

#B-2001

Tracking Evolutionary Genetic Events in Group A *Streptococcus* Using Whole Genome Mapping

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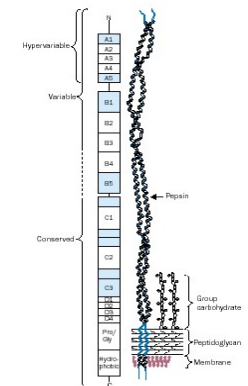
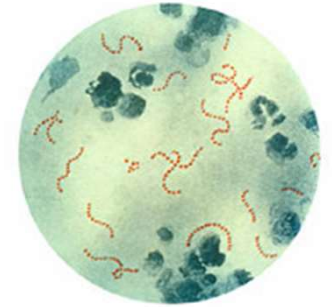
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Introduction

- **GAS**
 - Gram-positive bacterial pathogen
 - Causes human infections worldwide
 - Diverse clinical manifestations
 - Pharyngitis
 - Invasive infections
 - Streptococcal toxic shock syndrome
- *Emm1* gene
 - Encode for M protein
 - Antiphagocytic cell surface molecule



Aim of the study

Using whole genome mapping and whole genome sequencing to study longitudinal evolutionary events in *emm1* GAS strains collected in Belgium

- 1994-2013
- invasive strains
- non-invasive strains

Whole genome mapping (WGM)

WGM is an ordered, high-feature density whole genome restriction map generated from single DNA molecule

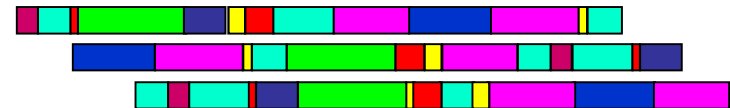


**DNA
Extraction**

**DNA Stretching
and Immobilization**

**RE Digest
Fluorescent Stain**

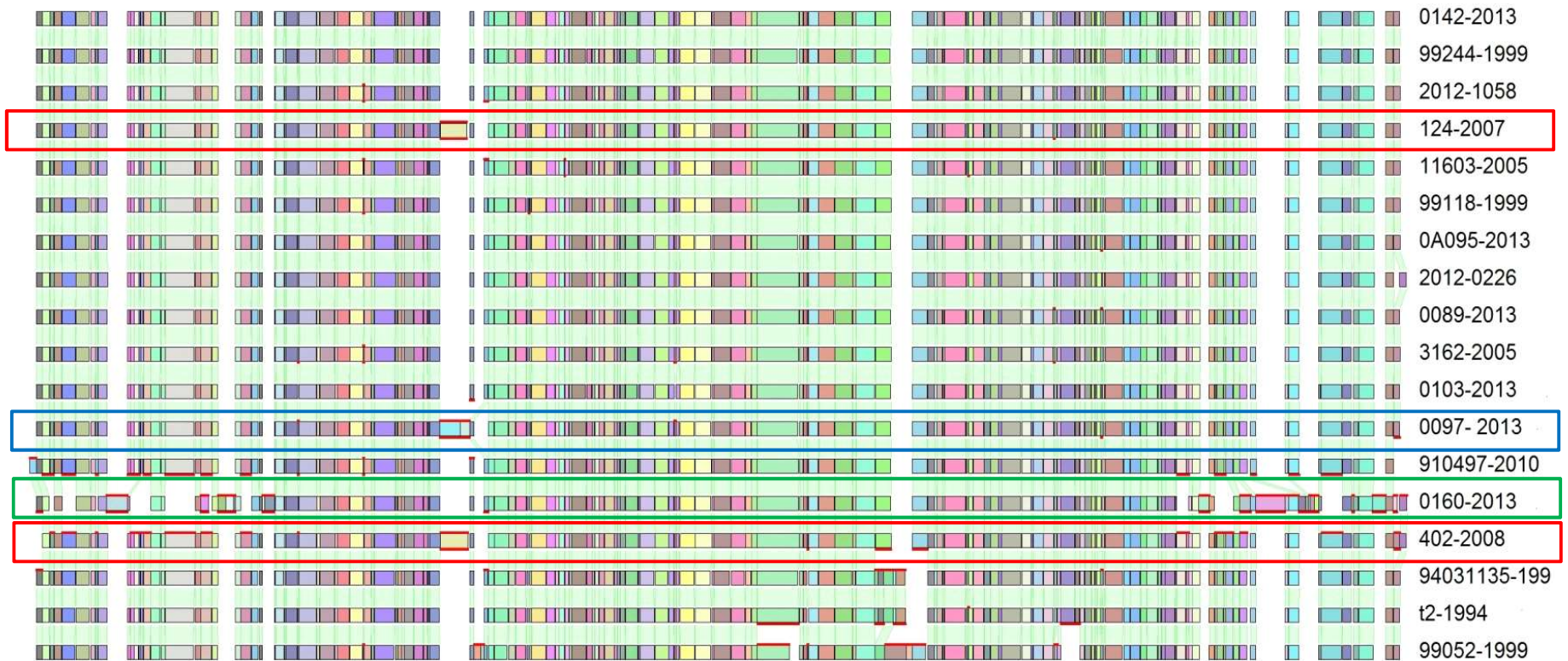
**Analysis
Bionumerics v7.1**



Longitudinal collection of *emm1* GAS

Year	Strains	Invasive	Source	MLST	PFGE
1994	2	Yes	Not determined	ST-28	03a
	0	No	Throat		03a
1999	3	Yes	Blood	ST-28	03a
	2	No	Throat		03a
2005	2	Yes	Blood	ST-28	03a
	1	No	Throat		03a
2007	1	Yes	Tissue	ST-28	03f
	3	No	Throat		03a (n=2); 03f (n=1)
2008	1	Yes	Blood	ST-28	03f
		No	Throat		03a (n=1); 03f (n=2)
2009	1	Yes	Blood	ST-28	03a
	2	No	Throat		03a
2010	1	Yes	wound, not sterile	ST-28	03a
	2	No	Throat		03a
2012	2	Yes	Blood (1), Tissue (1)	ST-28	03a
	2	No	Throat		03a
2013	5	Yes	Other invasive (3); blood (2)	ST-28	03a (n=4); 03f (n=1)
	3	No	Throat		03a

Whole genome maps of *emm1* invasive strains (1994-2013)



Whole genome maps of emm1 non-invasive strains (1994-2013)



Whole genome sequencing of invasive emm1 GAS

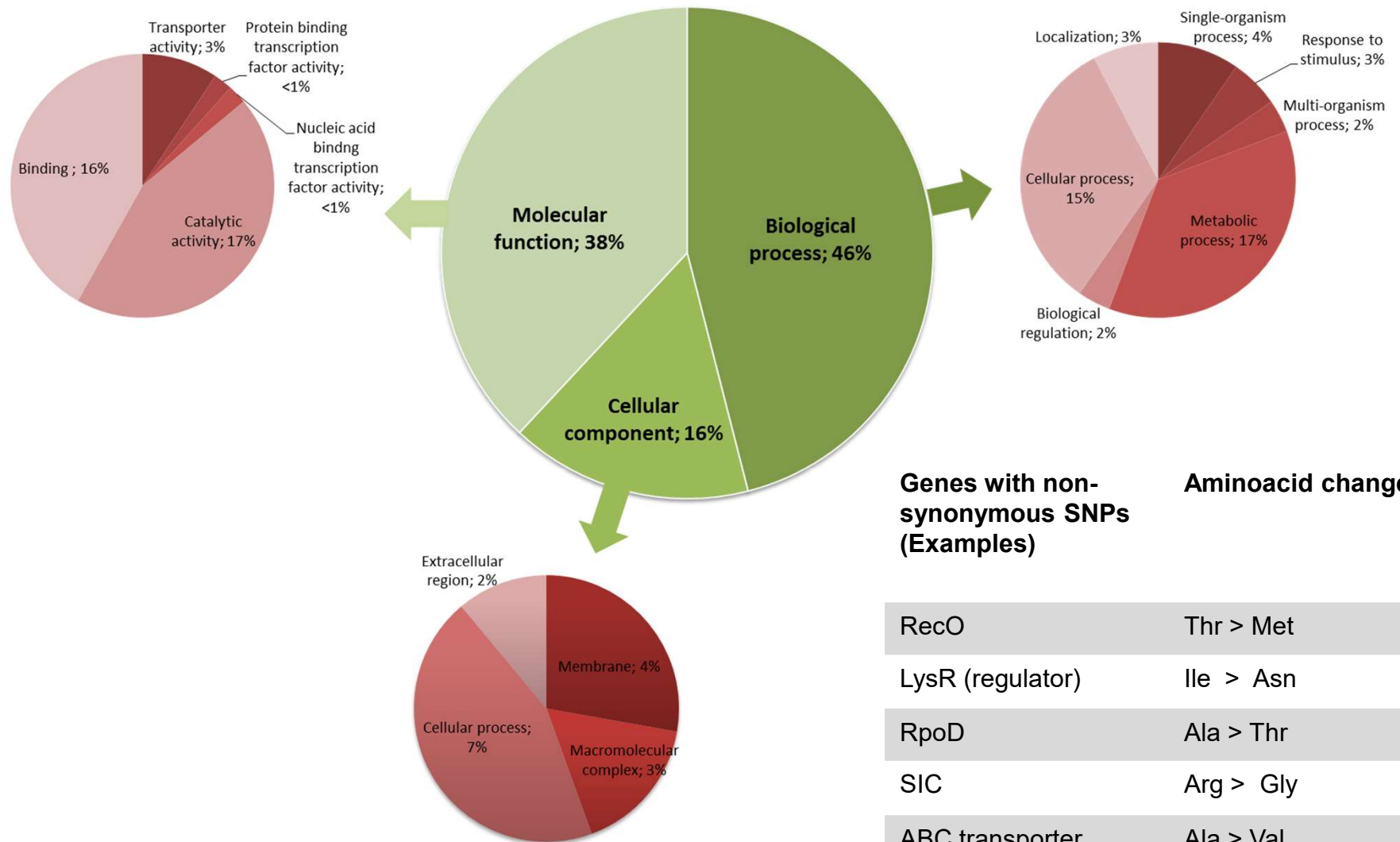
- MiSeq Illumina
- *De novo* Genome assembly (Velvet & SPAdes)
- Annotation (RAST Server <http://rast.nmpdr.org/>)
- Comparative genome analysis (MUMmer)



Average genome size (bp): ~1865579

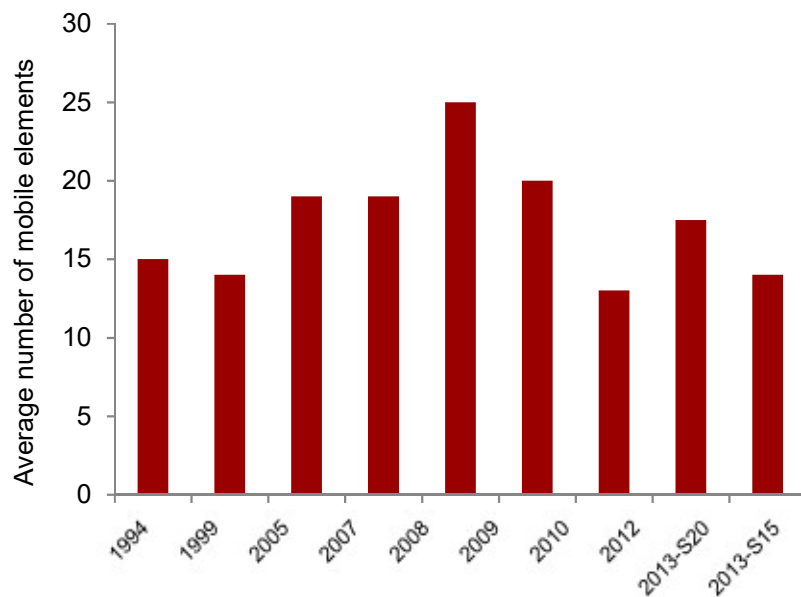
Average GC content (%): ~38

Non-Synonymous SNPs over years

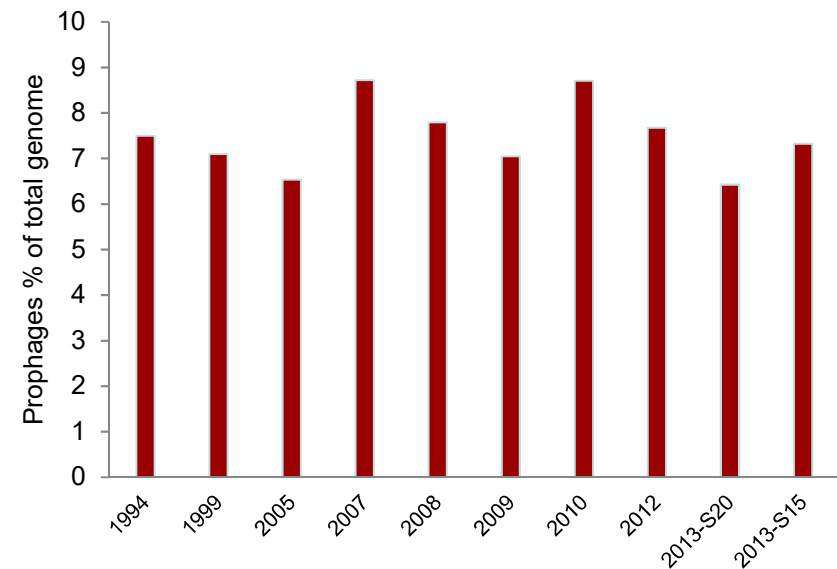


Prophages and other mobile elements in invasive *emm1* GAS during 1994-2013

Mobile elements (transposons)



Prophages



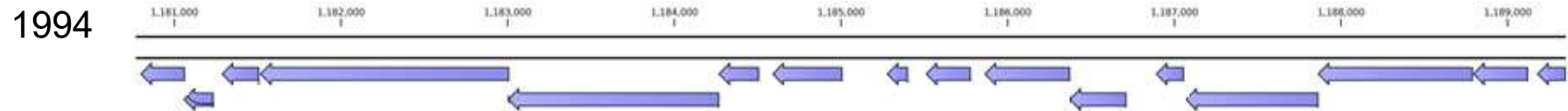
Prophages

	1994 (n=1)	1999 (n=2)	2005 (n=2)	2007 (n=1)	2008 (n=1)	2009 (n=1)	2010 (n=1)	2012 (n=1)	2013 (n=6)
PHAGE_Strept_pyogenes_315_5	2	2+3	2+3	3	3	2	3	2	2-4
PHAGE_Strept_P9	1	1+1	1	1	1	1	1	1	1-2
PHAGE_Enterococcus_G4_sensu_lato	0	0	0	0	0	0	0	1 (S2)	0
PHAGE_Streptococcus...									
PHAGE_Temp...									
PHAGE_Streptococcus...									

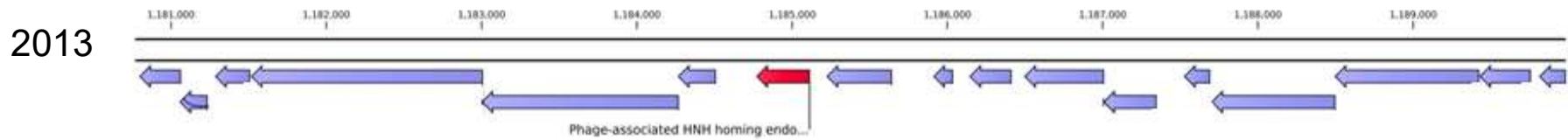
Our results show that SpeJ might be phage associated and thus transferable between GAS

	SpeG	SpeA	SpeB	SpeK	SmeZ	SpeJ	SpeJ-fragment
1994	1	1	1	0	0	1	0
1999	1	1	1	0	0	1	1
2005	1	1	1	0	0	1	0
2007	1	1	1	1	1	1	0
2008	1	1	1	1	1	1	0
2009	1	1	1	0	0	1	0
2010	1	1	1	0	0	1	1
2012	1	1	1	0	0	1	0
2013	1	1	1	0	0	1	0
s16	1	1	1	1	1	1	0

Gain of intron containing HNH-Homing endonucleases in phage 315



Our results show that spreading of prophage 315 might have influence from homing endonuclease



Correlation of CRISPR and Prophages

CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats

Strain	CRISPR	Prophage	Accession Number
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Our results show that there is no correlation between CRISPR/CAS genes and the gain/ loss of prophages

S5-1994	2 (4)	5	This study
S1-1999	2 (3)	3	This study
S8-2007	2 (3)	4	This study
S4-2008	2 (3)	4	This study
S16-2013	2 (4)	5	This study

Conclusion

- Genomic stability
- Selection pressure 2007 and 2008
- SNPs
 - *RecO* and *LysR* transcriptional regulator
 - *RpoD*, *SIC*
- Gain/ loss
 - Prophages along with HNH-Homing Endonuclease in phage 315.
 - No correlation as shown before between CRISPR and prophages
- Superantigens
 - *Phage associations of SpeJ*

Acknowledgement

- Funding
 - Platform foR European Preparedness Against (Re-)emerging Epidemics, EU-FP7 (PREPARE; 602525)
 - European public health initiative EUPHi (Opgen Inc., Gaithersburg, USA)
- The Belgian National Reference Centre for invasive Group A Streptococci is supported by the Belgian Ministry of Social affairs through a fund within the Health Insurance System
- Xavier Basil Britto for bioinformatics support
- Sabine Chapelle for technical assistance



Thank you for your attention