What do 'omic technologies have to offer periodontal clinical practice in the future?

Grant, Melissa

DOI:
10.1111/j.1600-0765.2011.01387.x

Document Version
Early version, also known as pre-print

Citation for published version (Harvard):
Grant, M 2012, 'What do 'omic technologies have to offer periodontal clinical practice in the future?', Journal of Periodontal Research, vol. 47, no. 1, pp. 2-14. https://doi.org/10.1111/j.1600-0765.2011.01387.x

Title: What do ‘omic technologies have to offer periodontal clinical practice in the future?

Authors: Melissa M Grant[1]

Affiliations:

[1] Periodontal Research Group, School of Dentistry, University of Birmingham, St Chads Queensway, Birmingham, B4 6NN, UK

Running title: ‘Omic technologies & periodontal research

Author for correspondence:

Dr. Melissa M Grant, Periodontal Research Group, School of Dentistry, University of Birmingham, St Chads Queensway, Birmingham, B4 6NN, UK.

Tel: +44 121 237 2884

Email: m.m.grant@bham.ac.uk

Keywords: Genomics, Transcriptomics, Proteomics, Metabolomics, Systems Biology, Periodontology

Abstract:

Background & Objective: Periodontal diseases are the most common chronic inflammatory diseases of humans and a major cause of tooth loss. Inflammatory periodontitis is also a complex multi-factorial disease involving many cell types, cell products and interactions. It is associated with a dysregulated inflammatory response, which fails to resolve, and which also fails to re-establish a beneficial periodontal microbiota. There is a rich history of biomarker research within the field of periodontology, but exemplary improvements in analytical platform technologies offer exciting opportunities for discovery. These include the ‘omic technologies, genomics, transcriptomics, proteomics and metabolomics, which provide information on global scales that can match the complexity of the disease. This narrative review focuses on the recent advances made in *in vivo* human periodontal research by use of ‘omic technologies.

Methods: The Medline database was searched to identify articles currently available on ‘omic technologies in regard to periodontal research

Results: 144 articles focusing on biomarkers of and ‘omic advances in periodontal research were analyzed for their contributions to the understanding of periodontal diseases.

Conclusion: The data generated by the use of ‘omic technologies have huge potential to inform paradigm shifts in our understanding of periodontal diseases, but data management, analysis and interpretation require a thoughtful and systematic bioinformatics approach, to ensure meaningful conclusions can be made.

Introduction

Periodontal diseases are the most common chronic inflammatory diseases of humans and a major cause of tooth loss (1). Diagnosis requires training, knowledge and dedicated clinical facilities, creating a need for those in non-specialist and/or non-dental environments (e.g. medical practice) for simple, objective diagnostic tools, to help identify patients with periodontitis. These would help in early diagnosis of disease onset, progression, or indeed resolution following treatment and may reduce both the healthcare and economic burdens arising from periodontitis, estimated as £2.78 billion in the UK in 2008 (2). Moreover, they may positively impact upon systemic inflammatory diseases, where periodontitis is recognised as a risk factor. The identification of biomarkers using 'omic' technologies, such as genomics, transcriptomics, proteomics and metabolomics, could deliver such diagnostic tests.

The official National Institute of Health (NIH, USA) definition of a biomarker is 'a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention'. Although this could be a physical trait, such as hair colour, for the purpose of this focussed review of *in vivo* biomarkers of human periodontitis only molecular biomarkers, and those determined in a genetic, proteomic or metabolomic profile will be discussed. As Bensalah et al (3) have recently documented, six different types of biomarker can be differentiated. These are:

- early detection of disease;
- diagnosis of presence or absence of disease;
- prognosis of disease outcome and possible patient stratification allowing for personalized medical interventions;

- prediction of treatment outcome;
- identification of patients who will respond well to a particular treatment;
- surrogate end points.

In addition for a biomarker, or a panel of biomarkers, to be successfully employed within the clinical environment, they must also be: objective; reproducible; easy to use; cheaper and; with greater sensitivity, specificity and diagnostic accuracy than existing tests (3-5). These hurdles are made higher still by the need for potential biomarkers to achieve a status akin to the rigorous governance processes through which drugs must pass for licensing; there is, however, currently no such mechanism in place for such evaluations(3).

In the past, the most useful biomarkers have either been found serendipitously or through careful evaluation of candidates generated through hypothesis driven research (6). Many potential biomarkers are developed using pre-clinical *in vitro* models and a few go onto the development of assays used in the evaluation of a small number of patients in the equivalent of phase 1 trials. Proof of biomarker efficacy cannot be established solely by statistics, there needs to be an evaluation akin to structured, phased trial testing (3, 6). Such independent validation and efficacy determination in large community dwelling populations, in the equivalent of phase 2 and 3 trials, is even scarcer than phase 1 studies. Thus to mine the proverbial biomarker iceberg and to leverage these novel biomarker technologies, larger multi-centre multi-'omic systems biology trials need to be performed.

Samples available for *in vivo* studies of periodontal diseases include: GCF, plaque, saliva, biopsies, peripheral blood cells and plasma (figure 1). Several excellent reviews discuss these compartments for targeted approaches to biomarker discovery (5, 7-10). In particular Loos & Tjoa (5) undertook a critical review of biomarkers in GCF and found only 8 out of 94, in the literature of the time, fulfilled any of the criteria for biomarker status. These included: alkaline phosphatase (11-17) , beta glucuronidase (15, 18-26) , cathepsin B (27-32) , MMP-8 &-9 (15, 33-45) , dipeptidyl peptidase II & IV (28, 29, 31, 46), neutrophil elastase (15, 24, 26-

29, 39, 47-66). Potential novel biomarkers have been described since using ‘omics driven discoveries as are discussed below.

The ‘omic technologies include genomics, transcriptomics, proteomics and metabolomics (figure 1) and each is discussed below. It should be noted that, in contrast to genomics, transcriptomics, proteomics and metabolomics assess the temporal expression of genes rather than the static encoding of the genome. Thus they take into account environmental influences, nurture as well as nature. As we progress from genomics, to transcriptomics, proteomics and metabolomics we also progress from what might happen to what actually did happen: with transcriptomics being influenced by translation and activation; proteomics elucidating changes to global protein expression, splice variants of proteins and post-translational modifications; and metabolomics demonstrating end products of reactions. All these technologies and assessments can be applied to both the host and the microbiota in periodontitis. Here, only the host contributions are discussed.

Drawbacks of all the functional genomics technologies include confounding issues such as age, gender, diet, smoking and likely many more. Where dynamic range is a problem the technology may be affected by the ‘usual suspects’ phenomenon (67) where similar species are found in a variety of unrelated studies and reflect the fact that some situations/treatments affect central signalling or metabolic hubs within cells, for example affecting energy generation. This is a problem that can mask less obvious biological perturbations, but can be overcome with much larger study populations where general “noise” can be removed and small changes can gain statistical significance due to increasing study power.

Genomics

Genomics is the study of whole genomes, i.e. all the DNA of a single organism. With improvements in sequencing, the dawn of genome-driven individualised medicine has arrived, where changes to multiple genes may be taken into account for diagnosis and

treatment. But with differences in more than 3 million nucleotides (0.1% of the whole genome) evident when comparing 2 individual genomes, it will likely take many years before such differences can be mapped to disease correlations (68, 69). However, for some time, changes in individual genes (gene polymorphisms) have been studied with reference to disease risk, severity and therapeutic outcome. These gene polymorphisms are highly prevalent in the population (70) and the most common type is the single nucleotide polymorphism (SNP) where an individual base pair is affected, by alteration within, insertion into, or deletion from the DNA sequence. Where these changes fall in promoter regions, exons, introns or untranslated regions, will differentially affect gene products (69).

The influence of SNPs on periodontal disease was reviewed in 2006 by Takashiba & Naruishi (69). They highlighted that nearly half of the research in this area has focused upon cytokines, with the rest investigating human leukocyte antigens, immuno-receptors, proteases, structural molecules and other proteins. However, of the 140 papers they used for their review the majority focused on only 6 genes: (Interleukin (IL) 1, Tumour necrosis factor (TNF) α, Fcγ receptors, matrix metalloproteins, cathepsin C and vitamin D receptor), indicating that this field is still in its infancy. IL-1 SNPs were suggested to be more associated with environmental interactions, such as with smoking, than with susceptibility to periodontitis, whereas TNFα showed a lack of association with inflammatory periodontal disease. However, polymorphisms in Fcγ receptors tend to be associated with both aggressive and chronic forms of periodontitis. For the other genes mentioned above, limited evidence makes it difficult to relate SNPs to periodontitis. In the past 5 years since the review by Takashiba & Naruishi (69), there have been at least an additional 37 articles published concerning SNPs in cytokines (71-107) . These small scale studies of individual SNPs are no longer in a position to contribute anything new to the literature and are of limited value.

Moving into wider ranging analysis, Suzuki et al (108) examined 637 SNPs in 19 healthy and 22 severe periodontitis cases, revealing 5 previously untargeted genes as potential

markers for periodontitis. Using an *ab initio* bioinformatic approach, Covani et al (109) predicted five leader genes from an investigation of 61 genes potentially involved in periodontitis, using published articles as the source of data. These genes were NFkB1, CBL, GRB2, PIK3R1 and RELA, and are predominantly involved receptor-mediated signalling and may reflect the stimulation of the host inflammatory-immune system by bacteria in periodontitis.

Overall the genetic basis of periodontitis accounts for approximately half the population variance in chronic periodontitis (110, 111). There is a need to progress to large scale genome wide association studies (GWAS) and the first of these has been published (111). Comparison of two cohorts of aggressive periodontitis patients independently identified 197 and 244 quality controlled SNPs from 141 and 142 patients respectively, examining 500,568 potential SNPs. However, when the results from both sets were compared only one remained significant, which was subsequently validated in a third set of patients (n=164). The gene identified was *GLT6D1*, which encodes for a glycosyltransferase 6 family protein. These enzymes are single pass transmembrane proteins which contribute to the synthesis of histo-blood related antigens in the golgi. *GLT6D1* was found to be highly expressed in the gingival connective tissues and may influence immune responses. Future studies using greater numbers of patients and controls may yield more associations, however the acquisition of even one unknown gene that may predict periodontal disease is potentially of great value.

Transcriptomics

The field of transcriptomics involves the study of messenger RNA (mRNA) production by cells under particular conditions. Unlike proteomics and metabolomics (below), this is typically studied in cell populations and thus in periodontal investigations either utilises biopsies of relevant oral tissues or peripheral blood leukocytes rather than oral fluids such as

GCF and saliva, which can be studied using proteomic and metabolomic platforms. There are two major advantages that this technique provides: 1) the ability to amplify the expressed gene products; and 2) the stability and uniformity of the platforms employed in identification of interesting and/or novel species. This is reflected in the far greater number of articles reporting transcriptomic studies than proteomic and metabolomic studies. Over the last 5 years Papapanou and colleagues have analysed whole tissue transcriptomes from the excised papillae of healthy and diseased patients in an attempt to re-classify periodontal disease biologically rather than clinically (112-114). A pilot study however could not differentiate between chronic and aggressive forms of periodontitis (112) but comparison of diseased and healthy papillae from patients with advanced periodontitis did detect differences in gene ontology groups for apoptosis, antimicrobial humoral responses, antigen presentation, regulation of metabolic groups, signal transduction and angiogenesis. The authors commented that the papillae are composed of a variety of cell types, these differences in composition may give rise to different transcriptome profiles and contribute to the heterogeneity of results. However, it was possible to identify genes that have not previously been linked with periodontal diseases, such as CXCL6 (granulocyte chemoattractant protein 6 (112, 115). In their latest paper, Papapanou et al (114) correlated the transcriptomes of chronic periodontitis patients with the subgingival microflora in those patients/sites. This interesting study coupled the two key drivers of periodontal disease expression, the host and microbial factors, to determine whether species of bacteria can cluster the large number of genes differentially expressed in periodontal disease, thus yielding information on how bacterial species might influence host gene expression. Gingival biopsies were also taken by Offenbacher et al (116) to investigate the temporal changes in gene expression during experimental gingivitis. Again, large numbers of genes were differentially expressed and novel gene ontology groups were reported including those of neural process, epithelial defences, angiogenesis and wound healing.

Beikler et al (117) investigated gene expression changes in periodontal tissues before and after treatment using a semi-targeted human inflammation microarray. They concluded that those gene profiles that were altered the most indicated an activation of pathways that regulate tissue damage and repair. Kim et al (118) examined sub-epithelial connective tissues from healthy controls and periodontal patients. They found these tissues also demonstrated transcriptomic increases in the immune response, tissue remodelling and apoptosis genes.

Looking at how periodontitis affects the peripheral blood system, Papapanou et al (119) took monocytes from periodontal patients undergoing treatment and examined mRNA expression using Affymetrix arrays. They found that a third of patients had substantial changes in genes relevant to innate immunity, apoptosis and cell signalling; and concluded that periodontal therapy had a systemic anti-inflammatory effect. Matthews et al (120, 121) have previously reported that neutrophils from periodontitis patients are both hyper-reactive to stimulation by *F.nucleatum* or Fcγ-receptors and also show baseline hyperactivity with respect to reactive oxygen species (ROS) production. Following these discoveries, the same group (122) utilised neutrophils from periodontitis patients to determine what genes were affected. They found significant increases in type-1 interferon-stimulated genes and this led to the discovery that patients had significantly greater concentrations of circulating interferon-alpha, which, upon successful periodontal treatment, decreased to the same levels as non-diseased controls. They concluded that periodontitis is a complex disease where increases in interferon-alpha may be one component of a distinct molecular phenotype in neutrophils, triggered potentially by viral priming or autoimmune responses. This latter concept is new to periodontology and may help explain the association between periodontitis and rheumatoid arthritis (123-125).

Advances have been made using transcriptomic approaches but there is a need to bring together the established datasets and also to conduct much larger, wide ranging studies that can take into account possible changes in cell type within periodontal tissues, to pinpoint

genes that may be useful in differentiating between disease types and address the criteria for biomarker research previously stated.

Proteomics

Proteomics, the study of all the proteins in a given sample, was revolutionised by advances in mass spectrometry in the 1990s. It became possible to identify the constituent protein species within biological samples and now many studies have used an ever expanding and complex array of techniques that are both qualitative and quantitative in their outputs. A feature of many biological/clinical samples is that they exhibit a very wide dynamic range of constituent protein species, for instance in plasma that range is 6 orders of magnitude. Without the advantages that DNA and RNA amplification strategies offer, it is often not possible to examine the entire proteome, and it is frequently necessary to try and remove or separate the most abundant proteins from a sample (e.g. albumin) prior to analysis. However, proteomics does address changes to proteins such as splice variants and post translational modifications. Targeted approaches to look at panels of cytokines, such as using the bead based Luminex platform, allow examination of proteins of low concentration, but such presumptive approaches are not discussed here.

In the study of periodontal diseases many proteomic approaches have been used. Top-down whole protein approaches to identify small molecular weight proteins have investigated the presence of human neutrophil peptides (HNPs) (126-128) in gingival crevicular fluid. However, the use of bottom-up approaches, where proteins are digested to individual peptides prior to identification by tandem mass spectrometry techniques, has yielded many more novel insights into the periodontitis proteome. Kojima et al (129) separated GCF proteins by 2-dimensional electrophoresis (2DE) and then identified proteins of interest by mass spectrometry. The addition of 2DE introduced a way to quantitatively assess protein levels between diseased and healthy subjects, although intra-individual variation swamped

the slight trend for more calprotectin subunits in periodontitis patients. Use of liquid chromatography (LC) mass spectrometry techniques to study periodontitis has recently been reported. Ngo et al (130) examined GCF samples by electrophoresis and LC-MS/MS to identify 66 proteins, which included a large number of serum and cell derived proteins reflecting the dual origin of the fluid. Wu et al (131) compared saliva proteomes from generalised aggressive periodontitis patients and controls using a similar technique. Whole saliva yielded differences in highly abundant proteins, such as albumin and amylase which were increased in the diseased samples, illustrating perhaps the need for prefractionation to dissect deeper down into the proteome. Quantitative LC-MS/MS has been used by Bostanci et al (132) and by Grant et al (133) to investigate GCF profiles from patients with generalized aggressive periodontitis and volunteers undergoing experimental gingivitis, respectively. Both studies, as with Ngo et al (130), found proteins of both serum and tissue origins, and more specifically found changes in common previously uninvestigated proteins, such as neutrophil Plastin-2, an actin bundling protein involved in Fcγ–receptor stimulation. With the inclusion of a quantitative aspect these studies allow for a more detailed investigation, where bioinformatic tools may be able to find composites of proteins that could be used as biomarkers. However, to date these biomarkers have not been validated.

Metabolomics

Metabolomics is a discipline that studies the quantities of all chemicals except DNA, RNA and proteins within a sample. No one experimental technique can analyse all chemical structures. Thus samples need to be analysed by a battery of techniques and separated by their chemical and physical properties and identified, principally, by nuclear magnetic resonance (NMR) and mass spectrometry. There is a vast number of potential metabolites and targeted approaches have elucidated some changes (22, 134-136), but there are very few articles that report on tackling the global metabolome in periodontal disease. Barnes et

al (137) used gas and liquid chromatographic separations coupled to mass spectrometry to investigate GCF samples from 22 chronic periodontitis patients, stratified for healthy, gingivitis and periodontitis sites. They identified 103 metabolites in comparison to a chemical reference library, finding that levels of metabolites from gingivitis sites fell between healthy and periodontitis sites. At disease sites, in comparison to healthy sites, antioxidant, glutamine and di-and tri-sacchride levels were decreased whereas amino acids (except glutamine), choline, glucose, polyamines, and purine degradation and urea cycle metabolites were increased. This study has expanded our knowledge of the sources of oxidative stress, which is already acknowledged as being of particular importance, in periodontal disease by the potential increase in activity of the xanthine oxidase-reactive oxygen species axis (137). NMR based approaches have not, as yet, been described for human GCF. This may be due to the larger concentrations of samples required.

Lipidomics is a particular subgroup of metabolomics that investigates the role of lipids in cellular function, because they integrate signalling and metabolic processes. The most common technique employs mass spectrometry, particularly using MS^n where n>1. Recently, Gronert et al (138) used a lipidomics approach to identify and quantify diacyl glycerol species in neutrophils from LAP patients, following a transcriptomics analysis that had identified DAG kinase from neutrophils as not being expressed, in comparison to disease free controls. Metabolomics is an area that could and should see intensive research to provide a clearer understanding of periodontitis. It will be able to reveal information about host and host-microflora interactions which may yield specific small molecule targets that have been over looked by other techniques.

Systems Biology

Systems biology is the integration of multiple omics platforms and data through the reconstruction of the complex networks involved (139). These complex networks

characterise particular systems, often cells, but in periodontitis it would need to address the whole disease – interactions not of one cell type, but many and also with the micro-organisms present in the disease state. Advances in network inference and analysis in other diseases, such as obesity, diabetes and atherosclerosis, are already highlighting that it may be necessary to target multiple (10-50) genes, in different tissues, simultaneously to treat a disease effectively(140). Such an approach would yield a holistic overview of the disease milieu. The complementary information from the different 'omic technologies needs to be coordinated and integrated, and several strategies are being progressed in other research areas (141). This still remains a major challenge to the periodontal field and there is still the requirement for fundamental understanding of the mechanisms taking place so that the data can be appropriately modelled. Using holistic approaches will have the advantage that they will address the synergistic qualities of multiple bacterial challenges and multiple cell types present at the diseased lesion. The bacterial challenge in particular should not be overlooked, with so many so called unculturable bacteria being present (142). Microbiome strategies to study the thousands of bacteria present will unite with the 'omic technologies (143). Nibali et al (144) have already termed the interaction between host genetic factors, such as SNPs, and the oral microbiome as "infectogenomics".

To conclude, as yet 'omic technologies have not yielded validated biomarkers for periodontal disease but they are identifying new routes for research to follow in relation to disease pathogenesis. It is unrealistic to think that one biomarker will be found, there is no more "low hanging fruit" (5). Periodontitis is acknowledged as a complex inflammatory disease, initiated by a plaque biofilm and with multiple component causes, and it is therefore much more likely that there is a multiplicity of biomarkers which together can: differentiate between health and disease; between disease onset and progression; improve the prognosis of disease outcomes and possible patient stratification allowing for personalized medical interventions; identify disease resolution/healing; predict treatment outcomes; identify patients who will

respond well to a particular treatment; or provide surrogate end points. The use of use 'omic techniques will play an important role in their discovery.

Conflict of Interest and Sources of Funding Statement

The author declares that they have no conflict of interest. The author gratefully acknowledges Unilever Oral Care UK (Unilever Oral Care, Bebington, UK) for funding.

References

(1) Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. *Lancet* 2005; **366**: 1809-1820.
(2) Consulting A. Adult periodontal disease cost analysis, a report commisioned by Listerine. 2008.
(3) Bensalah K, Montorsi F, Shariat SF. Challenges of cancer biomarker profiling. *Eur Urol* 2007; **52**: 1601-1609.
(4) Chapple IL. Periodontal disease diagnosis: current status and future developments. *J Dent* 1997; **25**: 3-15.
(5) Loos BG, Tjoa S. Host-derived diagnostic markers for periodontitis: do they exist in gingival crevice fluid? *Periodontol 2000* 2005; **39**: 53-72.
(6) Listgarten J, Emili A. Practical proteomic biomarker discovery: taking a step back to leap forward. *Drug Discov Today* 2005; **10**: 1697-1702.
(7) Zhang L, Henson BS, Camargo PM, Wong DT. The clinical value of salivary biomarkers for periodontal disease. *Periodontol 2000* 2009; **51**: 25-37.
(8) Oppenheim FG, Salih E, Siqueira WL, Zhang W, Helmerhorst EJ. Salivary proteome and its genetic polymorphisms. *Ann N Y Acad Sci* 2007; **1098**: 22-50.
(9) Ozmeric N. Advances in periodontal disease markers. *Clin Chim Acta* 2004; **343**: 1-16.
(10) Taba M, Jr., Kinney J, Kim AS, Giannobile WV. Diagnostic biomarkers for oral and periodontal diseases. *Dent Clin North Am* 2005; **49**: 551-571, vi.
(11) Chapple IL, Glenwright HD, Matthews JB, Thorpe GH, Lumley PJ. Site-specific alkaline phosphatase levels in gingival crevicular fluid in health and gingivitis: cross-sectional studies. *J Clin Periodontol* 1994; **21**: 409-414.
(12) Chapple IL, Socransky SS, Dibart S, Glenwright HD, Matthews JB. Chemiluminescent assay of alkaline phosphatase in human gingival crevicular fluid: investigations with an experimental gingivitis model and studies on the source of the enzyme within crevicular fluid. *J Clin Periodontol* 1996; **23**: 587-594.
(13) Chapple IL, Garner I, Saxby MS, Moscrop H, Matthews JB. Prediction and diagnosis of attachment loss by enhanced chemiluminescent assay of crevicular fluid alkaline phosphatase levels. *J Clin Periodontol* 1999; **26**: 190-198.
(14) Hanioka T, Takaya K, Matsumori Y, Matsuse R, Shizukuishi S. Relationship of the substance P to indicators of host response in human gingival crevicular fluid. *J Clin Periodontol* 2000; **27**: 262-266.
(15) Nakashima K, Giannopoulou C, Andersen E, et al. A longitudinal study of various crevicular fluid components as markers of periodontal disease activity. *J Clin Periodontol* 1996; **23**: 832-838.

(16) Nakashima K, Roehrich N, Cimasoni G. Osteocalcin, prostaglandin E2 and alkaline phosphatase in gingival crevicular fluid: their relations to periodontal status. *J Clin Periodontol* 1994; **21**: 327-333.
(17) Wright HJ, Chapple IL, Matthews JB. Levels of TGFbeta1 in gingival crevicular fluid during a 21-day experimental model of gingivitis. *Oral Dis* 2003; **9**: 88-94.
(18) Grbic JT, Lamster IB, Fine JB, et al. Changes in gingival crevicular fluid levels of immunoglobulin A following therapy: association with attachment loss. *J Periodontol* 1999; **70**: 1221-1227.
(19) Grbic JT, Singer RE, Jans HH, Celenti RS, Lamster IB. Immunoglobulin isotypes in gingival crevicular fluid: possible protective role of IgA. *J Periodontol* 1995; **66**: 55-61.
(20) Lamster IB. Evaluation of components of gingival crevicular fluid as diagnostic tests. *Ann Periodontol* 1997; **2**: 123-137.
(21) Lamster IB, Celenti R, Ebersole JL. The relationship of serum IgG antibody titers to periodontal pathogens to indicators of the host response in crevicular fluid. *J Clin Periodontol* 1990; **17**: 419-425.
(22) Lamster IB, Harper DS, Fiorello LA, Oshrain RL, Celenti RS, Gordon JM. Lysosomal and cytoplasmic enzyme activity, crevicular fluid volume, and clinical parameters characterizing gingival sites with shallow to intermediate probing depths. *J Periodontol* 1987; **58**: 614-621.
(23) Lamster IB, Holmes LG, Gross KB, et al. The relationship of beta-glucuronidase activity in crevicular fluid to probing attachment loss in patients with adult periodontitis. Findings from a multicenter study. *J Clin Periodontol* 1995; **22**: 36-44.
(24) Lamster IB, Oshrain RL, Harper DS, Celenti RS, Hovliaras CA, Gordon JM. Enzyme activity in crevicular fluid for detection and prediction of clinical attachment loss in patients with chronic adult periodontitis. Six month results. *J Periodontol* 1988; **59**: 516-523.
(25) Lamster IB, Wallenstein S, Sengupta S, Duffy T. Within-mouth correlations for indicators of the host response in gingival crevicular fluid. *Arch Oral Biol* 1990; **35**: 779-783.
(26) Wolff LF, Koller NJ, Smith QT, Mathur A, Aeppli D. Subgingival temperature: relation to gingival crevicular fluid enzymes, cytokines, and subgingival plaque micro-organisms. *J Clin Periodontol* 1997; **24**: 900-906.
(27) Chen HY, Cox SW, Eley BM. Cathepsin B, alpha2-macroglobulin and cystatin levels in gingival crevicular fluid from chronic periodontitis patients. *J Clin Periodontol* 1998; **25**: 34-41.
(28) Cox SW, Eley BM. Cathepsin B/L-, elastase-, tryptase-, trypsin- and dipeptidyl peptidase IV-like activities in gingival crevicular fluid. A comparison of levels before and after basic periodontal treatment of chronic periodontitis patients. *J Clin Periodontol* 1992; **19**: 333-339.
(29) Eley BM, Cox SW. The relationship between gingival crevicular fluid cathepsin B activity and periodontal attachment loss in chronic periodontitis patients: a 2-year longitudinal study. *J Periodontal Res* 1996; **31**: 381-392.
(30) Ichimaru E, Tanoue M, Tani M, et al. Cathepsin B in gingival crevicular fluid of adult periodontitis patients: identification by immunological and enzymological methods. *Inflamm Res* 1996; **45**: 277-282.
(31) Kennett CN, Cox SW, Eley BM. Investigations into the cellular contribution to host tissue proteases and inhibitors in gingival crevicular fluid. *J Clin Periodontol* 1997; **24**: 424-431.
(32) Kunimatsu K, Yamamoto K, Ichimaru E, Kato Y, Kato I. Cathepsins B, H and L activities in gingival crevicular fluid from chronic adult periodontitis patients and experimental gingivitis subjects. *J Periodontal Res* 1990; **25**: 69-73.
(33) Buduneli N, Vardar S, Atilla G, Sorsa T, Luoto H, Baylas H. Gingival crevicular fluid matrix metalloproteinase-8 levels following adjunctive use of meloxicam and initial phase of periodontal therapy. *J Periodontol* 2002; **73**: 103-109.
(34) Golub LM, Siegel K, Ramamurthy NS, Mandel ID. Some characteristics of collagenase activity in gingival crevicular fluid and its relationship to gingival diseases in humans. *J Dent Res* 1976; **55**: 1049-1057.

(35) Kiili M, Cox SW, Chen HY, et al. Collagenase-2 (MMP-8) and collagenase-3 (MMP-13) in adult periodontitis: molecular forms and levels in gingival crevicular fluid and immunolocalisation in gingival tissue. *J Clin Periodontol* 2002; **29**: 224-232.
(36) Kinane DF, Darby IB, Said S, et al. Changes in gingival crevicular fluid matrix metalloproteinase-8 levels during periodontal treatment and maintenance. *J Periodontal Res* 2003; **38**: 400-404.
(37) Lee W, Aitken S, Kulkarni G, et al. Collagenase activity in recurrent periodontitis: relationship to disease progression and doxycycline therapy. *J Periodontal Res* 1991; **26**: 479-485.
(38) Lee W, Aitken S, Sodek J, McCulloch CA. Evidence of a direct relationship between neutrophil collagenase activity and periodontal tissue destruction in vivo: role of active enzyme in human periodontitis. *J Periodontal Res* 1995; **30**: 23-33.
(39) Liu CM, Hou LT. Collagenase activity in the gingival crevicular fluid of periodontal patients. *J Formos Med Assoc* 1993; **92**: 157-164.
(40) Mancini S, Romanelli R, Laschinger CA, Overall CM, Sodek J, McCulloch CA. Assessment of a novel screening test for neutrophil collagenase activity in the diagnosis of periodontal diseases. *J Periodontol* 1999; **70**: 1292-1302.
(41) Nakamura T, Kido J, Kido R, et al. The association of calprotectin level in gingival crevicular fluid with gingival index and the activities of collagenase and aspartate aminotransferase in adult periodontitis patients. *J Periodontol* 2000; **71**: 361-367.
(42) Nomura T, Ishii A, Oishi Y, Kohma H, Hara K. Tissue inhibitors of metalloproteinases level and collagenase activity in gingival crevicular fluid: the relevance to periodontal diseases. *Oral Dis* 1998; **4**: 231-240.
(43) Persson L, Bergstrom J, Gustafsson A. Effect of tobacco smoking on neutrophil activity following periodontal surgery. *J Periodontol* 2003; **74**: 1475-1482.
(44) Romanelli R, Mancini S, Laschinger C, Overall CM, Sodek J, McCulloch CA. Activation of neutrophil collagenase in periodontitis. *Infect Immun* 1999; **67**: 2319-2326.
(45) Teng YT, Sodek J, McCulloch CA. Gingival crevicular fluid gelatinase and its relationship to periodontal disease in human subjects. *J Periodontal Res* 1992; **27**: 544-552.
(46) Gazi MI, Cox SW, Clark DT, Eley BM. Comparison of host tissue and bacterial dipeptidyl peptidases in human gingival crevicular fluid by analytical isoelectric focusing. *Arch Oral Biol* 1995; **40**: 731-736.
(47) Armitage GC, Jeffcoat MK, Chadwick DE, et al. Longitudinal evaluation of elastase as a marker for the progression of periodontitis. *J Periodontol* 1994; **65**: 120-128.
(48) Bader HI, Boyd RL. Long-term monitoring of adult periodontitis patients in supportive periodontal therapy: correlation of gingival crevicular fluid proteases with probing attachment loss. *J Clin Periodontol* 1999; **26**: 99-105.
(49) Darany DG, Beck FM, Walters JD. The relationship of gingival fluid leukocyte elastase activity to gingival fluid flow rate. *J Periodontol* 1992; **63**: 743-747.
(50) Figueredo CM, Areas A, Miranda LA, Fischer RG, Gustafsson A. The short-term effectiveness of non-surgical treatment in reducing protease activity in gingival crevicular fluid from chronic periodontitis patients. *J Clin Periodontol* 2004; **31**: 615-619.
(51) Figueredo CM, Gustafsson A. Activity and inhibition of elastase in GCF. *J Clin Periodontol* 1998; **25**: 531-535.
(52) Giannopoulou C, Andersen E, Demeurisse C, Cimasoni G. Neutrophil elastase and its inhibitors in human gingival crevicular fluid during experimental gingivitis. *J Dent Res* 1992; **71**: 359-363.
(53) Gustafsson A, Asman B, Bergstrom K. Altered relation between granulocyte elastase and alpha-2-macroglobulin in gingival crevicular fluid from sites with periodontal destruction. *J Clin Periodontol* 1994; **21**: 17-21.

(54) Gustafsson A, Asman B, Bergstrom K. Elastase and lactoferrin in gingival crevicular fluid: possible indicators of a granulocyte-associated specific host response. *J Periodontal Res* 1994; **29**: 276-282.
(55) Gustafsson A, Asman B, Bergstrom K, Soder PO. Granulocyte elastase in gingival crevicular fluid. A possible discriminator between gingivitis and periodontitis. *J Clin Periodontol* 1992; **19**: 535-540.
(56) Herrmann JM, Gonzales JR, Boedeker RH, Vonholdt J, Meyle J. Microassay for the detection of elastase activity in the gingival crevice. *J Clin Periodontol* 2001; **28**: 31-37.
(57) Ingman T, Sorsa T, Kangaspunta P, Konttinen YT. Elastase and alpha-1-proteinase inhibitor in gingival crevicular fluid and gingival tissue in adult and juvenile periodontitis. *J Periodontol* 1994; **65**: 702-709.
(58) Jin L, Soder B, Corbet EF. Interleukin-8 and granulocyte elastase in gingival crevicular fluid in relation to periodontopathogens in untreated adult periodontitis. *J Periodontol* 2000; **71**: 929-939.
(59) Jin LJ, Soder PO, Asman B, Bergstrom K. Granulocyte elastase in gingival crevicular fluid: improved monitoring of the site-specific response to treatment in patients with destructive periodontitis. *J Clin Periodontol* 1995; **22**: 240-246.
(60) Jin LJ, Soder PO, Leung WK, et al. Granulocyte elastase activity and PGE2 levels in gingival crevicular fluid in relation to the presence of subgingival periodontopathogens in subjects with untreated adult periodontitis. *J Clin Periodontol* 1999; **26**: 531-540.
(61) Murray MC, Mooney J, Kinane DF. The relationship between elastase and lactoferrin in healthy, gingivitis and periodontitis sites. *Oral Dis* 1995; **1**: 106-109.
(62) Nakamura-Minami M, Furuichi Y, Ishikawa K, Mitsuzono-Tofuku Y, Izumi Y. Changes of alpha1-protease inhibitor and secretory leukocyte protease inhibitor levels in gingival crevicular fluid before and after non-surgical periodontal treatment. *Oral Dis* 2003; **9**: 249-254.
(63) Palcanis KG, Larjava IK, Wells BR, et al. Elastase as an indicator of periodontal disease progression. *J Periodontol* 1992; **63**: 237-242.
(64) Persson L, Bergstrom J, Gustafsson A, Asman B. Tobacco smoking and gingival neutrophil activity in young adults. *J Clin Periodontol* 1999; **26**: 9-13.
(65) Persson L, Bergstrom J, Ito H, Gustafsson A. Tobacco smoking and neutrophil activity in patients with periodontal disease. *J Periodontol* 2001; **72**: 90-95.
(66) Smith QT, Harriman L, Au GS, et al. Neutrophil elastase in crevicular fluid: comparison of a middle-aged general population with healthy and periodontitis groups. *J Clin Periodontol* 1995; **22**: 935-941.
(67) Jones OA, Cheung VL. An introduction to metabolomics and its potential application in veterinary science. *Comp Med* 2007; **57**: 436-442.
(68) Steiner M, Hodes MZ, Shreve M, Sundberg S, Edson JR. Postoperative stroke in a child with cerebral palsy heterozygous for factor V Leiden. *J Pediatr Hematol Oncol* 2000; **22**: 262-264.
(69) Takashiba S, Naruishi K. Gene polymorphisms in periodontal health and disease. *Periodontol 2000* 2006; **40**: 94-106.
(70) Nielsen R. Population genetic analysis of ascertained SNP data. *Hum Genomics* 2004; **1**: 218-224.
(71) Kim YJ, Viana AC, Curtis KM, et al. Association of haplotypes in the IL8 gene with susceptibility to chronic periodontitis in a Brazilian population. *Clin Chim Acta*; **411**: 1264-1268.
(72) Scapoli C, Borzani I, Guarnelli ME, et al. IL-1 gene cluster is not linked to aggressive periodontitis. *J Dent Res*; **89**: 457-461.
(73) Cury PR, Horewicz VV, Ferrari DS, et al. Evaluation of the effect of tumor necrosis factor-alpha gene polymorphism on the risk of peri-implantitis: a case-control study. *Int J Oral Maxillofac Implants* 2009; **24**: 1101-1105.

(74) Shete AR, Joseph R, Vijayan NN, Srinivas L, Banerjee M. Association of single nucleotide gene polymorphism at interleukin-1beta +3954, -511, and -31 in chronic periodontitis and aggressive periodontitis in Dravidian ethnicity. *J Periodontol*; **81**: 62-69.
(75) Noack B, Gorgens H, Lorenz K, Schackert HK, Hoffmann T. TLR4 and IL-18 gene variants in chronic periodontitis: impact on disease susceptibility and severity. *Immunol Invest* 2009; **38**: 297-310.
(76) Lopez NJ, Valenzuela CY, Jara L. Interleukin-1 gene cluster polymorphisms associated with periodontal disease in type 2 diabetes. *J Periodontol* 2009; **80**: 1590-1598.
(77) Saleh TA, Stephen L, Kotze M, Pretorius A. The composite interleukin-1 genotype in South Africa. *SADJ* 2009; **64**: 170-173.
(78) Xiao LM, Yan YX, Xie CJ, et al. Association among interleukin-6 gene polymorphism, diabetes and periodontitis in a Chinese population. *Oral Dis* 2009; **15**: 547-553.
(79) Kim YJ, Viana AC, Curtis KM, Orrico SR, Cirelli JA, Scarel-Caminaga RM. Lack of association of a functional polymorphism in the interleukin 8 gene with susceptibility to periodontitis. *DNA Cell Biol* 2009; **28**: 185-190.
(80) Kaarthikeyan G, Jayakumar ND, Padmalatha O, Sheeja V, Sankari M, Anandan B. Analysis of the association between interleukin-1beta (+3954) gene polymorphism and chronic periodontitis in a sample of the south Indian population. *Indian J Dent Res* 2009; **20**: 37-40.
(81) Hu KF, Huang KC, Ho YP, et al. Interleukin-10 (-592 C/A) and interleukin-12B (+16974 A/C) gene polymorphisms and the interleukin-10 ATA haplotype are associated with periodontitis in a Taiwanese population. *J Periodontal Res* 2009; **44**: 378-385.
(82) Nibali L, D'Aiuto F, Donos N, et al. Association between periodontitis and common variants in the promoter of the interleukin-6 gene. *Cytokine* 2009; **45**: 50-54.
(83) Andreiotelli M, Koutayas SO, Madianos PN, Strub JR. Relationship between interleukin-1 genotype and peri-implantitis: a literature review. *Quintessence Int* 2008; **39**: 289-298.
(84) Trombone AP, Cardoso CR, Repeke CE, et al. Tumor necrosis factor-alpha -308G/A single nucleotide polymorphism and red-complex periodontopathogens are independently associated with increased levels of tumor necrosis factor-alpha in diseased periodontal tissues. *J Periodontal Res* 2009; **44**: 598-608.
(85) Noack B, Gorgens H, Lorenz K, Ziegler A, Hoffmann T, Schackert HK. TLR4 and IL-18 gene variants in aggressive periodontitis. *J Clin Periodontol* 2008; **35**: 1020-1026.
(86) Holla LI, Fassmann A, Augustin P, Halabala T, Znojil V, Vanek J. The association of interleukin-4 haplotypes with chronic periodontitis in a Czech population. *J Periodontol* 2008; **79**: 1927-1933.
(87) Claudino M, Trombone AP, Cardoso CR, et al. The broad effects of the functional IL-10 promoter-592 polymorphism: modulation of IL-10, TIMP-3, and OPG expression and their association with periodontal disease outcome. *J Leukoc Biol* 2008; **84**: 1565-1573.
(88) Fiebig A, Jepsen S, Loos BG, et al. Polymorphisms in the interleukin-1 (IL1) gene cluster are not associated with aggressive periodontitis in a large Caucasian population. *Genomics* 2008; **92**: 309-315.
(89) Ferreira SB, Jr., Trombone AP, Repeke CE, et al. An interleukin-1beta (IL-1beta) single-nucleotide polymorphism at position 3954 and red complex periodontopathogens independently and additively modulate the levels of IL-1beta in diseased periodontal tissues. *Infect Immun* 2008; **76**: 3725-3734.
(90) Cullinan MP, Westerman B, Hamlet SM, et al. Progression of periodontal disease and interleukin-10 gene polymorphism. *J Periodontal Res* 2008; **43**: 328-333.
(91) Akman A, Sallakci N, Kacaroglu H, et al. Relationship between periodontal findings and the TNF-alpha Gene 1031T/C polymorphism in Turkish patients with Behcet's disease. *J Eur Acad Dermatol Venereol* 2008; **22**: 950-957.

(92) Takeuchi-Hatanaka K, Ohyama H, Nishimura F, et al. Polymorphisms in the 5' flanking region of IL12RB2 are associated with susceptibility to periodontal diseases in the Japanese population. *J Clin Periodontol* 2008; **35**: 317-323.
(93) Komatsu Y, Galicia JC, Kobayashi T, Yamazaki K, Yoshie H. Association of interleukin-1 receptor antagonist +2018 gene polymorphism with Japanese chronic periodontitis patients using a novel genotyping method. *Int J Immunogenet* 2008; **35**: 165-170.
(94) Struch F, Dau M, Schwahn C, Biffar R, Kocher T, Meisel P. Interleukin-1 gene polymorphism, diabetes, and periodontitis: results from the Study of Health in Pomerania (SHIP). *J Periodontol* 2008; **79**: 501-507.
(95) Nibali L, Griffiths GS, Donos N, et al. Association between interleukin-6 promoter haplotypes and aggressive periodontitis. *J Clin Periodontol* 2008; **35**: 193-198.
(96) Mellati E, Arab HR, Tavakkol-Afshari J, Ebadian AR, Radvar M. Analysis of -1082 IL-10 gene polymorphism in Iranian patients with generalized aggressive periodontitis. *Med Sci Monit* 2007; **13**: CR510-514.
(97) Akman A, Ekinci NC, Kacaroglu H, Yavuzer U, Alpsoy E, Yegin O. Relationship between periodontal findings and specific polymorphisms of interleukin-1alpha and -1beta in Turkish patients with Behcet's disease. *Arch Dermatol Res* 2008; **300**: 19-26.
(98) Kara N, Keles GC, Sumer P, et al. Association of the polymorphisms in promoter and intron regions of the interleukin-4 gene with chronic periodontitis in a Turkish population. *Acta Odontol Scand* 2007; **65**: 292-297.
(99) Maria de Freitas N, Imbronito AV, Neves AC, Nunes FD, Pustiglioni FE, Lotufo RF. Analysis of IL-1A(-889) and TNFA(-308) gene polymorphism in Brazilian patients with generalized aggressive periodontitis. *Eur Cytokine Netw* 2007; **18**: 142-147.
(100) Gonzales JR, Mann M, Stelzig J, Bodeker RH, Meyle J. Single-nucleotide polymorphisms in the IL-4 and IL-13 promoter region in aggressive periodontitis. *J Clin Periodontol* 2007; **34**: 473-479.
(101) Tervonen T, Raunio T, Knuuttila M, Karttunen R. Polymorphisms in the CD14 and IL-6 genes associated with periodontal disease. *J Clin Periodontol* 2007; **34**: 377-383.
(102) Sumer AP, Kara N, Keles GC, Gunes S, Koprulu H, Bagci H. Association of interleukin-10 gene polymorphisms with severe generalized chronic periodontitis. *J Periodontol* 2007; **78**: 493-497.
(103) Savarrio L, Donati M, Carr C, Kinane DF, Berglundh T. Interleukin-24, RANTES and CCR5 gene polymorphisms are not associated with chronic adult periodontitis. *J Periodontal Res* 2007; **42**: 152-158.
(104) Babel N, Cherepnev G, Babel D, et al. Analysis of tumor necrosis factor-alpha, transforming growth factor-beta, interleukin-10, IL-6, and interferon-gamma gene polymorphisms in patients with chronic periodontitis. *J Periodontol* 2006; **77**: 1978-1983.
(105) Agrawal AA, Kapley A, Yeltiwar RK, Purohit HJ. Assessment of single nucleotide polymorphism at IL-1A+4845 and IL-1B+3954 as genetic susceptibility test for chronic periodontitis in Maharashtrian ethnicity. *J Periodontol* 2006; **77**: 1515-1521.
(106) Galicia JC, Tai H, Komatsu Y, Shimada Y, Ikezawa I, Yoshie H. Interleukin-6 receptor gene polymorphisms and periodontitis in a non-smoking Japanese population. *J Clin Periodontol* 2006; **33**: 704-709.
(107) Jansson H, Lyssenko V, Gustavsson A, et al. Analysis of the interleukin-1 and interleukin-6 polymorphisms in patients with chronic periodontitis. A pilot study. *Swed Dent J* 2006; **30**: 17-23.
(108) Suzuki A, Ji G, Numabe Y, Ishii K, Muramatsu M, Kamoi K. Large-scale investigation of genomic markers for severe periodontitis. *Odontology* 2004; **92**: 43-47.
(109) Covani U, Marconcini S, Giacomelli L, Sivozhelevov V, Barone A, Nicolini C. Bioinformatic prediction of leader genes in human periodontitis. *J Periodontol* 2008; **79**: 1974-1983.

(110) Michalowicz BS, Aeppli D, Virag JG, et al. Periodontal findings in adult twins. *J Periodontol* 1991; **62**: 293-299.
(111) Schaefer AS, Richter GM, Nothnagel M, et al. A genome-wide association study identifies GLT6D1 as a susceptibility locus for periodontitis. *Hum Mol Genet*; **19**: 553-562.
(112) Papapanou PN, Abron A, Verbitsky M, et al. Gene expression signatures in chronic and aggressive periodontitis: a pilot study. *Eur J Oral Sci* 2004; **112**: 216-223.
(113) Demmer RT, Behle JH, Wolf DL, et al. Transcriptomes in healthy and diseased gingival tissues. *J Periodontol* 2008; **79**: 2112-2124.
(114) Papapanou PN, Behle JH, Kebschull M, et al. Subgingival bacterial colonization profiles correlate with gingival tissue gene expression. *BMC Microbiol* 2009; **9**: 221.
(115) Kebschull M, Demmer R, Behle JH, et al. Granulocyte chemotactic protein 2 (gcp-2/cxcl6) complements interleukin-8 in periodontal disease. *J Periodontal Res* 2009; **44**: 465-471.
(116) Offenbacher S, Barros SP, Paquette DW, et al. Gingival transcriptome patterns during induction and resolution of experimental gingivitis in humans. *J Periodontol* 2009; **80**: 1963-1982.
(117) Beikler T, Peters U, Prior K, Eisenacher M, Flemmig TF. Gene expression in periodontal tissues following treatment. *BMC Med Genomics* 2008; **1**: 30.
(118) Kim DM, Ramoni MF, Nevins M, Fiorellini JP. The gene expression profile in refractory periodontitis patients. *J Periodontol* 2006; **77**: 1043-1050.
(119) Papapanou PN, Sedaghatfar MH, Demmer RT, et al. Periodontal therapy alters gene expression of peripheral blood monocytes. *J Clin Periodontol* 2007; **34**: 736-747.
(120) Matthews JB, Wright HJ, Roberts A, Ling-Mountford N, Cooper PR, Chapple IL. Neutrophil hyper-responsiveness in periodontitis. *J Dent Res* 2007; **86**: 718-722.
(121) Matthews JB, Wright HJ, Roberts A, Cooper PR, Chapple IL. Hyperactivity and reactivity of peripheral blood neutrophils in chronic periodontitis. *Clin Exp Immunol* 2007; **147**: 255-264.
(122) Wright HJ, Matthews JB, Chapple IL, Ling-Mountford N, Cooper PR. Periodontitis associates with a type 1 IFN signature in peripheral blood neutrophils. *J Immunol* 2008; **181**: 5775-5784.
(123) de Pablo P, Dietrich T, McAlindon TE. Association of periodontal disease and tooth loss with rheumatoid arthritis in the US population. *J Rheumatol* 2008; **35**: 70-76.
(124) de Pablo P, Chapple IL, Buckley CD, Dietrich T. Periodontitis in systemic rheumatic diseases. *Nat Rev Rheumatol* 2009; **5**: 218-224.
(125) Bartold PM, Marshall RI, Haynes DR. Periodontitis and rheumatoid arthritis: a review. *J Periodontol* 2005; **76**: 2066-2074.
(126) Lundy FT, Orr DF, Shaw C, Lamey PJ, Linden GJ. Detection of individual human neutrophil alpha-defensins (human neutrophil peptides 1, 2 and 3) in unfractionated gingival crevicular fluid--a MALDI-MS approach. *Mol Immunol* 2005; **42**: 575-579.
(127) Dommisch H, Vorderwulbecke S, Eberhard J, Steglich M, Jepsen S. SELDI-TOF-MS of gingival crevicular fluid--a methodological approach. *Arch Oral Biol* 2009; **54**: 803-809.
(128) Pisano E, Cabras T, Montaldo C, et al. Peptides of human gingival crevicular fluid determined by HPLC-ESI-MS. *Eur J Oral Sci* 2005; **113**: 462-468.
(129) Kojima T, Andersen E, Sanchez JC, et al. Human gingival crevicular fluid contains MRP8 (S100A8) and MRP14 (S100A9), two calcium-binding proteins of the S100 family. *J Dent Res* 2000; **79**: 740-747.
(130) Ngo LH, Veith PD, Chen YY, Chen D, Darby IB, Reynolds EC. Mass spectrometric analyses of peptides and proteins in human gingival crevicular fluid. *J Proteome Res*; **9**: 1683-1693.
(131) Wu Y, Shu R, Luo LJ, Ge LH, Xie YF. Initial comparison of proteomic profiles of whole unstimulated saliva obtained from generalized aggressive periodontitis patients and healthy control subjects. *J Periodontal Res* 2009; **44**: 636-644.
(132) Bostanci N, Heywood W, Mills K, Parkar M, Nibali L, Donos N. Application of label-free absolute quantitative proteomics in human gingival crevicular fluid by LC/MS E (gingival exudatome). *J Proteome Res*; **9**: 2191-2199.

(133) Grant MM, Creese AJ, Barr G, et al. Proteomic analysis of a noninvasive human model of acute inflammation and its resolution: the twenty-one day gingivitis model. *J Proteome Res*; **9**: 4732-4744.
(134) Brock GR, Butterworth CJ, Matthews JB, Chapple IL. Local and systemic total antioxidant capacity in periodontitis and health. *J Clin Periodontol* 2004; **31**: 515-521.
(135) Grant MM, Brock GR, Matthews JB, Chapple IL. Crevicular fluid glutathione levels in periodontitis and the effect of non-surgical therapy. *J Clin Periodontol*; **37**: 17-23.
(136) Chapple IL, Mason GI, Garner I, et al. Enhanced chemiluminescent assay for measuring the total antioxidant capacity of serum, saliva and crevicular fluid. *Ann Clin Biochem* 1997; **34 (Pt 4)**: 412-421.
(137) Barnes VM, Teles R, Trivedi HM, et al. Acceleration of purine degradation by periodontal diseases. *J Dent Res* 2009; **88**: 851-855.
(138) Gronert K, Kantarci A, Levy BD, et al. A molecular defect in intracellular lipid signaling in human neutrophils in localized aggressive periodontal tissue damage. *J Immunol* 2004; **172**: 1856-1861.
(139) Fukushima A, Kusano M, Redestig H, Arita M, Saito K. Integrated omics approaches in plant systems biology. *Curr Opin Chem Biol* 2009; **13**: 532-538.
(140) Schadt EE, Lum PY. Thematic review series: systems biology approaches to metabolic and cardiovascular disorders. Reverse engineering gene networks to identify key drivers of complex disease phenotypes. *J Lipid Res* 2006; **47**: 2601-2613.
(141) De Smet R, Marchal K. Advantages and limitations of current network inference methods. *Nat Rev Microbiol*; **8**: 717-729.
(142) de Lillo A, Ashley FP, Palmer RM, et al. Novel subgingival bacterial phylotypes detected using multiple universal polymerase chain reaction primer sets. *Oral Microbiol Immunol* 2006; **21**: 61-68.
(143) Keijser BJ, Zaura E, Huse SM, et al. Pyrosequencing analysis of the oral microflora of healthy adults. *J Dent Res* 2008; **87**: 1016-1020.
(144) Nibali L, Donos N, Henderson B. Periodontal infectogenomics. *J Med Microbiol* 2009; **58**: 1269-1274.

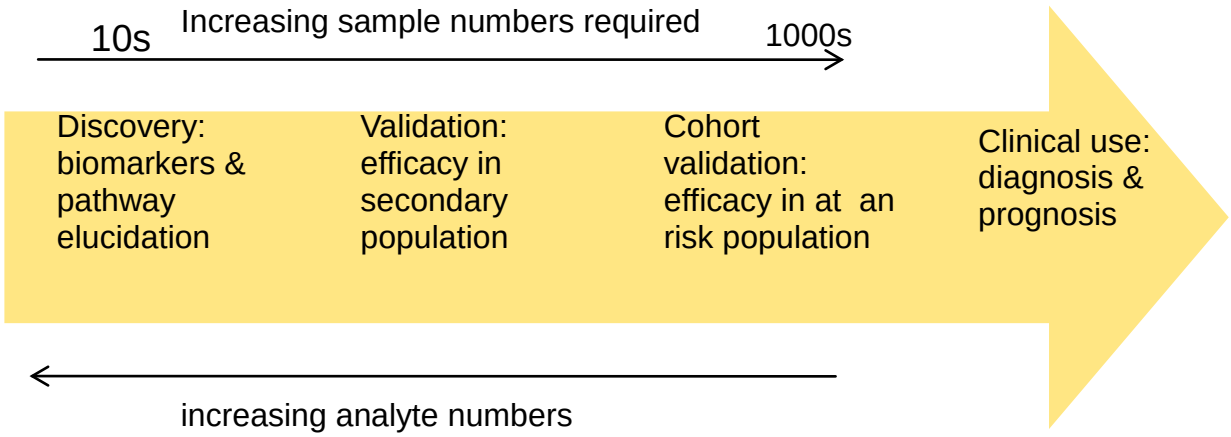

Figure 1. The path through which biomarkers must travel to be useful for the clinician. 'Omic technologies can be used at all stages but have most impact on the initial stages.

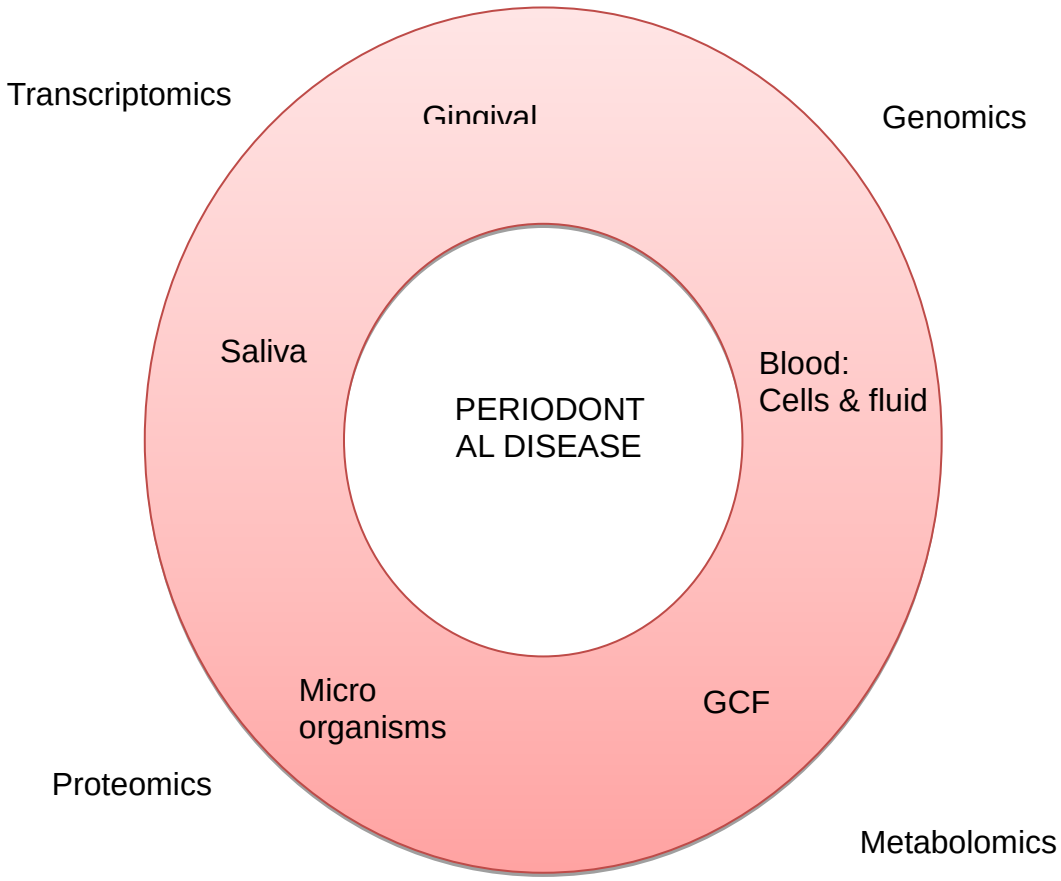

Figure 2. The compartments available for studying periodontal disease using 'omic technologies

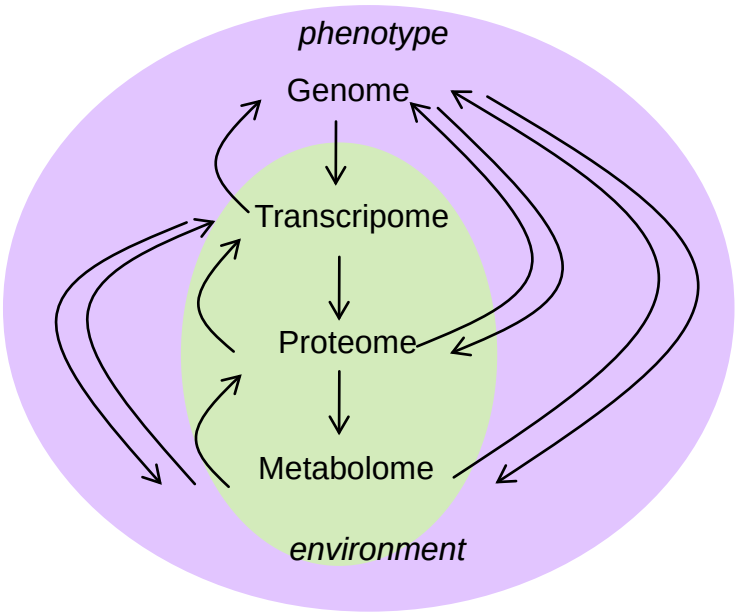

Figure 3. The interplay of the different compartments studied by ‘omic technologies.

Approach	Study population(s)	Platform of evaluation	Number of species investigated	Source of biological samples	Number of subjects in study	Conclusion	Reference
Genomics	Severe periodontitis comparison to healthy subjects	TaqMan PCR	637 SNPs	whole peripheral blood	n=22 severe periodontitis patients, n=19 controls	GNRH1, PIK3R1, DPP4, FGL2, and CALCR significantly associated with disease	114
	Aggressive periodontitis	Affymetrix Gene Chip Human Mapping 500K Array Set	GWAS of 500,000 SNPs	whole peripheral blood & gingival tissue	n=141, 142, 164 assessment in two cohorts by GWAS and validated in a third cohort (affected teeth 2-6%)	GLT6D1 was significantly associated with disease	116
Transcriptomics	Chronic & aggressive periodontitis	Affymetrix Human Genome U-133 A arrays	22,000 transcripts	Biopsies of gingival tissue	n=1 localised chronic periodontitis, n=6 generalised chronic periodontitis n=1 localised aggressive periodontitis, n=6 generalised	Patients were clustered by pathogen presence rather than by disease type	117

				aggressive periodontitis		
Chronic & aggressive periodontitis	Affymetrix Human Genome U-133 Plus 2.0 arrays	47,000 transcripts	Biopsies of gingival tissue	n=90 (63 chronic & 27 aggressive) periodontitis patients	Gene ontology groups increased included apoptosis, antimicrobial humoral response, antigen presentation, regulation of metabolic processes, signal transduction, and angiogenesis	118
Chronic & aggressive periodontitis	Affymetrix Human Genome U-133 Plus 2.0 arrays	47,000 transcripts	Biopsies of gingival tissue	n=120 (65 chronic & 55 aggressive) patients	Commonalities & differences were found between gene expression and bacterial species present	119
Experimental gingivitis	Affymetrix Human Genome U-133	47,000 transcripts	Biopsies of gingival tissue	n=14 healthy volunteers	Differences in GO groups: neural processes, epithelial defences,	121

		Plus 2.0 arrays				angiogenesis & wound healing	
	Periodontal disease compared with control	Agilent 2100 Bioanalyzer and a human inflammation microarray	160 genes	Biopsies of gingival tissue	n=12 severe generalized chronic periodontitis, n=11controls	Activation of pathways regulating tissue damage & repair after treatment	122
	Refractory periodontitis	Affymetrix Human Genome U-133 A arrays	22,000 transcripts	Subepithelial connective tissue	n=7 refractory periodontitis & n=7 periodontally well-maintained patients	Increases in immune response, tissue modelling & apoptosis in disease in refactory patients	123
	Periodontitis patients undergoing treatment	Affymetrix Human Genome U-133 Plus 2.0 arrays	47,000 transcripts	Peripheral monocytes	n=15 patients	Changes in innate immunity, apoptosis & cell signalling were seen	124
	Periodontitis	Affymetrix Human Genome U-133 A arrays	22,000 transcripts	Peripheral neutrophils	n=19 patients	Type-1 interferon stimulated genes were increased	127
Proteomics	Periodontitis in	Electrophoresis	Not given	GCF, serum &	n=10 periodontitis patients, n=4	S100A8 and S100A9 represented major	134

	comparison to control	& MS		saliva	controls	differences between GCF and saliva	
	Periodontitis patients in maintenance phase	Electrophoresis & MS	66 proteins identified	GCF	n=12 patients	Identification of serum and cell derived proteins	135
	Aggressive periodontitis in comparison to control	Electrophoresis & MS	Not given	Saliva	n=5 aggressive periodontitis patients, n=5 controls	6 proteins were increased in saliva of periodontitis subjects, while 5 were decreased	136
	Generalized aggressive periodontitis	Quantitative MS	154 human, bacterial, fungal & viral proteins	GCF	n=5 aggressive periodontitis patients, n=5 controls	Human plastin-2 and Microbial proteins increased in disease, Annexin A1 increased in health	137
	Experimental gingivitis	Quantitative MS	202 human and bacterial proteins	GCF	n=10 healthy volunteers	Identification of 186 proteins including serum and cell derived species including plastin-2. Novel structural proteins for cilia and ribbon synapses found.	138
Metabolomics	Chronic periodontitis	MS	103 metabolites identified	GCF	n=22 patients samples collected included diseased	At diseased sites antioxidant, glutamine, di-&trisaccharide levels	147

					and healthy sites	decreased; amino acids (except glutamine), choline, glucose, polyamines, purine degradation & urea cycle metabolites increased	
	Localised aggressive periodontitis	MS	7 diacylglycerol species	Neutrophils	n=11 localised aggressive periodontitis, n=4 asymptomatic family members	Increased diacylglycerol species in disease compared to control	148
Systems Biology	Periodontitis	Data mining and cluster analysis	61 genes	In silico	Not relevant	5leader genes (or hubs) (NFkB1, CBL, GRB2, PIK3R1, RELA) identified	152

Table 1. Summary of data rich 'omics studies. Abbreviations: MS mass spectrometry; GCF gingival crevicular fluid.