Molecular Markers for the Study of Streptococcal Epidemiology

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Abstract Diseases caused by *Streptococcus pyogenes* (Group A streptococcus, GAS) range from superficial infections such as pharyngitis and impetigo to potentially fatal rheumatic heart disease and invasive disease. Studies spanning emm-typing surveillance to population genomics are providing new insights into the epidemiology, pathogenesis, and biology of this organism. Such studies have demonstrated the differences that exist in the epidemiology of streptococcal disease between developing and developed nations. In developing nations, where streptococcal disease is endemic, the diversity of GAS emm-types circulating is much greater than that found in developed nations. An association between emm-type and disease, as observed in developed countries is also lacking. Intriguingly, comparative genetic studies suggest that emm-type is not always a good predictor of the evolutionary relatedness of geographically distant isolates. A view of GAS as a highly dynamic organism, in possession of a core set of virulence genes that contribute to host niche specialization and common pathogenic processes, augmented

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Current Topics in Microbiology and Immunology (2013) 368: 29–48
DOI: 10.1007/82_2012_278

Published Online: 24 November 2012

by accessory genes that change the relative virulence of specific lineages is emerging. Our inability to definitively identify genetic factors that contribute to specific disease outcome underscores the complex nature of streptococcal diseases.

Contents

1 Introduction

Diseases associated with infection by *Streptococcus pyogenes* (group A streptococcus, GAS) have been so prevalent in history that many have been given easily recognizable common names (e.g., strep throat, scarlet fever, school sores, childbed fever). While the incidence of most diseases has declined markedly in countries with high per capita income, countries and regions of low income continue to suffer a high burden of these and other GAS associated diseases. Infection by GAS has been estimated to result in half a million deaths each year (Carapetis et al. 2005), and thereby position GAS as one of the top ten bacterial killers of humans. The majority of these deaths follow the development of rheumatic heart disease (RHD) and occur in developing nations. In more affluent countries, with better access to healthcare and antibiotic treatment, the prevalence of RHD is much lower, the majority of deaths attributed to the clinical manifestations associated with streptococcal invasive disease.

The burden of GAS-related diseases, differences in the geographic occurrences of these diseases, environment and the preferred body sites colonized by the organism, and accompanying changes in disease outcomes have all impelled continuing molecular and epidemiological studies of this organism. The aims of these studies have been to develop a greater understanding of molecular differences that underpin relative differences in virulence of discrete GAS lineages, identify plausible microbiological based rationale for differences in the epidemiology of GAS in different geographic locations, and predict the effectiveness of putative vaccine candidates (Smeesters et al. 2009; Steer et al. 2009d). Almost all GAS epidemiological studies use the M-protein or its genetic counterpart, the *emm*-gene as the basis for discriminating between GAS lineages. Here, we

describe how these studies, the analysis of other genetic markers and genomics has changed our view of the epidemiology and biology of this organism.

2 *emm*-Gene-Based Molecular Epidemiology

Rebecca Lancefield first reported serotypic diversity in GAS more than 80 years ago. Unlike other bacterial species in which diversity was based on variation in capsular antigens, the serotypic variation that Lancefield observed was based on variation in the N-terminal region of the surface exposed M-protein (Cunningham 2000; Fischetti 1989). Subsequent work in the 1950s showed that the presence of type specific antibodies in animal and human serum was responsible for immunity against the homologous emm-type but did not protect against heterologous emm-types (Lancefield 1962). Thus, M-protein-based serotype diversity was given a functional credence, and became the basis of the GAS typing scheme (Lancefield 1962).

Serotype-based M-typing has given way to nucleotide-based procedures (emm-typing) that targets the nucleotide sequence corresponding to the hypervariable amino terminal region of the M-protein. More than 200 different emm-sequence types have now been reported (Beall et al. 1996; Facklam et al. 2002). Although undergoing several minor revision since it's introduction, the basic premise of emm-typing remains the same (http://www.cdc.gov/ncidod/biotech/strep/strepblast.htm). In both its serological and nucleotide-based incarnations, emm-typing has been extensively used to examine both geographic strain distribution and disease association. With the large number of emm-type-based surveillance studies carried out in developing countries over the past two decades it has become clear that the epidemiology of GAS differs between developing and developed regions. Excellent systematic reviews summarizing the global molecular epidemiology of GAS have been published separately by Smeesters and Steer in 2009 (Smeesters et al. 2009; Steer et al. 2009d). In low-income regions where streptococcal infections and disease are endemic, the diversity of circulating emm-types is high. This has been demonstrated globally in distinct area such as India, Fiji, Ethiopia, and Brazil (Abdissa et al. 2006; Dey et al. 2005; Smeesters et al. 2006, 2008, 2010a; Steer et al. 2009e; Tewodros and Kronvall 2005). These studies also demonstrate that no one emm-type is dominant in these regions. Steers review reported the greatest diversity of emm-types to be found in Pacific Regions and Africa. Figure 1, depicting the emm-type diversity as determined using Simpson Reciprocal Index, clearly demonstrates the differences in diversity between countries. The pool of emm-types recovered in separate studies in low-income regions also differs (Smeesters et al. 2009; Steer et al. 2009d). For example, only one third of the emm-types recovered in two Ethiopian studies (Abdissa et al. 2006; Tewodros and Kronvall 2005) were described in a separate Fijian study (Steer et al. 2009e). Taken together the data appear to fit a model where the number of circulating emm-types is high, and subject to temporal flux. Few longitudinal studies, examining the rate of emm-type replacement have been

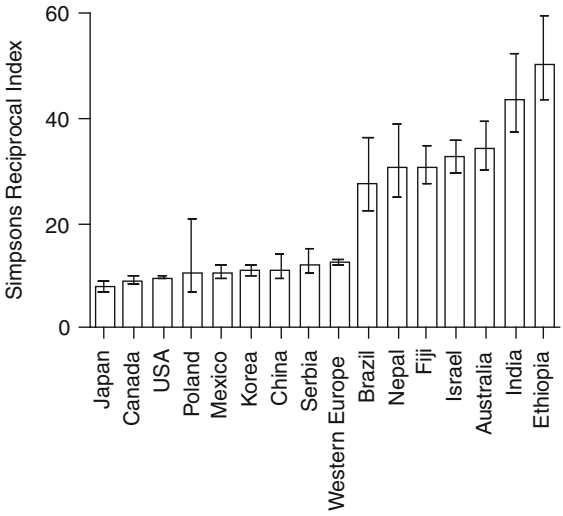

Fig. 1 Diversity of circulating emm-types in 15 countries and Western Europe. Diversity was calculated and presented as Simpsons Reciprocal Index with 95 % confidence intervals. (Adapted from Expert Review of Vaccines, December 2009, Vol. 8, No. 12, Pages 1705–1720 with permission of Expert Reviews Ltd)

carried out in endemic regions (Steer et al. 2009b). There is also lack of surveillance and prospective surveys in some areas of the world where the burden of streptococcal disease is high (Carapetis et al. 2005). This is particularly true for Africa, South America, and some locations in Asia (Smeesters et al. 2009; Steer et al. 2009d).

In developed countries, fewer emm-types appear to circulate, with even fewer dominant. Steer et al. (Steer et al. 2009d) reported that 25 emm-types accounted for more 90 % of all isolates recovered in developed nations, with 146 different emm-types accounting for the remaining 10 %. Recovery of these predominant emm-types in multiple studies also suggests that are consistently present in the wider population (McNeil et al. 2005; Shulman et al. 2009; Smeesters et al. 2006). Longitudinal studies carried out in smaller geographic region show that different emm-types are recovered at different time points (Shulman et al. 2009), demonstrating that local temporal flux in circulating emm-types occurs.

Molecular techniques, such as vir-typing (Gardiner et al. 1995) and emm pattern-typing (Hollingshead et al. 1993) represent alternate methods to categorize GAS based on variation in the emm-gene and surrounding DNA. Emm pattern typing categorizes emm-types into three distinct patterns based on chromosomal architecture (pattern types A-C, D, and E). The patterns are determined by the presence and arrangement of *emm* and *emm*-like genes, as determined by specific PCR reactions (Hollingshead et al. 1993). emm-type correlates well with specific emm pattern type (McGregor et al. 2004b). In general emm pattern-type correlates with host colonization site. Pattern A-C strains are usually associated with throat colonization, pattern D strains mainly recovered from superficial skin infection, while pattern E represents a "generalist" group associated with both tissue sites (Bessen and Lizano 2010). Similar to emm-typing, exceptions to the emm pattern type/tissue tropism have been reported, especially in low-income populations

(Bessen and Lizano 2010). Only about 20 % of the 223 known emm-types belong to pattern A-C type. These emm-types predominantly circulate in high-income countries. The remaining 80 % of emm-types are equally distributed between the pattern D and E groups, and are mostly found in developing countries. Of note, emm-types belonging to emm pattern A-C, which contain the so-called 'rheumatogenic' and 'invasive' M-types have been most extensively studied in relation to disease outcome (Erdem et al. 2005; Fischetti 1989; Smeesters et al. 2010b).

Certain emm-types, belonging to pattern A-C, are predominantly recovered from the throat rather than skin in developing countries (Shulman et al. 2004). As rheumatic fever (RF) is considered to follow an episode of pharyngitis, these types have also come to be known as 'rheumatogenic' emm-types (Bisno et al. 2003; Martin and Barbadora 2006; Stollerman 2001). Other emm-types are more commonly associated with skin infection and glomerulonephritis, and have come to be known as 'nephritogenic' emm-types. A third group of emm-types (e.g., emm1, emm12, emm3, and emm18) associated with the outbreak of severe invasive disease in the USA and Europe over the past two decades are known as 'invasive' emm-types (O'Loughlin et al. 2007; Vlaminckx et al. 2007). Interestingly recent epidemiological studies carried out in geographic regions where RHD and streptococcal infections are endemic have failed to recover significant numbers of these rheumatogenic emm-types (Bessen et al. 2000; Steer et al. 2009c). In fact, epidemiological studies in tropical regions, where the incidence of RF, but the incidence of pharyngitis is low is challenging the dogma that there is an association between GAS, throat infection, and RF (Bessen et al. 2000; Parks et al. 2012). Unique emm-types have also been implicated in RF in Hawaii (Erdem et al. 2007). Similarly, 'invasive' emm-types while present are not common in low income countries (Steer et al. 2009a).

Emm-typing has served the scientific community well for several decades, and is still relevant to tracking disease outbreaks. However, the differences in emm-type diversity and disease associations have created challenges for the streptococcal community when attempting to extrapolate data and interpretation of results from developed nations to developing nations. It should be acknowledged that comparisons of emm-type prevalence in different locations can be complicated by the different methods used to acquire isolates. Some studies represent single timepoint surveillance, whereas others analyses isolates collected over time (Shulman et al. 2009). Isolates recovered during epidemic outbreaks are also likely to differ from those recovered during non-disease related surveillance in the same location. Nevertheless, the data from different studies conducted in endemic and non-endemic regions collectively suggest differences in diversity observed between these groups are real.

Emm-type is also assumed to be marker for evolutionary relatedness, and by extension, similarity in pathogenic potential of isolates. While this is undoubtedly true for isoaltes collected during clonal outbreaks, it is less so for geographically or temporally unrelated strains. As described in more detail below, evidence for lateral gene transfer (LGT) and recombination of DNA, including the emm-gene is strong. The section of the emm-gene used for emm-typing encodes the region of

the corresponding protein that may be targeted by the host immune system, and is therefore likely to be under strong immune selection pressure. Replacement of this region, through recombination involving part or all of the emm-gene is an efficient method for escaping the host immune response (Panchaud et al. 2009; Whatmore and Kehoe 1994). In countries where streptococcal disease is endemic, and emm-type diversity is high, the conditions for LGT of the emm-gene is much greater than non-endemic countries.

3 Non-emm-Gene Virulence Factors and GAS Epidemiology

GAS pathogenesis depends on the coordinated regulation of a complex repertoire of virulence factors. While host factors undoubtedly contribute to variation in susceptibility to GAS, the frequent association of specific emm-types with disease in developed countries lends itself strongly to the argument that specific bacterial characteristics are associated with defined epidemiologies. The most comprehensive body of work examining association between non-*emm* genetic factors and GAS disease has occurred in the area of serious invasive disease. However, despite many years of research, the identification of a defined subset of invasive disease related virulence factors remains elusive.

There is increasing evidence that in populations where GAS is endemic, despite the diversity of emm-types, there is conservation of certain virulence factors linked to tissue tropism. While emm pattern typing has been useful for classification of GAS into these three patterns, several elegant epidemiological studies have led to the identification of a specific subset of genes which may confer GAS with a tissue specific phenotype (Fig. 2) (Bessen et al. 2005; Kalia and Bessen 2004; Kratovac et al. 2007). Linkages to tissue-specificity are particularly strong for genes encoding the colonization factors serum opacity factor (*sof*), collagen-binding protein *(cpa),* and fibronectin binding proteins *(prtF1)* and (*fbaA)*, which have a defined pattern of presence or absence in strains associated with distinct tissue sites (Kratovac et al. 2007). There is also significant historical evidence to implicate these colonization factors in tissue tropism, and most recently, comparative genomic hybridization analysis of 96 GAS isolates revealed that a major defining factor of tissue tropism in skin versus throat isolates is the presence or absence of genes encoding fibronectin binding proteins (Bessen et al. 2011).

Historically, SOF has provided the basis for an alternative GAS typing scheme. Streptococci of differing emm-types produce distinct variants of SOF, and type specific antibodies can be used to inhibit serum opacification by SOF variants (Maxted et al. 1973). There is a strong correlation between emm-type and SOF phenotype, and SOF antisera has been used as a method of GAS typing in the past. A comprehensive study of GAS isolates in 2000 showed that SOF is a useful predictor of emm-type, and that discordance between *sof* and *emm* is rare. However, GAS strains of the same emm-type do not always contain the same *sof* allele, and strains with diverse emm-types can have highly similar SOF genes (Beall et al.

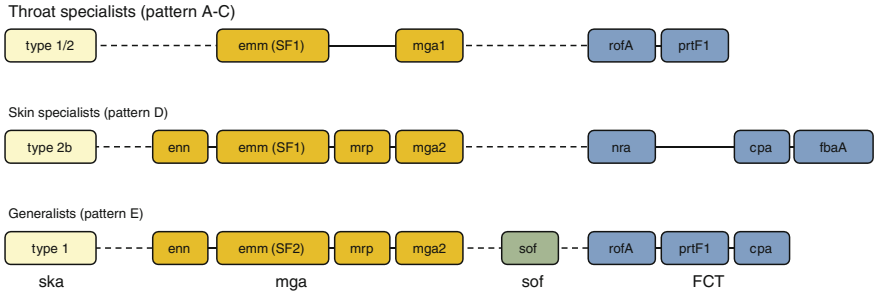

Fig. 2 Typical virulence gene repertoires of throat (pattern A-C), skin (pattern D) and generalist (pattern E) GAS isolates. Allelic variants of genes located in the streptokinase (ska), mga, serum opacity factor (sof) and FCT loci are shown. SF indicates emm gene subfamily as defined by Hollingshead et al. (1994)

2000; Johnson et al. 2006; Sakota et al. 2006). No correlation has been found between various *sof* alleles and different disease states, or geographical locations. A comparison of published studies from around the world shows that most reported *sof/emm* combinations are distributed globally (Dhanda et al. 2011; Goodfellow et al. 2000; Johnson et al. 2006; Sakota et al. 2006).

This lack of geographical segregation may, in part, be explained by the linkage between *sof* and the "generalist" tissue phenotype, that is, isolates which are equally associated with skin and pharyngeal infection, which represent approximately 50 % of GAS isolates globally (Bessen and Lizano 2010). Typically, pharyngeal specialists are opacity factor negative, and harbor a *prtF1* allele (Courtney and Pownall 2010; Cunningham 2000; Kratovac et al. 2007). In contrast, skin specialists are more likely to be endowed with *fbaA*. In a study of GAS isolates from the Northern territory of Australia, where skin infection predominates, 92 % of isolates were found to be *fbA* positive (Ramachandran et al. 2004). Similarly, *cpa* is more frequently associated with skin trophic isolates than strains which would be regarded as throat specialists (Kratovac et al. 2007). The differential distribution of these genes between skin versus throat versus generalist strains largely parallels the architecture of the overall *mga* regulon.

Additionally, a number of genes appear to have evolved into discrete phylogenetic lineages associated with tissue site preference. The regulatory gene *mga* is present in the GAS genome in one of two allelic forms, *mga1,* which is predominantly associated with throat specialist strains, and *mga2*, which appears to be found in all emm pattern D and E strains. These allelic forms are mutually exclusive (Bessen et al. 2005). Similarly, *rofA/nra* encodes as transcriptional regulator which regulates expression of pillus structural genes (Kreikemeyer et al. 2003). *rofA* is associated with emm pattern A-C and E strains (throat specialists and generalists), skin specialists typically harbor *nra* (Bessen et al. 2005).

The plasminogen activator, streptokinase, has been found in all GAS strains screened to date. Streptokinase is comprised of three distinct domains, α, β, and γ. There is significant variability within the β domain of *ska,* and 3 distinct *ska* alleles

have been described—type 1, type 2a, and 2b (Kalia and Bessen 2004). There is strong genetic linkage between the gene encoding the plasminogen binding M protein (PAM) and type 2b *ska* alleles, which appear to be almost exclusively coinherited (Kalia and Bessen 2004; McArthur et al. 2008). Isolates harboring this allele are typically regarded as "skin trophic". In contrast, cluster 2a alleles are almost exclusively associated with isolates that would be regarded as throat-trophic based on emm pattern A-C, while type 1 *ska* alleles are found in both emm pattern A-C and E backgrounds (Kalia and Bessen 2004).

These preferences for tissue site, and distribution of virulence factors, are not absolute. In geographical regions where GAS infection is endemic, the demarcation line between emm-type and tissue site preference is less well defined. Population surveillance of GAS isolates in Nepal found that 19 % of isolates associated with skin infection display a genetic architecture usually associated with pharyngeal specialists (Sakota et al. 2006), while in an Ethiopian study, 28 % of isolates associated with tonsillitis harbored an emm pattern D, or skin specialist genomic arrangement (Tewodros and Kronvall 2005). Similarly, GAS with an emm pattern D, or skin trophic chromosomal arrangement harboring *ska* type 1 alleles have been reported (Kalia and Bessen 2004). This underscores the high degree of genetic diversity among GAS isolates. In areas where GAS is endemic, recombination between skin and throat specialists appears to be common (Bessen et al. 2011; Kalia et al. 2002). Given these findings, it will remain important to monitor the relationships between virulence factor distribution and tissue tropism in epidemiological studies.

Significant intra-emm-type genetic variation, particularly with respect to regulatory gene profile, phage content, and toxin profile have been observed in the dominant emm-types associated with invasive disease in developed countries. Perhaps the most striking example of this is the reported difference in transcription profile between invasive and colonizing GAS strains of the same emm-type in outbreaks of GAS infection (Johnson et al. 1992; Marcon et al. 1988; Sumby et al. 2006). Expression microarray analysis of M1 isolates from the United States, Canada, and Finland revealed the existence of distinct non-invasive and invasive transcriptome profiles within a group of clinical M1 strains (Sumby et al. 2006). These transcriptome profiles have since been linked to mutations in the control of virulence regulatory sensor kinase (*covRS*; alternatively designated *csrRS*), which is responsible for the regulation of approximately 10 % of the GAS genome (Graham et al. 2002; Sumby et al. 2006). Acquisition of mutations in *covRS* which inactivate the ability of this system to negatively regulate the GAS genome during infection results in upregulation of capsule, loss of SpeB expression and increased disease severity in animal models of infection (Walker et al. 2007). Acquisition of these mutations appears to be dependent on the expression of specific virulence factor genes, including *emm*, *hasA*, and the phage encoded *sda1* (Cole et al. 2011). The presence of mutations in *covRS* associated with clinical invasive GAS isolates have been extensively reported in studies of isolates from developed nations, and these mutations are not restricted to emm1 GAS. A recent study of isolates from Canada that compared the genome sequences of GAS serotype M3 isolates from

human pharyngitis cases and from human invasive disease reported that *covS* mutations occur with a higher frequency in invasive-disease isolates than in pharyngeal isolates (Shea et al. 2011). Several studies report *covS* mutations in GAS isolates recovered from patients with severe STSS (Ato et al. 2008; Ikebe et al. 2010). Similarly, a Hawaiian emm81.0 GAS isolate from the throat that had spread to the bloodstream was shown to have acquired a mutation in *covS* (Garcia et al. 2010).

While these mutations can be linked to "hypervirulence", the association is not absolute. In a retrospective study of GAS clinical isolates from Chile, 77/110 isolates were found to have SNPs in the *covRS* regulon, irrespective of their site of isolation. However, only two of these isolates were found to express the high levels of capsule associated with hypervirulence as a result of mutations in *covS* (Wozniak et al. 2012). In contrast, screening of isolates from the Northern Territory of Australia, where skin infection predominates, found a much lower frequency of *covRS* mutation. Of the invasive ($n = 12$) and superficial ($n = 13$) Northern Territory isolates screened, only one was found to contain a *covS* mutation (Maamary et al. 2010). Whether this lower frequency of mutation is common in regions such as the Northern Territory, where emm-types are diverse, and skin infection is endemic, is unclear due to a lack of data on the *covS* status of clinical isolates from these and other similar regions.

Studies of other virulence markers have been used to track the origins of outbreak strains, and may prove useful in characterizing newly emergent strains of GAS. Exotoxin profile has been used as a means of differentiating GAS isolates in a number of studies. To date, 11 distinct superantigens have been identified. Three of these are chromosomally encoded (SpeG, SpeJ, and SMEZ), while the remaining eight (SpeA, SpeC, SpeH, SpeI, SpeJ, SpeK, SpeL, SpeM, and SSA) are located on temperate phage. Exchange of mobile genetic elements (MGE) such as phage contributes significantly to the genetic diversity seen within the GAS species, and is thought to be involved in the emergence of highly successful virulent clones (Lintges et al. 2010; Maamary et al. 2012; Maripuu et al. 2008). Superantigen profiling may therefore prove useful as a marker for the presence and transfer of prophages that encode additional genes playing a role in GAS pathogenesis or tissue tropism.

A high level of allelic variation within certain superantigen genes has been reported (Bianco et al. 2006; Talkington et al. 1993), and this, combined with the fact that studies of superantigen distribution from different groups often use different primer sets, confounds the comparison of superantigen profiling by different groups. However, a 2012 study of the distribution of superantigens amongst 480 diverse GAS isolates from Portugal has reported the use, for the first time, of a defined primer set enabling amplification of all reported superantigen alleles to date (Friaes et al. 2012). Using this technique, 11 different superantigen profiles were identified. Interestingly, this study found that none of the individual superantigen genes could be linked with a specific emm-type, but rather, superantigen profile shows a strong association with emm-type. Superantigen profile could not be accurately predicated using alternative typing methods (other than PFGE

subtyping), leading to the conclusion that superantigen profile may be a useful predictor of emm-type, but that superantigen profile cannot be inferred from emm-type (Friaz et al. 2012). This assertion is supported by previous findings from numerous studies undertaken in various countries suggesting a link between emm-type and superantigen profile (Commons et al. 2008; Le Hello et al. 2010; Schmitz et al. 2003; Vlaminckx et al. 2003).

The reported circulation of superantigens within the streptococcal population varies temporally. Screening of GAS isolates associated with a reemergence of invasive disease in Denmark between 1999 and 2002 found that the frequency of emm1 isolates harboring *speA* decreased from 94 % in 1999 to 71 % in 2002, while the emm1-specific prevalence of *speC* increased from 25 to 53 % over the same period (Ekelund et al. 2005). Similarly, a comprehensive study of GAS isolates from Melbourne, Australia indicates that strains before the mid-1980s do not typically harbor *speK*, which is found in the genome of a high proportion of contemporary emm3 isolates (Commons et al. 2008). This highlights the potential usefulness of superantigen profiling to monitor the emergence of highly successful GAS clones during epidemics. There is evidence to suggest that superantigen prevalence in the GAS population differs geographically (Commons et al. 2008; Proft et al. 2003). However, cross-study comparison is, as stated previously, confounded by the use of different primer sets between studies.

4 Multilocus Sequence Typing

As virulence factors may be under strong selection pressure, and as is the case with superantigens, be present on mobile genetic elements (MGEs), they are not an ideal target for determination of evolutionary relationships between GAS strains. Multilocus Sequence Typing (MLST) is nucleotide-based method for investigating relationships among bacteria of the same species that targets seven selection neutral house-keeping genes. The genes used in the GAS MLST scheme (http://spyogenes.mlst.net/) are glucose kinase (*gki*), glutamine transport ATP-binding protein (*gtr*), glutamate racemase (*murI*), DNA mismatch repair (*mutS*), transketolase (*recP*), xanthine phosphoribosyltransferase (*xpt*), and acetoacetyl-CoA thiolase (*yql*). The combination of seven allelic variants is used to denote a specific multilocus sequence type (ST). More than 600 STs are listed on the *S. pyogenes* MLST website, indicating that ST is more discriminatory than emm-type.

A strong linkage between emm-type and ST has been reported for isolates from endemic countries (Enright et al. 2001). However a subsequent study by McGregor et al. (McGregor et al. 2004a) examining STs of isolates recovered from the Indigenous population of a remote Australian island (representing a low income region) reported a weaker relationship between emm-type and ST combinations present in isolates from the island and emm-type/ST combinations from other populations. The data can be interpreted as providing strong circumstantial evidence for LGT of the *emm*-gene in regions where GAS diversity is high. Further

complicating interpretation of MLST data was the observation of LGT involving housekeeping present on the 'core' genome (McGregor et al. 2004b). The proportion of emm-types associated with distant genetic backgrounds, as determined by MLST, were found to be much high for skin specialists (pattern D strains) and generalists (pattern E strains) than throat specialists (pattern A-C), suggesting that recombination events in latter occur at a lower frequency (Bessen et al. 2008).

5 Genomic Studies of Group A Streptococcus

It is becoming clear that emm-typing provides limited value when attempting to identify genetic factors linked to niche specialization of specific disease outcomes. Other approaches, examining the presence or absence of multiple genetic factors has been one pathway taken in attempt to provide a more thorough analysis of streptococcal diseases (Bessen et al. 2011; McMillan et al. 2006). Genomic studies are also proving to be pivotal in understanding the level of diversity that exists in the streptococcal population. The first M1 GAS genome was published in 2001 (Ferretti et al. 2001), with genome from other emm-types following soon after (Beres et al. 2002; Green et al. 2005; Smoot et al. 2002). Comparative analyses of these genomes clearly demonstrated the importance of MGEs, LGT, and recombination in generating diversity between emm-types. The majority of GAS genomes possess multiple bacteriophage, which in turn often carry virulence genes predicted to change the virulence of a lineage. Integrative conjugative elements (ICE) and remnant MGEs are also common (Green et al. 2005; Maamary et al. 2012; Smoot et al. 2002; Sumby et al. 2005).

However, it has been the intra-emm-type genomic studies utilizing multiple isolates with defined clinical histories that are providing the greatest insight into how strains with altered capacity to cause disease may evolve (Ben Zakour et al. 2012; Fittipaldi et al. 2012; Maamary et al. 2012; Sumby et al. 2005; Tse et al. 2012). These studies suggest that the acquisition of new MGEs, resulting in the elaboration of novel virulence gene repertoire is an important facet underlying the changes in virulence (Maamary et al. 2012; Sumby et al. 2005). Genomic analysis of the recent invasive disease outbreak associated with GAS emm59 provides an illustrative example of the value of population-based genomics. Prior to 2005, emm59 was not considered to be an emm-type associated with major outbreaks of invasive disease. However, in the second half of the decade, more than 500 invasive disease cases associated with this emm-type were reported in Canada (Tyrrell et al. 2010). Comparison of the core genome of outbreak isolates with historical and temporally distinct clones found the outbreak isolates to form a genetically distinct group (Fittipaldi et al. 2012). However, differences in the core genome between outbreak and non-outbreak isolates were remarkably small. While differences in MGE content between the outbreak strains and non-outbreak strain were apparent, as were difference in biological properties, the identity of putative genes within the MGEs that contribute to the relative differences in virulence could not be identified.

The first genomic analysis of GAS emm12 isolates involved in a scarlet fever outbreak were also recently reported (Tse et al. 2012). Unlike the emm59 outbreak, this outbreak appeared to be multiclonal. A novel ICE and bacteriophage were found to be distributed throughout the different clonal lineages involved in the outbreak. The latter contains genes encoding superantigens, and were hypothesized to be one of the reason for the observed increase in virulence.

6 The Role of Other *β*-Hemolytic Bacteria in 'GAS-Associated Diseases

Streptococcus dysgalactiae subsp. *equisimilis* (SDSE) is generally considered as a commensal organism with occasional potential to cause diseases in humans. Over the past few decades there have been several reports of SDSE causing diseases that are normally attributed to GAS. The list of diseases includes tonsillitis, skin infections, post streptococcus arthritis, pleuropneumonia, meningitis, endocarditis, puerperal septicaemia, necrotizing fasciitis, toxic shock syndrome (Jensen and Kilian 2012).

However, a possible link between SDSE and RF/RHD is still deemed as circumstantial, and controversial, with a general recalcitrance among the medical community against the view that *β*-hemolytic streptococci other than GAS (for example SDSE) could also be associated with RF/RHD. A major problem in definitively demonstrating a link between SDSE and RF in clinical settings is the long lag between infection and RF, and possible requirement for multiple episodes of infection, both of which call for well-controlled follow up studies. Such studies are near impossible as SDSE and GAS do cocolonize at the same tissue sites. Animal models of RHD are also in their infancy (Lymbury et al. 2003).

Despite the resistance to the accommodation of alternative views, several observations have strongly supported possible involvement of SDSE in the pathogenesis of RF/RHD. We and others (Bramhachari et al. 2010; McDonald et al. 2007) have observed that the GAS throat isolation rate in some communities is not commensurate with the RF/RHD burden. However, in these same population SDSE isolation rates from the throats are high. Indeed SDSE, but not GAS, has been recovered from an Indigenous Australian child after recurrent severe pharyngitis which was followed by RF in this patient (Davies et al. 2005) suggesting a role for SDSE in the pathogenesis of RF. All SDSE strains also express the M protein which may elicit heart-tissue cross-reactive autoantibodies (Haidan et al. 2000). In this study purified F(ab')2 fragments from the Indigenous Australians reacted with surface protein extracts of SDSE, but not with those of GAS skin isolates (Haidan et al. 2000). RF/RHD associated GAS M types are also reported to possess collagen binding motifs in their M proteins suggesting a possible role for this interaction in the pathogenesis of RF/RHD (Dinkla et al. 2003) Interestingly, similar motifs were also seen in some SDSE M proteins (Dinkla et al. 2007). Collectively, these above

observations strongly suggest SDSE possessed many of the same characteristics as GAS that is linked to the pathogenesis of RF/RHD.

Phylogenically SDSE and GAS are closely related to each other (Lefebure et al. 2012). While there are conspicuous absences of some virulence genes in SDSE, up to half of GAS genes encoding virulence factors or surface associated proteins could be present in SDSE (Davies et al. 2007). Intra and Interspecies LGTs in GAS and SDSE have been long recognized (Sriprakash and Hartas 1996; Towers et al. 2004) with phages and ICEs contributing significantly to the population structure of these streptococci. Clonal relationships revealed that recombination far outweigh mutation in generating clonal variants. While both replacement and additive changes occur through LGT, characteristics that influence host-pathogen interactions are likely to be due to additive LGT (Choi et al. 2012).

Interestingly, cross-species LGTs between GAS and SDSE seem to be predominantly unidirectional; from the former to the latter (Choi et al. 2012). This seems to be true even for selective-neutral housekeeping genes (McMillan et al. 2010). However, although the same emmSTs were found in different clonal complexes of GAS and SDSE suggesting occurrence of LGT of the emm-gene within the species, cross-species replacement of this gene was rarely observed. Likewise, SDSE with group A carbohydrate was found rarely. All SDSE isolates are speB-negative (Bramhachari et al. 2010) and no instance of additive LGT for this locus was ever reported to our knowledge. These observations, and mechanisms such as general resistance to phage mediated changes in SDSE due to clustered regularly interspaced short palindromic repeats (Shimomura et al. 2011) suggest that despite extensive ongoing LGTs, species demarcation between SDSE and GAS is unlikely to blur. However, with extensive additive LGTs into SDSE and sharing of many virulence characteristics the epidemiology of GAS diseases may be blurred in regions of high endemicity.

7 Conclusion

In a relatively short time, studies of the molecular epidemiology of GAS have progressed from the study of single genes, to population-based genomic comparisons. These more comprehensive studies have led to a view of GAS as highly dynamic organism. In endemic regions, where the number of circulating emm-types is high, no one lineage is associated with disease. SDSE is also abundant in these regions. In non-endemic countries, a small pool of emm-types that fluctuates at the local level is present. Ongoing LGT and recombination occasionally give rise to new lineages whose novel genetic repertoire may increase their virulence and disease causing potential. Mutation of core genes is a second mechanism that can also change the virulence properties of streptococci. Our inability to identify or validate the role of genes is specific disease outcomes, highlights the complex nature of streptococcal virulence, and suggests that different genetic repertoires may be responsible for the same disease in different lineages. Changes in

accessory virulence gene repertoire occur against a background of core virulence genes that are associated with niche specialization and core pathogenic processes. For a complete understanding of streptococcal functional genomics of large global collections of streptococci will be essential.

References

Abdissa A, Asrat D, Kronvall G, Shittu B, Achiko D, Zeidan M, Yamuah LK, Aseffa A (2006) High diversity of group A streptococcal emm types among healthy schoolchildren in Ethiopia. Clin Infect Dis 42:1362–1367

Ato M, Ikebe T, Kawabata H, Takemori T, Watanabe H (2008) Incompetence of neutrophils to invasive group A streptococcus is attributed to induction of plural virulence factors by dysfunction of a regulator. PLoS One 3:e3455

Beall B, Facklam R, Thompson T (1996) Sequencing emm-specific PCR products for routine and accurate typing of group A streptococci. J Clin Microbiol 34:953–958

Beall B, Gherardi G, Lovgren M, Facklam RR, Forwick BA, Tyrrell GJ (2000) emm and sof gene sequence variation in relation to serological typing of opacity-factor-positive group A streptococci. Microbiology 146(Pt 5):1195–1209

Ben Zakour NL, Venturini C, Beatson SA, Walker MJ (2012) Analysis of a Streptococcus pyogenes puerperal sepsis cluster by use of whole-genome sequencing. J Clin Microbiol 50:2224–2228

Beres SB, Sylva GL, Barbian KD, Lei B, Hoff JS, Mammarella ND, Liu MY, Smoot JC, Porcella SF, Parkins LD, Campbell DS, Smith TM, McCormick JK, Leung DY, Schlievert PM, Musser JM (2002) Genome sequence of a serotype M3 strain of group A Streptococcus: phage-encoded toxins, the high-virulence phenotype, and clone emergence. Proc Natl Acad Sci U S A 99:10078–10083

Bessen DE, Lizano S (2010) Tissue tropisms in group A streptococcal infections. Future Microbiol 5:623–638

Bessen DE, Carapetis JR, Beall B, Katz R, Hibble M, Currie BJ, Collingridge T, Izzo MW, Scaramuzzino DA, Sriprakash KS (2000) Contrasting molecular epidemiology of group A streptococci causing tropical and nontropical infections of the skin and throat. J Infect Dis 182:1109–1116

Bessen DE, Manoharan A, Luo F, Wertz JE, Robinson DA (2005) Evolution of transcription regulatory genes is linked to niche specialization in the bacterial pathogen Streptococcus pyogenes. J Bacteriol 187:4163–4172

Bessen DE, McGregor KF, Whatmore AM (2008) Relationships between emm and multilocus sequence types within a global collection of Streptococcus pyogenes. BMC Microbiol 8:59

Bessen DE, Kumar N, Hall GS, Riley DR, Luo F, Lizano S, Ford CN, McShan WM, Nguyen SV, Dunning Hotopp JC, Tettelin H (2011) Whole-genome association study on tissue tropism phenotypes in group A Streptococcus. J Bacteriol 193:6651–6663

Bianco S, Allice T, Zucca M, Savoia D (2006) Survey of phenotypic and genetic features of streptococcus pyogenes strains isolated in Northwest Italy. Curr Microbiol 52:33–39

Bisno AL, Brito MO, Collins CM (2003) Molecular basis of group A streptococcal virulence. Lancet Infect Dis 3:191–200

Bramhachari PV, Kaul SY, McMillan DJ, Shaila MS, Karmarkar MG, Sriprakash KS (2010) Disease burden due to Streptococcus dysgalactiae subsp. equisimilis (group G and C streptococcus) is higher than that due to Streptococcus pyogenes among Mumbai school children. J Med Microbiol 59:220–223

Carapetis JR, Steer AC, Mulholland EK, Weber M (2005) The global burden of group A streptococcal diseases. Lancet Infect Dis 5:685–694

Choi SC, Rasmussen MD, Hubisz MJ, Gronau I, Stanhope MJ, Siepel A (2012) Replacing and additive horizontal gene transfer in Streptococcus. Mol Biol Evol 29:3309−3320

Cole JN, Barnett TC, Nizet V, Walker MJ (2011) Molecular insight into invasive group A streptococcal disease. Nat Rev Microbiol 9:724–736

Commons R, Rogers S, Gooding T, Danchin M, Carapetis J, Robins-Browne R, Curtis N (2008) Superantigen genes in group A streptococcal isolates and their relationship with emm types. J Med Microbiol 57:1238–1246

Courtney HS, Pownall HJ (2010) The structure and function of serum opacity factor: a unique streptococcal virulence determinant that targets high-density lipoproteins. J Biomed Biotechnol 2010:956071

Cunningham MW (2000) Pathogenesis of group A streptococcal infections. Clin Microbiol Rev 13:470–511

Davies MR, Tran TN, McMillan DJ, Gardiner DL, Currie BJ, Sriprakash KS (2005) Inter-species genetic movement may blur the epidemiology of streptococcal disease in endemic regions. Microbes Infect 7:1128–1138

Davies MR, McMillan DJ, Beiko RG, Barroso V, Geffers R, Sriprakash KS, Chhatwal GS (2007) Virulence profiling of Streptococcus dysgalactiae subspecies equisimilis isolated from infected humans reveals 2 distinct genetic lineages that do not segregate with their phenotypes or propensity to cause diseases. Clin Infect Dis 44:1442–1454

Dey N, McMillan DJ, Yarwood PJ, Joshi RM, Kumar R, Good MF, Sriprakash KS, Vohra H (2005) High diversity of group A Streptococcal emm types in an Indian community: the need to tailor multivalent vaccines. Clin Infect Dis 40:46–51

Dhanda V, Vohra H, Kumar R (2011) Virulence potential of Group A streptococci isolated from throat cultures of children from north India. Indian J Med Res 133:674–680

Dinkla K, Rohde M, Jansen WT, Kaplan EL, Chhatwal GS, Talay SR (2003) Rheumatic fever-associated Streptococcus pyogenes isolates aggregate collagen. J Clin Invest. 111:1905–1912

Dinkla K, Nitsche-Schmitz DP, Barroso V, Reissmann S, Johansson HM, Frick IM, Rohde M, Chhatwal GS (2007) Identification of a streptococcal octapeptide motif involved in acute rheumatic fever. J Biol Chem 282:18686–18693

Ekelund K, Skinhoj P, Madsen J, Konradsen HB (2005) Reemergence of emm1 and a changed superantigen profile for group A streptococci causing invasive infections: results from a nationwide study. J Clin Microbiol 43:1789–1796

Enright MC, Spratt BG, Kalia A, Cross JH, Bessen DE (2001) Multilocus sequence typing of Streptococcus pyogenes and the relationships between emm type and clone. Infect Immun 69:2416–2427

Erdem G, Ford JM, Kanenaka RY, Abe L, Yamaga K, Effler PV (2005) Molecular epidemiologic comparison of 2 unusual clusters of group a streptococcal necrotizing fasciitis in Hawaii. Clin Infect Dis 40:1851–1854

Erdem G, Mizumoto C, Esaki D, Reddy V, Kurahara D, Yamaga K, Abe L, Johnson D, Yamamoto K, Kaplan EL (2007) Group A streptococcal isolates temporally associated with acute rheumatic fever in Hawaii: differences from the continental United States. Clin Infect Dis (an official publication of the Infectious Diseases Society of America) 45:e20–e24

Facklam RF, Martin DR, Lovgren M, Johnson DR, Efstratiou A, Thompson TA, Gowan S, Kriz P, Tyrrell GJ, Kaplan E, Beall B (2002) Extension of the lancefield classification for group A streptococci by addition of 22 new M protein gene sequence types from clinical isolates: emm103 to emm124. Clin Infect Dis 34:28–38

Ferretti JJ, McShan WM, Ajdic D, Savic DJ, Savic G, Lyon K, Primeaux C, Sezate S, Suvorov AN, Kenton S, Lai HS, Lin SP, Qian Y, Jia HG, Najar FZ, Ren Q, Zhu H, Song L, White J, Yuan X, Clifton SW, Roe BA, McLaughlin R (2001) Complete genome sequence of an M1 strain of Streptococcus pyogenes. Proc Natl Acad Sci U S A 98:4658–4663

Fischetti VA (1989) Streptococcal M protein: molecular design and biological behavior. Clin Microbiol Rev 2:285–314

Fittipaldi N, Beres SB, Olsen RJ, Kapur V, Shea PR, Watkins ME, Cantu CC, Laucirica DR, Jenkins L, Flores AR, Lovgren M, Ardanuy C, Linares J, Low DE, Tyrrell GJ, Musser JM

(2012) Full-genome dissection of an epidemic of severe invasive disease caused by a hypervirulent, recently emerged clone of group A Streptococcus. Am J Pathol 180:1522–1534
Friaes A, Pinto FR, Silva-Costa C, Ramirez M, and Melo-Cristino J (2012) Superantigen gene complement of Streptococcus pyogenes-relationship with other typing methods and short-term stability. Eur J Clin Microbiol Infect Dis [Epub ahead of print]
Garcia AF, Abe LM, Erdem G, Cortez CL, Kurahara D, Yamaga K (2010) An insert in the covS gene distinguishes a pharyngeal and a blood isolate of Streptococcus pyogenes found in the same individual. Microbiology 156:3085–3095
Gardiner D, Hartas J, Currie B, Mathews JD, Kemp DJ, Sriprakash KS (1995) Vir typing: a long-PCR typing method for group A streptococci. PCR Methods Appl. 4:288–293
Goodfellow AM, Hibble M, Talay SR, Kreikemeyer B, Currie BJ, Sriprakash KS, Chhatwal GS (2000) Distribution and antigenicity of fibronectin binding proteins (SfbI and SfbII) of Streptococcus pyogenes clinical isolates from the northern territory. Australia. J Clin Microbiol 38:389–392
Graham MR, Smoot LM, Migliaccio CA, Virtaneva K, Sturdevant DE, Porcella SF, Federle MJ, Adams GJ, Scott JR, Musser JM (2002) Virulence control in group A Streptococcus by a two-component gene regulatory system: global expression profiling and in vivo infection modeling. Proc Natl Acad Sci U S A. 99:13855–13860
Green NM, Zhang S, Porcella SF, Nagiec MJ, Barbian KD, Beres SB, LeFebvre RB, Musser JM (2005) Genome sequence of a serotype M28 strain of group a streptococcus: potential new insights into puerperal sepsis and bacterial disease specificity. J Infect Dis 192:760–770
Haidan A, Talay SR, Rohde M, Sriprakash KS, Currie BJ, Chhatwal GS (2000) Pharyngeal carriage of group C and group G streptococci and acute rheumatic fever in an Aboriginal population. Lancet 356:1167–1169
Hollingshead SK, Readdy TL, Yung DL, Bessen DE (1993) Structural heterogeneity of the emm gene cluster in group A streptococci. Mol Microbiol 8:707–717
Hollingshead SK, Arnold J, Readdy TL, Bessen DE (1994) Molecular evolution of a multigene family in group A streptococci. Mol Biol Evol 11:208–219
Ikebe T, Ato M, Matsumura T, Hasegawa H, Sata T, Kobayashi K, Watanabe H (2010) Highly frequent mutations in negative regulators of multiple virulence genes in group A streptococcal toxic shock syndrome isolates. PLoS Pathog 6:e1000832
Jensen A, Kilian M (2012) Delineation of Streptococcus dysgalactiae, its subspecies, and its clinical and phylogenetic relationship to Streptococcus pyogenes. J Clin Microbiol 50:113–126
Johnson DR, Stevens DL, Kaplan EL (1992) Epidemiologic analysis of group A streptococcal serotypes associated with severe systemic infections, rheumatic fever, or uncomplicated pharyngitis. J Infect Dis 166:374–382
Johnson DR, Kaplan EL, VanGheem A, Facklam RR, Beall B (2006) Characterization of group A streptococci (Streptococcus pyogenes): correlation of M-protein and emm-gene type with T-protein agglutination pattern and serum opacity factor. J Med Microbiol 55:157–164
Kalia A, Bessen DE (2004) Natural selection and evolution of streptococcal virulence genes involved in tissue-specific adaptations. J Bacteriol 186:110–121
Kalia A, Spratt BG, Enright MC, Bessen DE (2002) Influence of recombination and niche separation on the population genetic structure of the pathogen Streptococcus pyogenes. Infect Immun 70:1971–1983
Kratovac Z, Manoharan A, Luo F, Lizano S, Bessen DE (2007) Population genetics and linkage analysis of loci within the FCT region of Streptococcus pyogenes. J Bacteriol 189:1299–1310
Kreikemeyer B, McIver KS, Podbielski A (2003) Virulence factor regulation and regulatory networks in Streptococcus pyogenes and their impact on pathogen-host interactions. Trends Microbiol 11:224–232
Lancefield RC (1962) Current knowledge of type-specific M antigens of group A streptococci. J Immunol 89:307–313
Le Hello S, Doloy A, Baumann F, Roques N, Coudene P, Rouchon B, Lacassin F, Bouvet A (2010) Clinical and microbial characteristics of invasive Streptococcus pyogenes disease in

New Caledonia, a region in Oceania with a high incidence of acute rheumatic fever. J Clin Microbiol 48:526–530

Lefebure T, Richards VP, Lang P, Pavinski-Bitar P, Stanhope MJ (2012) Gene repertoire evolution of Streptococcus pyogenes inferred from phylogenomic analysis with Streptococcus canis and Streptococcus dysgalactiae. PLoS One 7:e37607

Lintges M, van der Linden M, Hilgers RD, Arlt S, Al-Lahham A, Reinert RR, Plucken S, Rink L (2010) Superantigen genes are more important than the emm type for the invasiveness of group A Streptococcus infection. J Infect Dis 202:20–28

Lymbury RS, Olive C, Powell KA, Good MF, Hirst RG, LaBrooy JT, Ketheesan N (2003) Induction of autoimmune valvulitis in Lewis rats following immunization with peptides from the conserved region of group A streptococcal M protein. J Autoimmun 20:211–217

Maamary PG, Sanderson-Smith ML, Aziz RK, Hollands A, Cole JN, McKay FC, McArthur JD, Kirk JK, Cork AJ, Keefe RJ, Kansal RG, Sun H, Taylor WL, Chhatwal GS, Ginsburg D, Nizet V, Kotb M, Walker MJ (2010) Parameters governing invasive disease propensity of non-M1 serotype group A streptococci. J Innate Immun 2:596–606

Maamary PG, Ben Zakour NL, Cole JN, Hollands A, Aziz RK, Barnett TC, Cork AJ, Henningham A, Sanderson-Smith M, McArthur JD, Venturini C, Gillen CM, Kirk JK, Johnson DR, Taylor WL, Kaplan EL, Kotb M, Nizet V, Beatson SA, and Walker MJ (2012) Tracing the evolutionary history of the pandemic group A streptococcal M1T1 clone. FASEB J 26:4674−4684

Marcon MJ, Hribar MM, Hosier DM, Powell DA, Brady MT, Hamoudi AC, Kaplan EL (1988) Occurrence of mucoid M-18 Streptococcus pyogenes in a central Ohio pediatric population. J Clin Microbiol 26:1539–1542

Maripuu L, Eriksson A, Norgren M (2008) Superantigen gene profile diversity among clinical group A streptococcal isolates. FEMS Immunol Med Microbiol 54:236–244

Martin JM, Barbadora KA (2006) Continued high caseload of rheumatic fever in western Pennsylvania: possible rheumatogenic emm types of streptococcus pyogenes. J pediatr 149:58–63

Maxted WR, Widdowson JP, Fraser CA (1973) Antibody to streptococcal opacity factor in human sera. J Hyg (Lond). 71:35–42

McArthur JD, McKay FC, Ramachandran V, Shyam P, Cork AJ, Sanderson-Smith ML, Cole JN, Ringdahl U, Sjobring U, Ranson M, Walker MJ (2008) Allelic variants of streptokinase from Streptococcus pyogenes display functional differences in plasminogen activation. FASEB J 22:3146–3153

McDonald M, Towers RJ, Andrews RM, Carapetis JR, Currie BJ (2007) Epidemiology of Streptococcus dysgalactiae subsp. equisimilis in tropical communities. Northern Austral Emerg Infect Dis. 13:1694–1700

McGregor KF, Bilek N, Bennett A, Kalia A, Beall B, Carapetis JR, Currie BJ, Sriprakash KS, Spratt BG, Bessen DE (2004a) Group A streptococci from a remote community have novel multilocus genotypes but share emm types and housekeeping alleles with isolates from worldwide sources. J Infect Dis 189:717–723

McGregor KF, Spratt BG, Kalia A, Bennett A, Bilek N, Beall B, Bessen DE (2004b) Multilocus sequence typing of Streptococcus pyogenes representing most known emm types and distinctions among subpopulation genetic structures. J Bacteriol 186:4285–4294

McMillan DJ, Beiko RG, Geffers R, Buer J, Schouls LM, Vlaminckx BJ, Wannet WJ, Sriprakash KS, Chhatwal GS (2006) Genes for the majority of group a streptococcal virulence factors and extracellular surface proteins do not confer an increased propensity to cause invasive disease. Clin Infect Dis 43:884–891

McMillan DJ, Bessen DE, Pinho M, Ford C, Hall GS, Melo-Cristino J, Ramirez M (2010) Population genetics of Streptococcus dysgalactiae subspecies equisimilis reveals widely dispersed clones and extensive recombination. PLoS One 5:e11741

McNeil SA, Halperin SA, Langley JM, Smith B, Warren A, Sharratt GP, Baxendale DM, Reddish MA, Hu MC, Stroop SD, Linden J, Fries LF, Vink PE, Dale JB (2005) Safety and immunogenicity of 26-valent group a streptococcus vaccine in healthy adult volunteers. Clin Infect Dis 41:1114–1122

O'Loughlin RE, Roberson A, Cieslak PR, Lynfield R, Gershman K, Craig A, Albanese BA, Farley MM, Barrett NL, Spina NL, Beall B, Harrison LH, Reingold A, Van Beneden C (2007) The epidemiology of invasive group A streptococcal infection and potential vaccine implications: United States, 2000–2004. Clin Infect Dis 45:853–862

Panchaud A, Guy L, Collyn F, Haenni M, Nakata M, Podbielski A, Moreillon P, Roten CA (2009) M-protein and other intrinsic virulence factors of Streptococcus pyogenes are encoded on an ancient pathogenicity island. BMC Genomics 10:198

Parks T, Smeesters PR, Steer AC (2012) Streptococcal skin infection and rheumatic heart disease. Curr Opin Infect Dis 25:145–153

Proft T, Webb PD, Handley V, Fraser JD (2003) Two novel superantigens found in both group A and group C Streptococcus. Infect Immun 71:1361–1369

Ramachandran V, McArthur JD, Behm CE, Gutzeit C, Dowton M, Fagan PK, Towers R, Currie B, Sriprakash KS, Walker MJ (2004) Two distinct genotypes of prtF2, encoding a fibronectin binding protein, and evolution of the gene family in Streptococcus pyogenes. J Bacteriol 186:7601–7609

Sakota V, Fry AM, Lietman TM, Facklam RR, Li Z, Beall B (2006) Genetically diverse group A streptococci from children in far-western Nepal share high genetic relatedness with isolates from other countries. J Clin Microbiol 44:2160–2166

Schmitz FJ, Beyer A, Charpentier E, Normark BH, Schade M, Fluit AC, Hafner D, Novak R (2003) Toxin-gene profile heterogeneity among endemic invasive European group A streptococcal isolates. J Infect Dis 188:1578–1586

Shea PR, Beres SB, Flores AR, Ewbank AL, Gonzalez-Lugo JH, Martagon-Rosado AJ, Martinez-Gutierrez JC, Rehman HA, Serrano-Gonzalez M, Fittipaldi N, Ayers SD, Webb P, Willey BM, Low DE, Musser JM (2011) Distinct signatures of diversifying selection revealed by genome analysis of respiratory tract and invasive bacterial populations. Proc Natl Acad Sci U S A 108:5039–5044

Shimomura Y, Okumura K, Murayama SY, Yagi J, Ubukata K, Kirikae T, Miyoshi-Akiyama T (2011) Complete genome sequencing and analysis of a Lancefield group G Streptococcus dysgalactiae subsp. equisimilis strain causing streptococcal toxic shock syndrome (STSS). BMC Genomics 12:17

Shulman ST, Tanz RR, Kabat W, Kabat K, Cederlund E, Patel D, Li Z, Sakota V, Dale JB, Beall B (2004) Group A streptococcal pharyngitis serotype surveillance in North America, 2000–2002. Clin Infect Dis 39:325–332

Shulman ST, Tanz RR, Dale JB, Beall B, Kabat W, Kabat K, Cederlund E, Patel D, Rippe J, Li Z, Sakota V (2009) Seven-year surveillance of North American pediatric group a streptococcal pharyngitis isolates. Clin Infect Dis 49:78–84

Smeesters PR, Vergison A, Campos D, de Aguiar E, Miendje Deyi VY, Van Melderen L (2006) Differences between Belgian and Brazilian group A Streptococcus epidemiologic landscape. PLoS One 1:e10

Smeesters PR, Mardulyn P, Vergison A, Leplae R, Van Melderen L (2008) Genetic diversity of Group A Streptococcus M protein: implications for typing and vaccine development. Vaccine 26:5835–5842

Smeesters PR, McMillan DJ, Sriprakash KS, Georgousakis MM (2009) Differences among group A streptococcus epidemiological landscapes: consequences for M protein-based vaccines? Expert Rev Vaccines 8:1705–1720

Smeesters PR, Dramaix M, Van Melderen L (2010a) The emm-type diversity does not always reflect the M protein genetic diversity—is there a case for designer vaccine against GAS. Vaccine 28:883–885

Smeesters PR, McMillan DJ, Sriprakash KS (2010b) The streptococcal M protein: a highly versatile molecule. Trends Microbiol 18:275–282

Smoot JC, Barbian KD, Van Gompel JJ, Smoot LM, Chaussee MS, Sylva GL, Sturdevant DE, Ricklefs SM, Porcella SF, Parkins LD, Beres SB, Campbell DS, Smith TM, Zhang Q, Kapur V, Daly JA, Veasy LG, Musser JM (2002) Genome sequence and comparative microarray analysis

of serotype M18 group A Streptococcus strains associated with acute rheumatic fever outbreaks. Proc Natl Acad Sci U S A 99:4668–4673

Sriprakash KS, Hartas J (1996) Lateral genetic transfers between group A and G streptococci for M-like genes are ongoing. Microb Pathog 20:275–285

Steer AC, Jenney A, Kado J, Good MF, Batzloff M, Waqatakirewa L, Mullholland EK, Carapetis JR (2009a) Prospective surveillance of invasive group a streptococcal disease, Fiji, 2005–2007. Emerg Infect Dis 15:216–222

Steer AC, Jenney AW, Kado J, Good MF, Batzloff M, Magor G, Ritika R, Mulholland KE, Carapetis JR (2009b) Prospective surveillance of streptococcal sore throat in a tropical country. Pediatr Infect Dis J 28:477–482

Steer AC, Kado J, Jenney AW, Batzloff M, Waqatakirewa L, Mulholland EK, Carapetis JR (2009c) Acute rheumatic fever and rheumatic heart disease in Fiji: prospective surveillance, 2005–2007. Med J Aust 190:133–135

Steer AC, Law I, Matatolu L, Beall BW, Carapetis JR (2009d) Global emm type distribution of group A streptococci: systematic review and implications for vaccine development. Lancet Infect Dis 9:611–616

Steer AC, Magor G, Jenney AW, Kado J, Good MF, McMillan D, Batzloff M, Carapetis JR (2009e) emm and C-repeat region molecular typing of beta-hemolytic streptococci in a tropical country: implications for vaccine development. J Clin Microbiol 47(8):2502–2509

Stollerman GH (2001) Rheumatic fever in the 21st century. Clin Infect Dis 33:806–814

Sumby P, Porcella SF, Madrigal AG, Barbian KD, Virtaneva K, Ricklefs SM, Sturdevant DE, Graham MR, Vuopio-Varkila J, Hoe NP, Musser JM (2005) Evolutionary origin and emergence of a highly successful clone of serotype M1 group a Streptococcus involved multiple horizontal gene transfer events. J Infect Dis 192:771–782

Sumby P, Whitney AR, Graviss EA, DeLeo FR, Musser JM (2006) Genome-wide analysis of group a streptococci reveals a mutation that modulates global phenotype and disease specificity. PLoS Pathog 2:e5

Talkington DF, Schwartz B, Black CM, Todd JK, Elliott J, Breiman RF, Facklam RR (1993) Association of phenotypic and genotypic characteristics of invasive Streptococcus pyogenes isolates with clinical components of streptococcal toxic shock syndrome. Infect Immun 61:3369–3374

Tewodros W, Kronvall G (2005) M protein gene (emm type) analysis of group A beta-hemolytic streptococci from Ethiopia reveals unique patterns. J Clin Microbiol 43:4369–4376

Towers RJ, Gal D, McMillan D, Sriprakash KS, Currie BJ, Walker MJ, Chhatwal GS, Fagan PK (2004) Fibronectin-binding protein gene recombination and horizontal transfer between group A and G streptococci. J Clin Microbiol 42:5357–5361

Tse H, Bao JY, Davies MR, Maamary P, Tsoi HW, Tong AH, Ho TC, Lin CH, Gillen CM, Barnett TC, Chen JH, Lee M, Yam WC, Wong CK, Ong CL, Chan YW, Wu CW, Ng T, Lim WW, Tsang TH, Tse CW, Dougan G, Walker MJ, Lok S, Yuen KY (2012) Molecular characterization of the 2011 Hong Kong scarlet fever outbreak. J Infect Dis 206:341–351

Tyrrell GJ, Lovgren M, St Jean T, Hoang L, Patrick DM, Horsman G, Van Caeseele P, Sieswerda LE, McGeer A, Laurence RA, Bourgault AM, Low DE (2010) Epidemic of group A Streptococcus M/emm59 causing invasive disease in Canada. Clin Infect Dis 51:1290–1297

Vlaminckx BJ, Mascini EM, Schellekens J, Schouls LM, Paauw A, Fluit AC, Novak R, Verhoef J, Schmitz FJ (2003) Site-specific manifestations of invasive group a streptococcal disease: type distribution and corresponding patterns of virulence determinants. J Clin Microbiol 41:4941–4949

Vlaminckx BJ, Schuren FH, Montijn RC, Caspers MP, Fluit AC, Wannet WJ, Schouls LM, Verhoef J, Jansen WT (2007) Determination of the relationship between group A streptococcal genome content, M type, and toxic shock syndrome by a mixed genome microarray. Infect Immun 75:2603–2611

Walker MJ, Hollands A, Sanderson-Smith ML, Cole JN, Kirk JK, Henningham A, McArthur JD, Dinkla K, Aziz RK, Kansal RG, Simpson AJ, Buchanan JT, Chhatwal GS, Kotb M, Nizet V (2007) DNase Sda1 provides selection pressure for a switch to invasive group A streptococcal infection. Nat Med 13:981–985

Whatmore AM, Kehoe MA (1994) Horizontal gene transfer in the evolution of group A streptococcal emm-like genes: gene mosaics and variation in Vir regulons. Mol Microbiol 11:363–374

Wozniak A, Rojas P, Rodriguez C, Undabarrena A, Garate C, Riedel I, Roman JC, Kalergis AM, Garcia P (2012) M-protein gene-type distribution and hyaluronic acid capsule in group A Streptococcus clinical isolates in Chile: association of emm gene markers with csrR alleles. Epidemiol Infect 140:1286–1295

http://www.springer.com/978-3-642-36339-9

Host-Pathogen Interactions in Streptococcal Diseases
Chhatwal, G.S. (Ed.)
2013, VIII, 255 p., Hardcover
ISBN: 978-3-642-36339-9