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Molecular Epidemiology of *sil* Locus in Clinical *Streptococcus pyogenes* Strains

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***Streptococcus pyogenes* (group A *Streptococcus* [GAS]) causes a wide variety of diseases, ranging from mild noninvasive to severe invasive infections. Mutations in regulatory components have been implicated in the switch from colonization to invasive phenotypes. The inactivation of the *sil* locus, composed of six genes encoding a quorum-sensing complex, gives rise to a highly invasive strain. However, studies conducted on limited collections of GAS strains suggested that *sil* prevalence is around 15%; furthermore, whereas a correlation between the presence of *sil* and the genetic background was suggested, no link between the presence of a functional *sil* locus and the invasive status was assessed. We established a collection of 637 nonredundant strains covering all *emm* genotypes present in France and of known clinical history; 68%, 22%, and 10% were from invasive infections, noninvasive infections, and asymptomatic carriage, respectively. Among the 637 strains, 206 were *sil* positive. The prevalence of the *sil* locus varied according to the *emm* genotype, being present in >85% of the *emm4*, *emm18*, *emm32*, *emm60*, *emm87*, and *emm90* strains and absent from all *emm1*, *emm28*, and *emm89* strains. A random selection based on 2009 French epidemiological data indicated that 16% of GAS strains are *sil* positive. Moreover, due to mutations leading to truncated proteins, only 9% of GAS strains harbor a predicted functional *sil* system. No correlation was observed between the presence or absence of a functional *sil* locus and the strain invasiveness status.**

Streptococcus pyogenes (group A *Streptococcus* [GAS]) causes a wide variety of diseases, ranging from mild pharyngitis or impetigo to more severe invasive infections, including streptococcal toxic shock syndrome (STSS) and necrotizing fasciitis (NF) (1). Moreover, this exclusively human pathogen is responsible for a high rate of asymptomatic upper respiratory tract carriage (2). However, the origin of the switch from carriage to pathogen status remains mostly unknown. An increased frequency of invasive GAS infections has been reported since the late 1980s, resulting in a reinforcement of epidemiological surveillance (3). Sequencing of the variable extremity of the *emm* gene is at the basis of epidemiological surveys of GAS infections (4). The *emm* gene encodes the surface M protein, one of the main GAS virulence factors, and >225 different *emm* genotypes have been described (5, 6). The global distribution of the *emm* genotypes is variable by continents and also in time (7, 8). Moreover, variations are observed between countries of the same continent reflecting ongoing epidemic waves, herd immunity, or population immunity (9–12).

Hidalgo-Grass et al. identified the streptococcal invasion locus (*sil*) using the polymorphic tag-length transposon mutagenesis (PTTM) method on JS95, an *emm14* GAS strain isolated from a patient with NF (13). In this strain, the *sil* locus was shown to control GAS spread into deeper tissues in a mouse model of human soft tissue infection and to be involved in DNA transfer between two *emm*14 strains (13). This locus contains six genes, two of which (*silA* and *silB*) encode a two-component system (TCS), two others (*silD* and *silE*) encode an ABC transporter, and the last two (*silC* and *silCR*) are located between the *silA* and *silB* genes and the *silD* and *silE* genes, are divergent, and overlap. *silCR* encodes a 41-amino acid (aa) propeptide, which after cleavage yields a 17-amino acid pheromone, SilCR. The SilD/E system cleaves and then exports the signaling immature propeptide SilCR. Upon reaching a threshold concentration, the mature SilCR binds to the TCS. This in turn activates the transcription of *silCR* (autoregulation) and *silD* and *silE* and represses that of *silC* (14). The *silC* gene product is linked to GAS virulence in the mouse model of human NF (13, 15). SilCR is involved in the downregulation of the expression of the gene encoding the CXC chemokine protease ScpC, which impairs the recruitment and the activation of neutrophils to the soft tissue infection site (16, 17). Furthermore, and in contrast to subcutaneous injection of GAS strains, subcutaneous coinjection of the mature SilCR peptide and GAS strains into mice yielded strong neutrophil recruitment that prevented systemic GAS dissemination (17). However, this result may be GAS strain dependent (18). In the highly invasive strain JS95, the *sil* locus is inactive due to a point mutation in the start codon of *silCR* (ATA instead of ATG) (13, 19). Thereby, the state of the *sil* locus seems to account for the virulence of strains such as GAS JS95, which is important as no locus has been strictly associated with GAS virulence. For this reason, it was deemed important to study its in-

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TABLE 1 Clinical characteristics of the 637 GAS strains and prevalence of the *sil* locus

Isolate type	Clinical manifestation(s)	No. of strains	No. (%) of *sil* locus-positive strains	No. (%) of potential functional *sil* loci[a]
Invasive	Necrotizing fasciitis	91	32 (35)	20 (22)
	Other skin and soft tissue infections	50	22 (44)	12 (24)
	Bacteremia without focus	132	50 (38)	25 (19)
	Gynecological infections	66	20 (30)	13 (20)
	Joint and bone infections	36	11 (31)	6 (17)
	Pleuropulmonary infections	35	9 (26)	4 (11)
	Meningitis	12	4 (33)	3 (25)
	Peritonitis	8	2 (25)	1 (13)
	Others[b]	5	1 (20)	1 (20)
Total		435	151 (35)	85 (20)
Total representative[c]		324	57 (18)	28 (8)
Noninvasive	Superficial cutaneous infections	66	20 (30)	12 (18)
	Pharyngitis	20	2 (10)	2 (10)
	Scarlet fever	16	9 (56)	1 (6)
	Abscesses	11	4 (36)	4 (36)
	Vaginitis	9	3 (33)	1 (11)
	Endophthalmia	7	2 (29)	2 (29)
	Otitis	4	3 (75)	3 (75)
	Others[d]	5	0 (0)	
Total		138	43 (31)	25 (18)
Total representative[c]		76	12 (16)	11 (14)
Asymptomatic colonization	Pharynx	46	10 (22)	0 (0)
	Nose	7	1 (14)	0 (0)
	Skin	6	1 (17)	1 (17)
	Others[e]	5	0 (0)	0 (0)
Total		64	12 (19)	1 (2)
Total representative[c]		12	1 (8)	0

[a] The absence of a deletion, frameshift, or mutation leading to a stop codon in *silCR*, *silC*, and *silD* sequences. The percentage is relative to the number of strains.
[b] Two endocarditis, 2 postmortem samples, and 1 myocarditis.
[c] Taking into account strains from the most prevalent *emm* types in France; percentages are to the number of strains with the given clinical manifestation or invasiveness status.
[d] Three ethmoiditis and 2 urinary infections.
[e] Three newborn colonization and 2 vaginal colonization.

volvement in the virulence of other GAS isolates. Previous studies showed that most of the GAS invasive strains either lost *sil* completely or retained a mutated *sil* locus (19). A study conducted in Japan on noninvasive strains showed a prevalence of 12% for the *sil* locus (20). In China, a prevalence of 13% for the *sil* locus was found in both invasive and noninvasive strains (21). In France, Bidet et al., studying GAS strains causing pediatric invasive infections, detected the *sil* locus in 16% of the strains (22). Moreover, by sequencing the *sil* loci of three *emm4* strains, they identified a frameshift mutation in *silD* generated by the replacement of CTCAAA by TTTAG at position 436 to 441 (22). Thus, we wondered if the presence of a nonmutated putatively functional *sil* locus may be different in isolates from asymptomatic carriage, noninvasive infections, or invasive infections.

As the previous studies were carried out on small numbers of strains, we conducted a study to (i) assess the prevalence of the *sil* locus in a larger collection of GAS strains, (ii) determine whether its presence is related to the *emm* genotype, (iii) define whether the *sil* locus was predominantly detected in invasive, noninvasive, or colonization GAS strains, and (iv) assess whether the *sil* locus is mutated in the *sil* locus-positive strains. Hence we wished to determine the prevalence of a functional *sil* in GAS strains of various invasive status.

MATERIALS AND METHODS

Strains and clinical data. A total of 637 nonredundant GAS strains isolated from clinical samples collected between 2003 and 2009 were selected from the collection of the French National Reference Center for Streptococci (CNR-Strep [see https://www.cnr-strep.fr]). Strains were selected according to their *emm* genotype to have the widest variety of strains. All strains from infrequent *emm* genotypes (6 strains or less in that period) were included. Furthermore, to reflect the French epidemiology, a higher number of strains from the 12 most prevalent *emm* genotypes were selected (9–11). Finally, due to the results obtained with the *emm4*, *-25*, *-32*, *-43*, *-53*, *-58*, *-60*, *-63*, *-64*, *-71*, *-74*, *-87*, *-90*, *-93*, *-94*, *-101*, and *-102*, genotypes, all strains from these genotypes were included in this study (Table 1). Clinical characteristics were obtained from questionnaires sent with the isolates on a voluntary basis by a stable network of 233 laboratories located throughout the 22 French administrative regions. Data collected included the sex and date of birth of the patient, the date and origin of the sample, the geographical area, and the clinical manifestations (Table 1 and data not shown).

Case definition. GAS invasive infection was defined as the isolation of bacteria from a usually sterile site (e.g., blood, cerebrospinal fluid, bone, or joint fluid), or from samples obtained from a nonsterile site in combination with the clinical signs of NF or STSS (data not shown). STSS was defined according to the U.S. Working Group on Severe Streptococcal Infections definitions (23). Bacteremia was considered to be without focus when no focal symptoms were identified. GAS colonization strains

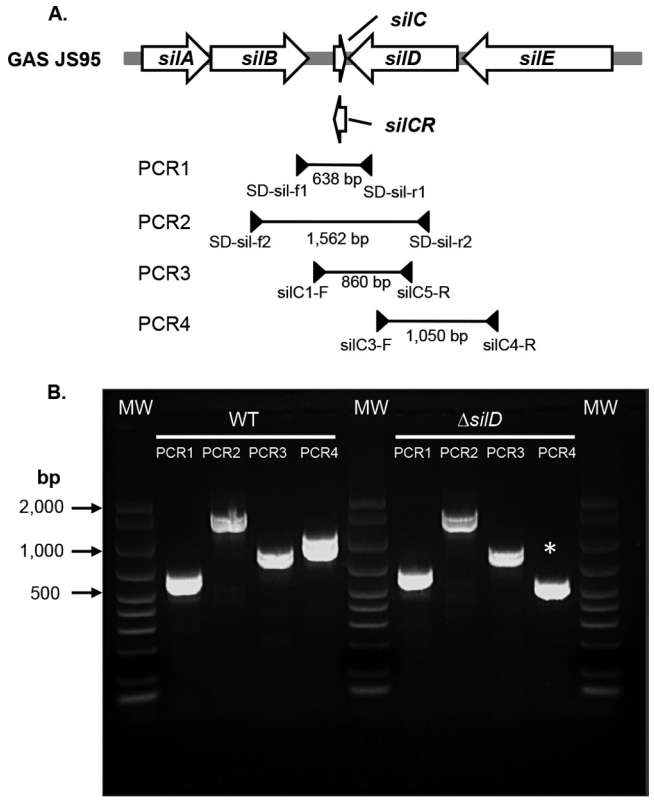

FIG 1 *sil* locus organization and PCR assays used to detect its presence and variability. (A) Schematic representation of the *sil* locus and PCR products obtained with the primers listed in Table 2. (B) Gel electrophoresis of the 4 PCR products obtained on a wild-type (WT) *sil* locus strain (CCH20080458) (PCR1, -2, -3, -4) and on a *silD*-deleted mutant strain (CCH20080441) (PCR1, -2, -3, -4). The PCR product deleted in the mutant strain is indicated by an asterisk. MW, molecular weight markers (Bio-Rad).

were sent to the CNR-Strep as part of the investigations conducted around clusters.

Strain identification and growth conditions. GAS isolates were confirmed to be *S. pyogenes* using morphological and growth characteristics, including beta-hemolysis on horse blood agar, the production of pyrrolidonyl arylamidase, and the presence of the Lancefield group A antigen. The strains were cultured on horse blood agar plates and were stored in 2% glycerol Todd-Hewitt broth at −80°C.

***emm* sequence typing.** The *emm* genotype was determined by sequencing the variable 5′-end of the *emm* gene and comparing sequences with the database of the Centers for Disease Control and Prevention (see http://www.cdc.gov/ncidod/biotech/strep/strepblast.htm).

Detection of the *sil* locus. PCR detection of the *sil* locus was performed as previously described, using two sets of primers, SD-sil-f1 and SD-sil-r1 or SD-sil-f2 and SD-sil-r2 (Fig. 1 and Table 2), amplifying, respectively, a fragment of 638 bp or 1,562 bp, encompassing *silB* and *silD* (14).

Sequence analysis. The primers silC1-F and silC5-R and silC3-F and silC4-R (Fig. 1 and Table 2) were used to amplify a 860-bp fragment encompassing *silC* and *silCR* and a *silD* gene, respectively. PCR products were sequenced and compared to the published sequence of the JS95 strain in order to identify mutations in this fragment, including the previously described *silCR* start codon mutation (13, 19).

The DNA fragments amplified using the R primers (Fig. 1 and Table 2) were sequenced and compared to the published sequence of the JS95 strain in order to detect mutations in *silD*, including the already described frameshift mutation (22). The sequence analysis and multiple alignments were performed using, respectively, BioEdit software and ClustalW.

Statistical analysis. The chi-square test was used for statistical analysis with a P value <0.05 considered significant.

RESULTS

Clinical and epidemiological data. The 637 nonredundant GAS strains selected were isolated between 2003 and 2009 from all regions of France (median, 86 strains/year; range, 40 to 164 strains/year). Among these 637 GAS strains, 435 (68%) were from invasive infections, 138 (22%) were from noninvasive infections, and the 64 remaining strains were asymptomatic colonization strains (10%) (Table 1). Most invasive strains were either from skin or soft tissue infections ($n = 141$; 32%), including 91 cases of NF, or from bacteremia without focus ($n = 132$; 31%). STSS was described in 23% of the cases, mostly associated with NF ($P < 0.001$) (data not shown). Among the 138 noninvasive strains, 66 were from superficial cutaneous infections (45%), and 20 were from pharyngitis (14%) (Table 1). Asymptomatic colonization strains were mostly isolated from the pharynx (68%) (Table 1). The sex ratio (male/female) was 1, and the distribution according to age groups, infants (<1 to 17 years) and adults (18 to 97 years), was 103 and 534 strains, respectively. Among these 637 GAS strains, 87 different *emm* genotypes were identified, but no *emm14* strain in which *sil* was discovered (13) was found (Table 3).

***sil* locus prevalence.** The *sil* locus prevalence was determined by performing PCR detection on the 637 strains (Fig. 1). The *sil* locus was detected in 206 GAS strains from 42 different *emm* genotypes (Tables 1 and 3). Of note, the prevalence of the *sil* locus varied according to the *emm* genotype (Table 3). The *sil* locus was detected in 95% ($n = 93$) of *emm4* GAS strains and in more than 85% of the *emm* genotypes for which there were more than 5 isolates (*emm18*, *emm32*, *emm60*, *emm87*, *emm90*, and *emm102*). Furthermore, the *sil* locus was absent from all *emm1* ($n = 60$), *emm28* ($n = 58$), or *emm89* ($n = 45$) strains, representing the three most frequent genotypes involved in invasive infections in France (9–11). The *sil* locus was detected in 151 (35%) invasive strains, 43 (31%) noninvasive strains, and 12 (19%) asymptomatic colonization strains of our collection (Tables 1 and 3). We analyzed a putative link between the presence of *sil* and the clinical manifestations. Among the invasive strains, the percentage of *sil*-harboring strains varied from 25% (peritonitis) to 44% (other skin and soft tissue infections). Among the noninvasive strains, the variation in the presence of *sil* was more dispersed but overall greater, ranging from 10% among pharyngitis isolates to 56% among scarlet fever isolates. The presence of *sil* appears to be associated with scarlet fever ($P < 0.05$). Of note, the *sil*$^+$ strains

TABLE 2 Primers used in this study

Name	Sequence (5′ to 3′)	Reference or source
SD-sil-f1	GGAGTTGGTTTATCAAATGTCAG	14
SD-sil-r1	ATCTGCCACAAAGACTGATCAAG	14
SD-sil-f2	TTATTGGATCGGAACTTACGC	14
SD-sil-r2	TGCTTCCCAACAACTTACCAC	14
silC1-F	GGCTAAACCTGCTAAAGACTCTTG	This study
silC5-R	TCTCTTCCAGACACTAGTCATAGG	This study
silC3-F	AGCTGAATATTGGCTTGCTC	This study
silC4-R	CGCGGACCAATCAAGTCATTGT	This study

TABLE 3 Prevalence of *sil* locus and of a predicted functional *sil* locus among the 87 different *emm* genotypes according to the invasiveness of the 637 GAS strains

emm genotype	No. of strains	No. of invasive strains with:			No. of noninvasive strains with:			No. of asymptomatic colonization strains with:		
		sil^{-a}	*sil*$^{+b}$	Potential functional *sil* locus[c]	*sil*$^{-}$	*sil*$^{+}$	Potential functional *sil* locus	*sil*$^{-}$	*sil*$^{+}$	Potential functional *sil* locus
emm1	60	42	0		7	0		11	0	
emm2	15	9	0		5	0		1	0	
emm3	25	17	1	0	3	0		4	0	
emm4	98	3	64	4	1	18	2	1	11	0
emm5	7	4	0		2	0		1	0	
emm6	19	11	0		5	0		3	0	
emm8	2	2	0		0	0		0	0	
emm9	6	3	1	1	2	0		0	0	
emm11	13	6	0		3	0		4	0	
emm12	24	14	0		6	0		4	0	
emm18	5	0	5	3	0	0		0	0	
emm22	7	4	0		3	0		0	0	
emm24	1	1	0		0	0		0	0	
emm25	3	1	2	2	0	0		0	0	
emm27	2	2	0		0	0		0	0	
emm28	58	39	0		14	0		5	0	
emm29	1	0	1	1	0	0		0	0	
emm30	1	0	0		0	1	1	0	0	
emm32	7	0	5	5	0	2	2	0	0	
emm33	1	1	0		0	0		0	0	
emm41	4	0	3	3	0	1	0	0	0	
emm42	1	1	0		0	0		0	0	
emm43	2	0	1	1	0	1	1	0	0	
emm44	4	2	0		2	0		0	0	
emm48	1	0	0		0	1	1	0	0	
emm49	6	5	0		1	0		0	0	
emm50	2	1	1	1	0	0		0	0	
emm53	3	0	2	2	0	1	1	0	0	
emm55	1	0	0		1	0		0	0	
emm58	7	2	1	1	3	1	1	0	0	
emm59	5	2	0		3	0		0	0	
emm60	11	1	5	5	0	5	5	0	0	
emm63	3	2	0		1	0		0	0	
emm64	4	2	0		1	0		1	0	
emm65	1	1	0		0	0		0	0	
emm66	1	1	0		0	0		0	0	
emm68	3	2	0		0	0		1	0	
emm69	1	1	0		0	0		0	0	
emm71	3	0	2	2	0	1	1	0	0	
emm73	2	2	0		0	0		0	0	
emm74	3	0	2	2	0	1	1	0	0	
emm75	9	5	0		0	0		4	0	
emm76	5	2	0		3	0		0	0	
emm77	22	13	3	3	2	3	3	1	0	
emm78	4	2	0		1	0		1	0	
emm81	12	9	1	1	2	0		0	0	
emm82	8	7	1	1	0	0		0	0	
emm83	15	120	0		3	0		0	0	
emm85	2	1	1	1	0	0		0	0	
emm87	20	1	14	14	0	4	4	0	1	1
emm88	1	1	0		0	0		0	0	
emm89	45	23	0		11	0		11	0	
emm90	7	0	7	7	0	0		0	0	
emm92	2	1	0		1	0		0	0	
emm93	2	0	2	2	0	0		0	0	
emm94	3	0	3	3	0	0		0	0	

(Continued on following page)

TABLE 3 (Continued)

emm genotype	No. of strains	No. of invasive strains with:			No. of noninvasive strains with:			No. of asymptomatic colonization strains with:		
		sil^{-a}	*sil*$^{+b}$	Potential functional *sil* locus[c]	*sil*$^{-}$	*sil*$^{+}$	Potential functional *sil* locus	*sil*$^{-}$	*sil*$^{+}$	Potential functional *sil* locus
emm100	1	0	0		0	1	1	0	0	
emm101	3	0	3	3	0	0		0	0	
emm102	7	1	4	3	0	2	1	0	0	
emm103	1	1	0		0	0		0	0	
emm104	1	1	0		0	0		0	0	
emm106	3	2	0		1	0		0	0	
emm108	1	0	1	1	0	0		0	0	
emm110	3	1	1	1	1	0		0	0	
emm112	2	1	1	1	0	0		0	0	
emm113	2	1	1	0	0	0		0	0	
emm116	3	2	0		1	0		0	0	
emm117	2	0	2	2	0	0		0	0	
emm118	4	2	2	2	0	0		0	0	
emm122	1	0	1	1	0	0		0	0	
emm124	2	2	0		0	0		0	0	
emm142	1	1	0		0	0		0	0	
emm147	1	0	1	1	0	0		0	0	
emm158	1	0	1	1	0	0		0	0	
emm168	1	1	0		0	0		0	0	
emm172	1	1	0		0	0		0	0	
emm174	1	0	1	1	0	0		0	0	
emm176	3	0	0		3	0		0	0	
emm179	1	0	1	0	0	0		0	0	
emm180	2	2	0		0	0		0	0	
emm182	2	2	0		0	0		0	0	
emm183	1	0	1	1	0	0		0	0	
emm187	1	1	0		0	0		0	0	
emm192	1	0	0		1	0		0	0	
emm217	2	1	1	1	0	0		0	0	
emm230	1	0	1	1	0	0		0	0	
stG1750	1	1	0		0	0		0	0	
Total no. (%)	637	284	151	85 (30)	88	43	25 (28)	52	12	1 (<1)
Total representative no. (%)[d]	430	267	57	28 (8)	64	12	11 (14)	11	1	0

[a] *sil*$^{-}$, *sil* locus absent.
[b] *sil*$^{+}$, *sil* locus present.
[c] Absence of a deletion, frameshift, or mutation leading to a stop codon in *silCR*, *silC*, and *silD* sequences. Percentages refer to the number of strains with the given invasiveness status.
[d] Taking into account strains from the most prevalent *emm* genotypes in France.

responsible for scarlet fever belonged to *emm4* ($n = 8$) and *emm87* ($n = 1$) genotypes, and those which had elicited pharyngitis were *emm87* strains ($n = 2$). Finally, among the *emm4* strains, a genotype that is far more prevalent in children (second or third most common) than in adults (6th encountered *emm* genotype), 96%, 95%, and 92% of strains harboring the *sil* locus were invasive, noninvasive, and asymptomatic colonization strains, respectively. There were no significant differences between the presence and absence of the *sil* locus, depending on the invasiveness status of the *emm4* strains. Furthermore, no correlation between age group and the presence of the *sil* locus was noted (data not shown).

Taking into account the frequency of the different *emm* genotypes in France in 2009 (9), we estimated that the *sil* locus prevalence was 16% overall frequency with 18%, 16%, and 8% in invasive, noninvasive, and asymptomatic colonization strains, respectively (Table 3). Thus, a trend for a lower frequency of the presence of the *sil* locus in asymptomatic colonization strains emerges, but, due to the low number of the latter in our survey (Table 1), it was not statistically significant.

Sequence analysis. To identify a possible mutation in the *silCR* translational start codon as previously described for the JS95 (*emm14*) strain, we sequenced *silCR* in the 206 *sil* locus-positive strains (13, 19). Among these strains, no mutation in the *silCR* start codon was observed; a functional translational start codon was present in *silCR* of all strains (Fig. 2). Furthermore, in 200/206 strains, the *silC* and *silCR* sequences were identical to those of the JS95 strain (except for the start codon) (Fig. 2). The remaining six strains had additional differences in the sequences (Fig. 2). In four strains, the guanine at position 17 in *silCR* or the cytosine at position 66 in *silC* was substituted by an adenine or a thymidine, leading to the replacement of a threonine by an isoleucine in SilCR and a valine by an isoleucine in SilC. Among these four strains,

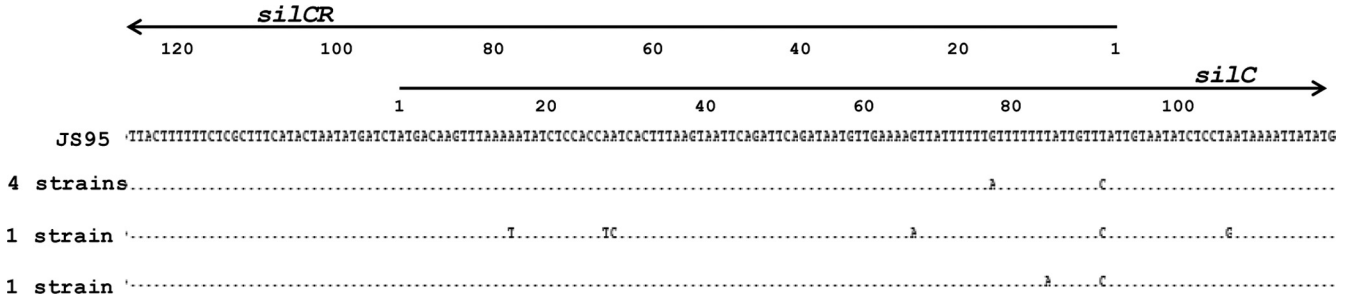

FIG 2 Mutation occurrences in *silCR* and *silC*. Both genes are symbolized by solid lines. Below the sequence of the JS95 gene all of the mutations found during this study are indicated; their occurrences are shown on the left.

three are *emm74* strains, including two invasive strains and one noninvasive strain, and one is an invasive *emm4* strain. Several single nucleotide polymorphisms (SNPs) were identified in *silCR* and *silC* of an *emm4* strain (Fig. 2), which led to a single amino acid change in SilCR and to several amino acid replacements in SilC: the isoleucine at position 22 of SilCR was replaced by an arginine, and in SilC, the lysine at position 5, the isoleucine at position 10, and the asparagine at position 36, were replaced by an asparagine, a leucine, and an aspartic acid, respectively. This strain harboring several SNPs was isolated from an asymptomatic pharyngeal colonization. Finally, in a noninvasive *emm41* strain, the replacement of the adenine at position 10 of *silCR* by thymine and consequently the thymine at position 83 of *silC* by adenine resulted in a stop codon in both *silC* and *silCR* (Fig. 2). It was the only strain among the 206 characterized which presented truncated SilCR and SilC. This strain was isolated from a superficial cutaneous infection.

Consequently, in all *sil*$^+$ strains, except one with a stop codon and a second one with nonconservative changes, SilC and SilCR are predicted to be functional. This contrasts with the situation in JS95, the strain in which the *sil* locus was originally described (13, 19).

The *silD* gene was sequenced to search for the frameshift mutations previously described in *emm4* and *emm18* strains or other mutations (22). *silD* was identical to that of JS95 in only 55 (27%) strains (Fig. 3). In 78 strains (38%), a substitution of CTCAAA at position 436 to 441 by TTTTAG gave rise to the replacement of a leucine at position 146 of SilD by a phenylalanine and to a stop codon in position 441 of *silD*. In 12 strains, a 516-bp deletion was found from adenine at position 235 to thymine at position 749, yielding a truncated SilD protein (Fig. 1 and 3). Three strains displayed the deletion of an adenine at position 479, generating the frameshift mutation previously described in the reference strain MGAS8232 (*emm18*) (24). In one strain, a stop codon was generated by the replacement of cytosine at position 883 by a thymine. Altogether, 94 of the 206 *sil*$^+$ strains (45.5%) encode a truncated SilD. Finally, in 57 strains (28%), 29 different SNP patterns were observed, ranging from 1 single amino acid substitution to 14 amino acid substitutions, of which 19 patterns displayed more than three SNPs (data not shown). SNPs affected *silD* from many *emm* genotypes; only 17 among the 42 genotypes analyzed were not affected.

Seventy-six *emm4* strains (82%) from the 93 *emm4 sil*$^+$ strains of our collection and the reference MGAS10750 strain harbored the CTCAAA by TTTTAG substitution at position 436 to 441, 11 strains (12%) harbored the 516-bp deletion, 3 strains (3%) harbored SNPs, and only 3 *emm4 sil*$^+$ strains (3%) possessed a *silD* sequence identical to that of JS95, thus probably functional (Fig. 3 and Table 3). From the 90 *emm4* strains harboring mutations, 60 were from invasive infections, 16 were from noninvasive infections, and 11 were from asymptomatic colonization, and the 3 strains possessing SNPs in *silD* were from invasive infections (Table 3); among the 3 *emm4* strains with a wild-type *silD*, 2 were from noninvasive infections and 1 was from an invasive infection (data not shown).

Interestingly, if we do not take *emm4* strains into consideration, the percentage of *sil*-positive strains harboring a putatively functional *sil* is much higher, reaching 93% (105/113) (Table 3). The two non-*emm4* strains with the CTCAAA by TTTTAG substitution at position 436 to 441 are the *emm102* noninvasive strain and the *emm113* invasive strain. The only non-*emm4* strain harboring the 516-bp deletion is an *emm3* strain (Fig. 3). The strains

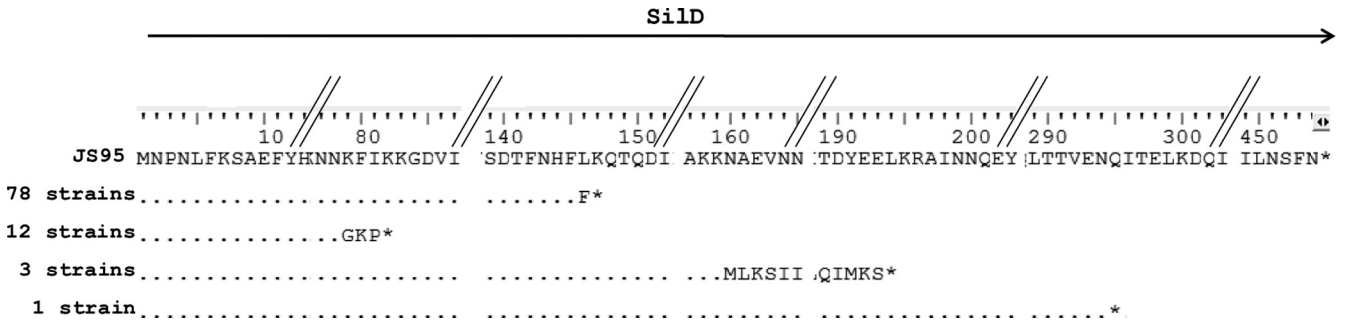

FIG 3 Mutation occurrences in SilD. The SilD sequence is symbolized by the solid line. Fragments of the JS95 SilD sequence are shown, separated by double oblique lines. The mutations leading to premature translation arrest or to deletions are indicated below the sequence; their occurrences are shown on the left.

with the frameshift mutations are two *emm18* strains and one *emm179* strain. Finally, the strain with the stop codon at position 883 is an invasive *emm102* strain. The 105 non-*emm4* strains with a potentially functional *sil* locus were isolated from 81 invasive infections, 23 noninvasive infections, and only 1 asymptomatic colonization.

Taking into account the frequency of the different *emm* genotypes in France in 2009 (9), we estimated that the *sil* locus was present and potentially functional in 8% of the invasive and 14% of the noninvasive strains but absent from all asymptomatic colonization strains.

DISCUSSION

In this study, we characterized the prevalence of the *sil* locus and sequenced the three genes of the *sil* locus in which mutations had been described (*silC*, *silCR*, and *silD*), among a large collection of clinical GAS strains from France.

Our data show that the presence of the *sil* locus is correlated with the *emm* genotype, and within the *emm* genotypes the prevalence was overwhelmingly high. This is in accordance with observations in previous studies, but our study expands this observation to other *emm* genotypes (Table 3) (20–22). Indeed, an overwhelming prevalence of the *sil* locus was detected for *Streptococcus dysgalactiae* subsp. *equisimilis*, a group G *Streptococcus* (GGS) (100%), which is a close relative of GAS (13, 19). GGS is usually regarded as a commensal organism, but recent studies showed that it can cause human invasive infections similar to those caused by GAS (25).

Nonetheless, within the *emm* genotypes in which *sil* is highly prevalent, only 6 (6%) (half from invasive and half from noninvasive infections) have a potentially functional *sil* locus. This was defined by the absence of deletions, frameshift mutations, or mutations leading to formation of a stop codon in *silCR*, *silC*, and *silD* sequences. While SNPs were mostly accountable for mutations in *sil*-positive strains of various *emm* genotypes, only 3% of *emm4* strains, respectively, responsible for a STSS associated with NF ($n = 2$) and pneumonia ($n = 1$), displayed SNPs in *sil*. Furthermore, the finding that 82% of the *emm4 silD* mutant strains shared the same 516-bp deletion, leading to a truncated 146-amino acid (aa)-long SilD, suggested that this mutation is clonal. Of note, none of our *emm4* strains nor the MAGS10750 reference strain displayed the frameshift mutation previously described for *emm4* (22).

Sequencing of *silCR* allowed us to identify 6 strains containing SNPs in the *silC-silCR* overlapping segment (Fig. 2). Yet the SNP introduced a stop codon mutation in only one strain of the *emm41* genotype. None of the strains in our collection was found to have the SilCR translation initiation codon mutation as found in the *emm14* strain JS95 isolated in Israel in which *sil* was discovered (13). This translation stop codon mutation may be restricted to *emm14* strains. Indeed, no *emm14* strain was present in our collection, which contained a very large number of different genotypes. In fact, whereas the *emm14* genotype is among the five most frequent *emm* genotypes in Israel, it is seldom encountered in Europe, North America, or Japan among isolates from invasive disease (9–11, 26, 27). Moreover, in the Pacific region, the *emm14* genotype represents only the 25th most common genotype, amounting to 2% of the strains (8). Furthermore, a systematic sequencing of the *silCR* gene in *emm14* strains is necessary to determine whether the described mutation is an exception or the rule among the *emm14* strains (13, 19). Nonetheless, a similar mutation was reported for an additional *emm14* strain isolated in the United States (15).

No correlation between the presence of a potentially active *sil* locus and the clinical manifestations or invasive status of the stains isolated in France was identified. However, interestingly, very few asymptomatic carriage strains possessed a functional *sil* locus and when the prevalence of the *emm* genotypes was taken into account, we found no asymptomatic *sil*$^+$ strain.

In conclusion, we show that (i) the prevalence of the *sil* locus varied according to the *emm* genotype, being present in more than 85% of the *emm4*, *emm18*, *emm32*, *emm60*, *emm87*, and *emm90* strains and absent from all *emm1*, *emm28*, and *emm89* strains, and only 9% of GAS strains harbored a predicted functional *sil* system and (ii) no correlation was observed between the presence or absence of a functional *sil* locus and the strain invasiveness status.

These results confirm and reinforce previous studies in France and Asia. However, because the epidemiology of GAS strains varies according to the geographical location, our findings cannot be extended to other continents.

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