Gene-Expression Differences in *Streptococcus pyogenes* Strains that Cause Infective Endocarditis

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Research Question

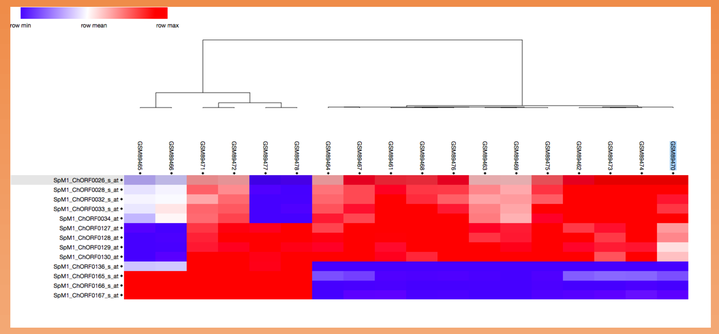
Are there any particular virulence genes which are expressed or over-expressed in *Streptococcus pyogenes* that causes it to attach to tissue of the inner surface of the heart upon entering the bloodstream, leading to Infective Endocarditis.

Abstract

Infective Endocarditis (IE) is a disease wherein bacteria that have entered the bloodstream infect the endocardium, the inner tissue of the heart [1]. 24.7% of cases of IE in the United States were attributed to Streptococci in a recent study [2]. We chose to focus on one rare causative pathogen of IE: Streptococcus pyogenes, which belongs to group A streptococci (GAS) [3]. Herein we analyzed pre-collected gene-expression data, with the assistance of a Bioinformaticist, in order to discover if the reason for the pathogen's attachment to the inner lining of the heart was related to the expression or over-expression of particular genes. Our pre-collected data, "Group A streptococcus (GAS) isolates from pharyngeal and invasive diseases," were gathered using micro-array technology [4]. The gene-expression data described two types of clinical isolates of S. pyogenes; the first was S. pyogenes in its most common form (pharyngeal), the second was a form that had surpassed a patient's immune system (invasive) [4]. We concluded in our data analysis that four over-expressed genes among the Invasive isolates possess significant enough (>4) fold changes to merit further study. Future research may be conducted to find if there is a correlation between the over expression of these particular genes in S. pyogenes which cause invasive disease and the expression of genes in S. pyogenes which cause IE.

Introduction

- There are so-called good bacteria which assists in maintaining overall wellbeing, called homeostasis [3].
- There are latent bad bacteria which occupy an individual's body and may take advantage of momentary vulnerabilities in their immune system to cause disease [4].
- Bacteremia has the potential to cause an intravascular infection known as Infective Endocarditis (IE) also referred to as Bacterial Endocarditis (BE) [5].
- IE is an infection of the membrane which lines the inside of the heart, the endocardium.
- Most patients are men, ages 50 and over, who possess cardiac devices and implants [2].
- An increase in the incidence of IE in the United States has been reported in the last two decades [7].
- Our research seeks to provide new insights on the genetic level concerning specifically why the pathogen *S. pyogenes* attaches to the endocardium upon entering the bloodstream [8].
- Pre-collected data was then analyzed with the help of a Bioinformaticist and GenePattern [11].
- In short, Bioinformatics is a discipline that seeks to understand and organize biological experimental data by applying methods from applied mathematics and computer science [12].



Heat map obtained using GenePattern software

Methods

- We collaborated with a Bioinformaticist and used the software program GenePattern to perform a gene-expression analysis on the pre-collected data.
- The DNA microarray data had to be processed using various software modules and turned into usable, comparable, information that we could make real sense of.
- The first step in the process of turning the publicly available data into data which could be compared and further analyzed was transformation.
- The transformed data then had to be normalized, and after that preprocessed.
- Filters were then applied to this mass of data to identify those genes which were of critical importance (fold change >4).

Materials

- GenePattern: an in-browser, public server, genomic analysis software

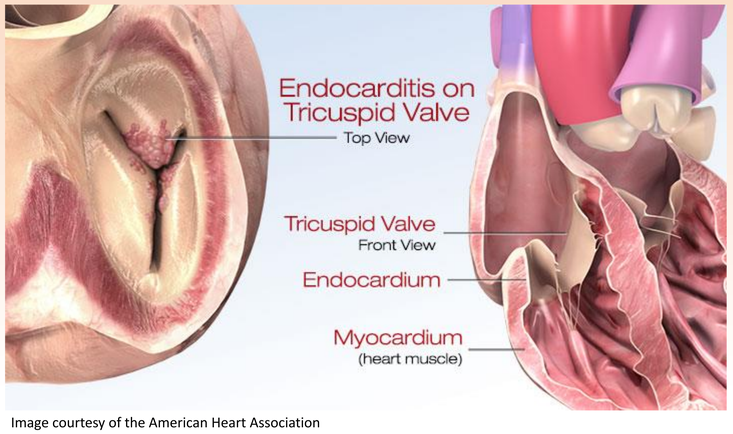


Image courtesy of the American Heart Association

Upregulated	Feature		T-Test	FDR(BH)	Fold Change
Inv	SpM1_ChORF0167_s_	8499 streptolysin O precursor SpyoM1_ChrORF0167	62.759	0.0004515226	4.052
Inv	SpM1_ChORF0165_s_	8497 nicotine adenine dinucleotide glycohydrolase prec	41.819	0.0004515226	4.249
Inv	SpM12_ChORF251-6_	10483 predicted open reading frame SpyoM12_ChrORF	41.671	0.0004515226	3.160
Inv	SpM1_ChORF0428_s_	8683 conserved hypothetical protein SpyoM1_ChrORF(	34.619	0.0004515226	2.499
Inv	SpM1_ChORF2009_at	9929 hypothetical protein SpyoM1_ChrORF2009	32.317	0.0004515226	2.569
Inv	SpM12_ChORF248-6_	10477 predicted open reading frame SpyoM12_ChrORF	31.074	0.0004515226	2.916
Inv	SpM1_ChORF2010_s_	9930 C5A peptidase precursor SpyoM1_ChrORF2010	26.371	0.0004515226	2.819
Inv	SpM1_ChORF2016_at	9932 inhibitor of complement-mediated lysis SpyoM1	25.356	0.0004515226	1.722
Inv	SpM1_ChORF1718_s_	9703 putative esterase SpyoM1_ChrORF1718	18.113	0.0004515226	2.436
Inv	SpM1_ChORF1832_s_	9792 hypothetical protein SpyoM1_ChrORF1832	16.825	0.0004515226	2.086
Inv	SpM1_ChORF0416_s_	8677 putative cell envelope proteinase SpyoM1_ChrO	14.006	0.0004515226	4.577
Inv	SpM18_ChORF2095_s	10430 hypothetical protein SpyoM18_ChrORF2095	13.109	0.0004515226	1.737

Results/Conclusion

In analyzing the pre-collected data and collaborating with a Bioinformaticist we were able to identify 13 genes, whose amount of fold changes (>4), FDR sores, and t-test scores (>13) evinced statistical significance. We narrowed these down to four relevant genes which were: C5A peptidase precursor, Inhibitor of complement mediated lysis, Putative esterase catalyst of hydrolysis of various bonds, and Putative cell envelope proteinase. The first, C5A peptidase precursor, cleaves the human serum chemotaxin destroying its ability to serve as a chemoattractant [13]. The second one was inhibitor of complement mediated lysis which allows the increase of *S. pyogenes* infections to be associated with strains of M1 stereotype, and blocks complement mediated hemolysis [14]. Normally, complement is a process whereby the body's immune system destroys cells. The third gene is Putative esterase catalyst of hydrolysis of various bonds; it is part of a reading frame, which has potential to code for a peptide. The last gene, putative cell envelope proteinase, is also relevant precisely because its exact function is unknown to science. This makes its over-expression an interesting base point for further research concerning how this gene's over-expression relates to *S. pyogenes'* behavior. The presence and significant over-expression of these four genes in isolates of *S. pyogenes* taken from patients who have contracted invasive disease somewhat confirms our hypothesis, because they show us that when *S. pyogenes* becomes invasive, particular genes are expressed that previously were not. If strains of *S. pyogenes* which cause IE behave similarly to *S. pyogenes* which causes invasive disease, then our project serves as a useful conclusion for further research to build upon.

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