A Comprehensive Metagenomic Approach to Determine the Relationship Between Periodontal Disease and Cardiovascular Disease in Young Adults

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ABSTRACT:
Periodontal Disease (PD) is shown to be associated with an increased risk of a number of systemic diseases – including atherosclerosis. We are investigating a possible causal relationship in a young population of individuals with PD (ages 20-30) to determine if they show early signs of atherosclerosis. Using powerful new culture-independent molecular approaches based on pyrosequencing technology, we are conducting a comprehensive metagenomic analysis to identify, classify, and quantify bacterial species associated with PD and atherosclerosis (both pre- and post-treatment for PD). This survey of microbial diversity is expected to cover between 350,000-500,000 16S sequences for 300 samples from 40 patients. The bioinformatics pipeline will include 1) database construction and data analysis using 16S rRNA sequence for species, 2) sequence alignment and phylogenetic analysis, and 3) computing distance metrics for differences between microbial communities (samples) at different stages of PD and with or without changes in brachial artery flow dynamics. Multivariate analyses, such as Principal Component Analysis (PCA), will be used to show the distribution of 16S community samples. Subsequently, functional annotation of the genes combined with relative abundance data for each species will allow for the prediction of important functional relationships among potential subgroups of species that support the persistence of PD and that could be involved in the mechanism of atherosclerosis. Ultimately, this knowledge could be used to develop an effective probiotic treatment for PD.

BACKGROUND AND SIGNIFICANCE:
Periodontal disease (PD) is a risk factor for atherosclerosis. Endothelial dysfunction is a sensitive and reliable indicator of vascular health. PD is accompanied by a local inflammatory response. The magnitude of inflammatory burden is related to the progression of atherosclerotic calcifications in patients with advanced disease (Li et al. 2007). Two studies implicated P. gingivalis as a particular risk factor for atherosclerosis (Chou et al. 2005; Yamazaki et al. 2007). Some bacteria secrete factors that limit growth of other species, likely substantially altering the entire ecosystem. Comprehensive characterization of the oral microbial ecosystem including many anaerobic species and those never before cultured (Lupp et al. 2008) will prove valuable in identifying those that are associated with PD, as well as oral flora specifically associated with atherosclerosis (as reflected by endothelial dysfunction) is highly problematic with new high-throughput molecular methods.

Culture-Independent Molecular Methods:
Culture-independent molecular methods, based on PCR and molecular cloning of small subunit ribosomal (16S) RNA gene sequences, revolutionized our ability to study previously uncultured microbial organisms. The 16S RNA gene is the “gold-standard” of markers for culture-independent microbial studies because: (1) it is found in all forms of cellular life: bacterial, archaeal and eukaryal; (2) Enormous databases of 16S sequences from cultured and uncultured microbes, allow for easy identification and phylogenetic analyses; (3) This molecule contains both highly conserved regions necessary for universal primer design, and highly variable regions necessary for identifying species and phylogenetic relationships (Pace 1997; Hueckelhoven et al. 1998). Our lab has studied microbial communities in sheep respiratory tracts (Safae et al. 2006), acid hot springs (Mathur et al. 2007), contaminated human environments (McManus and Kelly 2005; Lee et al. 2007) and oceanic waters (Breitbart et al. 2004; Argyle et al. 2006). Although we cannot completely rule out bias, particularly PCR bias, culture-independent diversity estimates, are considerably less biased than culturing.

Bioinformatics and Data Analysis Pipeline:

Figure 1: Flow of Analysis of Sequence Data
Phylogeny
Phylogeny Neighbor-joining (NJ) algorithm.

Sequence Similarity
Sequence Similarity BLAST, NAST, ClustalW Sequence-replication: Python script

Unifrac Analysis
Distance Matrix Calculation: Clustering: Hierarchical: PCA

Figure 2: Flow of Analysis of Sequence Data
Phylogeny
Phylogeny Neighbor-joining (NJ) algorithm.

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Sequence Similarity BLAST, NAST, ClustalW Sequence-replication: Python script

Unifrac Analysis
Distance Matrix Calculation: Clustering: Hierarchical: PCA

Patient Parameters for clustering analysis:
Baseline brachial artery diameter and reactive hyperemia (observed baseline blood flow);
Carotid artery intima-media wall thickness; 6-mode ultrasound images to measure carotid artery intima-media thickness (Selzer et al. 1994).

Bioinformatics:
1) Sequencing:
(a) Pyrosequencing
(b) 454 Life Sciences

Bladder
(a) High-resolution ultrasound
(b) 4D ultrasound

RESEARCH DESIGN AND METHODS:
Preliminary Results:

Figure 3: Microarray Analysis (left)
This image demonstrates the ability to rapidly detect the relative presence and quantities of a variety of different species of microorganisms that are present within samples or the oral microbial flora. Ultimately, this knowledge could be used to develop an effective probiotic treatment for PD.

Preclinical Results:
Cultured serum samples from PD patients will be stored for subsequent analysis of IL-6, TNF-α, CRP, and antibodies to LPS* for *Actinobacillus actinomycetemcomitans, #Porphyromonas gingivalis, Prevotella intermedia.

EXPECTED OUTCOMES:
This will be the first study to evaluate endothelial function in relation to periodontal disease in young adults aged 20-30. If periodontal disease contributes to vascular dysfunction and the development of atherosclerosis, the follow-up study will reveal whether vascular dysfunction is reversible in this age group and may provide insight into the role of poor oral hygiene as a contributing factor to health disparity. The most important information derived from this work will be the evaluation of the complex oral microbial flora in this patient population. Previously uncharacterized species of bacteria may be identified, some of which are expected to correlate with periodontal disease. We hypothesize that some organisms may correlate strongly with endothelial dysfunction. We also hypothesize that some organisms will correlate with good oral (and/or cardiovascular) health. Such organisms may act as factors that potentially suppress the overgrowth of oral pathogens and could benefit.

This study, which will demonstrate whether treating periodontal disease in young adults can normalize endothelial function, represents an important step towards demonstrating causality and will furthermore extend our understanding of microbial communities in the gastrointestinal tract and how they correlate with vascular health.

REFERENCES: