Longitudinal tracking of evolutionary genetic events in Group A *Streptococcus* using Whole Genome Mapping™

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Background: Group A *Streptococcus* (GAS) cause diseases ranging from throat to severe invasive infections. In the last two decades, there has been a global resurgence of severe invasive infections caused by serotype *Emm* protein 1 (M1) GAS. We studied invasive M1 GAS recovered during 1994 - 2013 for major evolutionary genetic events utilizing Whole Genome Mapping™ (WGM), a high-resolution mapping technique.

Methods: M1 GAS (n=18) isolated in 1994 (n=2), 1999 (n=3), 2005 (n=2), 2007 (n=1), 2008 (n=1), 2010 (n=1), 2012 (n=2), 2013 (n=6) from blood and other sterile sites from Belgian patients with toxic shock syndrome, sepsis, multiorgan failure or fasciitis were typed by WGM. *KpnI*-restriction maps generated using Argus® system (Opgen, Gaithersburg, USA) were analysed using MapSolver™ (Opgen). Prophages were identified by PHAge Search Tool.

Results: GAS isolated between 1994 and 2013 shared nearly identical maps with map distance ranging from 0.0 to 2.2%. These could be divided into 5 distinct sub-clusters (1st – 1994, 2010, 2013; 2nd – 2005, 2012; 3rd – 2008; 4th – 2009; 5th – 1999) (**Fig. A,C**). Compared to GAS isolated in 2013, genomic differences were detected in GAS from 1999 (16kb and 30kb insertions and 29kb deletion), 2007 and 2008 (42kb insertion) (**Fig. A**). The insertion in GAS of 2007 and 2008 encoded an intact prophage ϕNIH1 that was predicted to harbour the SpeC exotoxin C derived from other GAS (**Fig. B**). Comparison with published M1 GAS genome sequences showed the Belgian M1 GAS to be most similar to M1 476, a Japanese sepsis isolate (**Fig. C**).

Conclusion: This WGM-enabled study of Belgian M1 GAS illustrated their remarkable genomic stability over the last 20 years with minor genomic changes driven by prophages.

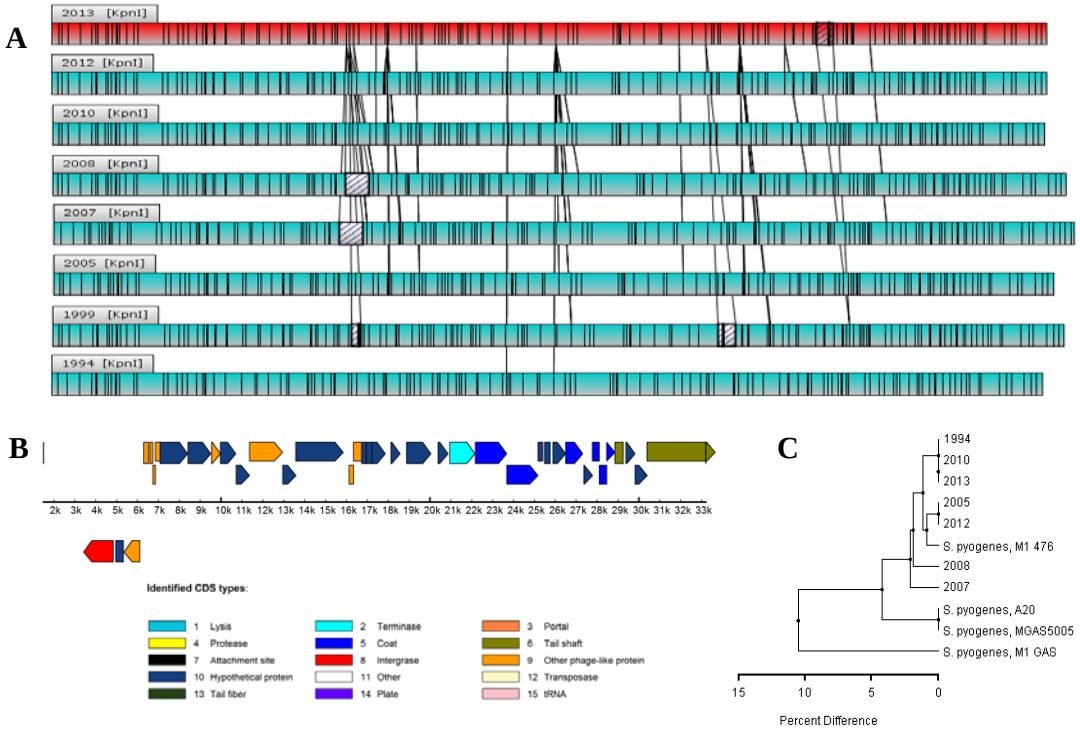

Figure: (A) Comparison of GAS strains isolated from Belgian hospitals between 1994 and 2013. Maps of representative isolates were aligned to the map of 2013. Fragments with perfect alignment are highlighted in green, map of 2013 is highlighted in red. Genomic differences are highlighted by diagonally hashed black lines; **(B)** Identification of φNIH1 in the maps of 2007 and 2008 by comparison to sequenced M1 GAS and MGAS2096; **(C)** Map similarity cluster of mapped as compared to genome sequenced GAS generated by unweighted-pair group method using arithmetic averages.