Genetic Characterization of a *Neisseria meningitidis* cluster in Queensland, Australia

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Abstract

Neisseria meningitidis serogroups B and C have been responsible for the majority of invasive meningococcal disease in Australia, with serogroup B strains causing an increasing proportion of cases in recent years. Serogroup Y has typically caused sporadic disease in Australia. In 2002, a cluster of four cases was reported from a rural region in Queensland. Three of these cases were serogroup C, with one case diagnosed by molecular detection only, and the fourth case was identified as a serogroup Y infection. Genomic analysis, including antigen finetyping, MLST and cgMLST demonstrated that the serogroup Y case, though spatially and temporally linked to a serogroup C disease cluster, was not the product of a capsule switch, and that one of the serogroup C isolates had a deletion of the entire *porA* sequence.

Neisseria meningitidis, a Gram negative commensal bacterium, causes invasive meningococcal disease (IMD) in humans. Strains of *N. meningitidis* undergo genetic recombination, through mechanisms such as recombination of flanking repeat regions or IS*1301* disruption of capsule biosynthesis genes, generating changes to the capsular serogroup, referred to as capsule switching (Hilse et al. 1996; van der Ende et al. 1999) . As the polysaccharide capsule is an important target of the host serogroup-specific immune response and a component of most *N. meningitidis* vaccines, capsule switching assists in immune evasion (Rishishwar et al. 2012). Strains may also lose outer membrane proteins such as PorA as an alternate mechanism for immune evasion (Harrison et al. 2006; van der Ende et al. 1999).

In 2002, four cases of IMD from a rural central Queensland region occurred over a 41 day period, which met the national guideline criteria of a disease cluster; a community vaccination program for the defined risk age group of 18-40 years old was subsequently implemented (Pugh et al. 2003). Of the four cases, three serogroup C and the other serogroup Y, one of the serogroup C cases was diagnosed by molecular detection therefore no isolate was available for further characterization. At the time of the disease cluster, serosubtyping determined the two serogroup C isolates were C:2a:P1.5 and C:2a:NST respectively, while the serogroup Y isolate was Y:NT:P1.5 (Programme 2003; Pugh et al. 2003). This information in combination with epidemiological linkages led to speculation that the serogroup Y isolate had arisen via a capsule switch from the serogroup C strain responsible for the other cases (Pugh et al. 2003). This study has revisited this disease cluster, using whole genome sequencing to characterize the genetic lineages and to establish if capsule switching had occurred.

DNA was prepared by the Ion Plus Fragment library kit and sequenced on an Ion Torrent PGM using the Ion PGM IC 200 Kit, Ion Chef and 316v2 chips (Life Technologies) according to the manufacturer's instructions. FASTQ sequences were *de novo* assembled using the built-in assembler in Geneious R7 Assemblies ranged from 122 to 361 contigs and 103-150 times average coverage. Sequences were deposited in ENA under study PREJEB13900, and isolate records in PubMLST isolate database (Table 1).

In silico molecular antigenic profiling and MLST analysis performed at pubMLST (http://pubmlst.org/neisseria/) (accessed 18/08/2016) revealed that the two serogroup C isolates shared MLST and antigen profiles, except that *porA* was absent in M35809 (Figure 1). M35044 was typed as Y:P1.5-1,10-4:F4-1:ST-23, sharing only the same PorA type as M35007 which was a C:P1.5-1,10-4:F3-6:ST-11 type.

High resolution gene typing was performed in RidomSeqSphere+ (Ridom GmbH, Germany) using the *N. meningitidis* cgMLST v1 scheme (Bratcher et al. 2014) (Table 1). As expected from the MLST and antigen profiling, the serogroup Y strain did not share significant core genome similarity to the serogroup C isolates (159/1605 loci alike). The serogroup C strains differed at 13/1605 core genes and fit with sublineage 11.2 (Lucidarme et al. 2015). At the time of writing, there are no other isolates on the pubMLST database with 25 or fewer cgMLST loci differences to either of these two strains.

M35809 sequences reads were mapped to the reference strain FAM18 (AM421808) using CLC Genomic Workbench 8, with no reads aligning to the *porA* gene region of FAM18. Alignment of the *de novo* assembly to the FAM18 genome, using Geneious 7 with the Mauve 2.3.1 plugin, demonstrated a 1013 bp deletion in the *porA* region of M35809. This deletion is flanked by

repeat regions and encompasses the entire *porA* gene as well as sequences upstream and downstream (Figure 1).

Antigenic profiling revealed that the serogroup Y isolate belongs to a different genetic lineage than the two serogroup C isolates and was not the result of a capsule switch during the outbreak as originally speculated (Pugh et al. 2003). Furthermore, although the serogroup C isolates, M35007 and M35809 were similar by antigenic profiling, analysis revealed that these strains not only differed in the *porA* locus but also at 13 other core genes. Comparatively, 6 isolates from a Canadian MSM outbreak of sublineage 11.2 differed by 1 to 6 cgMLSTV1.0 alleles and a French MSM outbreak strain LNP27256 (pubMLST ID 26733) was 36 alleles different from a fellow 'outbreak' isolate (pubMLST ID 26821), leading the authors to suggest that the level of genetic variation did not constitute an outbreak, but rather a close transmission network (Lucidarme et al. 2015; Tsang et al. 2003; Veyrier et al. 2013). We speculate that while the two serogroup C cases were considered sufficiently spatially and temporally linked to meet the criteria of a disease cluster, they are likely the result of a strain circulating in a geographical area, and do not represent a close transmission event.

The non serosubtypeable phenotype (C:2a:NST) of M35809 was due to the deletion of the *porA* region. Loss of *porA* has been reported in a low number of incidences and is thought to be either a host immune response evasion mechanism, or the result of multiple subcultures of the isolate *in vitro* (van der Ende et al. 1999, 2000). The M35809 strain was isolated 41 days after that for the M35007 specimen and stored at -80ºC within a few days. Repeat elements, present throughout the *N. menintigitidis* chromosome, have been demonstrated to play a role in deletion of genes, including *porA*, through recombination (Schoen et al. 2008; van der Ende et al. 1999). In

M35809, repeat sequences were identified upstream and downstream of *porA* (Figure 1), and it is likely that the deletion of *porA* occurred via recombination between these repeat regions.

This 2002 cluster of four IMD cases resulted in a significant public health response, including a targeted vaccination progam utilising a polysaccharide (ACW135Y) vaccine. Epidemiological information and phenotypic typing data available at the time lead to speculation that the serogroup Y case was caused by a capsule switch from a serogroup C strain. This study has utilised whole genome sequencing to demonstrate that the serogroup Y isolate was not derived by a capsule switch but most likely a sporadic case of IMD that occurred spatially and temporally related to a cluster of serogroup C disease. Furthermore, genetic analysis revealed that the two serogroup C cases were caused by isolates of the same lineage, with high genetic similarity but with variation in a small number of genes, including the loss of the entire *porA* gene in one isolate.

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Table 1: Serosubtype and antigenic allele profiles for isolates sequenced in this study.

Strain	PubMLST ID	Number of cgMLST loci used in analysis	Sero subtype	Genotype	ENA Sample number
M35007	41845	(1583/1605)	C:2a:P1.5	C:P1.5-1,10-4:F3-6:ST-11 (cc11)	ERS1146380
M35809	41847	(1584/1605)	C:2a:NST	C:P1.Δ:F3-6:ST-11 (cc11)	ERS1146381
M35044	41846	(1573/1605)	Y:NT:P1.5	Y:P1.5-1,10-4:F4-1:ST-23 (cc23)	ERS1146382

Note. NST = Not subtypeable, Δ= gene determined to be absent

178 Figure 1: Deletion of *porA* gene region including upstream and downstream repeat regions in M35809.

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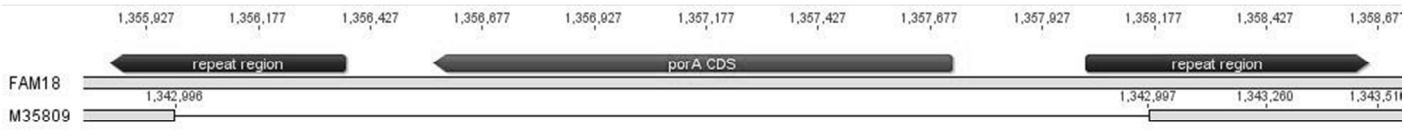
1,355,927
1,356,177
1,356,427
1,356,677
1,356,927
1,357,177
1,357,427
1,357,677
1,357,927
1,358,177
1,358,427
repeat region
porA CDS
repeat region
FAM18
M35809
1,342,996
1,342,997
1,343,260