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

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Overview

- Saliva is a complex biological fluid secreted from several glands. The main function of saliva is to initiate digestion, however; it is also antibacterial, protein rich and a source of biomarkers of disease.
- Here we ask: How does protein abundance in saliva vary between health, gingivitis, periodontitis and the return to health following treatment of periodontitis?

Introduction

- Saliva 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 saliva from healthy volunteers and volunteers at various points of disease performed on a Thermo Fisher Scientific LTQ-Orbitrap Velos mass spectrometer.
- 314 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

- Saliva was collected for 5 minutes by a stimulated method, from four groups of 10 volunteers: Pristine health, gingivitis, mild periodontitis and severe periodontitis. Samples were also collected three months post treatment from the two periodontitis groups.
- The samples were vortexed and centrifuged with the supernatant retained. A portion of the supernatant from each sample within the groups was 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% FA).
- 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 ontology analysis was performed with Panther online tools.
- Protein-Protein interaction networks were created in Cytoscape (v2.8.3) and Bisogenet plugin.

Conclusions

- We have identified 314 proteins from saliva with two or more peptides at a 1% FDR.
- Clustering analysis shows there are at several 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, differentiate between gingivitis to periodontitis and follow the return to health post treatment.

Acknowledgements

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Results I

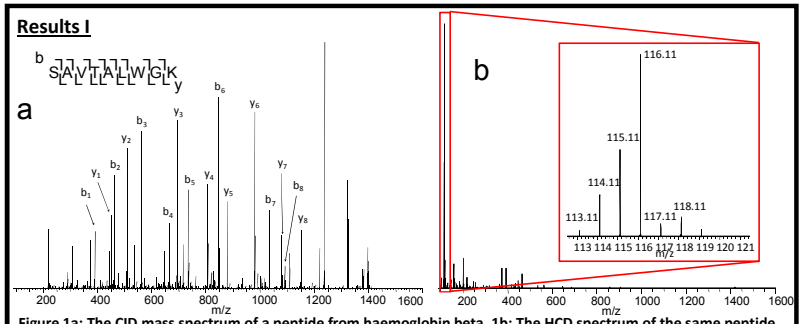

Figure 1a: The CID mass spectrum of a peptide from haemoglobin beta. 1b: The HCD spectrum of the same peptide, inset the low mass region of a HCD spectrum used for quantification.

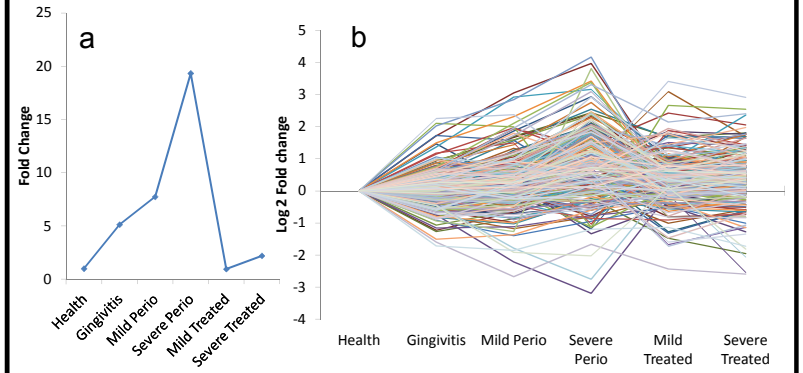

Figure 2a: The observed variation in haemoglobin beta abundance across the groups. 2b: The combined (normalised to volume and log 2 transformed) relative abundances of the 314 proteins across the disease groups.

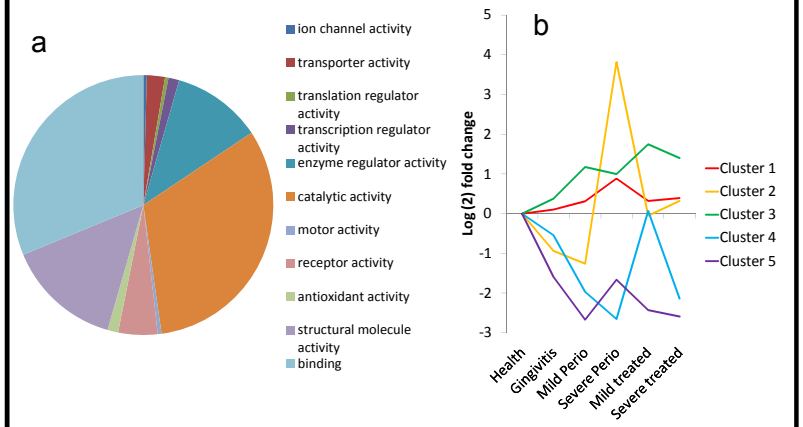

Figure 3a: The distribution of molecular functions observed from the proteins identified. 3b: The first round of clustering with PolySNAP 3, 297 proteins were in cluster 1.

Results II

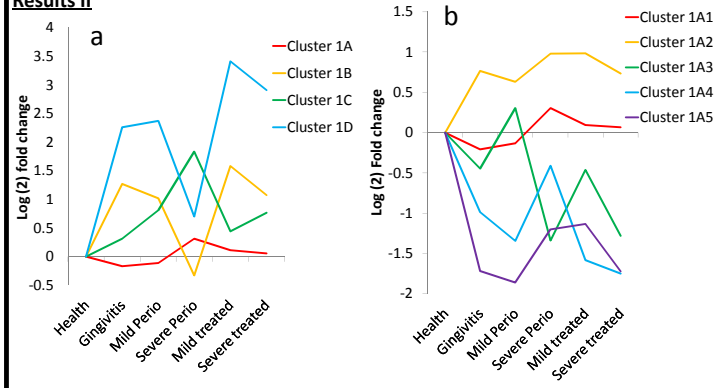

Figure 4a: Clustering of the largest group observed in figure 3b, clusters 1A and 1C contain 166 and 116 proteins. 4b: shows the re-clustering of cluster 1A.

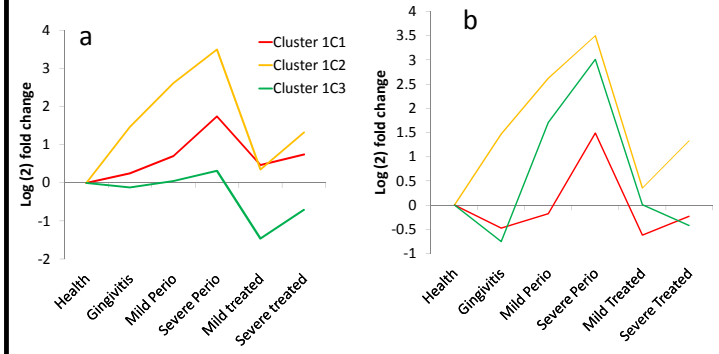

Figure 5a: Clustering of 116 proteins observed in cluster 1C. Further clustering was performed in 1C1 and 1A1. 5b: Three clusters which contain proteins with the potential to classify disease state and return to health post treatment.

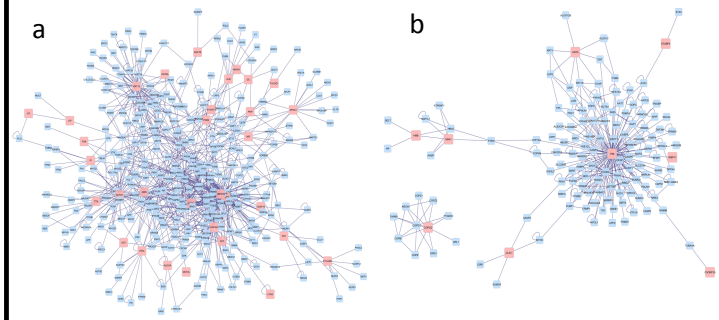

Figure 6: The Protein-Protein interaction networks for two of the three distinct clusters. Network a; proteins which increase with disease, these proteins are involved in apoptosis. Network b; proteins which increase only in periodontitis, these proteins are involved in oxygen transport.

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