Quantitative proteomic analysis of GCF to observe oral disease progression and treatment: From health through to severe periodontitis

Andrew J. Creese[1], Melissa M. Grant[1], Marcelo Aspiras[2], Marco de Jager[2], Iain L C. Chapple[1], Helen J. Cooper[3]

1. Periodontal research group, School of Dentistry, University of Birmingham, St Chads Queensway, Birmingham, B4 6NN, UK.
2. Clinical & Scientific Affairs, Philips Oral Healthcare, Snoqualmie, WA.
3. School of Biosciences, University of Birmingham, Birmingham, B15 2TT, UK.

Overview

- Gingival crevicular fluid (GCF) is a complex biological fluid exuded between the teeth and gums to keep the oral cavity healthy. It is a source of protein biomarkers for disease.
- Here we ask: How does protein abundance vary between health, gingivitis, periodontitis and the return to health?

Introduction

- GCF is a complex biological fluid with a large dynamic range. It is a rich source of proteins and collection is non-invasive. However the relative abundance of these proteins across disease states is currently poorly defined.
- We show quantitative online data-dependent liquid chromatography (LC) MS/MS analysis of GCF from healthy volunteers and volunteers at various points of disease performed on a Thermo Fisher Scientific LTQ-Orbitrap Velos mass spectrometer.
- 272 proteins were identified with 2 or more peptides. These proteins could be clustered into several different groups according to changes in relative abundance across disease.

Method

- GCF was collect on filter paper placed between the tooth and the gum for 30 seconds and snap frozen in ammonium bicarbonate (100 mM) from four groups of 10 volunteers. Pristine health, gingivitis, mild periodontitis and severe periodontitis. Samples were also collected post treatment from the two periodontitis groups.
- The samples were vortexed and centrifuged with the supernatant retained. The supernatant from each group were combined to give 6 samples.
- The proteins in both samples were reduced and alkylated prior to overnight digestion with Lys-C and trypsin. (Promega)
- The digests were labelled with six iTRAQ (ABSciex) labels from an 8-plex (113 to 118) and combined.
- The peptide mixture was fractionated by strong cation exchange (SCX) chromatography. SCX was performed with a Polysulfoethyl A column. Peptides were eluted over a 60 minute salt gradient from 0-50% 500 mM KCl (pH 3). 13 fractions were collected.
- The fractions were loaded onto a 75 µm C18 reversed phase analytical column (LC Packings) and peptides were separated over a 30 minute gradient from 3.2 to 44% acetonitrile (0.1% formic acid).
- Samples were infused by use of an Advion Triversa Nanomate nanospray ionization source into a Thermo Fisher Scientific LTQ-Orbitrap Velos hybrid mass spectrometer.
- The mass spectrometer performed an initial high resolution survey scan (15,000 at *m/z* 400) in the Orbitrap and the three most abundant multiply charged ions were selected for collision induced dissociation (CID) MS/MS, detected in the ion trap. The same three ions were then fragmented by higher energy collisional dissociation (HCD), detected in the Orbitrap.
- Analysed ions were placed on an exclusion list for 60 seconds.
- Data were collected with Xcalibur 2.1 (Thermo Fisher Scientific) and analysed using the SEQUEST and Mascot search algorithms and the IPI human database (v3.89) supplemented with known oral bacteria and concatenated with the reverse sequences.
- CID spectra were used to identify the peptides and HCD spectra were used for quantification.
- Clustering analysis was performed with PolySNAP3, Gene Cluster 3.0 and Treeview.
- Protein-Protein interaction networks were created in Cytoscape (v2.8.1) and Bisogenet plugin.

Conclusions

- We have identified 272 proteins from GCF with two or more peptides at a 1% FDR.
- Clustering analysis shows there are at least 4 distinct groups containing between 5 and 50 proteins within this dataset which show increases or decreases at different points during the different states of disease.
- The proteins in these groups may be used as biomarkers to define disease, predict patients that may progress from gingivitis to periodontitis and follow the return to health.

Acknowledgements

This work was funded by Philips Oral Healthcare. The Advion Triversa Nanomate, Dionex LC and Thermo Fisher Orbitrap mass spectrometer used in this research were funded through the Birmingham Science City Translational Medicine: Experimental Medicine Network of Excellence Project, with support for Advantage West midlands (AWM).

Results I

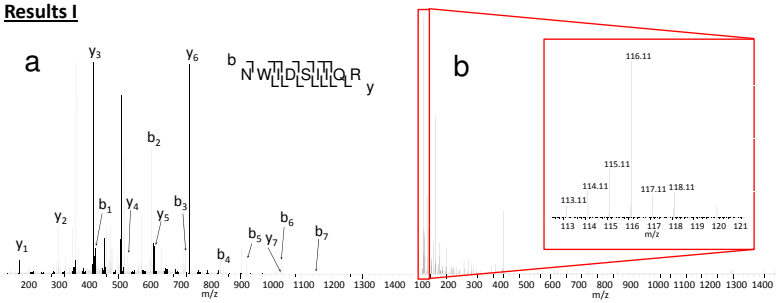

Figure 1: The CID mass spectrum of a peptide from neutrophil elastase (a) and the HCD spectrum of the same peptide (b), inset the low mass region of a HCD spectrum used for quantification.

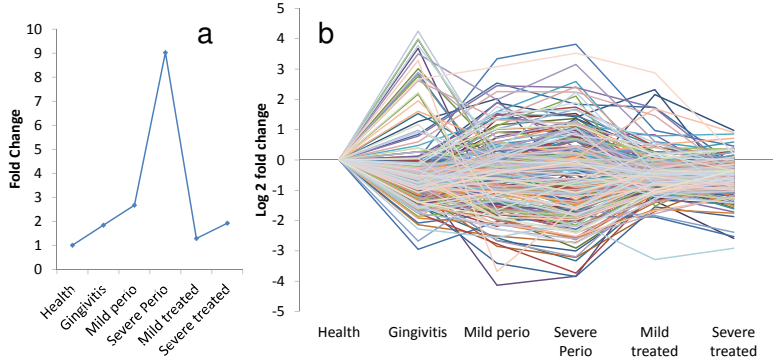

Figure 2a: The observed variation in neutrophil elastase abundance across the groups. 2b: The combined (normalised to volume and log 2 transformed) relative abundances of the 272 proteins across the disease groups.

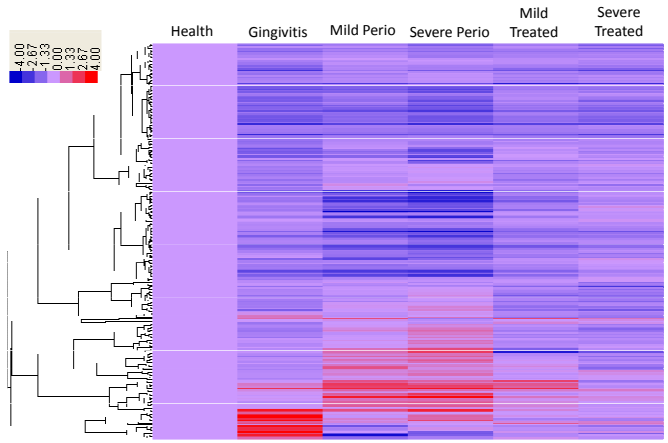

Figure 3: The Gene Cluster clustering of the 272 proteins (blue and red indicate decreases and increases in relative protein abundance respectively). Several groups with similar features can be seen.

Results II

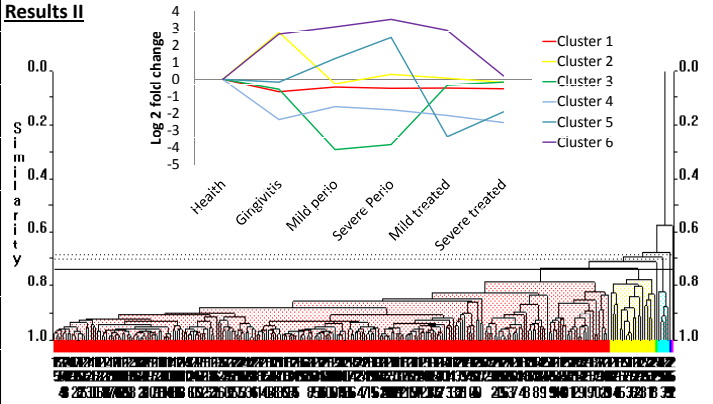

Figure 4: The dendrogram from the first round of PolySNAP3 clustering. The average abundance profiles for the clusters (inset).

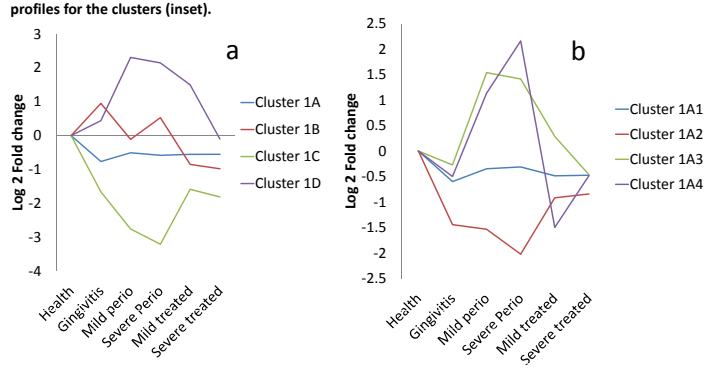

Figure 5a: Clustering of the largest group observed in figure 4. Figure 5b shows the re-clustering of cluster 1A. The PolySNAP3 clusters reveal 4 distinct groups which show interesting variation in abundance.

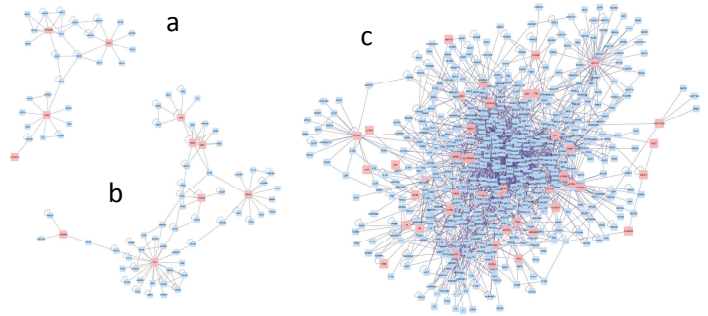

Figure 6: The Protein-Protein interaction networks for three of the 4 distinct clusters. Network a; proteins which increase with periodontitis. Network b; proteins which increase with gingivitis. Network c; proteins which decrease with gingivitis and periodontitis before returning to baseline.

Andrew Creese, Periodontal research group, School of Dentistry, University of Birmingham, St Chads Queensway, Birmingham, B4 6NN, UK. E-mail:a.creese.2@bham.ac.uk