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Association of the *prtF1* Gene (Encoding Fibronectin-Binding Protein F1) and the *sic* Gene (Encoding the Streptococcal Inhibitor of Complement) with *emm* Types of Group A Streptococci Isolated from Japanese Children with Pharyngitis

Xiaoming Ma,[1] Hideaki Kikuta,[1]* Nobuhisa Ishiguro,[1] Mikio Yoshioka,[1] Takashi Ebihara,[1] Teiko Murai,[2] Intetu Kobayashi,[3] and Kunihiko Kobayashi[1]

Department of Pediatrics, Hokkaido University School of Medicine, Sapporo,[1] and School of Nursing, Faculty of Medicine, Toho University,[2] and Department of Infectious Diseases, Mitsubishi Kagaku Bio-Clinical Laboratory,[3] Tokyo, Japan

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A total of 66 clinical isolates of group A streptococci (GAS) were obtained from 66 Japanese children with pharyngitis. The *prtF1* gene (encoding fibronectin-binding protein F1) and the *sic* gene (encoding the streptococcal inhibitor of complement) were present in 51 (77.3%) and 48 (72.7%) of the 66 isolates, respectively. These results indicated that a high prevalence of two virulence genes, *prtF1* and *sic*, is characteristic of GAS in Japan.

Group A streptococci (GAS; also referred to as S*treptococcus pyogenes*) are major etiological agents causing a wide variety of human diseases, ranging from pharyngitis to severe invasive diseases (3). The N-terminal region of the M protein is located distally to the surface of the bacterium and displays antigenic diversity. This heterogeneity provides the basis for identifying more than 100 different GAS serotypes (6). However, many GAS isolates are often nontypeable because of the lack of appropriate type-specific antisera or because of a loss of antigen expression under cultivation conditions. T typing has also been used as an alternative or supplement to M typing. The T antigen is a trypsin-resistant cell surface protein that exhibits extensive antigenic diversity but is not defined as a virulence factor (3). Recently, a genomic typing method based on the variable 5′ *emm* gene has become widely used, and more than 150 different types of the *emm* gene, encoding the N-terminal region of the M protein, have been characterized (13) (http://www.cdc.gov/ncidod/biotech/strep/strains.html). This methodology has enabled the recognition of several previously unknown GAS M types in different geographic areas. Additionally, *emm* typing has been shown to be useful for detecting genetic diversity among GAS isolates.

GAS cell surface proteins have been identified as virulence factors. More than 40 putative virulence-associated genes have also been identified (5). Some of these GAS cell surface proteins, such as fibronectin-binding proteins, function as adhesins. Adherence of GAS to pharyngeal or dermal epithelial cells is the first step in colonization of the host and development of infectious disease. Other GAS cell surface proteins, such as the streptococcal inhibitor of complement, are protective against the host defense system or trigger a severe nonspecific immunological response in the human host (3). The M protein, an important GAS virulence factor, has a dual role: it is a major antiphagocytic factor of GAS, and it is also an adhesin that binds fibronectin, fibrinogen, and albumin. Fibronectin-binding protein F1, encoded by the *prtF1* gene, is one of the adhesins; it has been shown to be important for binding of the bacterium to the extracellular matrix of respiratory epithelial cells and for promoting entry into these cells (8). Intracellular localization of GAS has been suggested as an explanation for the failure of penicillin treatment of GAS infections (4). However, the relationship between the presence of the *prtF1* gene and the failure of eradication is still controversial (2, 4, 11). The protein encoded by the *sic* gene is expressed extracellularly and inhibits in vitro the normal cytolytic function of the complement C5b-C9 membrane attack complex, which is responsible for target killing (1). The presence of the *sic* gene in GAS strains of 55 different M serotypes has been investigated; the genes have been reported to be present in M1- and M57-type strains but absent from other GAS strains (1). The purpose of this study was to determine the phenotypic (M and T types and azithromycin [AZM] sensitivity) and genotypic (*emm* type and the presence or absence of the *prtF1* and *sic* genes) characteristics of 66 GAS isolates from 66 pediatric patients (2 to 12 years old) with pharyngitis treated at eight hospitals in Hokkaido, the northernmost island of Japan, during the period from August 2000 to March 2001.

All 66 isolates were examined for M and T types by standard M protein precipitation in gel agar and by the T agglutination method, respectively (9). M-type-specific antisera were available for types 1, 2, 3, 4, 5, 6, 8, 9, 11, 12, 13, 14, 18, 19, 22, 23, 27, 28, 29, 39, 41, 49, 53, 56, and Matsuyama 2166 (a provisional type). The variable 5′ *emm* type was examined by sequence analysis of *emm* gene-specific PCR products (13). The primer sequences used were 5′-TATT(C/G)GCTTAGAAAA

* Corresponding author. Mailing address: Department of Pediatrics, Hokkaido University School of Medicine, N-15, W-7, Kita-ku, Sapporo 060-8638, Japan. Phone: 81-11-716-1161, ext. 5954. Fax: 81-11-706-7898. E-mail: hide-ki@med.hokudai.ac.jp.

TABLE 1. T Types, M types, and *emm* types of the isolates, and presence of *sic* and *prtF1* genes

emm type[a]	M/T type[a,b]	No. of strains with:								
		sic present	*sic* absent	*prtF1* present						*prtF1* absent
				Total	With RD2 repeat no. of:					
					1	2	3	4	6	
1 (1)	1/1 (1)	1								1
2 (4)	2–28/2–28 (4)	4								4
4 (17)	4/4 (16)	14	2	16	11	5				
	4/4–28 (1)	1		1	1					
12 (18)	12/12 (18)	13	5	18				18		
28 (5)	28/28 (4)	1	3	4		4				
	nt/28 (1)	1		1		1				
75 (4)	Mastuyama 2166/2 (3)	2	1	3			3			
	nt/6 (1)	1		1					1	
77 (2)	nt/28 (2)		2	2			2			
89 (4)	nt/B3265 (4)	2	2	4				4		
94 (10)	nt/B3264 (10)	7	3							10
112 (1)	nt/25-5-4 (1)	1		1	1					

[a] Numbers in parentheses are numbers of isolates.
[b] nt, nontypeable strain.

TTAA-3′ and 5′-GCAAGTTCTTCAGCTTGTTT-3′. The PCR-amplified products were sequenced directly by using a BigDye dye terminator cycle sequencing ready-reaction kit (Perkin-Elmer Applied Biosystems, Tokyo, Japan) with an ABI PRISM 310 genetic analyzer (Perkin-Elmer Applied Biosystems). Primer seq2, 5′-TATTCGCTTAGAAAATTAAAA ACAGG-3′, was used for sequencing. Nucleotide sequences of at least 160 bases were subjected to homology searches in the GenBank (http://www.ncbi.nlm.nih.gov/BLAST) and the Centers for Disease Control and Prevention (http://www.cdc.gov /ncidod/biotech/infotech_hp.html) databases. Table 1 summarizes the association of *emm* type with T type, M type, and the presence or absence of the *prtF1* and *sic* genes. The 66 isolates were of 10 T types and 6 M types, including one provisional T B3264 type and one provisional M Matsuyama 2166 type. Although all of the isolates were T typeable, six exhibited complex forms of T antigens. T types of most GAS isolates were associated with specific M and *emm* types. Commonly isolated strains among the 66 isolates were M4 and M12 types. Ten *emm* types were found among the 66 isolates. The prevalent *emm* types were *emm12* (18 isolates) and *emm4* (16 isolates). No new *emm* type was detected. M Matsuyama 2166-type GAS has been found in Japan, Thailand, and Malaysia, but the *emm* type(s) and its worldwide prevalence are not known (9). The M Matsuyama 2166-type strain was type T2 and type *emm75*. The T B3264 type was correlated with either *emm89* or *emm94*. The MIC of AZM was determined by the agar dilution method according to the National Committee for Clinical Laboratory Standard guidelines. AZM-resistant strains were defined as those for which the MIC of AZM was more than 2 μg/ml. Only 3 (4.5%) of the 66 isolates were resistant to AZM.

The presence of two virulence genes, *prtF1* and *sic*, was determined by PCR. The primer sequences used to amplify the *prtF1* gene and sequence the PCR-amplified products were 5′-TTTTCAGGAAATATGGTTGAGACA-3′ and 5′-TCGC CGTTTCACTGAAACCACTCA-3′. PCR-amplified products were subjected to electrophoresis through a 2.0% agarose gel. The *prtF1* gene was present in 51 (77.3%) of the 66 isolates, which were of various *emm* types, a somewhat higher percentage than previously reported for that gene (2, 7, 10). The *prtF1* gene was not detected in *emm94*-, *emm2*-, and *emm1*-type isolates. The association between the presence of the *prtF1* gene and the *emm* type of the isolates in this study is in agreement with results of previous studies of the distribution of the *prtF1* gene in GAS isolates of various *emm* types (2, 10). Gel electrophoresis revealed that the PCR-amplified products were of five different molecular sizes, ranging from approximately 100 to 750 bp. Repeat domain type 2 (RD2) is composed of 37 amino acid residues repeated fully four times and partially a fifth time (12). PCR-amplified products of 126, 237, 348, 459, and 745 bp corresponded to RD2 repeat numbers of 1, 2, 3, 4, and 6, respectively. The number of RD2 repeats was confirmed by direct sequencing of several representative PCR-amplified products and was constant among individual *emm*-type isolates except for those of type *emm4* or *emm75*. The primer sequences used to amplify the *sic* gene and sequence the PCR-amplified products were 5′-GATGAGACAGAAGA TAAAC-3′ and 5′-TTACGTTGCTGATGGTGTAT-3′. The presence of the *sic* gene was confirmed by Southern blot hybridization and direct sequencing of two representative PCR-amplified products. The *sic* gene was detected in GAS of various *emm* types, and 48 (72.7%) of the 66 isolates were *sic* gene positive.

In the present study, we have determined the phenotypic and genotypic characteristics of GAS isolates from patients with pharyngitis in Japan. Unexpectedly, the majority of GAS isolates possessed two virulence genes, the *prtF1* and *sic* genes. The high prevalence of these genes may be due to horizontal gene transfer and recombination. The results of this study should contribute to a better understanding of the pathogenesis of GAS as well as the epidemiology of GAS-associated disease and to the establishment of methods for prevention of diseases caused by GAS in Japan.

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