Phylogenetic and epidemiological analysis of *Neisseria meningitidis* using DNA probes

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SUMMARY

The genetic relationships between various serotypes and serogroups of meningococcal strains were investigated by restriction fragment-length polymorphism (RFLP) analysis using a number of random DNA probes and a probe containing a truncated copy of the meningococcal insertion sequence *IS1106*. The data were used to estimate genetic distance between all pairs of strains and to construct phylogenetic trees for meningococcal strains. B15:P1.16R strains isolated from cases of systemic meningococcal disease in two health districts with a high incidence of disease were clonal in contrast to similar strains from cases occurring in other parts of the UK. Strains from these areas, which contain a similar genomic deletion, were found to be derived from two distinct lineages within the B15:P1.16R phylogenetic group. RFLP data demonstrated that present serological typing systems for the meningococcus do not necessarily reflect true genetic relationships.

INTRODUCTION

Variations in the capsular polysaccharide permit the division of *Neisseria meningitidis* into a number of serogroups [1]. Strains may be further divided into serotypes on the basis of variation in the class 2 or 3 outer membrane protein (OMP) [2], and may be serosubtyped according to variations in the class 1 OMP [3]. Sulphonamide resistance is also utilized as an epidemiological marker. More recently multilocus enzyme electrophoretic typing (ET-typing) has been used to allocate strains to a number of ET types [4]. Strains of serogroup B have predominated in the UK and in Europe over the last decade; During the 1980s there was an increase in disease caused by group B type 15 subtype 16 (B15:P1.16), mainly sulphonamide-resistant (R) strains in the UK [5] and North Western Europe [6]. Although B15:P1.16R disease has occurred throughout the UK certain health districts including Gloucester [7] and Plymouth [8] have shown a prolonged increased incidence of endemic disease due to this strain.

Current vaccines based on meningococcal polysaccharides are unsatisfactory; those consisting of meningococcal polysaccharides A and C give only short lived

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immunity and group B polysaccharide is a poor immunogen. Considerable efforts are therefore being directed into the development of vaccines based on more immunogenic surface components [9]. The success of such vaccines will depend on detailed knowledge of the epidemiology of disease caused by *N. meningitidis* and on the relationship between serogroups, serotypes and subtypes.

Using a DNA probe pUS210 homologous to a repetitive element in the meningococcal genome, we were able to divide strains into a number of RFLP types. B15:P1.16R strains from two localities of increased endemic disease (Gloucester and Plymouth) gave identical banding patterns (termed 'outbreak-type') suggesting a single clone, possibly of high virulence, may have been responsible for the elevated rate of disease in these districts [10]. The outbreak-type banding pattern was shown to be due to an insertion or deletion event (for simplicity subsequently referred to as a deletion) downstream from the gene (*porA*) coding for the class 1 OMP. This deletion removed one copy of the repetitive element contained within pUS210; this element was found to be a truncated form of a novel neisserial insertion sequence IS1106 [11]. The same deletion was found in strains from the two districts with a high incidence of disease. However, Fox and colleagues [12], using a random DNA probe, typed a selection of UK meningococcal strains and found that B15:P1.16R strains were of two distinct RFLP types, with one type including Gloucester and the second type including Plymouth strains. In addition, McGuinness and colleagues [13] identified a point mutation in the *N. meningitidis porA* gene coding for one of two variable regions (VR2) determining sero-subtype; this mutation distinguished between B15:P1.16R strains which had predominated in Gloucester and Plymouth Health Districts. However the use of any single character for typing strains does not provide reliable phylogenetic data that may be used for examining the genetic relationship between strains. We therefore examined a selection of UK meningococcal strains with five further random probes in addition to the repetitive probe pUS210 and used this data to determine the phylogenetic relationships between strains in the UK.

MATERIALS AND METHODS

Strains

N. meningitidis G3 (NCIMB 40248) is an outbreak-type strain isolated from the Gloucester Health District. Additional meningococcal strains (Table 1) were supplied by Dr D. Jones, Meningococcal Reference Laboratory (MRL), Manchester and Dr R. G. Fallon, Ruchill Hospital, Glasgow. These strains were clinical isolates from a number of locations in the UK and were collected between 1980 and 1991. The strains used for diversity analysis were isolated between 1984 and 1990.

DNA manipulations

DNA extractions and Southern blotting of *N. meningitidis* DNA were performed as previously described [10].

DNA probes

Probes were obtained from *N. meningitidis* *EcoR* I genomic libraries constructed

Table 1. *Strain information*

Strains	Serotype*	Isolation	Date	Strains	Serotype*	Isolation	Date
P1	B15:P1.16R	Plymouth	04/84	P14	B15:P1.16R	Plymouth	05/88
G3	B15:P1.16R	Gloucester	06/84	P16	B15:P1.16R	Plymouth	11/88
A273	B15:P1.16	Lincoln	08/80	P18	B15:P1.16R	Plymouth	02/89
B376	B15:P1.16	Peterborough	08/81	L299	B15:P1.16R	London	01/90
C242	B15:P1.16	Leicester	08/82	L376	B15:P1.16R	London	01/90
C246	B15:P1.16	Belfast	08/82	J2007	B15:P1.16R	Birmingham	10/88
C457	B15:P1.16	Southend	08/82	J705	B15:P1.16R	Birmingham	03/88
C504	B15:P1.16	Shrewsbury	11/82	L744	B15:P1.16R	Birmingham	08/90
C225	B4:P1.15	Leicester	08/82	E74	B15:P1.16R	Norwich	01/84
S1	B15R	Scotland	1984	K992	B15:P1.16R	Norwich	04/89
S2	B15R	Scotland	1984	F921	B15:P1.16R	Westcliffe	10/85
S3	B15R	Scotland	1985	G1506	B15:P1.16R	Westcliffe	05/86
S4	B15:P1.16R	Scotland	1985	K1475	B15:P1.16R	Liverpool	05/89
S5	B4:P1.15R	Scotland	1987	G79	B15:P1.16R	Liverpool	01/86
S6	B4:P1.15R	Scotland	1988	F133	B15:P1.16R	Liverpool	02/85
S7	NG4:P1.15R	Scotland	1988	H1793	B15:P1.16R	Liverpool	07/87
S8	B4:P1.15R	Scotland	1988	H2347	B15:P1.16R	Liverpool	11/87
S9	B4:P1.15R	Scotland	1988	L274	B15:P1.16R	Liverpool	01/90
E781	Bnt:P1.15R	Birmingham	08.84	L61	B15:P1.16R	Liverpool	01/90
F971	B15R	Southampton	08/85	L273	B15:P1.16R	Liverpool	01/90
G7	C	Gloucester	12/85	L1571	B15:P1.16R	Liverpool	05/90
G8	C	Gloucester	12/85	L334	B15:P1.16R	London	01/90
G17	Cnt	Gloucester	04/86	G190	B15:P1.16R	Gloucester	04/91
G22	C2b	Gloucester	06/86	G177	B2b:P1.10S	Gloucester	04/91
G24	Cnt	Gloucester	06/86	G33	NG	Gloucester	08/86
G55	Cnt	Gloucester	06/86	G34	NGS	Gloucester	08/86
G57	CntR	Gloucester	04/87	G35	NG	Gloucester	08/86
G67	NG15	Gloucester	11/87	G38	Bnt:P1.12R	Gloucester	10/86
G96	C2bS	Gloucester	01/89	G84	NGntS	Gloucester	09/88
G97	C2bS	Gloucester	01/89	G107	B4:P1.15S	Gloucester	05/89
G98	C2aS	Gloucester	01/89	G110	B2bS	Gloucester	07/89
G99	C2aS	Gloucester	01/89	G119	Bnt:P1.5	Gloucester	12/89
G123	C2a:P1.15	Gloucester	12/89	G71	B15:P1.6	Gloucester	01/87
G126	B2bS	Gloucester	12/89	G106	B4S	Gloucester	05/89
G127	B2bS	Gloucester	12/89	G175	B15:P1.16R	Gloucester	04/91
G131	B2bS	Gloucester	12/89	G37	BntR	Gloucester	08/89
G132	B2bS	Gloucester	12/89	G39	B12R	Gloucester	08/89
G173	C2a:P1.12R	Gloucester	04/91	M2	B15:P1.15R	Suffolk	10/84
G136	B4:P1.7S	Gloucester	04/91	M3	B15:P1.16R	Kent	11/84
G148	C7S	Gloucester	04/91	M4	B15:P1.15R	Essex	12/84
G155	B15:P1.16R	Gloucester	04/91	M5	B15:P1.15R	Cheshire	01/85
G157	B15:P1.16R	Gloucester	04/92	M6	B15:P1.15R	Somerset	02/85
G162	C2a:P1.2R	Gloucester	04/91	M7	B15:P1.12R	London	02/85
G169	Bnt:P1.16R	Gloucester	04/91	M8	B15:P1.15R	Warwick	03/85
G171	C2a:P1.2R	Gloucester	04/91	M9	B15:P1.15R	Dorset	03/85
G133	B15:P1.16R	Gloucester	04/91	M10	B15:P1.15R	Lancashire	03/85
G120	NG4:P1.16	Gloucester	12/89	M11	B15:P1.16R	Cheshire	04/85
G6	B15:P1.16R	Gloucester	12/85	M12	B15:P1.16R	Lancashire	01/86
G112	B15:P1.16R	Gloucester	01/89	M13	B15:P1.16R	Suffolk	01/86
G65	B15:P1.16R	Gloucester	07/86	M14	B15:P1.16R	Sussex	01/86
G49	B15:P1.16R	Gloucester	10/86	M15	B15:P1.16R	Yorkshire	01/86
G48	B15:P1.16R	Gloucester	10/86	M16	B15:P1.16R	Lancashire	02/86
G108	B15:P1.16R	Gloucester	06/89	M17	B15:P1.16R	London	02/86
G122	B15:P1.16R	Gloucester	12/89	G74	Bnt	Gloucester	03/86
P2	B15:P1.16R	Plymouth	01/85	G87	Bnt:P1.2S	Gloucester	09/88
P4	B15:P1.16R	Plymouth	12/85	G28	29ES	Gloucester	08/86
P6	B15:P1.16R	Plymouth	07/86	G83	B4:P1.15S	Gloucester	09/88
P8	B15:P1.16R	Plymouth	11/86	G85	Bnt:P1.5S	Gloucester	09/88
P10	B15:P1.16R	Plymouth	03/87	G86	Bnt:P1.2S	Gloucester	09/88
P12	B15:P1.16R	Plymouth	10/87	G105	B4S	Gloucester	05/89

Table 1. (*cont.*)

Strains	Serotype*	Isolation	Date	Strains	Serotype*	Isolation	Date
G43	BntS	Gloucester	10/86	P15	B15:P1.16R	Plymouth	06/88
G54	Cnt	Gloucester	12/86	P17	B15:P1.16R	Plymouth	11/88
G53	NGS	Gloucester	12/86	P19	B15:P1.16R	Plymouth	03/89
G42	NG15	Gloucester	10/86	L107	B15:P1.16R	London	01/90
G51	NG15S	Gloucester	12/86	L163	B15:P1.16R	London	01/90
G63	NG15R	Gloucester	07/87	J2263	B15:P1.16R	Birmingham	11/88
G64	NGR	Gloucester	07/87	F104	B15:P1.16R	Birmingham	01/85
G32	NGnt:P1.12S.12	Gloucester	08/86	G2369	B15:P1.16R	Birmingham	06/86
G45	B15:P1.16R	Gloucester	04/84	F242	B15:P1.16R	Norwich	03/85
G10	B15:P1.15R	Gloucester	12/85	H6	B15:P1.16R	Norwich	01/87
G46	B15:P1.16R	Gloucester	10/86	F1113	B15:P1.16R	Westcliffe	12/85
G114	B15:P1.16R	Gloucester	10/89	K869	B15:P1.16R	Westcliffe	03/89
G26	B15:P1.16R	Gloucester	10/86	G919	B15:P1.16R	Liverpool	02/86
G50	B15:P1.16R	Gloucester	12/86	G54	B15:P1.16R	Liverpool	01/86
G121	B15:P1.16R	Gloucester	12/89	F485	B15:P1.16R	Liverpool	05/85
G123	B15:P1.16R	Gloucester	12/89	H1525	B15:P1.16R	Liverpool	05/87
P3	B15:P1.16R	Plymouth	07/85	J2063	B15:P1.16R	Liverpool	08/88
P5	B15:P1.16R	Plymouth	07/86	L443	B15:P1.16R	Liverpool	01/90
P7	B15:P1.16R	Plymouth	09/86	L73	B15:P1.16R	Liverpool	01/90
P9	B15:P1.16R	Plymouth	03/87	L1715	B15:P1.16R	Liverpool	01/90
P11	B15:P1.16R	Plymouth	04/87	L578	B15:P1.16R	London	02/90
P13	B15:P1.16R	Plymouth	01/88				

* R. sulphonamide resistance; S, sulphonamide sensitive.

in either pUC18 or pBR322. Randomly selected probes (with insert size) were: pUS250 (4.2 kb), pUS251 (3.2 kb), pUS252 (2.5 kb), pUS253 (6.2 kb) in pUC18 and pUS254 (12.5 kb) in pBR322. Probes were selected for this work on the basis of containing large DNA inserts and in giving polymorphic banding patterns when hybridized to digests of meningococcal DNA in Southern blots. The repetitive probe pUS210 [10] contained a truncated copy of IS1106 [11].

Analysis of RFLP data

Banding patterns were coded for each probe using the program ELBAMAP [14] in order to construct a binary code database dependent on the presence or absence of a specific band. This was then used to construct a similarity matrix [15]. The matrix was analyzed using the KITSCH program from the PHYLIP package of phylogeny inference programs using the SEQNET node at the SERC Daresbury Laboratory, to produce a phylogenetic tree. The KITSCH program uses the Fitch-Margoliash method [16, 17] to find the tree which minimizes:

$$\text{Sum of squares} = \sum_i \sum_j \frac{(\text{obs} - \text{exp})^2}{\text{obs}^2},$$

where obs is the observed distance between species *i* and *j* and exp is the expected distance, computed as the sum of the lengths (amounts of evolution) of the segments of the tree from species *i* to species *j*.

The mean isolation date for strains isolated from each health district was calculated as follows:

$$\bar{X} = \frac{\sum [Y + (D/365)]}{N},$$

Fig. 1. Representative Southern blot demonstrating banding patterns obtained after probing *Dra* I digested *N. meningitidis* DNA with pUS254. Lanes: 1, λ Hind III size markers; 2, G3 (B15:P1.16); 3, P1 (B15:P1.16); 4, G74 (Bnt); 5, G83 (B4:P1.15); 6, G85 (Bnt:P1.5); 7, G86 (Bnt:P1.12); 8, G105 (B4); 9, G43 (Bnt); 10, G28 (29E); 11, G54 (Cnt); 12, G32 (NGnt:P1.12); 13, G53 (NG); 14, G42 (NG15); 15, G51 (NG15); 16, G63 (NG15); 17, G64 (NG); 18, G67 (NG15); 19, G120 (NG4:P1.16); 20, λ Pst I size markers.

where Y is the year in which the strain was isolated, D is the number of days elapsed in that year before the strain was isolated, and N is number of strains studied in each health district. The differences in the means were analysed by *t* test.

Strain diversity (*h*) was estimated using the equation.

$$h = \frac{n(1 - \sum X_i^2)}{(n-1)}.$$

Standard errors due to sampling were calculated by the equation [18]:

$$Sd = \sqrt{\left(\frac{2}{2n(2n-1)} \{ 2(2n-2) [\sum X_i^3 - (\sum X_i^2)^2] + [\sum X_i^2 - (\sum X_i^2)^2] \right)},$$

where X is the proportion of each RFLP type in an area and n is the number of strains studied in each area.

RESULTS

Preliminary data obtained from DNA probes demonstrated that some probes gave identical banding patterns for closely related strains irrespective of the digest

Fig. 2.(A) Phylogenetic relationship between *N. meningitidis* strains derived using randomly selected probes. Strains designation and serotypes are shown. Strain G3 was from the Gloucester Health District; strain P1 was from the Plymouth Health District. G3 is indistinguishable to 16 additional strains from the Gloucester District and one strain from the Plymouth District. Strain P1 was identical to 17 strains from the Plymouth District and one strain from the Gloucester District. Strains designated outbreak-type (with pUS210/*Eco*RI) are indicated *.

Fig. 2(B) Phylogenetic relationship between *N. meningitidis* strains derived using pUS210 and pUS254. Strains designation and serotype are shown. Strain G3 was from the Gloucester Health District; and strain P1 was from the Plymouth Health District. G3 was indistinguishable to 16 additional strains from the Gloucester District and one strain from the Plymouth District. Strain P1 was identical to 17 strains from the Plymouth District and one strain from the Gloucester District. Strains designated outbreak-type (with pUS210/*EcoR* I) are indicated *.

Fig. 3. Phylogenetic relationships between strains belonging to different serogroups and serotypes based on data obtained from five independent probes.

used whereas other probes gave polymorphic patterns in each digest for the same strains. This might be expected for a species which, like the gonococcus, undergoes horizontal genetic transfer and recombination giving rise to genetic variation [19, 20]. This suggested that many of the polymorphisms were due to recombination events rather than to a low level of base substitution. Therefore in order to minimize bias due to recombination we used only a single enzyme *Dra* I for RFLP analysis. A representative blot probed with pUS254 is shown in Fig. 1.

In order to examine the robustness of the trees obtained using this method two phylogenetic trees were constructed for all strains. The first (Fig. 2A) was constructed using five randomly selected DNA probes (pUS250+pUS251+pUS252+pUS253+pUS254). The second (Fig. 2B), was constructed using data from the clone pUS210, containing the truncated form of *IS1106* [11] and also pUS254 which contains an unidentified, repetitive element (not homologous to pUS210). The two trees demonstrated similar branching orders with two main branches (a, b in Fig. 2) that are split into sub-branches (c, d, e, Fig. 2).

The B15:P1.16R strains examined were further identified as being either outbreak or non-outbreak type on the basis of hybridization to the probe pUS210 on *Eco*R I digested DNA. Since *IS1106* has no site for *Eco*R I, the difference between outbreak and non-outbreak strains is revealed by the presence (non-outbreak) or absence (outbreak) of a single band [10, 11]. The outbreak type strain, G3 was identified to 16 additional strains isolated in the Gloucester district and 1 strain from the Plymouth district. Similarly, the strain P1 was identical to 17 strains isolated from the Plymouth district and one strain from the Gloucester district. Analysis of all of the probe data demonstrates that B15:P1.16R strains could be divided into two phylogenetic groups with the first (G-type) predominating in Gloucester though also found elsewhere in the UK, and the second (P-type) occurring in Plymouth and elsewhere in the UK (Fig. 2A, B). Outbreak-type strains (* in Fig. 2) that contain the *IS1106* deletion downstream from the *porA* gene were found in both the P and G arms of the B15:P1.16R group. Each of the

Table 2. Genetic diversities from *pUS210/Dra I* and *pUS250/Dra I RFLPs* of *B15:PI,16 N. meningitidis* isolated from seven areas of England during 1984-91

Isolation places	No. of strains	pUS210 RFLP		Isolation time ($\bar{X} \pm \text{s.d.}$)	Genetic diversity of pUS210/ <i>Dra I</i> ($h_1 \pm \text{s.d.}_1$)	Genetic diversity of pUS250/ <i>Dra I</i> ($h_2 \pm \text{s.d.}_2$)	Out-break type (%)	pUS250 RFLP type		
		types	types					Gloucester (%)	Plymouth (%)	Others (%)
London	7	5	3	1989.63 $\pm$ 1.73*	0.904 $\pm$ 0.057†	0.667 $\pm$ 0.101†	14.32	28.57	57.14	14.29
Liverpool	17	11	6	1988.35 $\pm$ 2.05*	0.934 $\pm$ 0.021†	0.735 $\pm$ 0.051†	5.80	41.18	35.29	23.53
Plymouth	19	2	2	1986.79 $\pm$ 1.44*	0.107 $\pm$ 0.065	0.107 $\pm$ 0.065	100.00	5.26	94.74	0
Birmingham	6	4	2	1987.97 $\pm$ 1.86*	0.868 $\pm$ 0.071†	0.732 $\pm$ 0.090†	33.33	50.00	33.33	16.67
Gloucester	20	4	3	1988.22 $\pm$ 1.82*	0.284 $\pm$ 0.091	0.100 $\pm$ 0.062	100.00	95.00	5.00	0
Norwich	4	3	1	1986.44 $\pm$ 2.29*	0.833 $\pm$ 0.123†	0.000 $\pm$ 0.000	0	0	100.00	0
Westcliff-on-Sea	4	3	2	1986.85 $\pm$ 1.56*	0.833 $\pm$ 0.23†	0.500 $\pm$ 0.169	0	25.00	75.00	0

* No significant differences.

† Differences were significant comparing with the strains from Gloucester and Plymouth.

trees have a number of features in common and each conform most closely to a general branching order, as shown in Figure 3.

In order to investigate the epidemiological significance of probing results, B15:P1.16R strains were further analysed by their geographical distribution within the UK. These strains were divided into G, P and other types using a random probe pUS250, and also divided into outbreak and non-outbreak types using pUS210. Table 2 shows results for areas in the UK from which more than four strains were analysed. The overall genetic heterogeneity (calculated separately using either pUS210 or pUS250) of B15:P1.16R strains isolated in different areas of the UK was also examined, using 'gene diversity', as described by Nei [18] to estimate the diversity of RFLP types within B15:P1.16R strains (Table 2). Using this measure, the genetic diversity of strains from Gloucester and Plymouth was significantly lower than strains from Liverpool, London or Birmingham using either the random probe pUS250 or the repetitive probe pUS210 containing IS1106 (Table 2).

DISCUSSION

The validity of RFLP analysis for phylogenetic analysis of meningococcal strains is demonstrated by the similarity between Fig. 2(A and B); only minor variations were found between the branching orders obtained using probes derived from different genetic loci. The value of RFLP analysis for examining the relationships between the serogroups, serotypes and sero-subtypes of *N. meningitidis* is also supported by the similarity of the phylogenetic trees presented here and those obtained by ET-typing [4, 21]. RFLP analysis permits classification of strains which cannot be grouped or typed with current serological reagents. Fig. 1, for example, shows that two non-groupable strains, G63 and G64, gave identical banding patterns using pUS254/*Dra* I to some B15:P1.16R strains. Additional probes produced similar results. It is also possible to examine genetic diversity within sero-subtypes (e.g. amongst B15:P1.16R strains). RFLP analysis is simpler than ET-typing. A single Southern blot can be examined sequentially with a number of randomly cloned probes or probes containing repetitive elements in order to examine multiple genetic loci. The use of a single probe may not give epidemiologically reliable data; multiple probes and repetitive probes give more information. However repetitive probes such as pUS210 may be linked to more variable parts of the genome and undergo changes in copy number, both of which may be independent of clonal identity.

The phylogenetic trees obtained for *N. meningitidis* strains (Figs 2, 3) have two main branches, the first consisting mainly of B15:P1.16R strains and the second major branch consisting mainly of C2a/C2b and B2a/B2b and few B4 and NG strains. It is likely that the B15:P1.16R-related group of strains we identify corresponds to the ET-5 group [4]. Throughout the trees serogroup B strains are very closely related to some serogroup C strains, as phenomenon also found with ET-typing [4, 21, 22], suggesting that only minor genetic changes are responsible for the changes in the capsular polysaccharide antigen. This change is of great epidemiological significance [23]. It is also clear that strains of the same serogroup, serotype or serosubtype are not necessarily closely related. Conversely, some

strains of different serogroups, serotypes and sero-subsubtypes may be closely related. The close genetic relationship between strains of different serogroups, serotypes and sero-subtypes (e.g. B15:P1.16R, C15:P1.16, Bnt:P1.12, NG15, B4:P1.15 strains – see Fig. 2), demonstrates that variation of surface antigens occurs in these genetically closely-related strains and that serology may give an unrealistic picture of the true relationship between meningococcal strains, as suggested previously [4, 21, 24]. This is consistent with the complex epidemiology of meningococcal disease observed in some outbreaks using serological typing systems [7].

The division of UK B15:P1.16R strains into two types (G and P) as suggested by others [12, 13, 24, 25] is supported by this work. This differs from our earlier findings in which we were unable to differentiate Gloucester and Plymouth strains. The differentiation in this study is due to the use of the enzyme *Dra* I which cleaves meningococcal DNA at high frequency and therefore produces additional RFLPs not examined in our earlier work.

The assignment of each B15:P1.16R strain into either G or P groups on the basis of RFLPs was concordant for most of the probes used, indicating that the loci examined using the five randomly selected DNA probes have remained genetically linked throughout the divergence of B15:P1.16R meningococcal strains in the UK. This is perhaps surprising since *Neisserial* species are naturally competent for genetic transformation, and horizontal gene transfer is thought to be involved in antigenic variation of many proteins [19, 26]. The lack of a single recombination event that would have broken this linkage, despite the divergence of UK B15:P1.16R strains over the 6-year period, suggests that if recombination occurs, it may involve short regions of DNA [26], thereby not disrupting the overall linkage pattern of the genome.

The finding that outbreak-type strains were derived from both G and P arms of the B15:P1.16R phylogenetic group was surprising. Recent molecular analysis of the genetic locus responsible for the 'outbreak-type' banding pattern revealed by clone pUS210, [11] demonstrates that outbreak-type strains have a 2.7 kb deletion downstream of the *N. meningitidis* *porA* gene, when compared with non-outbreak type strains. This deletion removes a copy of the newly discovered insertion sequence *IS1106* and additional repetitive elements from this locus. The same deletion was found in outbreak-type strains from the two districts with a high incidence of disease. The results presented here indicate either that the deletion event occurred independently in each of the two (G and P) lineages or that the locus has been transferred between G and P strains. Alternatively, but less likely, the deleted locus could be the ancestral-type and the non-outbreak type strains could have derived by two independent insertional events at this locus, after the divergence of the G- and P-type strains.

Examination of the geographical distribution of RFLP-typed B15:P1.16R strains showed that G- and P-type strains (as typed with random probes) were distributed more or less randomly throughout the UK, whereas the outbreak type strains, of G and P types clustered strongly in Gloucester and Plymouth respectively. These two health districts in the UK have experienced a sustained high incidence of disease. Strains from both these districts also exhibited less variation as determined by measurement of genetic diversity (ie they were more

clonal in nature). This was found not only with pUS210 (identifying the IS1106-associated deletion), but also with random probes such as pUS250 that do not detect the outbreak deletion (Table 2). The populations of B15:P1.16R strains from these districts therefore differ in two respects from populations of strains elsewhere in the UK in showing a high prevalence of outbreak-types and a low degree of genetic diversity. The prolonged high incidence of disease due to both G- and P-outbreak types in these small areas of the UK adds weight to the hypothesis that outbreak-type strains with the IS1106-associated deletion are of increased virulence. The reason for the low rate of genetic divergence in these two clones compared with other UK B15:P1.16R strains, and its importance in relation to the high meningococcal disease attack rates in Gloucester and Plymouth Districts are not yet understood. It would clearly be of importance to determine the phenotypic consequence of the deletion responsible for the outbreak-type strains.

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