M-Protein Analysis of *Streptococcus pyogenes* Isolates Associated with Acute Rheumatic Fever in New Zealand

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We applied an *emm* cluster typing system to group A *Streptococcus* strains in New Zealand, including those associated with acute rheumatic fever (ARF). We observed few so-called rheumatogenic *emm* types but found a high proportion of *emm* types previously associated with pyoderma, further suggesting a role for skin infection in ARF.

Two of the most significant consequences of group A streptococcal (GAS) infection are acute rheumatic fever (ARF) and its sequelae, rheumatic heart disease (RHD). New Zealand has among the highest incidences of ARF in the developed world, with the greatest burden of disease in indigenous New Zealand (Māori) and Pacific populations (1).

Contemporary molecular typing of GAS is carried out by sequence analysis of the hypervariable region of the *emm* gene that encodes the M protein (2). Studies in the United States have suggested an association between distinct GAS *emm* types (so-called rheumatogenic strains, such as *emm*3, *emm*5, *emm*6, and *emm*18) and ARF (3). However, more recent epidemiological studies in areas where ARF is common today have found that ARF is not restricted to these rheumatogenic strains (4), raising questions around the concept of rheumatogenicity and *emm* type. It has further been postulated that certain GAS *emm* types (most notably *emm*3) may be rheumatogenic due to the presence of a specific collagen-binding motif, designated peptide associated with rheumatic fever (PARF), which elicits an immune response to type IV collagen (5, 6). To date, however, the presence of the PARF motif has not been systematically assessed in a large collection of GAS strains temporally associated with ARF.

Recently, an Australian and New Zealand GAS vaccine development program (the Coalition to Accelerate New Vaccines Against *Streptococcus* [CANVAS]) was formed with the aim of identifying suitable vaccine GAS candidates for both the Australian and New Zealand settings, and more widely (7). At present, the most clinically advanced GAS vaccine candidates are those that target the N-terminal region of the M protein, such as an experimental 30-valent M-protein vaccine (8). While this vaccine includes the classical rheumatogenic *emm* types, there have been few analyses to inform the coverage of contemporary ARF strains. Recently, an *emm* cluster-based typing system that classifies known *emm* types into 48 related *emm* clusters has been applied to several collections of GAS isolates and has shed new insights into the epidemiology of GAS and the potential vaccine coverage of M-protein-based vaccines (9, 10). The *emm* cluster system also predicts an *emm* pattern type that in turn correlates well with tissue tropism (pattern A-C for pharyngeal, pattern D for skin, and pattern E for either) (9).

Accordingly, the aims of this study were to (i) compare the

molecular epidemiology and theoretical vaccine coverage of GAS isolates associated with ARF in New Zealand with those of GAS isolates recovered from other GAS-related clinical syndromes, and (ii) identify GAS isolates containing the PARF motif and associate the presence of this motif with ARF and other GAS-related clinical syndromes.

We undertook a retrospective molecular epidemiological study of GAS *emm* types associated with ARF in New Zealand between January 2006 and December 2014. ARF is a notifiable disease in New Zealand, with surveillance performed by the Institute of Environmental Science and Research (ESR) (Wellington, New Zealand). A GAS isolate was deemed to be temporally associated with ARF if it was isolated from the pharynx of a patient with confirmed ARF in the 14 days preceding hospitalization or during admission. Confirmed ARF was defined as a case diagnosed using the New Zealand modification of the Jones criteria (11). In order to compare the collection of ARF-associated GAS strains with other GAS-related clinical syndromes, we included contemporaneous strains from invasive GAS infections and strains recovered from cases of presumptive GAS pharyngitis. The 2,681 invasive strains were collected over an 11-year period (2002 to 2012) and have been well described (12). GAS pharyngeal isolates were obtained from a prospective study conducted in Auckland, New Zealand, in 2013 (13).

emm typing was performed using a previously described protocol (2), and *emm* clusters, together with *emm* patterns, were extrapolated from the *emm* typing results (9). The *emm* gene se-

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TABLE 1 Group A *Streptococcus* strains temporally associated with acute rheumatic fever in New Zealand, 2006–2014

Yr	No. of strains (<i>n</i> = 74)	<i>emm</i> type (<i>emm</i> cluster)
2006	3	<i>emm</i> 12 (A-C4), <i>emm</i> 54 (D1), <i>emm</i> 123 (D3)
2007	1	<i>emm</i> 6 (M6)
2008	1	<i>emm</i> 33 (D4)
2009	12	<i>emm</i> 6 (M6) ^a , <i>emm</i> 12 (A-C4), <i>emm</i> 36 (D1), <i>emm</i> 39 (A-C4), <i>emm</i> 49 (E3), <i>emm</i> 59 (E6), <i>emm</i> 61/44 (E3), <i>emm</i> 65/69 (E6), <i>emm</i> 92 (E2), <i>emm</i> 93(D4), <i>emm</i> 108 (D4)
2010	8	<i>emm</i> 22 (E4); <i>emm</i> 36 (D1), <i>emm</i> 53 (D4), <i>emm</i> 71 (D2), <i>emm</i> 74(M74), <i>emm</i> 87 (E3), <i>emm</i> 113 (E3), <i>emm</i> 232 (E4)
2011	13	<i>emm</i> 11 (E6), <i>emm</i> 19 (M19), <i>emm</i> 54 (D1), <i>emm</i> 59 (E6), <i>emm</i> 61/44 (E3), <i>emm</i> 65/69 (E6), <i>emm</i> 68 (E2), <i>emm</i> 71 (D2) ^a , <i>emm</i> 74 (M74) ^a , <i>emm</i> 90 (E2), <i>emm</i> 92 (E2)
2012	11	<i>emm</i> 1 ^a (A-C3), <i>emm</i> 3 (A-C5), <i>emm</i> 41 (D4), <i>emm</i> 74 (M74), <i>emm</i> 77 (E4), <i>emm</i> 81 (E6), <i>emm</i> 104 (E2), <i>emm</i> 108 (D4), <i>emm</i> 218 (M218), <i>emm</i> 232 (E4)
2013	16	<i>emm</i> 8 (E4), <i>emm</i> 19 (M19), <i>emm</i> 41(D4), <i>emm</i> 53 (D4), <i>emm</i> 65 (E6), <i>emm</i> 74 (M74) ^a , <i>emm</i> 76 (E2) ^a , <i>emm</i> 81 (E6), <i>emm</i> 89 (E4), <i>emm</i> 95 (M95), <i>emm</i> 108 (D4), <i>emm</i> 116 (D4), <i>emm</i> 197 (A-C2), <i>emm</i> 232 (E4)
2014	9	<i>emm</i> 59 (E6), <i>emm</i> 74 (M74) ^a , <i>emm</i> 85 (E6), <i>emm</i> 90 (E2), <i>emm</i> 91 (D4), <i>emm</i> 103 (E3), <i>emm</i> 118 (E3), <i>emm</i> 238 (A-C3)

^a Two strains.

quence data were translated using the Geneious software (Bio-atters, Auckland, New Zealand) and searched for the presence of PARF motifs previously described by Reissmann et al. ([A/T/E]XYLXX[L/F]N) (5). Statistical analysis was performed using GraphPad Prism (version 6).

Seventy-four GAS strains temporally associated with ARF (ARF-associated GAS strains) were included, belonging to 43 *emm* types (Table 1). This diverse range of *emm* types includes very few of the classical rheumatogenic strains. Seventeen *emm* clusters were identified, but six clusters predominated and accounted for 68% of the ARF-associated GAS strains: D4 (15%), E6 (13%), E2 (11%), *emm* cluster M74 (11%), E3 (9%), and E4 (9%). There were notable differences in *emm* type and *emm* cluster distribution according to clinical syndrome (see Table S1 in the supplemental material). One *emm* type, *emm*74, accounted for 11% of all ARF-associated isolates but was uncommon among isolates causing invasive disease and pharyngitis. Cluster D4 was the dominant cluster among ARF-associated GAS strains in this study, despite the finding that *emm* clusters E4 and A-C3 were most frequently associated with pharyngitis (as is also the case in the United States) (14).

Strains belonging to *emm* pattern D (skin pattern) made up almost half (36/74 [49%]) of the ARF-associated GAS strains (see Table S1 in the supplemental material). This suggests a potential role for skin infection in these cases of ARF and adds to previous epidemiologic data relating skin disease with ARF (15). A limitation of this study is that we did not have information on skin disease in patients with ARF. It is therefore unknown whether pharyngeal strains from ARF patients represent strains from true GAS pharyngitis or from GAS pharyngeal colonization plus con-

current GAS skin infection. To date, ARF primary prevention strategies in New Zealand have focused almost predominantly on the timely treatment of GAS pharyngitis (16). Our findings suggest that further work is required in our setting to investigate the possible link between GAS skin disease and ARF.

We found no significant association between the presence of PARF motifs and ARF. Only one of the 74 ARF-associated strains (1.4%), belonging to *emm*3, contained a motif (Table 2). The proportions of invasive and pharyngeal isolates containing PARF motifs were also low (1.6% and 2.1%, respectively). In total, six different PARF motifs were detected from four *emm* types (*emm*3, *emm*31, *emm*55, and *emm*222), but as in previous analyses (5), the motifs were most commonly detected in *emm*3 strains. It is therefore highly likely that additional genotypic and phenotypic bacterial traits, together with host immune responses, contribute to the overall pathogenesis of ARF. A limitation of this work is that our genomic analysis was confined to the *emm* gene. Future work using whole-genome sequencing may be able to better characterize potential genomic associations between ARF-associated GAS strains and ARF.

The theoretical coverage of the experimental 30-valent M-protein vaccine was lower for the ARF-associated strains (31.1%) than that for invasive (58.2%) and pharyngeal strains (76%) (see Table S2 in the supplemental material). Theoretical coverage increased to 70.3% for ARF-associated strains when the potential bactericidal effect of cross-opsonic antibodies was considered (8, 17); however, further studies are needed to characterize the potential effect of *in vitro* cross-opsonization on vaccine efficacy in a clinical setting. Given the public health importance of ARF, it is vital that GAS vaccine design considers relevant molecular epidemiological data to ensure high coverage of ARF-associated strains.

TABLE 2 PARF motifs detected in group A *Streptococcus* isolates from New Zealand

<i>emm</i> type (<i>n</i>)	<i>emm</i> cluster	PARF motifs (no. associated with each <i>emm</i> type)
<i>emm</i> 3 (40) ^a	A-C5	AEYLKSLN (34) ^a ; AEYLKGLN (4); AEYLKGFN (2)
<i>emm</i> 31 (2)	A-C5	AEYLKALN (1); AEYLKGLN (1)
<i>emm</i> 55 (5)	<i>emm</i> cluster M55	ATYLKELN (5)
<i>emm</i> 222 (2)	<i>emm</i> cluster M222	ANYLKELN (2)

^a Includes one ARF-associated strain.

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