilin glycosylation in *Neisseria meningitidis*. *Microbiology* **146**:967–979.
17. Power, P. M., L. F. Roddam, K. Rutter, S. Z. Fitzpatrick, Y. N. Srikhanta, and M. P. Jennings. 2003. Genetic characterization of pilin glycosylation and phase variation in *Neisseria meningitidis*. *Mol. Microbiol.* **49**:833–847.
18. Schmidt, M. A., L. W. Riley, and I. Benz. 2003. Sweet new world: glycoproteins in bacterial pathogens. *Trends Microbiol.* **11**:554–561.
19. Tettelin, H., N. J. Saunders, J. Heidelberg, A. C. Jeffries, K. E. Nelson, J. A. Eisen, K. A. Ketchum, D. W. Hood, J. F. Peden, R. J. Dodson, W. C. Nelson, M. L. Gwinn, R. DeBoy, J. D. Peterson, E. K. Hickey, D. H. Haft, S. L. Salzberg, O. White, R. D. Fleischmann, B. A. Dougherty, T. Mason, A. Ciecko, D. S. Parksey, E. Blair, H. Cittone, E. B. Clark, M. D. Cotton, T. R. Utterback, H. Khouri, H. Qin, J. Vamathevan, J. Gill, V. Scarlato, V. Masignani, M. Pizza, G. Grandi, L. Sun, H. O. Smith, C. M. Fraser, E. R. Moxon, R. Rappuoli, and J. C. Venter. 2000. Complete genome sequence of *Neisseria meningitidis* serogroup B strain MC58. *Science* **287**:1809–1815.
20. Warren, M. J., and M. P. Jennings. 2003. Identification and characterization of *pptA*: a gene involved in the phase-variable expression of phosphorylcholine on pili of *Neisseria meningitidis*. *Infect. Immun.* **71**:6892–6898.

Editor: J. N. Weiser