

S1 Switching mutations observed in <i>covRS</i> in GAS from human isolates and murine models of invasive infections				
GAS serotype (strain)	Host*	Mutation ^{‡§}	Consequence	Reference
CovR missense				
M11 (NIH323-1)	H (lung)	nr	D10G	1
M89 (NIH157)	H (blood)	nr	D10Y	1
M59 (NIH301)	H (blood)	nr	I30F	1
M1 (NIH447)	H (blood)	nr	D53E	1
M4 (NIH406)	H (blood)	nr	D53Q	1
M3 (NIH212)	H (soft tissue)	nr	D60Y	1
M1 (MGAS2221)	M	Δ232-234	ΔM78	2
M1 (MGAS166)	M	nr	M86V	3
M1 (MGAS166)	M	nr	R94C	3
M89 (NIH345)	H (wound)	nr	R94C	1
M91 (NIH252-2)	H (muscle)	nr	R94C	1
M58 (NIH273)	H (blood)	nr	G95S	1
M1 (NIH381-1)	H (wound)	nr	A96V	1
M1 (5448)	M	G331A	A111T	4
M1 (NIH75)	H (blood)	nr	A111D	1
M3 (NIH1)	H (fascia)	nr	R118C	1
M81 (TK929)	H (blood)	nr	R118S	1
M1 (MGAS2221)	M	nr	R119H	2
M1 (NIH136)	H (blood)	nr	R119S	1
M3 (NIH300)	H (blood)	nr	R119L	1
M18 (NIH43)	H (effusion)	nr	S154P	1
M3 (NIH216)	H (fascia)	nr	W184C	1
M1 (MGAS166)	M	nr	R203S	3
M1 (MGAS166)	M	nr	G204D	3
M1 (MGAS166)	M	nr	ΔK211-G220	3
M3 (NIH9)	H (blood)	nr	Q216P	1
M1 (NIH44)	H (blood)	nr	ΔE252	1
CovR nonsense/frameshift				
M3 (NIH259)	H (blood)	nr	Truncation at aa 45	1
M3 (TK283)	H (fascia)	nr	Truncation at aa 134	1
M3 (NIH404)	H (soft tissue)	1bp insert	Truncation at aa 146	1
M1 (MGAS166)	M	nr	Truncation at aa 159	3
CovS missense				
M87 (NIH283-1)	H (blood)	nr	P16L	1
M89 (NIH238)	H (soft tissue)	nr	S204R	1
M1 (NIH222)	H (soft tissue)	nr	A206S	1
M1 (NIH75)	H (blood)	nr	P220K	1
M28 (NIH35)	H (blood)	nr	E226G	1
M6 (NIH296)	H (blood)	nr	M228I, G357D	1
M89 (NIH421)	H (blood)	nr	R229C	1
M1 (MGAS166)	M	nr	R241S	3
M81 (NIH268)	H (soft tissue)	nr	R241C	1
M87 (NIH437)	H (blood)	nr	S246P	1
M1 (MGAS166)	M	nr	S254F	3
M1 (MGAS166)	M	nr	M260T	3
M77 (NIH297)	H (soft tissue)	nr	T266I	1
M1 (5448)	M	C838T	H280Y	4
M1 (MGAS294)	H (invasive)	nr	V286F	2
M1 (NIH243-1)	H (blood)	nr	G291R	1
M1 (NIH286)	H (blood)	nr	I381Y, H437R	1
M12 (NIH263-2)	H (blood)	nr	N384K	1

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M1 (NIH205)	H (soft tissue)	nr	Q388R	1
M1 (MGAS6184)	H (invasive)	nr	A397V	2
M89 (NIH58)	H (joint fluid)	nr	V423A	1
M3 (NIH18)	H (effusion)	nr	A456Y ^{II}	1
M1 (NIH220-1)	H (blood)	nr	G457V	1
M49 (NIH200-4)	H (blood)	nr	G461S	1
M113 (NIH433)	H (blood)	Δ1408-1410	ΔD470	1
CovS nonsense/frameshift				
M1 (MGAS5005)	H (cerebrospinal fluid)	Δ80	Truncation at aa 35	2
M1 (5448)	M	Δ83	Truncation at aa 35	4
M1 (MGAS166)	M	Δ76-83::T	Truncation at aa 35	3
M1 (NIH202-2)	H (blood)	1bp deletion	Truncation at aa 35	1
M22 (NIH160)	H (blood)	1bp deletion	Truncation at aa 35	1
M28 (NIH422)	H (soft tissue)	1bp deletion	Truncation at aa 35 ^{II}	1
M81 (NIH156-1)	H (blood)	1bp deletion	Truncation at aa 35	1
M3 (NIH249)	H (blood)	1bp deletion	Truncation at aa 35	1
M1 (NIH314)	H (rubor site)	11bp insert	Truncation at aa 39	1
M3 (NIH424-1)	H (blood)	11bp insert	Truncation at aa 39	1
M49 (NIH269)	H (soft tissue)	11bp insert	Truncation at aa 39	1
M53 (NIH389)	H (soft tissue)	11bp insert	Truncation at aa 39	1
M89 (NIH250-2)	H (blood)	11bp insert	Truncation at aa 39 ^{II}	1
M112 (NIH287-1)	H (soft tissue)	11bp insert	Truncation at aa 39	1
M1 (MGAS166)	M	218::GAAAA	Truncation at aa 75	3
M1 (NIH102)	H (ascites)	1bp deletion	Truncation at aa 76	1
M1 (MGAS166)	M	321::AA	Truncation at aa 110	3
M1 (MGAS166)	M	342::IS1548	Insertional inactivation at aa 114	3
M3 (TK280)	H (blood)	nr	Truncation at aa 131	1
M1 (5448)	M	Δ406-1503	Truncation at aa 136	4
M22 (NIH236)	H (blood)	TTTT→GAGG	Truncation at aa 158 ^{II}	1
M3 (NIH152-3)	H (blood)	nr	Truncation at aa 160	1
M3 (NIH453)	H (effusion)	1bp deletion	Truncation at aa 180	1
M1 (MGAS166)	M	492::T	Truncation at aa 181	3
M11 (NIH49)	H (soft tissue)	nr	Truncation at aa 184	1
M98.1 (NS88.2)	H (blood)	G581A	Truncation at aa 193	5
M87 (NIH372)	H (blood)	nr	Truncation at aa 193 ^{II}	1
M1 (5448)	M	803::A	Truncation at aa 268	6
M60 (NIH317)	H (blood)	nr	Truncation at aa 282	1
M1 (5448)	M	877::A	Truncation at aa 300	4
M22 (NIH403)	H (blood)	nr	Truncation at aa 369	1
M1 (MGAS166)	M	1105::T	Truncation at aa382	3
M1 (MGAS166)	M	1136::GC	Truncation at aa 390	3
M1 (NIH73)	H (blood)	5bp deletion	Truncation at aa 407	1
M22 (TK76)	H (soft tissue)	5bp deletion	Truncation at aa 407	1
M23 (A20)	H (blood)	Δ1215-1219	Truncation at aa 407	5
M87 (NIH371)	H (blood)	5bp deletion	Truncation at aa 407 ^{II}	1
M11 (NIH325-1)	H (blood)	nr	Truncation at aa 450	1
M1 (MGAS2221)	M	Δ1250	Truncation at aa 457	2
M1 (NIH253-1)	H (blood)	1bp deletion	Truncation at aa 457	1
M89 (NIH5)	H (blood)	5bp insert	Truncation at aa 459	1
M1 (5448)	M	G1441T	Truncation at aa 480	6
M49 (NIH230)	H (blood)	GTTCTTTTT→ TCTGCATTTTC	Truncation at aa 39	1

*M, isolate from murine invasive disease study; H, human isolate. [†]nr, not reported. [§]Under *in vitro* conditions of general stress, secondary mutations in *covR* can be identified in *covS* mutant M6 GAS²; however, these do not appear to occur during human selection of *covS* mutants, as illustrated in this Table. ^{II}GAS isolate also contained a mutation in the gene encoding Rgg, a positive regulator of SpeB expression.

SUPPLEMENTARY INFORMATION

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5. Maamary, P. G. *et al.* Parameters governing invasive disease propensity of non-M1 serotype group A streptococci. *J. Innate Immun.* **2**, 596–606 (2010).
6. Cole, J. N. *et al.* M protein and hyaluronic acid are essential for *in vivo* selection of *covRS* mutations characteristic of invasive M1T1 group A *Streptococcus*. *mBio* **1**, e00191-10 (2010).
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